



PhD THESIS in Bio-engineering

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First *Polymyxa betae* reference genome enabling the
characterization of the tripartite interaction with *Beet
necrotic yellow vein virus* and sugar beet.

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Mahillon, M., **Decroës, A.**, Lienard, C., Bragard, C., & Legrève, A. (2019). Full genome sequence of a new polymycovirus infecting *Fusarium redolens*. *Archives of Virology*, 164, 2215-2219. doi :10.1007/s00705-019-04301-1

Decroës, A., Bagayoko, I., Mahillon, M., Verhaegen, H., Lienard, C., Legrève, A., & Bragard, C. (2017). Detection of the *Rice stripe necrosis virus* Causing Rice Crinkle Disease and its Vector *Polymyxa graminis* f. sp. *colombiana* in Mali. *Plant Disease*, 101, 2155-2155. doi :10.1094/PDIS-06-17-0801-PDN

Clinckemaillie, A., **Decroës, A.**, van Aubel, G., Carrola Dos Santos, S., Renard, M-E., Van Cutsem, P., & Legrève, A. (2016). The novel elicitor COS-OGA enhances potato resistance to late blight. *Plant pathology*, 1-8. doi :10.1111/ppa.12641

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ABBREVIATIONS

aa - amino acid
AAI - amino acid identity
ABA - Absciscic acid
ABP - auxin binding protein
ACC - 1-aminocyclopropane-1-carboxylate
ANI - average nucleotide identity
Anks - proteins containing ankyrin repeats
APXs - ascorbate peroxidases
Avr - avirulence gene
BaMMV - Barley mild mosaic virus
BdMoV - Burdock mottle virus
BGAL - beta-galactosidase
BNYVV - Beet Necrotic Yellow Vein Virus
BSBMV - Beet soil-borne mosaic virus
BSBV - Beet soil-borne virus
BSMV - Barley stripe mosaic virus
BVQ - Beet virus Q
CatSper - cation channel sperm-associated proteins
CAZy - Carbohydrate-Active enZymes
CC-NB-LRR - coiled-coil nucleotide binding leucine rich repeat
cDNA - complementary DNA
CK - cytokinin
COG - clusters of orthologous groups
CP - coat protein
CP-RT - Coat Protein Read-Through
CTAB - cetyltrimethylammonium bromide
cv - cultivar
DAMPs - damage-associated molecular patterns
DEGs - differentially expressed genes
DNA - deoxyribonucleic acid
dNTP - deoxyribonucleotide triphosphate
dpi - days post-inoculation
ER - Endoplasmic reticulum
ERF - ethylene response factor 1
EST - Expressed Sequence Tags
ET - ethylene
ETI - effector triggered immunity
ETS - effector-triggered susceptibility
EXP - expansin
EXT - extensins
FA - fatty acid
GO - Gene ontology
GPCR - G protein-coupled receptor
GPI - Glycosylphosphatidylinositol
GRXs - glutaredoxins
GST - Glutathione-S-Transferase

ABBREVIATIONS

HEL - helicase
HSP - heat shock protein.
ISR - induced systemic resistance
ITS - internal transcribed spacer
JA - jasmonic acid
Kb - kilobase
kDa - kilo Dalton
KEGG - Kyoto Encyclopedia of Genes and Genomes
KO - KEGG Ortholog
LBD - lateral organ boundary domain
LD - lipid droplets
LLGs - Lorelei-like glycosylphosphatidylinositol-anchored
LOX - lipoxygenases
LR - lateral roots
LRR - leucine rich repeat
LSU - large ribosomal subunit
LZ - leucine zipper
M - molar
MAMPs - microbe-associated molecular patterns
MAPKs - mitogen-activated protein kinases
MeSA - methyl salicylate
mRNA - messenger RNA
MTI - MAMPS-triggered immunity
NBS - nucleotide-binding site
ncRNA - non-coding RNA
NES - nuclear export sequence
NLS - nuclear localization signal
NoLS - nucleolar localization signal
NPR1 - Nonexpressor of Pathogenesis-Related Genes 1
Nt - nucleotide
ORF - open reading frame
PAMPs - pathogen-associated molecular patterns
PB - *P. betae* alone
PBBN - *P. betae* and the BNYVV
PbBSMT - *P. brassicae* SABATH-type methyltransferase
PCR - polymerase chain reaction
PCV - Peanut clump virus
PGIP - pathogen polygalacturonase inhibitor gene
PME - pectin methylesterases
PMTV - Potato mop top virus
PR - pathogenesis-related
Pro - protease
proPEP - precursor of signal peptide
PRRs - pattern recognition receptors ()
PTI - PAMPs triggered immunity
PVX - Potato virus X
QS - quality score
R - resistance gene
RALFs - Arabidopsis Rapid Alkalinization Factors

RB - resistance breaking
 Rboh - respiratory burst oxidase homologs
 rDNA - ribosomal DNA
 RDR6 - RNA-dependent RNA polymerase 6
 RdRp - RNA-dependent RNA-polymerase
 RGAs - Resistance Genes Analogues
 RKs or RLKs - receptor(-like) kinases
 RLCKs - receptor-like cytoplasmic kinases
 RLP - receptor-like proteins
 RNA - ribonucleic acid
 RNQP - Regulated Non-Quarantine Pests
 ROS – reactive oxygen species
 RSNV - Rice stripe necrosis virus
 RT - read-through
 Or in “RT- PCR”- reverse transcription-polymerase chain reaction
 SA - salicylic acid
 SAMT - SA methyltransferases
 SAR - systemic acquired resistance
 SBWMV - Soil-borne wheat mosaic virus
 SCF - Skp1-Cullin-F-box
 siRNA - small interfering RNA
 SP - signal peptide
 SSU - small ribosomal subunit
 STK - serine-threonine kinase
 SUT/SUC - sucrose transport protein
 TE - transposable elements
 TF - transcription factors
 TGB - triple gene block
 TIR - Toll/Interleukin-1 receptor
 TM - transmembrane
 TNV - Tobacco necrosis virus
 UBQ - Polyubiquitin
 UTR - untranslated region
 VIGS - virus-induced gene silencing
 vRNP - viral ribonucleoprotein
 VSR - viral suppressor of RNA silencing
 wpi - of weeks post inoculation
 WSSMV - Wheat spindle streak mosaic virus
 wt - wild-type
 XTH - xyloglucan endo-transglycosylases
 ZF - zinc finger

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	III
SCIENTIFIC COMMUNICATIONS	V
CREDIT	VII
ABBREVIATIONS	IX
TABLE OF CONTENTS	XIII
TABLE OF FIGURES.....	XVI
TABLE OF TABLES.....	XVIII
GLOSSARY	XIX
GENERAL INTRODUCTION	1
1. Problem statement.....	1
2. Thesis outline	3
LITERATURE REVIEW	5
CHAPTER I. <i>Polymyxa betae</i> and the Plasmodiophorida Order	7
1. General considerations about plasmodiophorids.....	8
2. Taxonomic position of <i>Polymyxa betae</i>	9
3. Life cycle and Morphology	12
4. Host range.....	20
5. Genetics and diversity.....	22
6. <i>Polymyxa betae</i> - <i>Beta vulgaris</i> , a tangled relationship	24
7. Other plasmodiophorids parasites of major crops	25
CHAPTER II. Tripartite interaction in rhizomania: review	29
1. General considerations about rhizomania	30
2. The BNYVV and its interactions with host plants.....	33
3. Interactions of <i>Polymyxa</i> spp. with vectored viruses	53
4. Interactions of <i>Polymyxa</i> spp. with plants	60
5. Interactions of other plasmodiophorids with plants.....	62
CHAPTER III. The sugar beet and plant defenses	69
1. The sugar beet.....	70
2. Plant immunity.....	72
OBJECTIVES.....	77

TABLE OF CONTENTS

RESULTS.....	79
CHAPTER IV. The <i>Polymyxa betae</i> genome	81
First Draft Genome Sequence of a <i>Polymyxa</i> Genus Member, <i>Polymyxa betae</i> , the Protist Vector of Rhizomania	82
1. ABSTRACT	82
2. ANNOUNCEMENT	82
Metagenomics Approach for <i>Polymyxa betae</i> Genome Assembly Enables Comparative Analysis Towards Deciphering the Intracellular Parasitic Lifestyle of the Plasmodiophorids	85
1. ABSTRACT	86
2. INTRODUCTION.....	86
3. MATERIALS AND METHODS	89
4. RESULTS.....	96
5. DISCUSSION.....	111
6. CONCLUSIONS.....	115
CHAPTER V. Molecular interactions in the <i>Beta vulgaris</i> - <i>Polymyxa betae</i> -BNYVV tripartite interaction	117
1. ABSTRACT	119
2. INTRODUCTION.....	120
3. MATERIALS AND METHODS	123
4. RESULTS AND DISUSSION	129
5. CONCLUSIONS.....	152
GENERAL DISCUSSION, CONCLUSIONS AND PERSPECTIVES	155
CHAPTER VI. General discussion.....	157
1. Defense response of <i>Beta vulgaris</i> to <i>Polymyxa betae</i> infection....	158
2. Is <i>Polymyxa betae</i> an ancient pathogen?	168
3. The interaction between BNYVV and <i>Polymyxa betae</i>	170
CHAPTER VII. Conclusion and perspectives	177
1. Main conclusions.....	177
2. More phytomyxids genomes	178
3. Future studies are needed to complement transcriptomic approach	179
4. Identification of <i>Polymyxa betae</i> effectors	180
5. Clarify the mechanisms of virus transmission by <i>Polymyxa</i> spp.	181

REFERENCES.....	183
APPENDIX.....	229
1. Methodology adopted for document search in Chapter II.....	229
2. Supplementary information – Chapter IV.....	229
3. Supplementary information – Chapter V.....	256

TABLE OF FIGURES

Figure I-1. The current eukaryotic tree of life	10
Figure I-2. Diversity of the eukaryotic tree of life and sequencing projects ongoing or completed in February 2017	11
Figure I-3. Schematic representation of <i>P. betae</i> life cycle and representative pictures of each life stage observed in transmitted Nomarski microscopy ...	14
Figure I-4. TEM micrographs showing the penetration of a host cell by a <i>P. betae</i> zoospore	15
Figure I-5. Neighbor-Joining tree of plasmodiophorids ITS1, 5.8S and ITS2 rDNA sequences	23
Figure I-6. Comparison of the symptoms of rhizomania induced by BNYYV vectored by <i>P. betae</i> , clubroot induced by <i>P. brassicae</i> , and powdery scab induced by <i>S. subterranea</i>	28
Figure II-1. Representative pictures of the main rhizomania symptoms.....	32
Figure II-2. Representation of the genomic organization of the BNYYV	34
Figure II-3. Electron micrographs of BNYYV and PMTV particles labeled with anti-RT-immunogold conjugate.....	36
Figure II-4. Schematic representation of the organization of TGB1 in hordei-like viruses	38
Figure II-5. Schematic representation of the P25 protein showing the positions of important functional domains.....	40
Figure II-6. Transmission of soil-borne rod-shaped virions requires internalization by plasmodiophorids biflagellate zoospores.....	54
Figure IV-1 Schematic representation of the workflow of metagenomics analysis.. ..	91
Figure IV-2. Syntenic comparison between the mitochondrial genomes of <i>P. betae</i> , <i>P. brassicae</i> and <i>S. subterranea</i>	98
Figure IV-3. Comparison of COG categories predicted for <i>P. betae</i> (Pbet) draft genomes A26-41 and RES F41, <i>P. brassicae</i> (Pbra) e3 and <i>S. subterranea</i> (Ssub) SSUBK13 with COG from <i>B. natans</i> (Bnat), <i>Plasmodium falciparum</i> (Pfal) et <i>P. infestans</i> (Pinf)	101
Figure IV-4. Protein clustering from <i>P. betae</i> (Pbet) draft genomes A26-41 and RES F41, <i>P. brassicae</i> (Pbra) e3 and <i>S. subterranea</i> (Ssub) SSUBK13 with OrthoVenn2	104
Figure IV-5. Assignments of proteins predicted as secreted and as membrane proteins to selected general GO terms (level 2)	107
Figure V-1. Relative quantification of <i>P. betae</i> in sugar beet roots in three successive bioassays and visual assessment of <i>P. betae</i> life cycle in sugar beet roots	134

Figure V-2. Cell walls related genes expression in sugar beet in response to <i>P. betae</i> +- BNYVV inoculation.....	137
Figure V-3. Redox homeostasis in response to <i>P. betae</i> +- BNYVV inoculation.....	146
Figure V-4. Barplot of the mean fluorescence derived from LD in <i>B. vulgaris</i> and representative images of LD content observed in various <i>P. betae</i> life stages, without BNYVV.....	149
Figure V-5. RT-qPCR expression quantification of five genes related to oxidative stress regulation and seven plant defenses genes at 1, 7, 14 and dpi	151
Figure VI-1. Theoretical model of cell-wall-integrity sensing in sugar beet and effect of <i>P. betae</i> infection	163
Figure VI-2. Recapitulative theoretical model of the molecular events occurring in a compatible <i>P. betae</i> -sugar beet interaction	167
Figure VI-3. Overview of the transcriptional changes of defense-related genes in <i>P. betae</i> or <i>P. betae</i> + BNYVV sugar beet plants at 28 dpi	171

TABLE OF TABLES

Table I-1. Classification of the plasmodiophorids according to Cavalier-Smith et al. (2018)	9
Table I-2. Comparative table of the sporosori cell wall architectures reported for <i>P. betae</i> and for <i>P. graminis</i>	18
Table I-3. Plant species reported to be host of <i>P. betae</i>	21
Table II-1. Current BNYVV strains and their original types classified according to the sequences of CP, P25 and P31 genes as well as the presence or absence of RNA5 and their geographical distribution	43
Table II-2. Summary of principal omics-based studies addressing the plant response during infection by BNYVV	47
Table II-3. Viruses transmitted by <i>P. betae</i> and <i>P. graminis</i> and references of transmission assays	56
Table III-1 Taxonomy of the cultivated beets and wild relatives as proposed by Kadereit et al. (2006)	71
Table III-2. The 17 recognized families of pathogenesis-related (PR) protein	75
Table IV-1 Comparison of <i>P. betae</i> draft genomes A26-41 and RES F41 with other fully sequenced plasmodiophorids: <i>P. brassicae</i> e3, eH, ZJ-1 and <i>S. subterranea</i> SSUBK13	100
Table IV-2. Secretome and membranome prediction pipeline results for <i>P. betae</i> draft genomes A26-41 and RES F41, <i>P. brassicae</i> e3 and <i>S. subterranea</i> SSUBK13	105
Table V-1. Most enriched KEGG pathways in the up-regulated and/or down-regulated DEGs from <i>P. betae</i> alone (PB) and <i>P. betae</i> + BNYVV (PBBN) infected plants in comparison to mock-inoculated plants	131
Table V-2. DEGs belonging to pathogenesis-related proteins encoding genes in response to <i>P. betae</i> +- BNYVV inoculation	140
Table V-3. DEGs belonging to resistance genes analogues (RGAs) in response to <i>P. betae</i> +- BNYVV inoculation	141
Table V-4. DEGs belonging to defense related TF families in response to <i>P. betae</i> +- BNYVV inoculation	143

GLOSSARY

Some terms widely used in biosciences can have slightly different meaning depending of the field in which they are used. For example, ecologists have not necessary the same meaning of symbiosis as plant pathologists. Even in plant pathology, authors sometimes don't share the same exact meaning for some concepts. It's therefore useful here to define some important concepts used all throughout this thesis.

Plasmodiophorids. In this work, we'll refer to "plasmodiophorids" when speaking of the *Plasmodiophorida* order members (Hittorf et al., 2020).

Endophyte. "Microorganism (archaea, bacteria, fungi, oomycetes, protista) inhabiting the interior of plants (endosphere) irrespective of the function in association with the plant" (Brader et al., 2017).

Mutualism. "mutual beneficial relationship between host and microorganism" (Brader et al., 2017)

Commensal microorganism. "organism living on exudates and nutrients provided by the plant host without having a negative effect on the host" (Brader et al., 2017).

Pathogenicity. Ability for a microorganism to infect and produce a disease (D'Arcy et al., 2001). It supposes a harmful effect of the pathogen on the host.

Parasite. "an organism that lives in intimate association with another organism on which it depends for its nutrition; not necessarily a pathogen (contrasts with saprophyte)" (D'Arcy et al., 2001).

With respect to these definitions, an endophytic microorganism could be involved in a mutualistic, a commensal or a pathogenic relationship with its plant host.

GENERAL INTRODUCTION

1. Problem statement

The sugar beet (*Beta vulgaris* subsp. *vulgaris*) is the main mean of sugar production in temperate zones. It represents a gross product of more than 184 million tons per year, accounting for a large proportion of the sugar market in Europe (CEFS, 2021; FAOSTAT, 2021). Notwithstanding its high production in northern countries, this crop is threatened by competition from the production of sugar cane in tropical and sub-tropical zones, which is more productive and less expensive to process (European Commission, 2021). Nevertheless, given the high consumption of sugar in northern countries, the socio-economic impact of the sugar industry, the importance of sugar beet in rotations as a head crop and the renewed interest in local production and food security, there is a strategic need to maintain this crop in Europe. In addition, sugar beet byproducts can be incorporated into livestock feed (Evans and Messerschmidt, 2017) or be used for energy production (Panella, 2010). However, production losses should be particularly avoided, which means that disease-related challenges must be addressed.

One of these challenges is the control of rhizomania, a viral disease caused by the *Beet Necrotic Yellow Vein Virus* (BNYVV). Before the introduction of resistant varieties in the 1990's, rhizomania was the most important disease, hampering sugar beet production (Scholten and Lange, 2000). Since there are no approved crop protection products which are effective against plant viruses, the management of viral diseases is mainly based on the development of resistant varieties as well as on prophylactic methods aimed at avoiding transmission (Bragard et al., 2013; Hull, 2013). However, the major resistance gene introgressed in sugar beet varieties to control BNYVV is bypassed by an increasing number of new BNYVV variants, as developed in Chapter II. In the absence of short-term genetic solutions, new sustainable control strategies are needed. This could be achieved through interference with vector transmission. Even though some vectors of plant viruses are well documented, such as arthropods, others are largely understudied such as protists (Bragard et al., 2013; Schwelm et al., 2015a). This is the case for *Polymyxa betae*, the plasmodiophorid vector of BNYVV.

After the first reports relating the discovery of rhizomania in 1952 (Canova, 1966), its causal agent, the BNYVV (Tamada et al., 1971), and *P. betae* (Keskin, 1964), most research effort aimed to describe the life cycle of rhizomania protagonists using microscopy and immunological techniques as well as to study their epidemiology and to assess their impacts on sugar beet and other hosts (reviewed in Chapter I for *P. betae* and Chapter II for BNYVV). Sources of genetic resistance to BNYVV and *P. betae* were also investigated (Panella and Biancardi, 2016).

GENERAL INTRODUCTION

The development of molecular biology techniques in the middle 1980's, especially the availability of cDNA infectious clones for all BNYVV genomic RNAs, has enabled in depth studies of the molecular biology of plant-virus and vector-virus interactions, from the BNYVV "point-of-view". As reported in Chapter II, several research groups have taken advantage of reverse genetics approaches to characterize the BNYVV multipartite genome, including genes involved in vector transmission (i.e. the Coat Protein Read-Through [CP-RT] and P31). Notably, two transmembrane domains located on the read through domain of CP-RT were shown to be essential for efficient virus transmission by *Polymyxa* spp.

Such techniques were not applicable to study viral transmission from the protist "point-of-view". Since the 1990's, the diversity of *Polymyxa* spp. has been characterized by rDNA amplification and sequencing of as well as by determining host range and ecological requirements of several isolates (reviewed in Chapter I). The specific interactions of *Polymyxa* special forms (subclades defined within species) with a limited number of viruses and hosts was reported. However, the molecular background of these interactions is mostly unknown (reviewed in Chapters II). So far, very few genomic resources for *Polymyxa* spp. are available, limiting progresses toward a better understanding of these obligate parasites. The recent publication of the *Plasmodiophora brassicae* genome, another plasmodiophorid, highlighted the unique evolutionary status of these eukaryotes, in view of the low sequence homology with well characterized plants, animals, fungi and oomycetes. As opposed to *P. betae*, the release of *P. brassicae* genome and from an increasing number of host plants, including the research model plant *Arabidopsis thaliana*, have led to a significant research effort toward deciphering the molecular interactions of this plasmodiophorid with plants.

In the late 2000's, the plant metabolic pathways involved in plant-BNYVV interactions have also been progressively unraveled thanks to the development of transcriptomics and proteomics (reviewed in Chapter II). The fully sequenced model plants *A. thaliana* and *Nicotiana benthamiana* as well as the BNYVV systemic host *Beta macrocarpa* were initially exploited for that purpose. Then, the release of the *sugar beet* reference genome in 2014 allowed direct investigation of the mechanisms responsible for lateral roots proliferation, the primary symptom of rhizomania. However, studies taking account of both *P. betae* and BNYVV are still scarce despite the availability of sugar beet genome. This could be explained by the absence of *P. betae* genomic resources and the difficulty to simultaneously work with both the virus and its vector in a soil-free, controlled environment. Indeed, the intracellular obligate parasite nature of both *P. betae* and BNYVV has made tripartite interactions studies more complex, since BNYVV can only be acquired *in vivo* by *P. betae* thalli.

2. Thesis outline

In this thesis, we firstly introduce the reader to *P. betae* and its plasmodiophorids relatives in Chapter I. We highlight the paucity of genomic data available for *P. betae* and other members of the Rhizaria supergroup despite their ecological importance. Next, we present the rhizomania disease of sugar beet and review the current knowledge on the interactions occurring between *P. betae*, the BNYVV and host plants (including sugar beet) in Chapter II. The main gaps of knowledge on this tripartite interaction are highlighted. In Chapter III, we finish the introduction with some considerations on sugar beet and notions on plant defense, important to interpret the plant-parasite interactions which are addressed in the general discussion. In the first part of the results (Chapter IV), we present the draft genomes of two *P. betae* isolates as well as an innovative metagenomic-based method to recover eukaryotic genomes (here, *P. betae*) from DNA datasets highly “contaminated” with host and microbial sequences. A comparative genomics approach of *P. betae* with other plasmodiophorids, *S. subterranea* and *P. brassicae* is also achieved. Next a protocol allowing the soil-free transmission of BNYVV to sugar beet by *P. betae* zoospores of one *P. betae* sequenced isolate is described in Chapter V. This Chapter also presents the implementation of this protocol in a transcriptomic analysis aiming to study the molecular interactions between the protist, the virus and the plant. Chapter VI is devoted to a general discussion. First, we build a theoretical model of the molecular interactions between *P. betae* and the sugar beet. Second, we discuss the impact of the BNYVV on plant reaction and the potential implications for *P. betae*. Moreover, we discuss the mutual benefits of the interaction between BNYVV and *P. betae*. Finally, we conclude this work in Chapter VII. On the basis of the results and resources obtained during this thesis, we propose directions for future research.

LITERATURE REVIEW

CHAPTER I.

***Polymyxa betae* and the Plasmodiophorida Order**

The first chapter of this manuscript is devoted to introducing the reader to *Polymyxa betae* and the fascinating eukaryotic order of plasmodiophorids. This understudied branch of protist-like organisms encompasses, among others, plant parasites in which at least four species are known to cause direct or indirect losses to major crops all around the world. This chapter presents the current state of the art on the biology of *P. betae*, its life cycle, morphology, host range and ecology. Some useful information on *P. graminis*, *Plasmodiophora brassicae* and *Spongospora subterranea* is also reported. An appropriate awareness of current knowledge on *P. betae* and, by extension, on the biology of plasmodiophorids is important in order to grasp the new results presented in this thesis. The state of the art of the molecular interactions between *P. betae*, plants and transmitted viruses is covered by Chapter II.

1. General considerations about plasmodiophorids

Polymyxa betae was first described by Keskin (1964) as a soil-inhabiting protist, obligate root parasite of sugar beet (*Beta vulgaris* subsp. *vulgaris*) and other chenopodiaceous plants. It belongs to the order of Plasmodiophorida in the Rhizaria “supergroup”. The term plasmodiophorids is commonly used to refer to organisms included in the Plasmodiophorida. Early research interest was drawn to these organisms because some of these are able to cause diseases in major agronomic crops such as sugar beet, rice, barley, wheat as well as potato, rape, cabbage and other Brassicaceae, resulting in significant economic losses. Damage to plants is either caused by the protists themselves or by vectored phytopathogenic viruses. For example, *P. betae* asymptotically infects field-grown sugar beet (*Beta vulgaris*) but carries certain viruses, including *Beet necrotic yellow vein virus* (BNYVV), the causal agent of a major sugar beet disease called rhizomania (see Chapter II). Other examples of well-known plant-parasitic plasmodiophorids are *Polymyxa graminis*, the vector of multiple viruses (Dieryck et al., 2011), *Plasmodiophora brassicae*, the causal agent of clubroot (Dixon, 2014) and *Spongospora subterranea*, the causal agent of root/stolon galls and powdery scab of potato and the vector of the *Potato mop top virus* (Falloon et al., 2015). Two additional members of this order, *Sorosphaerula viticola* and *Hillenburgia nasturtii* (formerly *S. subterranea* f. sp. *nasturtii*, or *S. nasturtii*), are able to infect grapevine and watercress, respectively, but their impact on plants remains unclear (Neuhauser et al., 2010, 2014). Plasmodiophorids all share common features including their intracellular lifestyle, cruciform nuclear divisions, production of multinucleate plasmodia, biflagellate zoospores and perennial resting spores (Braselton et al., 1975; Neuhauser et al., 2010). The obligatory intracellular lifestyle of these organisms hampered research progresses aiming to better explain their biology which remain therefore poorly known. However, the evolution of sequencing technologies is expected to give a new lease of life to the research on these organisms (Schwelm et al., 2015a).

Although *Polymyxa* spp. do not produce significant losses in infected crops, their ability to transmit viruses represent a serious threat to crop productions (Barr, 1979; Kanyuka et al., 2003). They are transmitted through soils in the form of swimming short-lived zoospores and long-lasting resistant survival sporosori (clusters of resting spores) and are distributed worldwide. Therefore, they threaten many crops even if some evidence showed a certain degree of environmental adaptation and host specialization (Legrève et al., 2002; Dieryck et al., 2011; Smith et al., 2013). With climate change, increasing deployment of agricultural machinery and global trade in plant products, the spread of *Polymyxa* spp. and transmitted viruses is expected keep growing (Jiang et al., 2020a).

2. Taxonomic position of *Polymyxa betae*

- a. *Polymyxa betae* belongs to the Rhizaria, a large group of protists

To date, the genus *Polymyxa* is divided in two species: *P. graminis* Ledingham (Ledingham, 1939) and *P. betae* Keskin (Keskin, 1964), each subdivided in ribotypes (Ward and Adams, 1998) and/or *Formae speciales* (Barr, 1979; Legrève et al., 2002). *Polymyxa betae* and its sister species *P. graminis* are included in the eukaryotic order Plasmodiophorida. In a recent update of the Rhizaria classification, shown in Table I-1, Cavalier-Smith et al. (2018) placed the order Plasmodiophorida in the phylum Retaria, outside of the Cercozoa.

Table I-1. Classification of the plasmodiophorids according to Cavalier-Smith et al. (2018).

Ranks	Classification
KINGDOM	Chromista
INFRAKINGDOM	Rhizaria
PHYLUM	Retaria
SUBPHYLUM	Endomyxa
SUPERCLASS	Proteomyxia
CLASS	Phytomyxea
ORDER	Plasmodiophorida

This order mainly includes obligate intracellular parasites of plants but also of stramenopiles (Neuhauser et al., 2014), the heterokont clade that contains oomycetes (Bass et al., 2018; Hittorf et al., 2020). The members of the terrestrial order Plasmodiophorida (plasmodiophorids) are grouped with the marine Phagomyxida (phagomixids, which infect brown algae, diatoms and oomycetes) in the Class Phytomyxea (phytomyxids) (Neuhauser et al., 2014). Up to now, this class contains at least 42 species from 12 genera (Neuhauser et al., 2011; Hittorf et al., 2020) but environmental sequencing studies bring more and more phytomyxids-related sequences, grouping in new clades sometimes lacking data from a characterized isolate (Bass et al., 2018; Hittorf et al., 2020). Phytomyxids belong to the phylum Endomyxa (Bass et al., 2018; Hittorf et al., 2020), embedded in the Rhizaria included in the Telonemia, Stramenopila, Alveolata, and Rhizaria (TSAR) clade, one of the main eukaryotic clade defined by Burki et al., (2020). The position of Rhizaria in the recently updated tree of life is visible in Figure I-1.

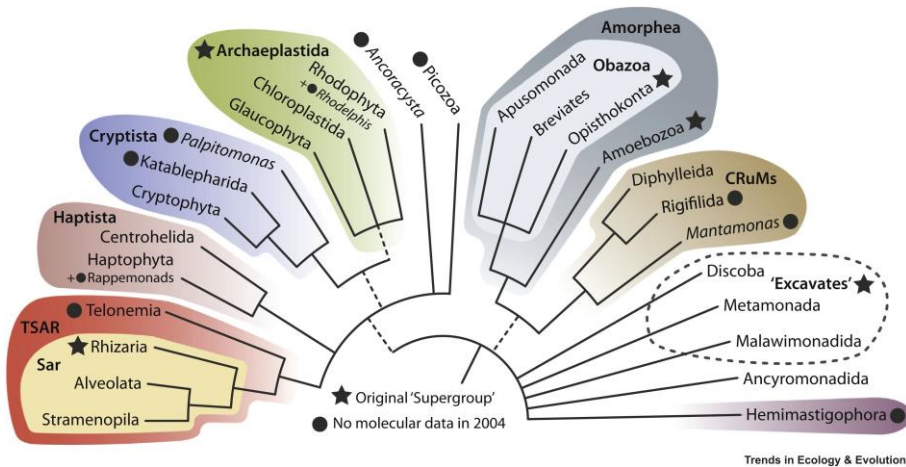


Figure I-1. The current eukaryotic tree of life described by Burki et al., 2020. Broken lines refer to groups in which the monophyletic origin remains precarious. Stars correspond to clades from the previous “supergroup model” utilized since 2005 (Simpson and Roger, 2004; Adl et al., 2005). Circles indicate groups for which no molecular data was available in 2004.

b. Rhizaria is one of the most poorly understood eukaryotic clade

In the last decade, with the drop in the price of DNA/RNA sequencing, a huge effort was granted to expand the number of available sequenced genomes and transcriptomes from non-model organisms. For example, the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP) identified genes from 396 unique strains within 210 genera belonging to un- or under sampled marine microbial eukaryotes, thus including many protists (Keeling et al., 2014). Concomitantly, initiatives have been taken to summarize and share genomic information and metadata in a joint effort using common controlled vocabulary. The Genomes OnLine Database (GOLD) (<https://gold.jgi.doe.gov/>) was created for that purpose and contains daily updated, manually curated genome projects held around the world (Mukherjee et al., 2021). Partly thanks to these efforts, a revised classification of the eukaryotes retaining emphasis on protists has been recently established (Adl et al., 2019). Unfortunately, the few phylogenomic studies encompassing rhizarian protists did not include *P. betae* sequences (Sierra et al., 2016; Cavalier-Smith et al., 2018), due to the lack of genetic information on the *Polymyxa* genus. Even though genomic information on eukaryotic organisms is increasing rapidly, it is still biased to specific groups of organisms, due to scientific interests and the amenability of these organisms to the application of sequencing. As a matter of fact, the great majority of known eukaryotic genomes belongs to animals, plants and fungi, representing only two of the at least eight main eukaryotic groups

summarized by Burki et al. (2020). It is obvious that the number of known genomes in some protist groups, including the Rhizaria, remains insignificant in comparison to these groups as illustrated by Figure I-2 (Burki and Keeling, 2014; Sibbald and Archibald, 2017). Before the start of our project, only one plasmodiophorid species was completely sequenced : *P. brassicae* (Schwelm et al., 2015b). The draft genome of *S. subterranea* has since been published (Ciaghi et al., 2018). Three other rhizarian species complete genomes are also available: the Chlorarachnea *Bigelowiella natans* (Curtis et al., 2012), the Foraminifera *Reticulomyxa filosa* (Glöckner et al., 2014) and the Imbricatea *Paulinella micropora* (accession no.: PRJDB3528, no paper published). However, the swelling diversity of organisms assigned to the Rhizaria is enormous and environmental surveys revealed that most of rhizarian diversity has yet to be found. These organisms are present in all ecosystems and could play a major role in controlling carbon fluxes in soils and oceans (Burki and Keeling, 2014). The extreme paucity of genomic data on single-celled protists was especially highlighted in the *Tara* Oceans Project which reported a tremendous biodiversity in unstudied/uncultured groups (Bork et al., 2015; Flegontova et al., 2016). Broadly speaking, more protist genomes are needed to fully embrace the microbial eukaryotic diversity present in all ecosystems on earth (Sibbald and Archibald, 2017).

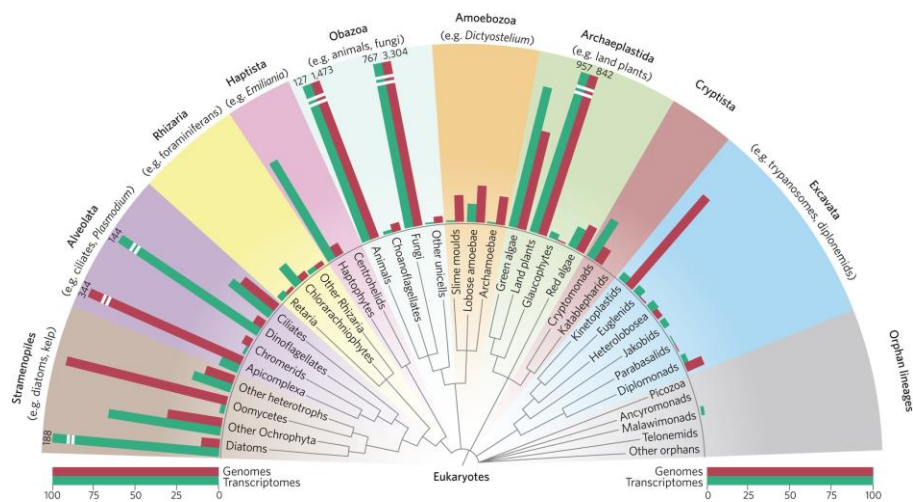


Figure I-2. Diversity of the eukaryotic tree of life and sequencing projects ongoing or completed in February 2017 (Sibbald and Archibald, 2017). The histograms show the number of genome and transcriptome sequencing projects reported in each displayed clades in the Genomes Online Database (Mukherjee et al., 2021) and in the Transcriptome Sequencing Project (Keeling et al., 2014) as of February 2017.

3. Life cycle and Morphology

P. betae shares the main features of its life cycle with other plasmodiophorids (Tamada and Asher, 2016b): 1) they are all heterotrophic obligate intracellular parasites 2) they are heterokonts, producing zoospores with two unequal whiplash flagella; 3) they grow inside host cells as multinucleate plasmodia following multiple nuclear divisions; 4) they have a characteristic type of mitotic nuclear division, “cruciform division” (Keskin, 1971); 5) they survive as long-lived resting spores. The general cycle of plasmodiophorids is described below. These common features are key in the epidemiology of *Polymyxa* spp. and transmitted viral diseases. As far as we know, *P. betae* and *P. graminis* share the same life cycle. Besides, under bright field microscopy, it is barely possible to distinguish *P. betae* from *P. graminis* (D’Ambra, 1967; Barr, 1979; Tamada and Asher, 2016b) even though some electronic microscopy studies suggested some minor differences. Studies on both species complement one another. We therefore decided to report results obtained with both *P. betae* and *P. graminis*, in the following sections.

a. Life cycle

The life cycle of *P. betae* and representative pictures of each life stage is shown in Figure I-3. The life cycle of plasmodiophorids was summarized by Braselton (1995) and Kanyuka et al. (2003). It can be divided in two major phases: a sporangial phase leading to the production of mobile infectious propagules called zoospores and the sporogenic phase, leading to the production of thick-walled resting spores or sporosori (=clusters of resting spores). Zoospores are the only plasmodiophorid structure actively released from host cells. They initiate each of these phases by encysting at the surface of epidermal or root hair cells. Infection and subsequent release of *P. graminis* zoospores from barley mutants defective for root hairs formation was possible but less effective than in wild type plants, demonstrating root hairs account for most new infections (McGrann and Mutasa-Göttgens, 2009). Infection starts by encystment of a zoospore at the surface of a potential host. an adhesive outgrowth (the adhesorium) and a germination tubular structure (the “Rohr”) containing a dense bullet-like structure (the “Stachel”) are formed. The Stachel emerges from the zoospore in the adhesorium and puncture the host cell wall, passing through the Rohr (Keskin, 1964; Keskin and Fuchs, 1969). Afterwards, the contents of the zoospore, including the Stachel is injected in host cell cytoplasm and start to grow as a plasmodium. After encystment, this infection process, illustrated in Figure I-4, is very quick: about one minute or less (Fuchs and Madelin, 1966; Keskin and Fuchs, 1969; Aist and Williams, 1971). The time required for the entire process from swimming, attachment and encystment to establishment of infection was estimated at 3-4h for *P. graminis* (Adams and Swaby, 1988) and 1-6h for *P. betae* (Barr et al., 1995). Then the zoospore contents start to enlarge in infected cell. Soon after infection, the protist appears as a well-

defined more or less circular structures, sometimes called “meronts”, with a distinct nucleolus in the middle. A proto-mitosis (cruciform division) occurs leading to the development of 2 nuclei which undergoes several synchronous cycles of cruciform divisions. A plurinuclear sporangial or sporogenic plasmodia eventually forms (Dahm and Buchenauer, 1993; Barr and Asher, 1996). The sporangial plasmodia are then usually compartmentalized by septa into multiple lobes that continue to enlarge. Nonmeiotic noncruciform divisions occur at this stage and secondary zoospores start to differentiate (Barr, 1979; Dahm and Buchenauer, 1993). The release of secondary zoospores (Figure I-3.4) occurs through exit tubes that form from zoosporangia and are dissolve openings in the host cell wall (Littlefield et al., 1998). Secondary zoospores then infect new cells in the same or in adjacent roots from the same or from different plants, inducing either the sporogenic or the sporangial phase. The meiosis takes place after some proto-mitosis (cruciform divisions) in sporogenic plasmodia (Figure I-3.5), where synaptonemal complexes are visible (Braselton, 1984). The sporogenic plasmodia are then cleaved to form small or large aggregates of resting spores called sporosori (Figure I-3.4). Sporosori are released into the soil after root tissue breakdown and used by *P. betae* to maintain itself in the soil for years in the absence of a suitable host (Richard-Molard, 1985; Abe and Tamada, 1986; Asher and Blunt, 1987). Thanks to these survival structures, it is considered that a field infested by *P. betae* sporosori will remain permanently contaminated (Wisler and Duffus, 2000). When a host plant is present, each individual resting spore in a sporosorus is able to germinate independently and release a primary zoospore capable of initiating a new infection. The determinants driving the occurrence of sporangial or sporogenic life stage are still unknown (Tamada and Asher, 2016b) but it could be related to environmental conditions and/or physiological state of the host plant. Details about morphology and appearance of each life stage are developed in the following sections.

CHAPTER I. *Polymyxa betae* and the Plasmodiophorida Order

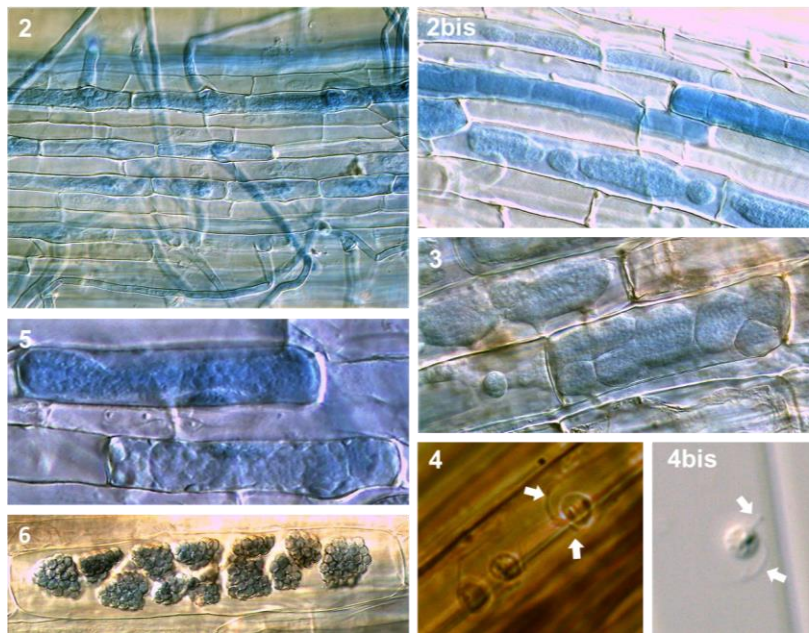
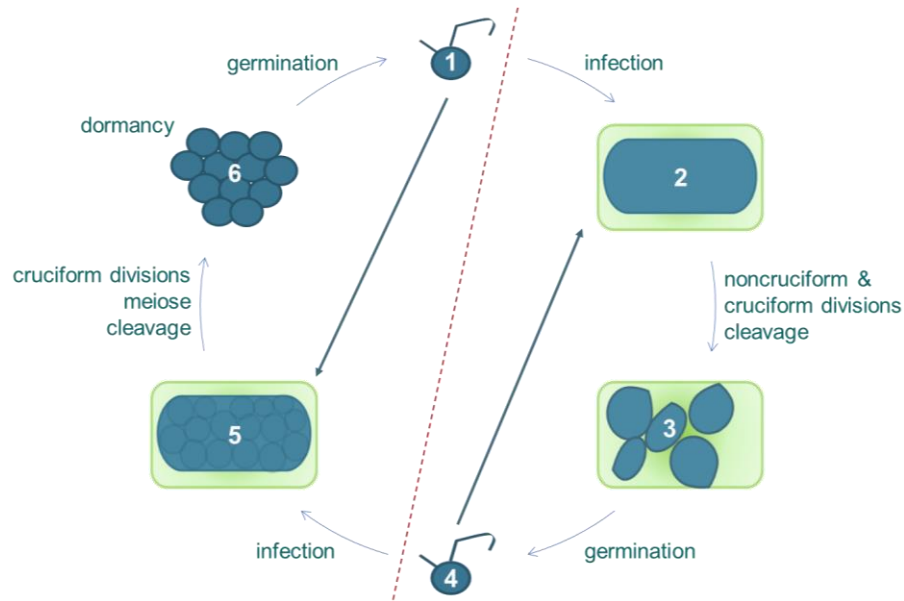


Figure I-3. Schematic representation of *P. betae* life cycle and representative pictures of each life stage observed in transmitted Nomarski microscopy. Numbers reported in the diagram correspond to those shown in pictures. (1) Primary zoospore, (2) rows of cells infected by plasmodia, (2bis) zoo sporangial plasmodia, (3) zoo sporangia, (4 and 4bis) secondary zoospores inside and outside the roots, respectively, (5) sporogenic plasmodia, (6) sporosori. The dotted red line separates the two major phases of the cycle: the sporogenic and the sporangial phases. White arrows point flagella.

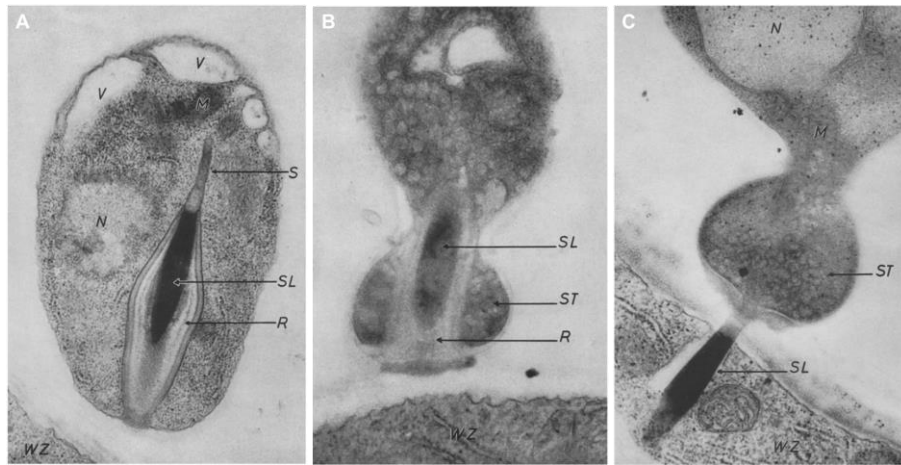


Figure I-4. TEM micrographs taken from Keskin and Fuchs, (1969) showing the penetration of a host cell by a *P. betae* zoospore. (A) Zoospore, attached to the host cell, (B) beginning of the infection, showing the stylet, (C) penetration of the host cell wall by the “Stachel” ER: endoplasmic reticulum, Gl: Golgi apparatus, M: mitochondria, N: nucleus, R: “Rohr”, S: tube, SL: “Stachel”, ST: stylet, V: vacuole, WZ: host cell.

b. Plasmodia

After infection, young *Polymyxa* spp. plasmodia start to develop in epidermal cells (including root hairs), primarily of lateral roots (LR ; PO:0020121), and firstly remain localized in the outermost cell layer of the root. An inward infection into cortical cell layers was sometimes observed at later stages, but the protist was never observed in the endodermis or in the stele (Barr and Asher, 1996). Young plasmodia quickly start to differentiate into zoosporangial or sporogenic plasmodia. As soon as 48-72H after zoospore inoculation (at 20°C), the first plasmodia can be observed in root sections (Dahm and Buchenauer, 1993; Barr and Asher, 1996). Their number increases for 6 days and then start to drop (Dahm and Buchenauer, 1993). Undifferentiated plasmodia (Figure I-3.2) are firstly rounded and connected to some part of the cell wall. Then, they gradually invade the host cells which often give them an elongated shape surrounded by a thin layer of host cytoplasm (Barr and Asher, 1996). Zoosporangial plasmodia, visible in Figure I-3.2bis, look like elongated lobbed or irregular masses with small dark exit papillae (Abe and Ui, 1986). They are often septate and may extend through the entire cell length (~200 µm). At first bordered by a thin membrane, they are rapidly surrounded by a distinct cell wall (Barr, 1979; Barr and Asher, 1996; Littlefield et al., 1998). Unlike mature sporogenic plasmodia, they never fill the cross sectional area of host cells (Littlefield et al., 1998). At early stages, sporogenic plasmodia of *Polymyxa* spp. are nearly identical to immature zoosporangial plasmodia but they are usually smaller and denser. To the difference of sporangial plasmodia, sporogenic

CHAPTER I. *Polymyxa betae* and the Plasmodiophorida Order

plasmodia are compartmentalized by septa and are only separated from host cytoplasm by an outer limiting membrane (Barr, 1979; Littlefield et al., 1998). As they mature, they take on a globular to amorphous appearance with a granulated surface (Figure I-3.5). The first sign of differentiation into sporosori is the appearance of cleavage planes within plasmodia, which are rapidly replaced by bilayer membranes compartmentalizing the plasmodium. A thick wall is then progressively formed between the bilayers (Barr and Asher, 1996; Littlefield et al., 1998). Initially coarsely structured, sporogenic plasmodia gradually differentiate into numerous hexagonal resting spores of 4-7 μm , giving rise to sporosori (Barr, 1979; Dahm and Buchenauer, 1993; Littlefield et al., 1997).

c. Zoosporangia

Mature zoosporangia are delimited by a distinct cell wall and can extend up to 200 x 40 μm , entirely filling root cells. Barr and Asher (1996) estimated that they appear in eight days at 20°C in *B. vulgaris*. They are often, but not necessarily, segmented by septa. When septate, zoosporangia look like a set of contiguous bags stacking in the cell, sometimes called sporangiosorus (Figure I-3.3). Zoospores delimitation inside zoosporangium occur by fusion of vesicles shaping the external membranes (D'Ambra and Mutto, 1975; Barr and Asher, 1996). At maturity, the septa degenerate leaving the zoospores circulating throughout the thallus (D'Ambra and Mutto, 1975, 1977; Barr, 1979). Multiple (up to 17) protuberant compartments extending 5-8 μm towards host cell walls are differentiated from mature zoosporangia during ripening (D'Ambra and Mutto, 1977; Barr and Asher, 1996). These compartments are separated from the zoosporangia by a cell wall and correspond to uninucleate cells which does not differentiate into zoospores (D'Ambra and Mutto, 1977). They form discharge tubes which anchor into plant cell wall in order to release secondary zoospores from the host cell. Depending on the presence of septa, on the discharge protuberances and on host cell morphology, sporangia can adopt variable irregular shapes. Therefore, morphology of zoosporangia does not allow differentiating *P. betae* and *P. graminis* (Barr, 1979).

d. Zoospores

During maturation in zoosporangia, the biflagellate zoospores of *P. betae* (Figure I-3.4,4bis) condense and the flagella appear. Mature primary and secondary zoospores are uninucleate and rounded amoeboid, with a diameter of about five micrometers. They are propelled by two flagella oriented in opposite directions. Numerous peripheral vesicles were observed in zoospores by TEM (Barr and Asher, 1996). Moreover, it should be noted that if some zoospores are released in rhizosphere solution, others encyst inside root cells (Barr, 1987; Dahm and Buchenauer, 1993) to contribute to propagation of the infection into the roots. It is supposed to permit the cortex invasion observed at later stages (Aist and Williams, 1971; Barr, 1987; Barr

and Asher, 1996). The propagation of the protist from cell to cell inside the root results in clusters of infected cells visible in bright field microscopy (Figure I-3.2). Alternatively, active movement of small myxamoeboid plasmodia through breaks in root cell wall could occur, as suggested for *P. brassicae* (Mithen and Magrath, 1992) and *H. nasturtii* (Claxton et al., 1996). This mechanism would be based on enzymatic digestion of cell wall components (Mithen and Magrath, 1992) and could be facilitated by the presence of plasmodesmata. Primary zoospores can fuse together (Keskin, 1964) and would be the stage where karyogamy occurs in plasmodiophorids. However, karyogamy has never really been observed and the evidence for its existence at this stage remains controversial, as discussed by Braselton (1995).

e. Sporosori

Sporosori (or cystosori) are clusters of resting spores, also called cysts (Figure I-3.6). According to Karling (1981), the terms “resting spores” and “sporosori” should be preferred to “cysts” and “cystosori” in order to avoid confusion with encysted zoospores (Braselton, 1995). *P. betae* sporosori are composed of 4 to 300 rounded thick-walled resting spores, each 4-7 µm diameter (Barr, 1979; Tamada and Asher, 2016b). Their shape and size are highly variable even though similar to *P. graminis* sporosori (Barr, 1979; Langenberg and Giunchedi, 1982). They often adopt a plate-like or boat-like shape, depicting host cell shapes and organization in smaller cells (Barr, 1979; Barr and Asher, 1996). Multiple small sporosori are often seen in root cells (Figure I-3.6) but sometimes, bigger ones invade nearly entire cells. When maturing, color of resting spore evolves from hyaline pale brown to medium-to-dark brown. Entire root section may appear brown even if not rotted in case of high infection. The surface of maturing resting spores become irregularly roughened or verrucose and adjacent walls seem cemented together. Individual resting spore of *P. betae* are polyhedral, firstly hollowed in a pole and become sub-spherical when mature (Ciafardini and Marotta, 1988; Ciafardini et al., 1995). Further studies on *P. graminis* confirm that angular immature resting spores become rounded when maturing and reported interstitial matrix and angular empty spaces between mature resting spores (Littlefield et al., 1997; Chen et al., 1998). Similar observation was made for *P. betae* (Barr and Asher, 1996). Unlike those from *Ligniera pilorum*, *Polymyxa* spp. sporosori are not observed in root hairs (Barr, 1979). However, it should be noted that overall morphology can be influenced by the host. For example, the same isolate of *P. betae* showed “typical” spores on *Chenopodium album* but on spinach, individual cysts appeared separated like *P. brassicae*, only rarely fused together (Barr, 1979). The number of sporosori start to increase from eight days after inoculation (Dahm and Buchenauer, 1993). This is the pivotal moment where the protist shift from a first multiplication phase to the initiation of a survival stage (Asher and Blunt, 1987; Dahm and Buchenauer, 1993).

Cell wall

Mature cell walls of sporosori are multilayered and 200-300 nm thick (Ciafardini et al., 1995). First studies showed that cyst wall had two layers: one outer electron-dense surface and one electron-transparent inner part (D'Ambra and Mutto, 1977; Langenberg and Giunchedi, 1982). Later authors reported that the cell wall of sporosori consists of five layers. Studies on resting spore wall architecture in *P. betae* and *P. graminis* reported a similar global structure but the authors have different interpretation of inner layers development, as summarized in Table I-2 (Ciafardini et al., 1995; Chen et al., 1998; Littlefield et al., 1998). It is unclear whether these differences are biologically relevant or simply due to subjective interpretations, the degree of maturation of sporosori observed at the time of analyses, or differences in specimen preparation protocols.

Table I-2. Comparative table of the sporosori cell wall architectures reported by Ciafardini et al., 1995 for *P. betae* and Chen et al., 1998 for *P. graminis*

Layer	<i>P. betae</i> (Ciafardini et al., 1995)	<i>P. graminis</i> (Chen et al., 1998)
(out) 1	30 nm thick, very electron-opaque, protrusions giving the wall an irregular external surface.	25-30 nm thick, extremely electron-dense.
2	30 to 70 nm thick, electron-opaque, extended into irregular spines.	60-80 nm thick, Appearance of a matrix containing electron-dense granules.
3	100 nm uniform thickness, less electron dense, pressed against layer 2.	25 nm thick, less electron dense. Thicker (70 nm) in the region of a "plug" (see layer 5).
4	30 nm thick, highly electron-dense. Polysaccharidic in nature.	60-80 nm thick, fibrillar.
5 (in)	<u>Immature</u> : 80-100 nm, continuous, electron-opaque. <u>At maturity</u> in the upper area of the spore: internal electron-transparent layer that protruded towards the protoplasm forming a papilliform internal discoid region, up to 240 nm thick oriented towards the surface of the roots. This structure disappears prior to germination.	60-80 nm thick, darker than the fourth layer. May represent an intermediate stage in the deposition of the inner spore wall. Prominent unstained plug within the fourth/fifth layer of the wall faced towards the outside. 100-120 nm thick, extending up to one third of the circumference of the spore.

The first two layers are tightly interconnected and adjacent cysts are at least partially fused to this outer coat (Ciafardini et al., 1995; Littlefield et al., 1998). In the case of *P. graminis*, resting spores are joined only with spines of this coat (Chen et al., 1998) and can be easily separated (Delfosse et al., 2000). It is not the case with *P. betae*. Another major difference between the

studies presented in Table I-2 is that (Chen et al., 1998) do not think that the papillate electron-transparent area attributed to the fifth layer by Ciafardini et al. (1995) evolves from this layer. They related a transparent structure (referred as “plug”) extending between the fourth and the fifth layers and a thicker third layer (about 70 nm) in the “plug” region. Both studies agree that this region would be associated to germination. In addition, earlier studies already suggested that germination occurs in localized weak areas of the multilayer wall which are gradually modified during spore maturation (Ciafardini and Marotta, 1988, 1989). During germination, this thickened area would be destroyed, weakening the wall (Ciafardini et al., 1995). However, this theory remains controversial. Indeed, Littlefield et al. (1998) suggested that this area would increase with maturation and not decrease. Moreover, the same authors questioned the interpretation of the fifth layer as a real cell wall layer. The exact architecture of the cell wall of *Polymyxa* spp. remains therefore mysterious. It should also be noted that different maturation levels can co-exist in the same sporosori and germination of some resting spores could already occur within host roots (Chen et al., 1998).

Ciafardini et al. (1995), also studied the cell wall composition of *P. betae* spores. They found that it was not processed by pronase (a proteolytic enzyme), lipase, and phospholipase but became spongy overall when treated with lysozyme, probably indicating the presence of peptidoglycans (Ciafardini et al., 1995). However, it should be noted that these results haven't been confirmed by others (Adams et al., 1988; Sayama et al., 2006). It was also shown that walls of *P. betae* contain chitin (Waterhouse, 1973; Sayama et al., 2006), probably in the third layer, in combination with polysaccharide components. The two first layers are supposedly made of proteins perhaps associated with lipids, in regard of their protective role against other microorganisms (Ciafardini et al., 1995). Cyst wall architecture differs somewhat from that of other plasmodiophorids such as *Ligniera verrucosa* (Miller et al., 1985) and *Plasmodiophora brassicae* (Buczacki and Moxham, 1983; Moxham and Buczacki, 1983).

Cell content

The cellular content of resting spores is close to the content of primary zoospores, which are produced within the spores. We should also note that microscopical observations also revealed that secondary and primary zoospores are very close (Barr and Allan, 1982). Chen et al. (1998) observed abundant mitochondria, vacuoles, strands of rough ER, Golgi bodies, vesicles containing a granular matrix and numerous free ribosomes in mature uninucleate spores. As a sporosorus consists of many cemented resting spores, cavities are left inside sporosorus after the release of primary zoospores from individual spores. These are invaded by bacteria. Cytoplasmic osmiophilic bodies are observed in immature spore cytoplasm. They migrate to the periphery as the spore mature driven by a membrane system (Chen et al., 1998).

Germination

Germination of resting spores of *P. betae* occurs in soils when temperatures are over 10°C (optimal : 25°C) with a pH over 5.3 and high soil moisture, depending on rains and soil water reserves (Horak and Schlösser, 1980; Abe, 1987; Blunt et al., 1991; Legrève et al., 2005b). The influence of temperature and moisture on *P. graminis* was also reported in several studies and differs between special forms and from *P. betae* (Adams and Swaby, 1988; Legrève et al., 1998; Adams and Ward, 1999; Delfosse et al., 2002). From the germination of resting spores, *P. graminis* zoospores take approximately 3-3.5 hours to initiate a new infection, probably the time needed to swim toward a host root cell and encyst to its surface (Adams and Swaby, 1988). An unknown chemical stimulus from root exudates is thought to trigger the germination, even if resting spore germination was already observed in distilled water and can be stimulated by preheating soils to 40 °C (Beemster and de Heij, 1987; Schlosser, 1988).

4. Host range

The host range of *P. betae* (European isolates) was firstly found to be limited to plants belonging to the former Chenopodiaceae, now included in Amaranthaceae in modern phylogenetics (Keskin, 1964). Unlike *P. graminis*, *P. betae* cannot induce heavy infection on Poaceae (with possible exceptions). This distinction led to the differentiation of two *Polymyxa* species despite their morphological similarity. After the first report published by Keskin, several studies addressed the host range of *P. betae*, as shown in Table I-3. Barr (1979) found that Canadian isolates can be divided into two special forms: *P. betae* f. sp. *betae* which have a similar host range as European isolates previously studied and *P. betae* f. sp. *amaranthi* which only infected *Amaranthus retroflexus*. Furthermore, the *P. betae* f. sp. *betae* could be divided into two biotypes: the European biotype commonly found on *B. vulgaris* and *Chenopodium album* and the Ontario biotype that grew on *C. album* but rarely on *B. vulgaris*. The ability to easily infect sugar beet was interpreted as a local adaptation of *P. betae* in areas where the sugar beet is extensively cultivated for many years (Barr, 1979). Later, Abe and Ui (1986) confirmed results from Barr (1979) and proposed a third special form, *P. betae* f. sp. *portulacae*, only capable of infecting plants belonging to the family Portulacaceae. Only isolates from the special form *betae* would be associated with the incidence of rhizomania (Abe and Ui, 1986). In 2005, Legrève and colleagues reported the RT-PCR detection of both *P. betae* and the BNYVV in a much larger host range including plants from additional families: Asteraceae, Brassicaceae, Caryophyllaceae, Papaveraceae, Poaceae, and Urticaceae. The proposed host range was further enlarged by Mouhanna et al. (2008), to more Poaceae and includes members of Convolvulaceae. However, the strict identification of *P. betae* (and not *P. graminis*) was not achieved in this last study (monocotyledonous plants *Alopecurus myosuroides*, *Lolium multiflorum*, *Sorghum vulgare*, *Sorghum*

halepense and dicotyledonous plants *Calystegia sepium*, *Capsella bursa-pastoris*, *Centaurea cyanus*, *Convolvulus arvensis*, *Galinsorga parviflora*, *Matricaria inodora*, *Stellaria media*, not included in Table I-3). Simultaneous reports of *P. betae* infection of *Arabidopsis thaliana* were published in 2011 (Desoignies et al., 2011; Smith et al., 2011). However, infection levels in this host were much lower than in *B. vulgaris* and the protist phenotype differed from its phenotype in sugar beet, likely revealing a distinct host-parasite interaction. More recently, Kutluk Yilmaz et al. (2016) detected both *P. betae* and the BNYVV on *Raphanus raphanistrum* a wild radish (Brassicaceae). As illustrated above, host specificity differs between isolates recovered from various plant species and different geographical regions.

Table I-3. Plant species reported to be host of *P. betae* (and special form).

<i>P. betae</i> (+ f. sp.)	Host species name and family	Reference
<i>P. betae</i> f. sp. <i>betae</i> (f. sp. deduced here)	Amaranthaceae <i>Atriplex halimus</i> , <i>A. hortensis</i> , <i>A. undulata</i> , <i>Beta vulgaris</i> , <i>Chenopodium album</i> , <i>C. bonus - henricus</i> , <i>C. polyspermum</i> , <i>C. quinoa</i> , <i>C. rubrum</i> , <i>C. vulvaria</i> , <i>C. amaranticolor</i> , and <i>Spinacia oleracea</i>	Keskin (1964)
<i>P. betae</i> f. sp. <i>betae</i> (European and Ontario biotypes)	Amaranthaceae <i>A. hortensis</i> , <i>C. album</i> , <i>C. capitatum</i> , <i>Beta vulgaris</i> (sugar beet and garden beet), <i>S. oleracea</i>	Barr (1979)
<i>P. betae</i> f. sp. <i>amaranthi</i>	Amaranthaceae <i>Amaranthus retroflexus</i>	Barr (1979)
<i>P. betae</i> f. sp. <i>betae</i> (European and Ontario biotypes)	Amaranthaceae <i>Beta vulgaris</i> (sugar beet, swiss chard), <i>C. album</i> , <i>C. capitatum</i> , <i>C. ficifolium</i> , <i>C. murale</i> , <i>C. quinoa</i> , <i>S. oleracea</i>	Abe and Ui (1986)
<i>P. betae</i> f. sp. <i>amaranthi</i>	Amaranthaceae <i>A. retroflexus</i> , <i>A. blitum</i>	Abe and Ui (1986)
<i>P. betae</i> f. sp. <i>portulacae</i>	Portulacaceae <i>Portulaca oleracea</i> , <i>P. grandiflora</i>	Abe and Ui (1986)
<i>P. betae</i> f. sp. <i>betae</i> (f. sp. deduced here)	Amaranthaceae <i>A. retroflexus</i> , <i>Atriplex halimus</i> , <i>A. hortensis</i> , <i>A. undulata</i> , <i>Beta vulgaris</i> , <i>C. album</i> , <i>C. bonus-henricus</i> , <i>C. murale</i> , <i>C. polyspermum</i> , <i>C. quinoa</i> , <i>C. rubrum</i> , <i>C. vulvaria</i> , <i>C. amaranticolor</i> , <i>S. oleracea</i> , <i>Gomphrena globosa</i> Apiaceae <i>Aethusa cynapium</i>	Legrève et al. (2005)

CHAPTER I. *Polymyxa betae* and the Plasmodiophorida Order

Asteraceae

Achillea millefolium, *Anthemis arvensis*,
Chrysanthemum segetum

Brassicaceae

Capsella bursa-pastoris, *Thlaspi arvense*

Caryophyllaceae

Silene alba, *Silene noctiflora*,
Stellaria media, *Stellaria graminea*

Papaveraceae

Papaver rhoeas

Poaceae

Apera spica-venti, *Lolium perenne*,
Digitaria sanguinalis, *Elymus repens*,
Hordeum vulgare, *Poa pratensi*

Solanaceae

Solanum nigrum

Urticaceae

Urtica urens

<i>P. betae</i> f. sp. <i>betae</i> (f. sp. deduced here)	Brassicaceae <i>A. thaliana</i>	Desoignies et al. (2011) & Smith et al. (2011)
<i>P. betae</i> (f. sp. nd)	Brassicaceae <i>Raphanus raphanistrum</i>	Kutluk Yilmaz et al. (2016)

5. Genetics and diversity

P. betae and *P. graminis* were primarily distinguished by their host ranges (as mentioned above) and environmental requirements (Barr, 1979). Genetic diversity in the *Polymyxa* genus, studied using restriction fragment length polymorphisms and ribosomal gene sequencing, confirmed the separation of these two species (Ward et al., 1994; Legrève et al., 1998, 2002, 2005a; Ward and Adams, 1998; Morales et al., 1999). Current phylogenetics studies are based on the sequencing of partial ribosomal RNA genes (rDNA) extending from the conserved 18S rDNA 3'-end to the conserved 28S rDNA 5'-end and containing the highly variable internal transcribed spacer (ITS) 1, ITS2 as well as the conserved 5.8S rDNA. Analyses of these ITS and 5.8S regions (Figure I-5) led to the definition of subgroups (or ribotypes) of *P. graminis* (Smith et al., 2013; Neuhauser et al., 2014) but not of *P. betae* (Smith et al., 2013; Tamada and Asher, 2016b).

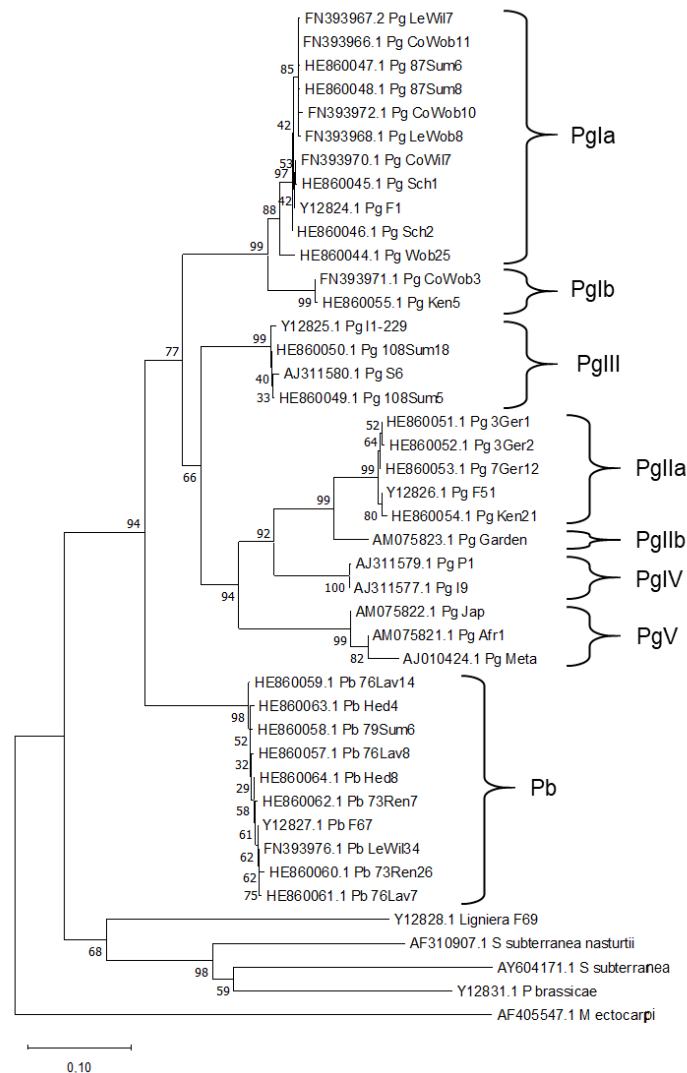


Figure I-5. Neighbor-Joining tree of plasmodiophorids ITS1, 5.8S and ITS2 rDNA sequences. The tree represents the relationships between *P. betae* (Pb) isolates, representative members of *P. graminis* (Pg) ribotypes (Ia-V) and other Phytomyxids: *Ligniera* sp., *Spongospora subterranea* f. sp. *subterranea*, *Spongospora subterranea* f. sp. *nasturtii* (now *Hillenburgia nasturtii*), *Plasmodiophora brassicae*, *Maullinia ectocarpii*. The sequences used to compute this tree were selected according to Smith et al. (2013). Positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons. Numbers on branches were computed according to a bootstrap test (10000). The tree was computed and drawn with the MEGAX software.

The integration of specific combination of host range, temperature requirements and rRNA sequences prompted Legrève et al. (2002) to define five special forms (and associated ribotypes) of *P. graminis* : *P. graminis* f.

CHAPTER I. *Polymyxa betae* and the Plasmodiophorida Order

sp. *temperata* (Pg-I), *P. graminis* f. sp. *tepida* (Pg-II), *P. graminis*, f. sp. *tropicalis* (Pg-III), *P. graminis* f. sp. *subtropicalis* (Pg-IV) and *P. graminis* f. sp. *colombiana* (Pg-V). Since then, this classification has been used to study the diversity within *P. graminis* from diverse parts of the world such as Australia (Cox et al., 2014), Germany (Ziegler et al., 2016), Japan (Ohki et al., 2013, 2017), China (Xu et al., 2018) and south Africa (Terefe et al., 2021). In particular, it was proposed to add a sixth ribotype (Pg-VI) and to subdivide ribotype I (Pg-Ia and Pg-Ib) and II (Pg-IIa and Pg-IIb) to accurately represent the diversity of newly sequenced isolates (Cox et al., 2014). The high genetic diversity found in *P. graminis* isolates compared to the poor diversity observed in *P. betae* raises the question of whether it would be appropriate to modify taxonomy. Even though, for both species, special forms assignment considers the susceptibility and multiplication rate in different hosts, it can't be ignored that the underlying genetic variability is far more extended in *P. graminis* compared to *P. betae*. More genetic information, relying on the sequencing of other loci, could help to better embrace the real diversity in *Polymyxa* genus and to redefine subclades or even to define new species in genus *Polymyxa*.

6. *Polymyxa betae* - *Beta vulgaris*, a tangled relationship

a. Parasitism in *Polymyxa betae* – *Beta vulgaris* interaction

It is known that a pathogenic interaction between plants and bacteria, fungi or some oomycetes could evolve to a non-pathogenic/asymptomatic interaction or even to a mutualistic interaction and *vice versa* (Kemen and Jones, 2012; Brader et al., 2017). Some organisms can even switch lifestyle in function of their life stage and depending on their abiotic and biotic environment.

Among plasmodiophorids, some species such as *P. brassicae* and *S. subterranea* are associated with the induction of cell proliferation and hypertrophy in infected plant roots and underground stems (Graaf et al., 2007; Ludwig-Müller et al., 2009). Since they produce such symptoms, these species are considered as pathogens. *P. betae* is able to “infect” the root cells of its hosts but, most of the time, it doesn't induce obvious disease symptoms or stunting. In rare cases, *P. betae* was reported to induced deleterious effects on its hosts (Barr, 1979; Gerik and Duffus, 1988). Field plants infected by nonviruliferous *Polymyxa* spp. appear normal, except that light brown discoloration of infected roots may occur with massive accumulation of sporosori. In light of this, we could say that *P. betae* is an asymptomatic endophytic parasite but not a pathogen. However, symptoms (sometimes severe) appear on some plants infected by *Polymyxa*-transmitted viruses. In the case of rhizomania, this compatible interaction between BNYVV and plants was not reported to naturally occur without *P. betae*. In this case, we could say that *P. betae* is an integral part of

rhizomania pathobiome and thus a BNYVV-carrying *P. betae* could be considered as a pathogen.

The question remains if *P. betae*-plant interaction (without the virus) should be considered as commensalism or mutualism. Commensalism is defined as a relationship between two species in which one gets an advantage from living closely with the other and the other is not affected by it. Mutualism supposes fitness benefit for both partners (Kogel et al., 2006). It means that *P. betae* must have a positive effect on the plant. Such an effect was observed by Desoignies (2012), who reported an enhanced protection of *B. vulgaris* against *Cercospora* leaf spot after root inoculation with *P. betae*. Apart from this improved protection against a foliar pathogen (*C. beticola*), Desoignies (2012) observed a poor defense reaction in the sugar beet roots, directed against *P. betae*. In the absence of symptoms induced by *P. betae*, the benefits obtained from better foliar protection wouldn't be balanced by equivalent fitness costs. These observations prompted Desoignies to suggest that we should describe the interaction between *P. betae* and the sugar beet as commensalism, possibly evolving to mutualism in the presence of pathogens such as *C. beticola*. However, as intracellular parasite, *P. betae* entirely depends on the cytoplasm of its host's cells to get carbohydrates and other nutrients. The fact that, most of the time, *P. betae* has no impact on *B. vulgaris* growth, even though it clearly represents an energy sink, strongly suggests that the protist should compensate for this dependence with benefits to the plant. This advantage could be the activation of systemic acquired resistance (SAR). Nevertheless, the constitutive activation of plant defenses would a second energy sink for the sugar beet. If the activation of systemic defenses is the only advantage for the plant, one should expect to observe a reduction of plant growth in healthy plants maintained in growth chamber, which is not obvious. Therefore, the nature of this benefit remains unclear.

7. Other plasmodiophorids parasites of major crops

a. *Plasmodiophora brassicae*

Plasmodiophora brassicae is the causal agent of clubroot disease of cruciferous vegetables (Figure I-6B). This widespread disease causes important economic losses in cabbage, rapeseed and other Brassicaceae crops (Hwang et al., 2012). *P. brassicae* is, by far, the most studied plasmodiophorid. This is probably related to its natural ability to infect *A. thaliana*, the most popular plant model for studying plant physiology and molecular biology. Although not reported as such in the literature, *P. brassicae* could therefore be considered as the model plasmodiophorid for studying the interactions of these obligate eukaryotic parasites with plants. Clubroot disease has been reported in more than 60 countries round the world, but is most prevalent in Europe, Australia and North America, where

CHAPTER I. *Polymyxa betae* and the Plasmodiophorida Order

Brassica species are cultivated. Average losses of 10 % are usual in oilseed rape in the UK (AHDB, 2021) but certain authors reported cases where losses reached 50-96% (Dixon, 2009). There are a few pathotypes of *P. brassicae* differing in their host range. For example, the pathotype 3 is highly virulent on rapeseed (*Brassica napus*) but also infects other *Brassica* species while the pathotype 6 infects only vegetable species (Xue et al., 2008). Both pathotypes along with pathotypes 2, 5 and 8 were sequenced and compared. Genomes are compact (about 24,2 – 25,5 Mb) with few repetitive elements and transposons and a high GC content (~59%) (Schwelm et al., 2015b; Rolfe et al., 2016). The mitochondrial genome of two isolates was also reported. The latter is quite large (>100 Kb), rich in introns, intergenic regions and contains large repeats (Daval et al., 2019; Stjelja et al., 2019). Between 9k and 11k genes were predicted on nuclear genome, depending the strains and sequencing project which would represent > 90 % of single-copy Core Eukaryotic Genes (Schwelm et al., 2015b; Rolfe et al., 2016; Bi et al., 2019; Daval et al., 2019; Stjelja et al., 2019). Gene prediction and RNA-seq analysis revealed narrow intergenic distances (often <800pb), with sometimes overlapping UTRs (potentially indicating shared regulatory elements) as it has been reported for other eukaryotes such as the unicellular algae *Guillardia theta* and *Bigeloviella natans* and the fungi *Antonospora locustae* (Williams et al., 2005; Rolfe et al., 2016). Compact genomes are common in biotrophic pathogens, due to reduced intergenic distance, gene loss, and low percentage of repetitive elements compared to non-obligate plant pathogens (Brader et al., 2017). In contrast, free-living Rhizaria seems to have big repeat rich genomes as *R. filosa* (~320Mb) (Glöckner et al., 2014). Even if several isolates have been sequenced, the proteome of *P. brassicae* remains cryptic. For example, Schwelm et al. (2015) have not been able to assign functions to half of the predicted protein coding genes. Similarly, Rolfe et al. (2016) found no homology for more than 30% of the predicted proteins on the Pb3 isolate genome using BLAST searches against the non-redundant protein NCBI database (nr) with a large cutoff (E-value < 10⁻³). In particular, 290 of the 341 paralogous protein families discovered in *P. brassicae* did not match the database nr. Similarly, 132 out of the 221 predicted small secreted proteins (>60%) were not matched to NCBI. Small secreted proteins are proteins potentially excreted by the parasite in its host's cytoplasm and are therefore considered as putative effectors. These results highlight the poor representation of Rhizaria sequences in public databases, stressing the understudied status of this eukaryotic group.

b. Spongospora subterranea

Spongospora subterranea is the causal agent of powdery scab of potato tubers (Figure I-6C) and the vector of the *Potato mop top virus* (PMTV) (Merz and Falloon, 2009; Tamada and Kondo, 2013). The symptoms of powdery scab on potato tubers are skin lesions progressively evolving in enlarged pustules which may rupture and release a powdery mass consisting of numerous sporosori. These symptoms are mostly cosmetic,

causing scabs restricted to the skin of affected tubers which remain edible (Johnson and Cummings, 2015). However, the pathogen is economically important since entire batches of potatoes (especially seeds) may be rejected if infected. *S. subterranea* is indeed included in the “Regulated Non-Quarantine Pest” Annex IV list. Furthermore, the disease may alter the industrial processing of the tubers and reduces their aesthetic quality for supermarket sale (Falloon et al., 2004; Johnson and Cummings, 2015). Other symptoms, such as hyperplasia (galls) on root, stolon and tubers as well as root membrane dysfunctions are reported. These symptoms can be associated with the reduction of water uptake and plant growth (Shah et al., 2012; Falloon et al., 2016). Moreover, the risk associated with the presence of *S. subterranea* is also related to its ability to transmit PMTV. This pomovirus is distributed worldwide and can produce internal symptoms to the potato tubers, making them not marketable, notably for processing (Merz and Falloon, 2009). *Spongospora subterranea* shares the same general life cycle than *P. betae* and *P. brassicae*. It is originated from South America, the cradle of potato, from where it was probably imported in Europe and subsequently dispersed in all potato-growing regions (Gau et al., 2013). Actual losses due to *S. subterranea* are difficult to estimate because of the possible presence of other pathogens in soils, possibly favored by *S. subterranea*, which confounds the results of the various studies that attempted to assess them (Brown et al., 2007; Gilchrist et al., 2011; Johnson and Cummings, 2015). Overall, the devaluation attributed to severe *S. subterranea* infections is estimated to be over 20% (Schwelm et al., 2018). Disease management relies on the use of uninfected seed tubers to avoid field infestation with long-lasting sporosori. Some potato cultivars were found less susceptible to the development of root galls and powdery scab symptoms but no resistance gene has yet been reported (Falloon et al., 2003; Bittara et al., 2016; Schwelm et al., 2018). Zoosporangia of *S. subterranea* were observed in many plant species but root galls occur only on Solanaceae. However, sporosori were rarely observed on non-solanaceous hosts. Moreover, the infection phenotype may also differ between solanaceous hosts (Qu and Christ, 2006b; Falloon et al., 2016), probably indicating a highly specialized interaction between the protist and its hosts. Even though the germination of resting spores is induced by potato root exudates, Balendres et al. (2016) showed that it was mostly due to the presence of l-glutamine and tyramine, which are not specific to potato root exudates. Moreover, a constitutive dormancy within the resting spore population was also evidenced even in germination-stimulating environment (Balendres et al., 2017). Thus, the germination of resting spores would not be host-specific, probably explaining that early infection was detected in a range of non-solanaceous hosts (Merz and Falloon, 2009). Recently, proteomics and transcriptomics analyses of germinating resting spores revealed the regulation of genes related to cell wall cytoskeleton, energy and fatty acid metabolism transcription and translation, synthesis of amino acids, ion transport, response to oxidative stress and DNA repair (Balotf et al., 2021a, 2021b). The nuclear and mitochondrial genomes as well as the transcriptome of *S. subterranea* were recently published (Schwelm et al., 2015b; Gutiérrez et al., 2016; Ciaghi et al., 2018). The draft nuclear genome

CHAPTER I. *Polymyxa betae* and the Plasmodiophorida Order

has a length of 28.08 Mb and encodes 10,780 predicted genes. In particular, it contains intron-rich genes and is enriched with chitin-related enzymes, like the one of *P. brassicae*. However, unlike of *P. brassicae*, it is also enriched with transposable elements and virus-related genes (Bulman et al., 2011; Schwelm et al., 2015b; Ciaghi et al., 2018). The mitochondrial genome is quite compact (~38Kb) compared to the one of *P. brassicae* and less rich in intron and repeats (Daval et al., 2019). A total of 697 proteins were predicted to be secreted, of which 102 were classified as potential effectors (Ciaghi et al., 2018).

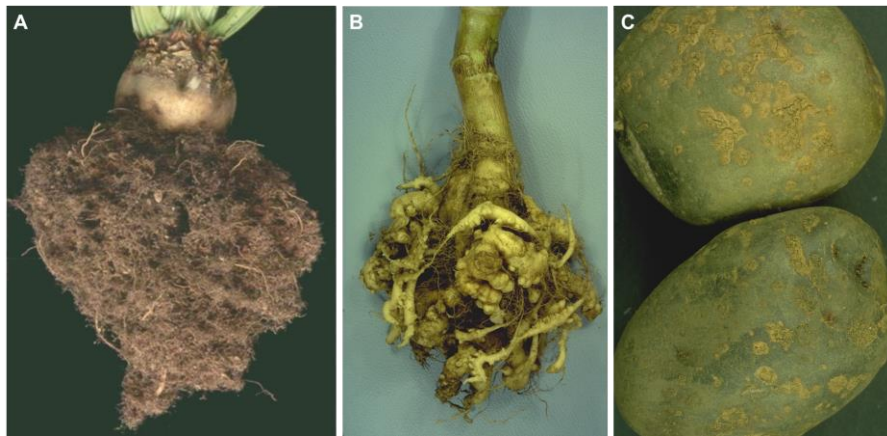


Figure I-6. Comparison of the symptoms of rhizomania induced by BNYYV vectored by *P. betae* (A), clubroot induced by *P. brassicae* (B), and powdery scab induced by *S. subterranea* (C).

CHAPTER II.

Tripartite interaction in rhizomania: review

Rhizomania is one of the most devastating diseases of sugar beet crops (Biancardi and Tamada, 2016). The causal agent of rhizomania is the *Beet necrotic yellow vein virus* (BNYVV). Despite the first reports dating back to the early 1950s, this virus remains a huge burden to the sugar beet crop. Indeed, it causes significant yield losses, even on resistant cultivars, due to virus populations associated with the breakdown of plant resistance (Hanse et al., 2011). The BNYVV is a single stranded RNA virus member of the *Benyvirus* genus, in the family *Benyviridae*. Its multipartite genome is composed of two main RNAs (RNA1 and RNA2) essential for virus cycle completion and three complementary RNAs primarily involved in pathogenicity, RNA silencing suppression and vector transmission (Gilmer and Ratti, 2017). BNYVV is transmitted naturally by only one known vector: the plasmodiophorid protist *Polymyxa betae* Keskin, an obligate intracellular parasite of *B. vulgaris* roots (Keskin, 1964; Tamada and Asher, 2016b). This protist greatly influences rhizomania epidemiology because of its obligate parasite nature, its ability to survive as resting spores (sporosori) in infested soils for decades and the fact that it infects plants through swimming zoospores. Other species of plasmodiophorid can transmit plant viruses: *Spongospora subterranea* is the vector of *Potato mop-top virus* (Jones and Harrison, 1969; Reavy et al., 1998) and *P. graminis* is the vector of beny- (Bagayoko et al., 2021), bymo- (Vaïanopoulos et al., 2006), furo- (Vaïanopoulos et al., 2005) and pecluviruses (Dieryck et al., 2009) associated with major diseases mainly on cereals (Dieryck et al., 2011).

Regarding the strict endoparasite nature of both the BNYVV and its vector it is clear that the rhizomania disease is the outcome of a fine tripartite interplay between the virus, the vector and their common host, *B. vulgaris*. In this chapter, we therefore achieved a systematic review of current knowledge on interactions between the three main rhizomania protagonists. According with the aim of this thesis, emphasis is placed on studies aiming to improve knowledge on the interactions between the three main protagonists of rhizomania than on studies focusing mainly on a single organism. The gaps of knowledge existing in this complex tripartite interaction are highlighted. Selected recent studies on other plant-plasmodiophorid(-virus) interactions are also considered here as they are helpful to grasp the rhizomania model.

1. General considerations about rhizomania

Sugar beet production is threatened by numerous pathogens. One of the most important is the *Beet necrotic yellow vein virus* (BNYVV), a multipartite single-stranded RNA virus causing the Rhizomania disease of sugar beet (Tamada and Baba, 1973; Rush et al., 2006; Biancardi and Tamada, 2016). Symptoms of rhizomania are presented in Figure II-1. The main symptoms of the disease are visible on the roots such as a reduced taproot size with “T-shape” aspect, a proliferation of LR giving a bearded aspect and a browning of the vascular system. Leaf chlorosis, wilting and vein necrosis are also sometimes observed. In susceptible varieties, the disease usually causes a 50-60% reduction in sugar yields, sometimes up to 80% (McGrann et al., 2009).

The rhizomania distribution is global, occurring in every zone of sugar beet cultivation (Tamada and Asher, 2016a; Dehnen-Schmutz et al., 2020). The first reports of rhizomania date back to the 1950s in the Padan Plain (the Pô valley) in the North of Italy (Canova, 1959). The disease observed at that time was called “rhizomania” (Alghishi and D’Ambra, 1966), an Italian term meaning “root madness”, because the main symptom was a strong proliferation of LR. The rhizomania syndrome was then reported in many other countries (Canova et al., 2016), including Belgium. Since the first reports in the Polders in 1984, the disease has spread to all sugar beet production areas (Meunier et al., 2002).

The vector of BNYVV was early identified to be *Polymyxa betae* Keskin (Fujisawa and Sugimoto, 1977; Vuittenéz et al., 1977; Tamada and Asher, 2016b), a plasmodiophorid protist belonging to the Rhizaria supergroup (Neuhauser et al., 2014) (see Chapter I). This protist is also the vector of other beny- et pomovirus infecting sugar beet: *Beet soil-borne mosaic virus* (BSBMV) (Heidel et al., 1997; Lee et al., 2001), *Beet soil-borne virus* (BSBV) (Mahillon et al., 2021) and probably also *Beet virus Q* (BVQ) (Meunier et al., 2003; Crutzen et al., 2009b). Their implication in the production of rhizomania symptoms remains enigmatic (Kutluk Yilmaz et al., 2018b) but some authors suggested that some positive or negative interactions occur between those viruses (Rush, 2003; Crutzen et al., 2009b, 2009a; Crutzen, 2010).

Although cultural practices, such as drainage or long rotations, can help reduce the impact of rhizomania on sugar beets somewhat, the best way to avoid yield losses is to use resistant cultivars (Ratti and Biancardi, 2016). A limited number of resistance genes against the BNYVV were identified (Panella and Biancardi, 2016). Two of them, *Rz1* associated or not with *Rz2* were introgressed in most commercial cultivars (Biancardi et al., 2002; Biaggi et al., 2011). Unfortunately, a resurgence of the disease was reported on *Rz1* cultivars in most sugar beet growing regions (Liu et al., 2005; Acosta-Leal et al., 2009; Pferdmenges et al., 2009; Chiba et al., 2011; Bornemann et al., 2015; Galein et al., 2018; Kutluk Yilmaz et al., 2018b;

Weiland et al., 2019). Two key viral proteins were associated with avirulence and resistance-breaking (RB): the P25 and P26 encoded in RNA3 and RNA5, respectively. More specifically, a hypervariable sequence of four amino acids (aa) located at positions 67-70 of P25 would be involved in the RB phenotype (see Chapter II.2.d). The *Rz2* resistance gene encodes a CC-NB-LRR protein (Capistrano-Gossmann et al., 2017). Little is known about the resistance mechanisms mediated by *Rz1*. Recently, a reverse genetic approach revealed that *Rz1*-mediated resistance is restricted to roots, and that RB ability conferred by RNA5 is independent of the RB ability conferred by the P25 variants (Liebe et al., 2020). Sources of resistance to *P. betae* were also identified in *Beta vulgaris* subsp. *maritima*, *B. patellaris* and *B. procumbens* and may provide an additional target for plant breeders. However, their introgression to cultivated beet accessions has not yet been achieved (Barr et al., 1995; Asher et al., 2009; Panella and Biancardi, 2016). It was proposed that partial resistance to *P. betae* would be associated with a decrease of the virus load (Barr et al., 1995; Asher et al., 2009; McGrann et al., 2009) and thus that a protection against the vector could be beneficial to reduce rhizomania pressure. However, other authors reported that an increased resistance to *P. betae* would not necessarily predict an increased tolerance to rhizomania, limiting the perspectives in sugar beet breeding (Paul et al., 1992, 1993).



Figure II-1. Representative pictures of the main rhizomania symptoms. (a) Comparison of taproot sizes between susceptible cultivars and a tolerant cultivar, **(b)** rootlets proliferation with a beard aspect, **(c)** “T-shaped” taproots showing a constriction and necrosis of the vascular tissues (arrows), **(d)** comparison of leaf chlorosis of a susceptible cultivar with leaves of a tolerant cultivar, **(e)** wilting and **(f)** leaf vein necrosis symptom (arrows) (courtesy of Y. Galein).

2. The BNYVV and its interactions with host plants

a. Genomic organization of BNYVV

BNYVV is the type member of the *Benyvirus* genus, in the family *Benyviridae* (Gilmer and Ratti, 2017). Its genome is constituted of four to five linear positive sense ssRNAs (Figure II-2) that are packed separately in rigid rod-shaped particles with a helical symmetry. Virions are 20 nm large, and 65-80, 85, 100, 265, 390 nm long depending on the encapsulated RNA length (Putz, 1977; Steven et al., 1981; Tamada et al., 2016). Each RNA is 5'-capped and 3'-poly-adenylated. The cap is constituted of a 7-methylguanosine linked by a triphosphate to the 5' hydroxyl group of viral RNAs (m7G5'ppp5') (Putz et al., 1983). It belongs to the *Benyvirus* genus, along with *Beet soil-borne mosaic virus* (BSBMV), *Rice stripe necrosis virus* (RSNV) and *Burdock mottle virus* (BdMoV). The benyviruses harbor two mandatory genomic RNAs carrying the functions of replication, packaging, vector transmission, movement in the plants and suppression of post-transcriptional gene-silencing. If RSNV and BdMoV apparently only rely on two RNAs (Lozano and Morales, 2009; Kondo et al., 2013) to fulfill their cycle, the BSBMV contain four RNAs (Heidel et al., 1997) and BNYVV isolates have four to five genome components of about 6.7, 4.6, 1.8, 1.4 and 1.3 Kb, respectively (Kiguchi et al., 1996; Saito et al., 1996). The BNYVV RNA1 and RNA2 are always found in natural field isolates (Schirmer et al., 2005; Chiba et al., 2011; Galein et al., 2018). Even if they are sufficient to establish infection after mechanical inoculation on multiple hosts, and thus contains all housekeeping genes, the two to three complementary RNAs are involved in increased pathogenicity, silencing suppression and vector transmission (Peltier et al., 2008; Gilmer and Ratti, 2017). Interestingly, BNYVV and BSBMV share the same vector, a similar host range and are sometimes found in the same plant. Their genomic components are largely exchangeable (Laufer et al., 2018b) but a spatial separation occurred in *N. benthamiana* after coinfection or sur-infection with both species (Laufer et al., 2018a). The roles of each genomic component in the interactions with plants and vector attracted much research attention since first reports on BNYVV. In this section, we review principal findings on the function of the different genomic features of BNYVV.

CHAPTER II. Tripartite interaction in rhizomania

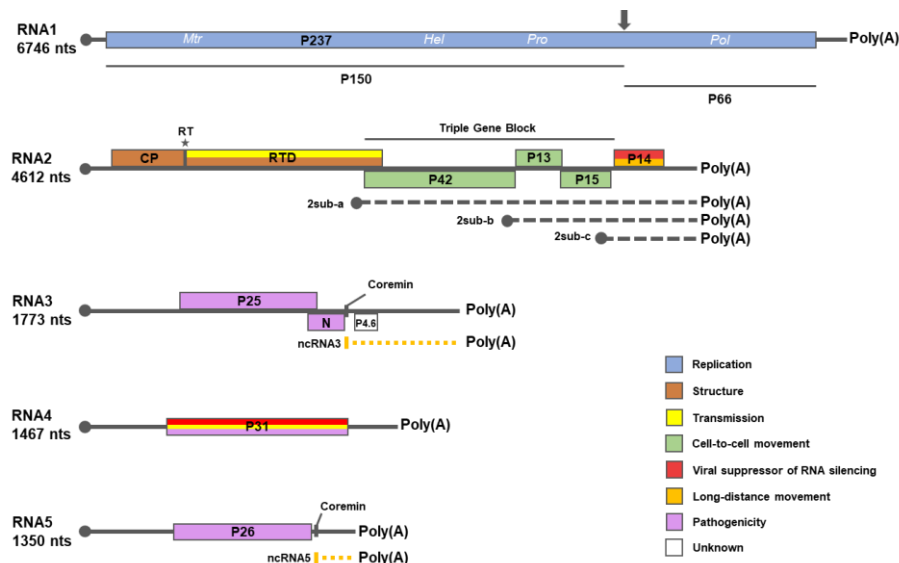


Figure II-2. Representation of the genomic organization of the BNYVV, adapted from Crutzen (2010) and Gilmer and Ratti (2017). All RNAs have an m7G5'ppp5' cap (circles) and a 3'-poly-adenylated tail (Poly(A)). The replicase (P237) contains methyltransferase (Mtr), helicase (Hel), protease (Pro), and RNA-dependent-RNA polymerase (Pol) motifs. ORFs encoding proteins are represented by the colored boxes. Colors are associated with the function(s) of each protein or non-coding RNA and specified in the legend. Polyfunctional proteins are associated to more than one color. The star indicates the leaky termination codon of the coat protein (CP) allowing the expression of a CP extended by a read-through domain (RTD). The arrow shows the autocatalytic site in P237.

RNA1

The first RNA is 6,746 nucleotides (nts) long and contains one single ORF (shown in Figure II-2) that encodes two large polyproteins of 220 and 237 kDa (P237), depending on the start codon used (AUG₁₅₄ and AUG₄₉₆) (Bouzoubaa et al., 1987). Both contains the three domains associated with viral translation: methyltransferase domain (MTR), NTP-binding/helicase domain (HEL) and RNA-dependent RNA-polymerase domain (RdRp) (Bouzoubaa and Scheidecker, 1990). Both polyproteins are autocatalytically cleaved by a papain-like protease domain into two proteins of 150 kDa (P150) and 66 kDa (P66) involved in viral genome replication (Hehn et al., 1997). To assemble the functional replicase complex, the P150 N-terminal domain would interact with itself and with the P66-RdRp internal domain and would be translocated to the endoplasmic reticulum through an interaction mediated by the N-terminal part of P237 protein (Pakdel et al., 2015).

RNA2

The second RNA is 4,612 nts long and encodes six proteins (Figure II-2) using different translational strategies (Bouzoubaa et al., 1986). The first ORF is directly translated from RNA2 and encodes two variants of the coat protein (CP). Three subgenomic RNAs (2sub-a, 2sub-b, 2sub-c), collinear in their 3'-end with the end of RNA2, are produced from genomic RNA2. They contain a triple gene block (TGB) encoding 42 kDa (P42), 13 kDa (P13) and 15 kDa (P15) proteins as well as an ORF encoding a 14 kDa (P14) viral suppressor of RNA silencing (VSR) (Gilmer et al., 1992; Chiba et al., 2013).

The most abundant CP is 21 kDa in size and is the major packaging component in the formation of virions. Occasionally (~10% of time), the amber stop codon is overlooked in a translational read-through (RT) mechanism, leading to the expression of an extended 75 kDa protein called CP-RT (Ziegler-Graff et al., 1985). The CP-RT is involved in virion assembly (Schmitt et al., 1992) and vector transmission by *P. betae* (Tamada and Kusume, 1991). It was shown to be protruding from BNYVV virions extremities (Haeberlé et al., 1994). Similarly, CP-RT of PMTV, a pomovirus transmitted by *S. subterranea*, was also observed at the extremities of virions (Cowan et al., 1997). Electron micrographs showing BNYVV and PMTV virions for which the RT has been labelled with gold particles are visible in Figure II-3. The RT domain of BNYVV CP-RT contains two transmembrane domains and a KTER motif, indispensable for efficient vector transmission (Tamada et al., 1996b). It is probably involved in the anchorage of virions in a host organelle membrane and/or in a membrane of their plasmodiophorid vectors (Adams et al., 2001). The structure and implication of CP-RT and its role in transmission is further developed in Chapter II.3.c.

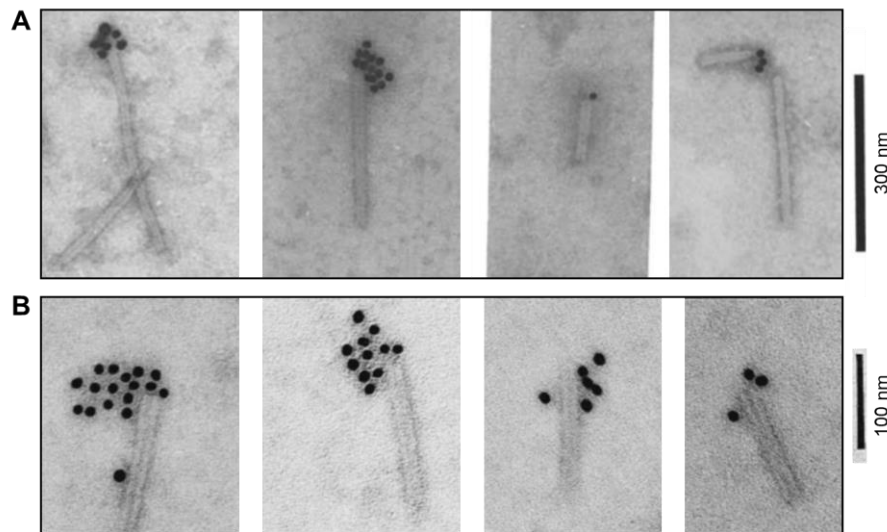


Figure II-3. Electron micrographs of BNYVV (A) and PMTV (B) particles labeled with anti-RT-immunogold conjugate. The localization of gold particles (black dots) suggests that RT is protruding from the extremities of the viral particles. Images have been selected from Haeberlé et al. (1994) for BNYVV and Cowan et al. (1997) for PMTV.

The TGB P42 is produced from 2sub-a whereas the P13 and the P15 are both produced from 2sub-b due to a leaky scanning mechanism (Gilmer et al., 1992). These proteins are involved in cell-to-cell movement of non-encapsidated viral RNAs through plasmodesmata (Verchot-Lubicz et al., 2010). The 42 kDa protein possess RNA/DNA binding activity in N-terminal region and ATP/GTP-dependent helicase motif in C-terminal region and is able to form viral ribonucleoprotein (vRNP) complexes with viral RNAs (Bleykasten et al., 1996). The P13 contains a hydrophilic region surrounded by two hydrophobic domains and was found associated with membrane structures (ER and plasma membranes) whereas P15 is principally hydrophobic (Niesbach-Klösigen et al., 1990; Lauber et al., 1998a). A highly specific interaction between the three cognate components of BNYVV TGB is sufficient and essential for activity *in planta* (Lauber et al., 1998a; Erhardt et al., 2005). More specifically, the P13 and P15 hydrophobic polypeptides would provide a plasmodesmata docking site to the P42 driven ribonucleoprotein RNAs complexes following the ER network (Erhardt et al., 2000, 2005) where the P42 would influence the plasmodesmatal size exclusion limit, allowing the movement of viral RNAs through the plasmodesmata (Lauber et al., 1998a; Verchot-Lubicz et al., 2010). The stoichiometry of BNYVV TGB components is essential for proper function (Bleykasten-Grosshans et al., 1997). In a recent report, Jiang et al. (2020b) highlighted the importance of the ATPase activity (ATPase/helicase domain) in TGB1 of *Barley stripe mosaic virus* (BSMV), *Potato virus X* (PVX), and BNYVV for the formation of vRNP complex assembly, plasmodesma and actin targeting, and cell-to-cell movement. It was also shown that TGB1

organization has been maintained in hordei-like viruses. Curiously, an in-frame insertion containing a nucleolar localization signal (NoLS) and a nuclear localization signals (NLS) was recently detected in TGB1 of several RSNV isolates and could play a role in cell-to-cell and/or long-distance movement (Bagayoko et al., 2021). The organization is represented in Figure II-4.

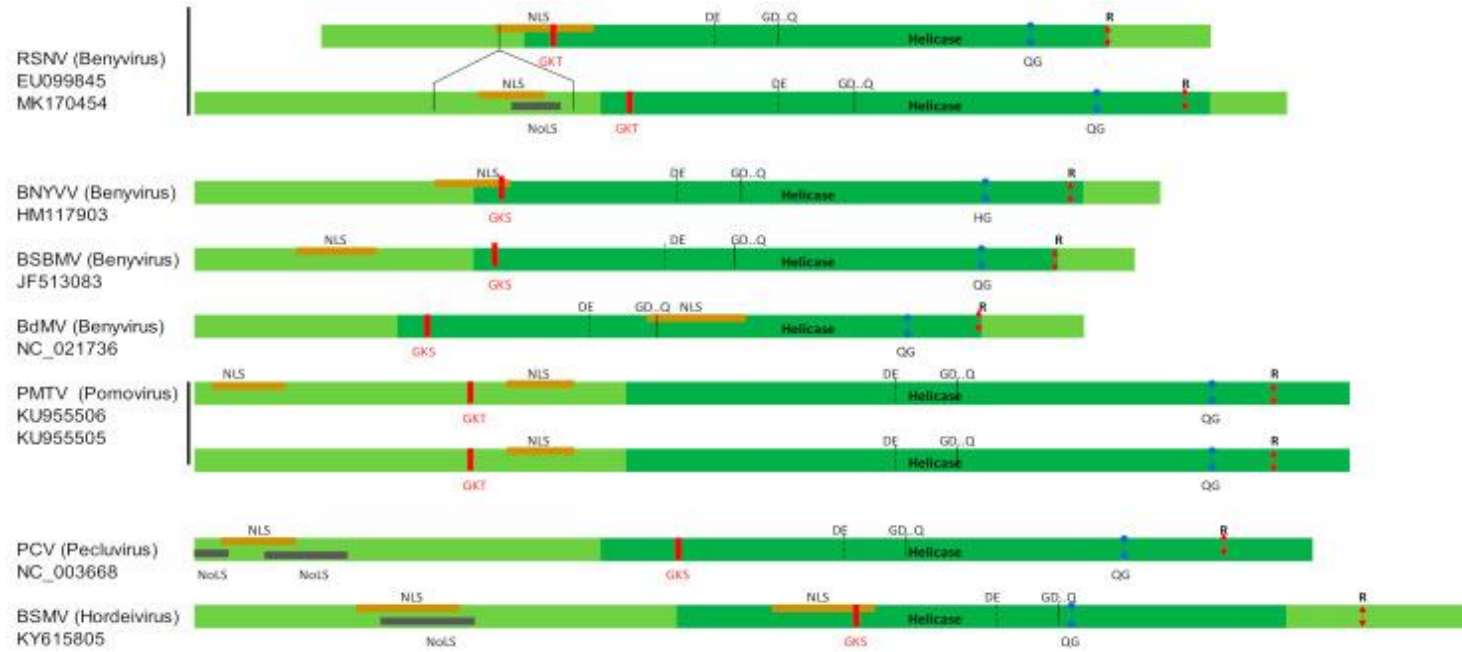


Figure II-4. Schematic representation of the organization of TGB1 in hordei-like viruses (Bagayoko et al., 2021). NoLS and NLS are represented in grey and brown, respectively. The helicase domain is shown in dark green. Insertion in recently sequenced RSNV isolates is indicated by black lines.

The 14 kDa cysteine-rich protein harbors a Zinc-finger domain and a NoLS domain. It was shown to suppress RNA silencing in host roots, likely interfering with AGO-siRNA loading (Hehn et al., 1995; Zhang et al., 2005; Andika et al., 2012; Chiba et al., 2013). It accumulates in both the cytoplasm and the nucleolus but localization in the nucleolus is not essential for VSR activity. Chiba et al. (2013) showed that P14 VSR activity is not directly involved in BNYVV replication and cell-to-cell movement but is required for long-distance movement in the presence of CP. Flobinus et al. (2016) confirmed the role of the P14 VSR activity in BNYVV long-distance movement and showed that this role was supported by the accumulation of non-coding RNA3 (ncRNA3; linked to the coremin sequence, see below) during the infection. Thus, the systemic spread of BNYVV would be linked to a functional silencing suppression activity, at least in *N. benthamiana* and *B. macrocarpa*. The P14 would influence the RNA-dependent RNA-polymerase 6 (RDR6) silencing pathway while ncRNA3 accumulation acts as a second VSR (Flobinus et al., 2016).

RNA3

The third RNA is 1,773 nts long and contains three ORFs, as illustrate in Figure II-2) (Bouzoubaa et al., 1985). It is involved in the development of symptoms in several mechanically inoculated hosts (*Tetragonia expansa*, *B. vulgaris*, *B. macrocarpa* and *Chenopodium quinoa*), yield reduction and increased virus content in taproots of susceptible *B. vulgaris* cultivars as well as in systemic spread in *B. macrocarpa* (Tamada et al., 1989; Koenig et al., 1991; Lauber et al., 1998b; Tamada, 2007).

The first ORF encodes a 25 kDa protein (P25). Its complete sequence has been associated with the strong symptom development in leaves of *T. expansa* (Jupin et al., 1992; Klein et al., 2007), and *Beta* species (Chiba et al., 2008). It also participates in the induction of the rhizomania-like root symptoms in *B. vulgaris* and *A. thaliana* (Koenig et al., 1991; Peltier et al., 2011). Besides being involved in pathogenicity in susceptible hosts, P25 acts as an avirulence factor in resistant cultivars of *B. vulgaris* subsp. *maritima* and in *B. vulgaris* subsp. *vulgaris* carrying the *Rz1* resistance gene (Chiba et al., 2003, 2008; Tamada, 2007). As illustrated in Figure II-5, the P25 sequence contains a NLS and a nuclear export sequence (NES) allowing nucleocytoplasmic localization of the protein (Haeberle and Stussi-Garaud, 1995; Vetter et al., 2004). It may be synthesized at an early infection stage compared to CP (Haeberle and Stussi-Garaud, 1995). Vetter et al. (2004) reported that symptom phenotype is altered when subcellular localization of P25 is modified. Since P25 contains a zinc finger domain, it was proposed that its effect on plants could be related to a transcriptional factor activity. Transcription activation was confirmed by yeast one hybrid experiment (Klein et al., 2007). It was also proposed that the P25 could interact with plant ubiquitin/proteasome system (Thiel and Varrelmann, 2009; Thiel et al., 2012). An hypervariable four amino acid motif, the so-called “tetrad”, was shown to be involved in resistance breaking (RB)

abilities of some BNYVV isolates (Schirmer et al., 2005; Galein et al., 2018). The role of P25 in plant-BNYVV interactions is further developed in Chapter II.2.d.

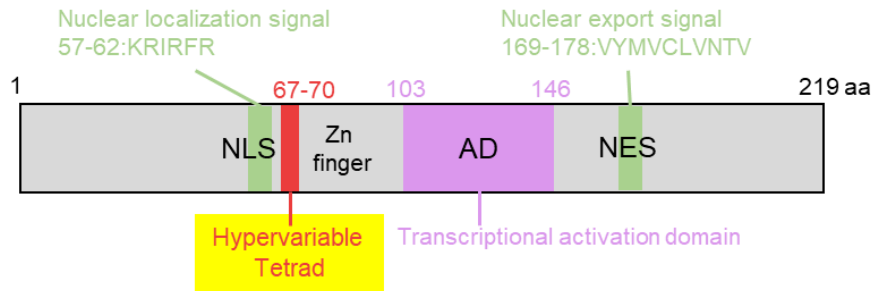


Figure II-5. Schematic representation of the P25 protein showing the positions of important functional domains (adapted from Chiba et al., 2011).

The second ORF overlaps the 3'-terminal portion of the 25 kDa ORF. It is expressed only in RNA3 deletion mutants and induces strong necrosis symptoms, even outside a BNYVV context (Jupin et al., 1992; Wang et al., 2011). In *N. benthamiana*, spontaneous deletion mutants occur and are more stable wild type (wt) RNA3. The N protein may have a role in systemicity (Wang et al., 2011). The last ORF encodes a 4.6 kDa protein that was never observed in natural conditions (Gilmer, 2016)

The effect of RNA3 on systemic movement of BNYVV in *B. macrocarpa* is promoted by a non-coding RNA3 sequence rather than by a protein (Laufer et al., 1998b). This characteristic was linked to the “coremin” sequence embedded in the “core” domain. It involves the accumulation of a ncRNA extending from the coremin sequence to the 3' end of the RNA3. This feature is also present in BSBMV (Ratti et al., 2009; Peltier et al., 2012; Flobinus et al., 2016). RNA3 of both viruses can complement each other for efficient long-distance movement in *B. macrocarpa* (Laufer et al., 2018b). The yeast 5'-to-3' exoribonuclease Xrn1 and plant Xrn4 were shown to process RNA3, producing the ncRNA3 species by stalling at the level of coremin sequence (Flobinus et al., 2018). The saturation of exoribonuclease could in turn impact RNA silencing machinery (Flobinus et al., 2016, 2018). The stalling of 5'-3' exoribonuclease activity involves 2 hairpins of 3 and 4 base pairs respectively and a highly conserved 10-nucleotides spacer that separates the hairpins (Dilweg et al., 2019).

RNA4

The fourth RNA is 1,467 nts long and contains one single ORF (Bouzoubaa et al., 1985). It encodes a soluble cysteine rich 31 kDa polyfunctional protein (P31) (Niesbach-Klösger et al., 1990) shown to participate to the enhancement of the symptom expression (slightly and severely in *Beta sp.*

and *N. benthamiana*, respectively) and silencing suppression in roots but does not influence viral RNA accumulation. It is also involved in efficient vector transmission, along with the RNA2 encoded CP-RT (Tamada and Abe, 1989; Rahim et al., 2007). A schematic representation of RNA4 is visible in Figure II-2).

P31 was shown to induce the transcription of the Pathogenesis-Related 10 gene (PR-10) in *N. benthamiana* (Wu et al., 2014). Transcriptomic analysis suggested that the strong symptom elicitation induced by RNA4 in *N. benthamiana* would result from the interaction of P31 with the host transcription network, since P31 accumulates primarily in the nuclei of *N. benthamiana* epidermal cells (Fan et al., 2014). The deregulation of hormone signaling pathways (auxin, ethylene, gibberellin) would be involved in symptom development (Liu et al., 2020a).

RNA5

A fifth, 1,350 nts long, RNA was described in Japan and contains a single ORF (Figure II-2) coding for a 26 kDa protein (P26) (Richards and Tamada, 1992; Kiguchi et al., 1996). It was also associated with European BNYVV P-type isolates (Koenig et al., 1997). Later it was also further found in China (Li et al., 1999), Japan (Miyanishi et al., 1999), Kazakstan (Koenig and Lennefors, 2000) and England (Harju et al., 2002). The P26 is considered as a second pathogenicity factor, and share characteristics with RNA3-encoded P25 (Koenig et al., 1997). It is not essential for the virus life cycle but involved in strong symptom expression in sugar beet (Kiguchi et al., 1996; Tamada et al., 1996a; Liu et al., 2003) and in *C. quinoa* (Link et al., 2005). As P25, P26 was observed in cytosol and nucleus of infected cells, was shown to self-interact and was shown to have a transcription activator activity in yeast one hybrid assay (Link et al., 2005; Covelli et al., 2009). As RNA3, RNA5 possesses a coremin sequence responsible for the accumulation of noncoding RNA5 (ncRNA5). This noncoding RNA can complement the ncRNA3 for long-distance movement in *B. macrocarpa* (Peltier et al., 2012). Scab-like symptoms were produced on roots by a laboratory isolate lacking RNA3 but harboring RNA5, differing from the symptoms produced by isolates containing RNA3. It was thus proposed that RNA3 and RNA5 play different roles in the development of symptoms in sugar beet roots (Tamada et al., 1996a). The P26 and P25 pathogenicity factors would therefore have a complementary effect and both could be involved in the RB events observed on sugar beet containing the *Rz1* resistance gene. Recently, Tamada et al. (2021) confirmed in field and laboratory studies that the BNYVV RNA5 is involved in RB on *Rz1* sugar beet plants. Isolates carrying a fifth RNA showed increased accumulation of RNA3 and produced scab-like symptoms on roots.

b. Host range

The host range of BNYVV was thought to be very narrow, including naturally infected *B. vulgaris*, *B. macrocarpa* and *Spinacia* sp. In laboratory tests, systemic hosts include *N. benthamiana*, *Spinacia oleracea* and *B. macrocarpa*. *Chenopodium* sp. can also be infected experimentally by foliar rub-inoculation, but infection remains local (Gilmer and Ratti, 2017). A broader host range study was conducted by Legrève et al. (2005) using cultivation of a wide range of potential host plants on soil infested with *P. betae* carrying BNYVV. They showed, by back inoculation tests on *B. vulgaris*, that the host range of *P. betae* and BNYVV could be wider, including other Chenopodiaceae (*Atriplex hortensis*, *Chenopodium polyspermum*, *S. oleracea*), Caryophyllaceae (*Silene alba* and *S. noctiflora*) and Poaceae (*Apera spica-venti* and *Poa pratensis*) and even more if taking account of plants where both organisms were detected by RT-PCR but for which back-inoculation test failed.

c. BNYVV diversity

Two major variants of the BNYVV were initially determined: the A-, B-type (Kruse et al., 1994; Koenig et al., 1995; Saito et al., 1996), harboring small changes in the CP and P25 sequences (Gilmer and Ratti, 2017). The presence of a fifth RNA in A-type-like isolates in France lead to the description of a third variant called P-type (Koenig et al., 1997). Different types of BNYVV showed differences in their pathogenicity. For example, in controlled greenhouse trials, the BNYVV B-type was less damaging than A- and P-types. The virus content in the taproots and the viral movement between lateral roots and taproots were higher in sugar beets infected with P-type than with A- and B-types (Heijbroek et al., 1999). Even if the RNA5 containing isolates are restricted in small areas in Europe, this additional genome component is widespread in China and Japan (Miyanishi et al., 1999; Li et al., 2008). Asian isolates containing RNA5 were very different from European isolates containing a fifth RNA (Miyanishi et al., 1999; Koenig and Lennefors, 2000), leading to the proposition of a Japanese type (J-type) (Tamada et al., 2016). In the last decade, conjoint analyses of the sequences of genes encoding the CP, P25 and P31 refined BNYVV isolate clustering accounting also for the geographical distribution, the presence or absence of RNA5 and the sequence of the P26 gene (Schirmer et al., 2005; Chiba et al., 2011; Zhuo et al., 2015; Tamada et al., 2016). In phylogenetic trees, the CP gene clustered in two branches (A and B), the P25 gene was divided in three branches (I, II, III) and P31 gene clustered in four groups (I, II, III, IV). The current phylogenetic characterization was summarized by Tamada et al. (2016) and presented in Table II-1.

Table II-1. Current BNYVV strains and their original types classified according to the sequences of CP, P25 and P31 genes as well as the presence or absence of RNA5 and their geographical distribution (adapted from Tamada et al., 2016).

Original type	Strain	CP	p25	p31	RNA5	Distribution
A-I	China-H	A or B	I	I	+	China, Japan, UK
A-I	China-Y	A or B	I	I	+	China
A-II	France-P (=P type)	A	II	II	+	France, Kazakhstan, UK, Iran
A-II	Japan-D	A	II	II	+	Japan
A-II	Japan-O	A	II	I or II	+	Japan
A-II	China-B	A or B	II	II	+	China, Germany
A-II	China-L	B	II	I	+	China
A-III	Italy (=European A type)	A	I	III	-	Europe, USA, Middle East
B	China-X	A	III	I or III	+	China
B	Germany (=B type)	B	III	IV	-	Germany, France, Belgium, Austria, Switzerland, Czech Republic, China

d. The RNA3-encoded P25. A key component of plant-virus interactions.

As mentioned above, the P25 protein encoded on BNYVV RNA3 is a key pathogenicity factor for the virus, sometimes complemented by the P26 protein from RNA5 (Bornemann and Varrelmann, 2013; Tamada et al., 2021). However, it was also proposed that this protein would act as an avirulence factor on resistant plants (Chiba et al., 2008). Partially deleted P25 mutants inoculated through *P. betae* to sugar beet roots were unable to produce symptoms on susceptible or resistant varieties where wild-type does on a susceptible variety (Tamada et al., 1999). In the same study, the translocation of the wt virus to the taproot was inhibited in the partially resistant cultivar. More generally, it was suggested that multiplication of the BNYVV in sugar beet roots and translocation of the virus from the rootlets to the taproot was inhibited in the partially resistant varieties (reviewed in Panella and Biancardi, 2016). Similarly, different isolates of the BNYVV showed different abilities to produce symptoms and to accumulate in *B. vulgaris* subsp. *vulgaris* and *B. vulgaris* subsp. *maritima* after foliar mechanical rub-inoculation (Tamada, 2007; Chiba et al., 2008). The sequence of the P25 elicitor, especially 3 aa residues at positions 68, 70 and 179, appeared determinant in the virus strain–cultivar-specific resistance interaction, resulting in no symptoms, local necrosis with reduced viral accumulation or local bright yellow lesions accompanied by an abundant viral accumulation (Chiba et al., 2008). Analysis of worldwide P25 diversity confirmed that a four aa hypervariable region, called “tetrad”, is present at positions 67-70 of the P25 and that it is associated with the ability of BNYVV

CHAPTER II. Tripartite interaction in rhizomania

isolates to overcome *Rz1*-resistance in sugar beet roots (Schirmer et al., 2005; Klein et al., 2007; Chiba et al., 2011; Galein et al., 2018).

After the emergence and dispersion of variants able to break *Rz1* resistance in USA (Liu et al., 2005; Liu and Lewellen, 2007), Acosta-Leal and Rush, (2007) reported that mutations in the P25 at position 67 (Alanine to valine [A>V]) and 135 [D>E] are associated with RB phenotype in Imperial Valley. Even if a high variability was obtained at positions 67 and 68 (aa₆₇₋₆₈) between RB and non-RB isolates of the BNYVV, Liu and Lewellen (2007) did not find a rule to differentiate RB and non-RB isolates based only on P25 sequence. The aa₆₇₋₆₈ of non-RB isolates found in that study were always AC whereas AF, AL, SY, VC, VL, and AC were found in RB isolates. By sequencing P25 cDNA clones from multiple BNYVV field populations Acosta-Leal et al. (2008) found that populations isolated from susceptible plants had the same P25 dominant haplotype, whereas a different major haplotype and a spectrum of mutants was determined in isolates infecting resistant cultivars. They suggest that when wt BNYVV population infect *Rz1*-resistant plants, the P25 would be firstly under positive selection for changes involved in RB, increasing intra-population sequence diversity (and thus a mix of tetrads are presents). In a second time, a purifying selection would occur, decreasing intra-population diversity (Acosta-Leal et al., 2008, 2010a). Further investigations in controlled conditions revealed that US isolates and a Spanish A-type isolate with VL and VC at positions 67 and 68, respectively, were RB whereas an Italian and a German A-type strains with AH and AY, respectively, were not (Pferdmenges et al., 2009) and that RB was independent of viruliferous *P. betae* content in soils (Pferdmenges and Varrelmann, 2009; Bornemann and Varrelmann, 2011). Moreover, the small sequence variation found in the *P. betae* ITS region, probably linked to its limited host range, could indicate that rhizomania RB is not likely due to changes in *P. betae* populations (Smith et al., 2013). The importance of the amino acid at position 67 of the P25 for the outcome of the interaction was further confirmed by reverse genetics and mechanical inoculations (i.e. without *P. betae*) and field observations in which the virus was transmitted by the vector (Koenig et al., 2009; Acosta-Leal et al., 2010b). Chiba et al. (2011) reported a positive selection pressure on *P25* gene and confirmed that a V at position 67 is likely associated with stronger symptoms in *Rz1*-resistant varieties. Moreover, they also highlighted that exchanges of aa at position 68 from F/Y to C, H, L, or Q in Italian BNYVV isolates is involved in symptom development in *Rz1*-resistant varieties. However, the RB ability of aa₆₇₋₆₈ variants seems to differ between isolates from distinct geographical areas, suggesting that other features may influence RB. For example, the VCHG tetrad was not associated with RB events in Greece (Pavli et al., 2011b). More recently, no clear association of P25 with RB ability was found in commercial genotypes growing areas in North Dakota and Minnesota (Weiland et al., 2019). Conversely, a new strain with AYPR at positions 67-70 of the P25 was shown to be able to overcome *Rz1*-mediated resistance but not *Rz1*+*Rz2*. This tetrad was detected in Netherland, England, France and Belgium (Bornemann et al., 2015; Decroës et al., 2015; Galein et al., 2018). Recently, a deleted form of the p25 (67 or 68) was identified in RB A-

and J-type BNYVV populations in Turkey (Kutluk Yilmaz et al., 2018a). An RB BNYVV strain also showed an increased incidence on spinach in a cultivar dependent manner (Mou et al., 2012), suggesting an extended role of P25 factors in various plant species. It should also be noted that, in susceptible plants, RB variants may display a relative fitness cost (Bornemann and Varrelmann, 2013). Recently, Liebe et al. (2020) showed by reverse genetics using infectious clones (vector-free) of an A-type BNYVV isolate that *Rz1*-mediated resistance is root-specific in sugar beet and that the P25 tetrad sequence and the presence of RNA5 can be independently involved in increased virus multiplication and RB ability in *Rz1*-resistant plants.

As suggested by Tamada et al. (2016), the P25-plant interaction is more comparable to a gene-for-gene relationship, between a bacterial/fungal effector and a plant, than to the recognition of essential proteins typically reported in plant-virus systems (CP, replicase, etc.). It is for example the case for *Rz2* which encodes a CC-NB-LRR protein able to recognize the TGBp1 of a range of soil-borne viruses in a root specific manner (Capistrano-Gossman et al., 2017; Wetzel et al., 2021). P25 acts indeed as a pathogenic effector, dispensable for infection. In this respect, *B. vulgaris* genes potentially interacting with BNYVV P25 were screened using a yeast double hybrid approach. Among interacting candidates, genes involved in ubiquitin/proteasome system, in phytohormones metabolism (auxin- and ethylene-responsive gene products) and in plant defense responses to stress (LRR-extensin) were uncovered. Some candidates interacting proteins were predicted as part of cell-cycle, calcium-signaling, cell-structure, gene expression, translation, protein modification and metabolism components (Thiel and Varrelmann, 2009). Further research showed that P25 interacts with a Kelch repeats containing F-box protein, members of the Skp1-Cullin-F-box (SCF), leading to interference with the ubiquitin proteasome system of its host. This would lead to the suppression of host resistance response, possibly abolishing hypersensitive response. P25 would have a direct effect on (SCF) complex formation or function (Thiel et al., 2012). However, no influence of host genotype (*Rz1* or *Rz2*) or tetrad mutation (*V67*) was shown on F-box interaction with P25.

The P25 interaction with plants was also studied using ectopic expression in *B. vulgaris* and transgenic *A. thaliana* plants constitutively expressing the protein (Peltier et al., 2011). Ectopic expression of P25 in *B. vulgaris* leaves induced yellowing. Roots of *A. thaliana* mutants developed a root branching phenotype, compared to wt plants. The P25 protein-expressing *A. thaliana* plants showed higher auxin contents and were more sensitive to auxin treatments than wild-type plants. Conversely, they showed lower jasmonic acid (JA) content and were less sensitive to JA and ABA treatments. An induction of Jasmonoyl-L-amino acid synthetase *JAR1*, involved in JA signaling (Staswick et al., 2002), and of phenylalanine ammonia-lyase (*PAL1*), involved in salicylic acid (SA) mediated defense response (Kim and Hwang, 2014), were reported in transgenic plants. These genes were previously reported by Schmidlin et al. (2008) as induced in sugar beet roots

CHAPTER II. Tripartite interaction in rhizomania

inoculated using soils infested with *P. betae* and BNYVV in comparison to plant infected with *P. betae* alone. Higher levels of conjugates of the ethylene (ET) precursor 1-Aminocyclopropane-1-carboxylic acid (ACC) were also measured in P25 expressing *A. thaliana* and in BNYVV infected *B. macrocarpa* leaves than in the absence of P25 whereas amounts of active ACC remained constant. An up-regulation of expansins and pectin esterase-encoding genes, both linked to cell-wall remodeling processes (Wu et al., 2018) was also recorded in transgenic roots expressing P25. In those plants, a down-regulation of R genes and an up-regulation of peroxidases encoding genes involved in oxygen species limitation was also reported. These results stressed the determinant role of P25 in eliciting plant defenses upon infection by the BNYVV.

e. Omics studies of BNYVV-plant interactions

The literature reviewed above mostly explain BNYVV-plants interactions focusing on the characterization of the function of viral genes. Some studies also took advantage of proteomic and transcriptomic approaches to investigate the molecular and cellular activities influenced by BNYVV in rhizomania-infected plants. A summary of the principal omics studies of BNYVV-plant interaction and main results are presented in Table II-2.

Table II-2. Summary of principal omics-based studies addressing the plant response during infection by BNYVV. Information about experimental procedures are given in the two first columns: R = root, L = leaves, e.g. L>R means that the inoculation was performed on the leaves and subsequent analysis was performed in the roots. Res = presence of resistance gene in sugar beet (Rz1 or no gene, N). Pol = presence (Y) or absence (N) of *P. betae*. The types of BNYVV isolates and their ability to break (RB) or not (NRB) Rz1-mediated resistance are given in fifth column. The columns six to nine show the effect of inoculation on the expression of selected genes/proteins involved in plant-pathogen interactions. Down-regulations are underlined, up-regulated genes/pathways are not. Terms were italicized when few related genes were significantly regulated but where no obvious trend could be found (e.g. one up- and one down-regulated gene). The numbers in parentheses are the number of weeks post-inoculation (wpi) when the corresponding regulation was recorded. *Beta vulgaris*, *Beta macrocarpa*, *Nicotiana benthamiana*, PR = pathogenesis-related, GST = Glutathione-S-Transferase, UBQ10 = Polyubiquitin 10, HSP70 = 70 kilodalton heat shock protein.

Technique	Plant	Res	Pol	BNYVV	PR & defenses	Oxidative response	Hormonal pathways	Cell walls	Reference
Proteomics (3, 6 wpi)	<i>B. vulgaris</i> (R>R)	N	Y	A-type <u>NRB</u>	chitinase (3,6), protease (-,6), peroxidase (3,6), <u>beta-1,3- glucanase</u> (-,6)	<i>polyphenol oxidases</i> , superoxide dismutase (-,6), germin-like oxalate oxidase (3,6), ascorbate peroxidase (3,6)	auxin (-,6), ethylene (3,6), abscisic acid (-,6)		Larson et al. (2008)
Proteomics (3, 6 wpi)	<i>B. vulgaris</i> (R>R)	Rz1	Y	A-type <u>NRB</u>	chitinase (-,6), protease (-,6), peroxidase (3,6), defensin (3,-), beta-1,3- glucanase (3,6)	<i>polyphenol oxidases</i> , superoxide dismutase (3,6), 2 x germin-like oxalate oxidase (3,6), ascorbate peroxidase (3,6)	<u>auxin</u> (3,6), abscisic acid (-,6)		Larson et al. (2008)
Transcriptomics: RFDD-PCR, reverse Northern, RT-PCR (1, 3, 8 wpi)	<i>B. vulgaris</i> (R>R)	N	Y	B-type <u>NRB</u>	UBQ10 (1,8), beta-1,3- glucanase (1), chitinase (1)	GST (8), G6PDH (1,8), germin-like oxalate oxidase (1,-)	auxin (1)	expansin (1), extensin (1)	Schmidlin et al. (2008)

CHAPTER II. Tripartite interaction in rhizomania

Transcriptomics: RFDD-PCR, reverse Northern, RT-PCR (1, 3, 8 wpi)	<i>B. macrocarpa</i> (L>R)	N	N	B-type NRB	UBQ10 (2), beta- 1,3-glucanase (1), chitinase (1)	G6PDH (2), germin- like oxalate oxidase (2) (GST: nd)	auxin (2)	expansin (2), extensin (2)	Schmidlin et al. (2008)
	<i>B. vulgaris</i> (R>R)	N	Y	P-type	HSP70 (8), UBQ10 (1,8), peroxidase (8) (nd for PR-2, PR-3) <u>polyubiquitin-like</u> , PR-1a, <u>beta-1,3-</u> <u>glucanase</u> , chitinase, PR-5, proteinase, <u>proteinase</u> <u>inhibitor</u> , defensin	GST (8), G6PDH (1,8), germin-like oxalate oxidase (1,8)	nd	nd	
Transcriptomic: mRNA-seq (2wpi)	<i>B. macrocarpa</i> (L>L)	N	N	type nd; RNA 1- 5		GST, germin-like <i>oxalate oxidase</i>	<u>auxin</u> , ethylene	expansin, <u>pectinesterase</u>	Fan et al. (2015)
Transcriptomic: miRNA-seq (2wpi)	<i>B. macrocarpa</i> (L>L)	N	N	type nd; RNA 1- 5			<u>auxin</u> , <u>gibberellin</u> , JA		Liu et al. (2017)
Transcriptomic: mRNA-seq, miRNA-seq (2wpi)	<i>N.</i> <i>benthamiana</i> (L>L)	N	N	type nd; RNA 1- 4	<u>26S proteasome</u> <u>regulatory subunit</u> , ubiquitination genes, <u>beta-1,3-</u> <u>glucanase</u> ,	<u>umecyanin</u>	<u>auxin</u> , <u>gibberellin</u> , ethylene	<u>cellulose</u> <u>synthase A</u> , <u>expansin A1</u> , <u>endoglucanase</u> , pectin methylesterase	Fan et al. (2014), Liu et al. (2020)
Transcriptomic: mRNA-seq (6wpi)	<i>B. vulgaris</i> (R>R)	N	N	A-type NRB	PR-1, PR-5, peroxidases		auxin, SA, <u>ethylene</u>	expansins, lateral organ boundaries domain	Gil et al. (2020)

Hormone signaling pathways

The infection of sugar beet with BNYVV induce large modulations of the total protein complement as initially shown in 2D IEF/SDS PAGE (Coutts et al., 1994). Early reports investigating the histological and hormonal changes induced in sugar beet by infection with BNYVV suggested that auxin regulation is involved in rhizomania symptoms (Pollini and Giunchedi, 1989; Pollini et al., 1990). Since then, the role of auxin signaling in the control of “normal” plant LR formation was demonstrated (Lavenus et al., 2013). Larson et al. (2008) used a quantitative proteomic approach to study the reaction of two *B. vulgaris* quasi-isogenic lines, one susceptible to BNYVV, the other carrying *Rz1* resistant gene, to the inoculation with a soil infested with *P. betae* carrying a wild type BNYVV A-type strain. Several differentially expressed proteins in the susceptible interaction but not in the resistant sugar beet line suggested the implication of auxin, ET and abscisic acid (ABA) pathways in rhizomania development. The probable involvement of auxin signaling in rhizomania development was confirmed with a transcriptomic approach (Schmidlin et al., 2008). Recently, it was reported, in a study including *P. betae* and the BNYVV, that P25 inhibits nuclear localization, and thus the repressor activity, of the AUX/IAA protein (BvAUX28) leading to the induction of lateral organ boundary domain (*LBD*), transcription factors (TF) and expansin (*EXP*) genes during rhizomania symptoms development (Fernando Gil et al., 2018). The expression of *LBD* is regulated by AUX/IAA-ARF modules and is key in LR development. *EXP* are cell-wall loosening proteins involved in many plant developmental processes, including LR formation (Cosgrove, 2015). The higher expression of auxin biosynthesis genes in the presence of BNYVV but without *P. betae* was confirmed by Fernando Gil et al. (2020) using a RNA-seq approach at 6wpi. They identified TF, *LBD* and *EXP* genes putatively involved in rhizomania and compared their expression in plants inoculated with BSBMV which does not induce strong root proliferation. However, highly dynamic changes in *LBD* & *EXP* expression under natural inoculation was noticed (Fernando Gil et al., 2018, 2020) and the accumulation of active auxin in diverse sugar-beet-BNYVV strain interactions was not observed at 25 dpi in a recent metabolomic study (Webb et al., 2020). This could be due to the sampling time and/or to an effect of *P. betae*, present in the last study. Further studies are needed to clarify the mechanisms underlying control of LR formation. The role of other hormones regulation in sugar beet roots infected by BNYVV is still unclear. Larson et al. (2008) reported the induction of proteins participating in ABA and ET signaling in susceptible interaction between sugar beet and BNYVV. However, Fernando Gil et al. (2020) recorded a strong down-regulation of ethylene response genes and didn't reported changes in genes related to ABA signaling. This difference could be due to the absence of *P. betae* in the study from Fernando Gil and colleagues.

The plant response to BNYVV seem organ- and species-dependent. If auxin pathway induction was also reported in roots of *B. macrocarpa* (Schmidlin et

al., 2008) the opposite regulation was observed in leaves of rub-inoculated *B. macrocarpa* (Fan et al., 2015a; Liu et al., 2017) and *N. benthamiana* plants (Fan et al., 2014; Liu et al., 2020a) using mRNA-seq and miRNA-seq approaches. This is not contradictory since the main BNYVV symptoms observed on these plants were stunting and deformations of leaves while root symptoms are characterized by an excessive LR growth. The repression of gibberellin was also recorded in symptomatic *B. macrocarpa* and *N. benthamiana* leaves and is probably also linked to symptom expression. Furthermore, the silencing of three plant genes (*LAC11*, *NAC21/22*, *GRF1*) involved in these hormonal responses was performed using virus-induced gene silencing (VIGS) assays (Liu et al., 2020a). The results confirm their implication in stunting symptom induction in *N. benthamiana*.

Finally, the implication of SA- or JA- mediated defenses in susceptible or resistant interaction with BNYVV is still unclear. The overexpression of several pathogenesis-related (PR) protein genes suggests the activation of SA- and/or JA- and/or ET- mediated defense response (Larson et al., 2008; Wu et al., 2014; Fernando Gil et al., 2020). Moreover, an increased SA and SA-glucoside contents was reported in susceptible and resistant (*Rz1* or *Rz2*) sugar beet cultivars when infected with *P. betae* and BNYVV (Webb et al., 2020). Particularly, the up-regulation of PR-1 is usually considered as a SA pathway response signature (van Loon et al., 2006; Sels et al., 2008; Ali et al., 2018). However, a transcriptomic analysis of sugar beet roots infected by BNYVV without *P. betae* (Fernando Gil et al., 2020) revealed the down-regulation of salicylic acid-binding protein 2 (*SABP2*) and ET response factor 1 (*ERF1B*), respectively involved in defense responses mediated by the SA (Kumar and Klessig, 2003) and ET (Lorenzo et al., 2003) pathways. Conversely, activation of ET and JA pathways was shown in *B. macrocarpa* leaves (Fan et al., 2015a; Liu et al., 2017) and the activation of ET was reported in *N. benthamiana* leaves (Fan et al., 2014; Liu et al., 2020a). Further studies are therefore needed to clarify the implication of phytohormones in sugar beet defense responses to BNYVV in the presence of *P. betae* and in different varieties containing or not known resistance genes.

Defense reaction: involvement of PR proteins, secondary metabolites and oxidative stress

Pathogenesis-related proteins are induced during plant infection with BNYVV. An early report showed that a β -1,3-glucanase (PR-2) and a chitinase (PR-3) are accumulated in roots infected with BNYVV-carrying *P. betae* zoospores (Burketová et al., 2003). With their proteomic approach, Larson et al. (2008) found some PR among proteins up-regulated by the inoculation: chitinase, protease, glucanase, peroxidase and defensin. Enzymes associated to redox homeostasis and plant defenses were also induced, such as polyphenol oxidases, a superoxide dismutase, a germin-like oxalate oxidase and an ascorbate peroxidase. A reduced number of proteins, such as a defensin and a beta-1,3-glucanase, were only induced in

the *Rz1* line and not in the susceptible one. However, a faster and stronger induction of several defense proteins was recorded in the resistant line suggesting that the timing of defenses induction is important in the resistance response. Similarly, transcriptomic approaches (Schmidlin et al., 2008; Fan et al., 2014, 2015a; Fernando Gil et al., 2020) highlighted the regulation of genes encoding PR-1, beta-1,3-glucanases, chitinases, PR-5 (thaumatin-like), proteinases as well as oxidative stress related proteins such as GST, G6PDH, germin-like oxalate oxidase, umecyanin (visible in Table II-2). Again, some organ- and species-dependent differences are reported. Interestingly, the down-regulation of the *N. benthamiana umecyanin* gene (involved in O₂⁻ scavenging) resulted in higher O₂⁻ concentration in plants which in turn is thought to induce primary defense response against viruses. The silencing of *umecyanin* reduced BNYVV CP content in the systemically infected leaves (Liu et al., 2020a). Using RNA-seq analysis, Fan et al. (2014) found that BNYVV also alters RNA silencing and ubiquitin-proteasome pathways in *N. benthamiana* leaves. However, the induction of silencing machinery was not confirmed in *Beta* species, using mRNA-seq approaches (Fan et al., 2015a; Fernando Gil et al., 2020). The up-regulation of genes coding for plant defense-related TF and of genes involved in secondary metabolite biosynthesis pathways such as phenylpropanoids and flavonoids pathways was also evidenced (Fernando Gil et al., 2020) and results in higher secondary metabolites contents in BNYVV infected plants including flavonoids and alkaloids (Webb et al., 2020). Phenylpropanoids are likely involved in enhanced production of lignin and could be involved in the formation of physical barrier in plant defense reaction (Vogt, 2010; Miedes et al., 2014). Flavonoids are compounds with multiple roles in defense and signaling between plant and microorganisms (Mathesius, 2018). The chalcone synthase, a key enzyme from the flavonoids pathway, is responsible for the accumulation of flavonoids and phytoalexins in response to the induction of SA signaling pathway (Dao et al., 2011). Therefore, it won't be surprising that synthesis of those secondary metabolite is involved in plant-BNYVV interaction. Since most studies have addressed compatible interactions, it's difficult to separate the effective activation of plant defense to mitigate the infection by the virus and the potential manipulation of plant immune system by viral features. Interestingly, it was reported that BNYVV could enhance fungal growth in sugar beet during storage (Strausbaugh et al., 2015), indicating that the virus compromise, at least partially, primary defenses.

Comparisons of compatible and resistant sugar beet-BNYVV interactions

In two proteomics studies, Webb and colleagues attempted to compare the effect of compatible and incompatible interactions in sugar beet leaves of three near isogenic sugar beet lines (susceptible or carrying *Rz1* or *Rz2* resistance genes) naturally inoculated with soil containing the same RB and non-RB BNYVV isolates. Slightly different proteins spectra were obtained from susceptible cultivar infected by the RB and the non-RB BNYVV strains.

CHAPTER II. Tripartite interaction in rhizomania

Particularly, a higher expression of a SOD and HSP70 was also recorded with the non-RB BNYVV isolate (Webb et al., 2014). Similarly, SOD was more abundant in the compatible interaction of the RB isolate with susceptible line than *Rz1* line. An ascorbate peroxidase was also more abundant in compatible interaction of the susceptible line with the non-RB than with the RB isolate (Webb et al., 2015). This suggests that, even in susceptible interaction, different interactions could occur in function of the BNYVV isolates. Proteins involved in proteasome were more abundant in incompatible interaction (*RZ1* vs non-RB) than in compatible (susceptible vs non-RB). HSP70 was up-regulated in compatible interaction (*Rz1* vs RB) in comparison to incompatible (*Rz1* vs non-RB). Generally, proteins involved in photosynthesis and energy metabolism are down-regulated in compatible interaction. Those results suggest that pathways related to reactive oxygen species generation/scavenging and pathways associated with programmed cell death in plants are differentially regulated during compatible and incompatible interactions of the sugar beet (susceptible, *Rz1*, *Rz2*) with the BNYVV (RB and non-RB). Supporting this hypothesis, it was previously reported that transgenic *N. benthamiana* and *B. vulgaris* hairy roots expressing the HrpZ gene from *Pseudomonas syringae* pv. *phaseolicola* were more resistant to BNYVV than WT, probably due to the induction of an HR reaction upon inoculation (Pavli et al., 2011a, 2012).

3. Interactions of *Polymyxa* spp. with vectored viruses

Current knowledge about *P. betae*-BNYVV molecular interaction are limited. To get a general overview of viral transmission by *Polymyxa* spp., we therefore considered other chosen pathosystems in the following sections.

a. Soil-borne viral transmission by plasmodiophorids and chytrids as viral vectors

Seven species of endoparasite zoosporic agents are known to transmit plant viruses: the plasmodiophorids *P. betae*, *P. graminis*, *S. subterranea* and *H. nasturtii* and the fungal chytrid species *Olpidium brassicae*, *O. bornovanus* and *O. virulentus* (Bragard et al., 2013). Plasmodiophorids and chytrids are a great example of convergent evolution. They share a highly similar life cycle but are found in completely different branches of eukaryotic tree of life: plasmodiophorids are bikonts, possessing zoospores with two flagella, whereas chytrids are unikonts, with monoflagellated zoospores (Figure II-6). Despite their profound genetic differences, plasmodiophorids and chytrids both became the vectors of soil-borne viruses, probably because of their similar life style (plant endoparasites producing free zoospores and long-lived survival spores). Two distinct modes of transmission have been distinguished based on the mode of virus acquisition by zoospores: the *in vivo* mode, which requires the internalization of viral particles by the vector inside the host roots, and the *in vitro* mode which is characterized by the adsorption of viral particles on the surface of zoospores flagella occurring outside the plant roots (Rochon et al., 2004; Bragard et al., 2013).

Plasmodiophorid-transmitted viruses are rod-shaped or filamentous (except *Watercress yellow spot virus* or WYSV [Clay and Walsh, 1996]) and carried inside zoospores and sporosori, thus following the *in vivo* mode. The process of transmission therefore requires the internalization of the viruses and is further developed in the sections below. Most viruses transmitted by *Olpidium* spp., are icosahedral and carried adsorbed on the surface of fungal zoospores, probably due to the interaction with glycoprotein receptors (Kakani et al., 2003). It was for example shown that transmission of *Tobacco necrosis virus* (TNV) by *O. brassicae* can be enhanced simply by incubating TNV purified virions with an *Olpidium* zoospores suspension (Kassanis and Macfarlane, 1964), and TNV virions were observed at the surface of zoospores (Temmink et al., 1970). On the contrary, transmission is decreased when the zoospore suspension is incubated with an antibody directed against the viral particles. Laboratory experiments with *P. graminis* and SBWMV showed that it is impossible to block the viral transmission by washing or incubating zoospores with a virus-directed antiserum. Similarly, sporosori retained their capacity to transmit viruses after surface treatments with NaOH, HCl and virus-directed antiserum (Rao and Brakke, 1969; Abe and Tamada, 1986). Some cases of *in vivo* transmissions were observed with *Olpidium* spp. Such as viruses transmitted by plasmodiophorids, the

CHAPTER II. Tripartite interaction in rhizomania

viruses involved in *in vivo* transmission by *Olpidium* spp. harbors rod-shaped or flexuous particles (Rochon et al., 2004; García et al., 2018; Walker et al., 2018).

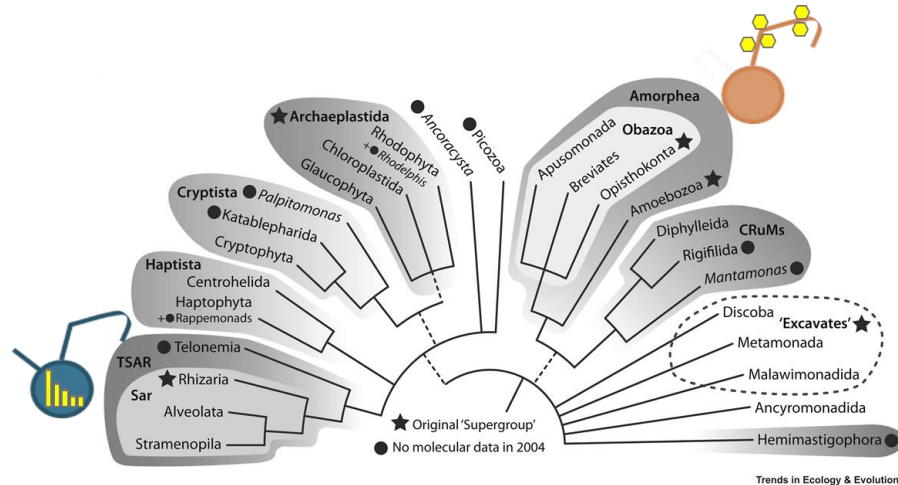


Figure II-6. Transmission of soil-borne rod-shaped virions requires internalization by plasmodiophorids biflagellate zoospores (shown in blue). Icosahedral soil-borne viruses are transmitted adsorbed on chytrids monoflagellated zoospores (shown in orange). The eukaryotic tree of life was taken from by Burki et al., 2020 and described in Chapter I. Not shown are the filamentous viruses transmitted by plasmodiophorids and rare cases of *in vivo* transmission by chytrids.

b. Viruses transmitted by *Polymyxa* spp.

The ability of *Polymyxa* spp. to transmit plant viruses has drawn much attention in 80s, 90s and early 2000s. At that time, in the absence of genomic resources, most research on plasmodiophorids consisted of ultrastructural and karyological observations (see chapter I) as well as rDNA-based and immuno-based detection on different hosts and co-occurrence with viruses (Legrève et al., 2000, 2002; Bulman and Neuhauser, 2017). As listed by Dieryck et al. (2011) and updated in Table II-3, effective transmission by *Polymyxa* spp. zoospores or sporosori was demonstrated for some viruses. For other viruses, the transmission was deduced based on co-occurrence with *Polymyxa* spp. in infected plants along with the fact that closely related viruses (from the same genus) have been shown to be transmitted by *Polymyxa* spp.

The epidemiology of *Polymyxa*-transmitted viruses is influenced by their interactions with *Polymyxa* species and special forms. Several *Polymyxa*-transmitted viruses can occur in the same plants (e.g. BNYYV, BSBV, BVQ), and the same soils can be infested by *Polymyxa* spp. involved in different pathosystems (Vaïanopoulos et al., 2007; Legrève et al., 2011). It is clear

that host specificity of *Polymyxa* spp. renders some virus-vector interactions unlikely. For example, the restriction of *P. betae* mainly to Amaranthaceae naturally limits its capacity to transmit cereal viruses (Legrève et al., 2011). However, viral transmission by *Polymyxa* spp. seems also to result from a fine-grained interaction between the virus and the vector. Some evidence show a certain specificity of the interaction between *Polymyxa* spp. and f. sp. isolates and viruses (Dieryck et al., 2011). For example, the *Peanut clump virus* (PCV) was transmitted with different efficiencies by *P. graminis* f. sp. *tropicalis* and *subtropicalis* zoospores and was not by *P. graminis* f. sp. *temperata*. Interestingly, the authors detected the PCV by RT-PCR in the zoospore suspensions of *P. graminis* f. sp. *temperata* produced on PCV infected sugar canes. These suspensions were then inoculated on cereal plants (host for both, the virus and the vector) and were unable to transmit the virus (at a lower temperature suitable for the development of the f. sp. *temperata*). This demonstrated that the virus could be acquired in sugar cane but could not be released in other cereals (i.e. wheat, barley, pearl millet sorghum) by this special form. This suggests the presence of distinct mechanisms for virus acquisition and release and underline the influence of plant-virus-*Polymyxa* specific interactions on transmission, upstream and downstream the production of viruliferous zoospores. In the same line, three virus-carrying *P. betae* isolates obtained from sugar beet, spinach and *C. murale* lost their viruliferous status after being grown in *Chenopodium ficifolium* even though this plant is considered as a suitable host for both *P. betae* and BNYVV. The same isolates could then re-acquire their viruliferous status after being propagated on sugar beet mechanically inoculated with BNYVV (Abe and Tamada, 1986). In *C. ficifolium*, the BNYVV was thus released from *P. betae* thalli but not re-acquired. The specificity of viral transmission could results from co-evolution of the vector and the virus on plants cultivated in certain areas, as illustrated by the high transmission rate of the PCV isolate from Burkina Faso by an isolate of *P. graminis* f. sp. *tropicalis* from Niger (Dieryck et al., 2011).

CHAPTER II. Tripartite interaction in rhizomania

Table II-3. Viruses transmitted by *P. betae* and *P. graminis* and references of transmission assays. If no reference is provided, the transmission was deduced from co-occurrence. Adapted from Dieryck et al. (2011)

<i>Polymyxa</i> Species	Virus		Transmission assay studies	
	Name	Genus	Sporosori or soil	Zoospores
<i>P. betae</i>	Beet necrotic yellow vein virus	Benyvirus	(Tamada et al., 1975)	(Tamada et al., 1975)
	Beet soil-borne mosaic virus	Benyvirus	(Wisler et al., 1994)	/
	Beet soil-borne virus	Pomovirus	(Ivanovic et al., 1983 ; Henry et al., 1986)	/
	Beet virus Q	Pomovirus	/	/
<i>P. graminis</i>	Barley mild mosaic virus	Bymovirus	/	/
	Oat mosaic virus	Bymovirus	/	/
	Rice necrosis mosaic virus	Bymovirus	/	/
	Wheat spindle streak mosaic virus	Bymovirus	(Slykhuis and Barr, 1978)	/
	Wheat yellow mosaic virus	Bymovirus	(Canova, 1966)	/
	Barley yellow mosaic virus	Bymovirus	(Adams et al., 1988)	(Adams et al., 1988)
	Chinese wheat mosaic virus	Furovirus	/	/
	Oat golden stripe virus	Furovirus	/	/
	Soil-borne cereal mosaic virus	Furovirus	(Vaïanopoulos, 2008)	/
	Soil-borne wheat mosaic virus	Furovirus	(Rao and Brakke, 1969)	(Rao and Brakke, 1969)
	Sorghum chlorotic spot virus	Furovirus	/	/
	Rice stripe necrosis virus	Benyvirus	/	/
	Wheat stripe mosaic virus	Benyvirus	/	/
	Indian peanut clump virus	Pecluvirus	(Ratna et al., 1991)	/
	Peanut clump virus	Pecluvirus	(Thouvenel and Fauquet, 1981)	(Dieryck et al., 2011)

c. Viral transmission by *Polymyxa* spp.: what do we know?

After several rounds of mechanical transmission of BNYPV (e.g. on *C. quinoa*) and other plasmodiophorid-transmitted viruses, including the PMTV transmitted by *S. subterranea*, some spontaneous mutants that are no longer transmissible by *Polymyxa* may appear. The characterization of such mutants along with complementary reverse genetic experiments led to the identification of viral proteins involved in transmission: the readthrough domain of the coat protein (CP-RT) of beny-, pomo- et furoviruses, P2 protein of bymoviruses as well as P39 from pecluviruses (Tamada and

Kusume, 1991; Tamada et al., 1996b; Reavy et al., 1998; Adams et al., 2001; Tamada and Kondo, 2013).

The similarity between those proteins is often weak (e.g. pairwise score between BNYVV and BSBV RNA2 protein sequences = 10). However, they share structural characteristics linked with the ability of these viruses to be transmitted by plasmodiophorids. Notably, two transmembrane domains of a similar length are predicted in the readthrough domain of CP-RT as well as in C-terminal of the P2 protein (downstream a “pseudo” CP domain). The sequences analysis of these domains and flanking regions suggested a compatible conformation of both transmembrane helices, including ridge/groove arrangements (Diao et al., 1999; Adams et al., 2001). This would allow their oligomerization and anchorage in membranes (Tamada et al., 1996b; Adams et al., 2001). In spontaneously deleted mutants that can't be transmitted by plasmodiophorids, the second transmembrane domain is often absent or disrupted. Strong electrostatic interactions between residues at the inner side of the membrane are thought to stabilize the protein conformation. For example, a “KTER” motif in the BNYVV RT domain toward the inside end of TM 2 was shown essential for transmission of BNYVV by *P. betae* via interaction with arginine residue (Tamada et al., 1996b; Koenig, 2000). Actually, the tight alignment of the two TM helices was predicted to be compromised in a non-transmissible substitution mutant affecting the KTER motif (KTER > ATAR) (Tamada et al., 1996b; Adams et al., 2001). The readthrough domains of BNYVV and PMTV were both observed at the extremity of virions of both viruses, as shown in Figure II-3 (Haeberlé et al., 1994; Cowan et al., 1997). A similar situation was suggested for BSBV CP-RT (Barbarossa et al., 1992; Crutzen, 2010).

At the beginning of infection, it was shown that RT domain enable the anchorage of BNYVV virions in the cytoplasmic side of plant mitochondria outer membranes (Tamada et al., 1996b; Erhardt et al., 2001). This was supported by the discovery of mitochondrial targeting domain in the RT of BNYVV (Valentin et al., 2005). Subsequently, the virions would however be released in the cytosol (Erhardt et al., 2001; Valentin et al., 2005). As discussed below, the exact mechanism by which viral particles then migrate between host and *Polymyxa* cytoplasms remains elusive. The fact that TM domains are indispensable for transmission suggests that the virion anchorage in *Polymyxa* membranes is probably involved.

It is generally thought that virus acquisition by *Polymyxa spp.* would take place during early infection of virus-infected cells, when zoospores transfer their cytoplasm directly into the host cell, and/or in immature plasmodia where the plant and the protist cytoplasms are only separated by a thin membrane (Kanyuka et al., 2003). BNYVV virions were observed in plasmodia, zoosporangia and mature zoospores using immunolabelling approaches (Abe and Tamada, 1986; Rysanek et al., 1992; Dubois et al., 1994). They were notably detected inside numerous vacuoles or vesicles of immature zoosporangia, suggesting that pinocytosis could be responsible for

CHAPTER II. Tripartite interaction in rhizomania

virus acquisition. Reinforcing this hypothesis, food vacuoles were observed in plasmodia of *H. nasturtii*, the plasmodiophorid vector of WYSV. These vacuoles seem to be the result of the internalization of host cytoplasm by invagination of the plasmodial membrane. It can therefore be postulated that pinocytosis could be used by plasmodiophorid-transmitted viruses to enter their vectors. Studies using immunolabelling techniques revealed that the presence of BNYSV in root cells is strongly correlated with the presence of sporosori even if rarely associated with the observation of virions in these structures. Authors suggested that poor labelling inside sporosori would reveal that viral particles are rarely present within *P. betae* resting spores, at least in the form of viral particles, and that multiplication of the virus does not occur at this stage of development. Regarding *P. graminis*, *Barley mild mosaic virus* (BaMMV) particles were seen by TEM and immunolabelling within zoosporangial plasmodia and zoospores but not in sporosori (Chen et al., 1991), probably due to the impermeability of their electron-dense thick wall which could interfere with sample preparation and/or TEM observations (Chen et al., 1991; Rysanek et al., 1992). Despite these limitations, Chen et al. (1998) observed *Barley yellow mosaic virus* (BaYMV)-like particles in sporosori of *P. graminis*. Regarding *Soil-borne wheat mosaic virus* (SBWMV) furovirus, movement proteins and viral RNA were detected in sporosori but no coat protein, implying that the virus is not encapsidated in its vector and thus, that it is putatively transmitted in the form of a ribonucleoprotein complex (Driskel et al., 2004). Conversely, the same study revealed the presence of coat protein from *Wheat spindle streak mosaic virus* (WSSMV) bymovirus in *P. graminis* sporosori. Distinct mechanisms of acquisition might therefore occur for furoviruses and bymoviruses. No information concerning the presence of pomoviruses in *P. betae* has been reported, to the best of our knowledge. However, based on similarities between benyviruses and pomoviruses CP-RT and the HIV Gag protein, Crutzen (2010) also hypothesized that viruses could enter *Polymyxa* spp. cell by budding, relying on a mechanism similar to the HIV export mechanism. The putative ubiquitination of the RT-domain of coat protein supports this hypothesis. Interestingly, the CP-RT characteristics are not shared by the P39 of *Peanut clump virus* (PCV), a pecluvirus transmitted by *P. graminis*. Distinct mechanisms could be responsible for PCV transmission by *P. graminis*. In addition to the BNYSV CP-RT, the RNA4-encoded P31 is also required for efficient vector transmission (Tamada and Abe, 1989; Rahim et al., 2007). The BSBMV RNA4-encoded P32 has the same function. BSBMV P32 and BNYSV P31 expressed from replicons of RNA5 were indeed sufficient to complement viral transmission by *P. betae* in the absence of RNA4, whereas a sample inoculated a p32 frame shift protein was not transmissible (D'Alonzo et al., 2012). The mechanism underlying the role of P31/P32 in transmission is not fully understood, yet.

In conclusion pinocytosis is, for now, the predominant hypothesis to explain the internalization of viruses by *Polymyxa* spp. It probably involves the anchorage of virions in host or vector membranes most likely aided by the TM domains encoded by the CP-RT and P2 proteins. However, the actual process was never observed. Nevertheless, some elements indicated that it

should involve other vector and/or other host factors. First, the preferential transmission of some viruses by some *P. graminis* special forms reflects a certain specificity of virus-vector interaction. Second, the transmission of multipartite viruses would require the simultaneous compartmentalization of all viral components in the same vector structures. Even if it is possible that this is only due to chance, one may argue that a kind of unknown specific “*Polomyxa* targetting” of viral particles could occur. Third, it seems that acquisition and/or release of viruses from *P. betae* rely on different mechanisms, probably involving host factors. It was for example demonstrated that *P. betae* was not able to acquire BNYVV in *C. ficifolium* despite the detection of both BNYVV and *P. betae* in roots of this host (Abe and Tamada, 1986). Lastly, different mechanisms seem to occur with different virus genera. Moreover, the example of P31/P32 suggest that other viral factors can also facilitate transmission through a still elusive mechanism.

In view of these reports, it is so far not possible to propose a general model explaining molecular mechanisms involved in viral transmission by *Polomyxa* spp.

d. Host or only vector?

It is well established that *Polomyxa* spp. are virus vectors. Plant virus transmission by insects is generally classified in four categories: i) non-circulative & non-persistent, ii) non-circulative & semi-persistent, iii) circulative & non-propagative, iv) circulative & propagative. This classification is based on acquisition/retention times as well as the ability of the virus to move and to multiply in the vector (Bragard et al., 2013). Because *Polomyxa* sporosori can retain their viruliferous status for years, the transmission can reasonably be described as “persistent”. We could also qualify the transmission as circulative, since the transmitted-viruses are internalized by *Polomyxa* spp. However, the circulative criterion is not really relevant here because, unlike insect vectors, *Polomyxa* spp. are not organized into complex tissues that can be crossed by the virus. However, the question about the multiplication of viruses inside *Polomyxa* spp. is controverted. On one hand, it was shown that BaMMV-carrying isolates of *P. graminis* lost their capacity to transmit the virus after being grown in the roots of a virus-resistant host. This is indirect evidence that BaMMV cannot spread exclusively within *P. graminis* structures, protected from the plant’s defense response (Adams et al., 1987; McGrann and Adams, 2003). Tuitert et al. (1994) also reported that large quantities of *P. betae* sporosori were formed in roots of sugar beet cultivars partially resistant to rhizomania but that soil BNYVV infectivity potential was lower after cultivation of a resistant cultivar. Similarly, no correlation was found between the development of *P. graminis* and PCV in host plants (Dieryck et al., 2011). This suggests that *Polomyxa*-transmitted viruses must reach the plant cytoplasm to complete their cycle and then be reacquired for transmission. Moreover, it was shown that BNYVV can induce strong symptoms and persist for a long time in sugar

beet roots in the absence of *P. betae* (Koenig and Burgermeister, 1989) and that similar BNYVV titers were measured in plants infected with increasing viruliferous zoospores dilutions (Dirven and Peters, 1995). The protist is therefore not essential for virus replication and movement *in planta*. All this evidence are indirect clues that viruses do not replicate in *Polymyxa* spp., or at least that the plant is required at some stage of the viral cycle. On the other hand, Driskel et al. (2004) detected movement proteins and RNA from the SBWMV in sporosori of *P. graminis*. Similarly Lubicz et al. (2007) reported the detection of BNYVV proteins involved in replication and movement inside zoosporangia and zoospores of *P. betae*. These findings suggest that the protist could be a propagative host for the virus. However, there is no evidence that these proteins were synthesized in the *Polymyxa* thalli and not by the host cell machinery, questioning this hypothesis. An impact of the viruses on *Polymyxa* spp. fitness could also bring additional clues of its host status. One would expect that viral multiplication inside *Polymyxa* should have a fitness cost for the later. BNYVV but not BSBV, another pomovirus transmitted by *P. betae*, inhibits subsequent development of *P. betae* in sugar beet (Schlösser, 1990). However, these observations are not necessarily resulting from a direct adverse effect of the virus on its vector. It could also be related to an indirect effect mediated by the plant cell response to viral infection. It was also shown that preinfection with BSBV, suppress the development of BNYVV but stimulates colonization by *P. betae* (Prillwitz and Schlösser, 1992, 1993). The question of the multiplication of *Polymyxa*-transmitted virus in their host is therefore not resolved and deserves complementary studies.

4. Interactions of *Polymyxa* spp. with plants

For a long time, molecular studies of the plasmodiophorids interactions with plants were restricted by the lack of genomic data on these protists. The separation of *Polymyxa* into two species, *P. betae* and *P. graminis*, was made according to their distinct host ranges, in spite of their high morphological resemblance. At infra-species level, the multiplication rates of different *P. graminis* in distinct compatible hosts depends on the specific host-special form interactions (Vaïanopoulos et al., 2007). Moreover, the host from which zoospores are produced also seems to affect infectivity of *P. graminis* (Dieryck et al., 2011). These studies suggested that specific molecular interactions might also occur between *Polymyxa* spp. and their host.

In the early 2000s, Mutasa-Gottgens et al. (2000) sequenced 11 *P. betae* cDNA clones using phagemid library generated from infected sugar beet roots. The sequences of 76 *P. brassicae* genes were obtained a few years later by suppression subtractive hybridization strategy (SSH) (Bulman et al., 2006, 2007). Using a similar approach, Šubr et al. (2002) generated four EST from *P. graminis*. The use of in-vitro dual culture also enabled the creation of a *S. subterranea* cDNA library (Qu and Christ, 2007; Bulman et

al., 2011). Regarding *P. betae*, Desoignies et al. (2014) also obtained 76 ESTs using SSH. Later, Burki et al. (2010) sequenced cDNA from five rhizarian species including *P. brassicae* and *S. subterranea*. In the last five years, the publication of *P. brassicae* (Schwelm et al., 2015b, 2015a), *S. subterranea* (Ciaghi et al., 2019) and *P. betae* (Decroës et al., 2019) genomes, as well as of several hosts (sugar beet, potato, cereals...), opened the way for transcriptomic analyses of plant-plasmodiophorids interactions. Until now, studies were mainly conducted with *P. brassicae* (Bi et al., 2016, 2019; Rolfe et al., 2016; Ciaghi et al., 2019; Shah et al., 2020). Since the first publication of the *P. brassicae* genome, several *P. brassicae* isolates have been now resequenced (Bi et al., 2016; Rolfe et al., 2016; Daval et al., 2019; Sedaghatkish et al., 2019; Stjelja et al., 2019).

Before 2015, the lack of enough plasmodiophorid genomic information naturally led scientists to focus on the plant side of the plant-plasmodiophorid interaction (Neuhauser et al., 2010). Until now, several studies have addressed the interactions between intracellular plasmodiophorids and plants. Most of them focused on host reaction to the infection with *P. brassicae* (see Chapter II.5). However, a few transcriptome profiling studies report on interactions between *Polymyxa* spp. and their hosts. In early 2000's, two xyloglucan endo-transglycosylases (XTH) were found highly expressed in sugar beet roots infected with *P. betae* (5-6 weeks post-inoculation) and could be involved in root damage repair (Mutasa-Gottgens et al., 2000; Dimmer et al., 2004). The XTH are indeed known to have a role in the secondary cell wall formation and root damage repair. A few years later, early changes (15min to 420min after elicitation) in sugar beet and barley gene expression during non-host and host interactions with *P. betae* and *P. graminis* were monitored through representational difference analysis (sugar beet) and microarray analysis (barley) (McGrann et al., 2007, 2009). It was found that *P. betae* and *P. graminis* induced the same basal response in barley, with less than 20 genes showing increased expression. Genes encoding PR-1a, PR-5, cysteine proteases, a chitinase, a glutathione S-transferase, lipid transfer proteins, thionins, xyloglucan endo-transglycosylase (XTH) and extensin-like proteins were found among induced transcripts (McGrann et al., 2009). Resistance to *Polymyxa* spp. would therefore occur after the infection relying on a second defense barrier. Supporting this hypothesis, several authors reported early development or detection of *P. graminis* (Thouvenel and Fauquet, 1981; Dieryck et al., 2011) and *P. betae* (Barr et al., 1995; Legrève et al., 2005b; Desoignies et al., 2011; Kutluk Yilmaz et al., 2016) in unusual or non-compatible hosts in which sporosori presented an atypical phenotype or couldn't be observed. Barr et al. (1995) proposed that the resistance mechanism to *P. betae* in wild *Beta* species occurs soon after infection of root hairs, probably relying on the induction of a hypersensitive response (HR). Later, Desoignies et al. (2014) reported that a fine interplay would occur during the successive stages of the *P. betae* life cycle in its host, enabling *P. betae* to "hide" from the plant immune system. They observed an overexpression of sugar beet genes encoding PR proteins and stress resistance proteins mainly during the early plasmodial stage of the *P. betae*

CHAPTER II. Tripartite interaction in rhizomania

life cycle. Less gene expression changes occurred during the sporangial and sporogenic stages. According to their results, Desoignies et al. (2014) postulated that systemic acquired resistance (SAR) would be induced in plasmodial growth (5dpi) then systemic defenses could switch to jasmonate response. Moreover, JA-mediated defenses induced by *Bacillus amyloliquefaciens* lipopeptides in sugar beet seems to reduce the infection by *P. betae* (Desoignies et al., 2013). Desoignies et al. (2014) also noticed the probable implication of a galactose-binding lectin encoded by *P. betae* in host recognition as well as of a profilin and a Von Willebrand factor domain-containing protein, in early infection process. The probable implication of a protist polysaccharide deacetylase in cell wall remodeling and of a transmembrane G protein receptor in pathogenicity was also reported during the plasmodial stage.

Even though the above-mentioned studies brought interesting information, the plant-*Polymyxa* interactions remains poorly understood. A better knowledge of the molecular pathways underlying the specificity of interaction between plant and *Polymyxa* spp. would be helpful to better appreciate the epidemiological characteristics of viral diseases vectored by *Polymyxa* species and to develop new control strategies for these soil-borne diseases.

5. Interactions of other plasmodiophorids with plants

a. Plant response to *P. brassicae* and *S. subterranea* infections

The rapid evolution of next generation sequencing techniques as well as the development of *A. thaliana* microarrays, among others, naturally led scientists to turn to study the effective *A. thaliana*-*P. brassicae* pathosystem. This model plant species is indeed a good host of the clubroot disease agent. Therefore, transcriptomics during the infection of *A. thaliana* root by *P. brassicae* was monitored in many studies. Although the inoculation of *A. thaliana* by *Polymyxa* spp. is technically possible, the use of this model with *P. betae* was restrained because of the lower infection levels in this plant species compared to *B. vulgaris* and the altered phenotype of the protist, likely revealing a distinct interaction (Desoignies et al., 2011; Smith et al., 2011).

In *A. thaliana*, the expression of a reduced number of genes was impacted during the initial root hairs infection by *P. brassicae*, between 4 and 10 dpi (Siemens et al., 2006; Agarwal et al., 2011). In later the stages, however, a greater proportion of genes were differentially regulated, including genes involved in auxin homeostasis and cytokinin (CK) biosynthesis (Siemens et al., 2006; Schwelm et al., 2015b; Malinowski et al., 2016). Especially, several genes involved in CK biosynthesis/degradation are mainly down-regulated during gall formation but some are up-regulated depending on the infection stage and cell types (Siemens et al., 2006; Schuller et al., 2014;

Malinowski et al., 2016). An increase of active CK content/response in the early stage of infection would be necessary to induce cell division (Agarwal et al., 2011). At a later stage auxin responses would be associated with cell elongation in galls (Ludwig-Müller et al., 2009). This dynamic hormonal regulation was confirmed by Rolfe et al. (2016) in *B. napus*. In *A. thaliana*, genes encoding nitrilase (an enzyme involved in auxin biosynthesis), auxin transport proteins, auxin receptors and auxin conjugates biosynthesis are up-regulated during club formation (Siemens et al., 2006; Jahn et al., 2013; Schuller et al., 2014). *A. thaliana* mutants defective for auxin receptors from TIR family and for downstream auxin conjugate synthetases (GH3) were more susceptible to *P. brassicae* (Jahn et al., 2013) while a mutant for nitrilase was shown more resistant (Grsic-Rausch et al., 2000). Lines overexpressing a CK oxidase/dehydrogenase (enzymes degrading CK) were resistant to *P. brassicae* (Siemens et al., 2006). Globally, auxin and CK balance manipulation during *P. brassicae* – *A. thaliana* interaction has been shown essential for the pathogenicity of *P. brassicae* and for symptom development (reviewed by Ludwig-Müller et al., 2009, 2017).

Siemens et al. (2006) reported a mostly down-regulation of SA and ET biosynthesis genes in infected roots during compatible interaction. GA-, ABA- and brassinosteroid-related (BR) genes were mostly up-regulated, in a well-established infection at 23 dpi. They also pointed out the importance Jasmonic acid (JA) synthesis and signaling up-regulation in compatible interaction. Using microdissection of cells infected with *P. brassicae* plasmodia, Schuller et al. (2014) obtained slightly different results: ET synthesis genes were up-regulated in cells containing large plasmodia whereas genes related to SA and ABA metabolism and signaling were less transcribed. In the same study, JA synthesis genes were mainly up-regulated while genes related to JA signaling seemed down-regulated in the central cylinder, thus showing a differential regulation depending on root tissue. GA-associated transcripts didn't show clear regulation. Interestingly the authors observed an up-regulation of most transcripts associated with BR biosynthesis and signaling pathways in cells containing plasmodia. The inoculation of mutants defective for a BR receptor as well as of wild type plants treated with a BR inhibitor resulted in reduced gall formation, confirming the implication of this hormone in clubroot development (Schuller et al., 2014). Agarwal et al. (2011) also reported that SA treatment suppresses clubroot in *A. thaliana*. In the case of partial resistance, it was found that JA pathway was repressed, whereas SA and ET pathways were induced, further supporting the hypothesis that SA would be involved in resistant interaction (Jubault et al., 2013). In these transcriptomic studies, PR genes were found induced, probably in response to the activation of SA signaling pathway after *P. brassicae* inoculation, even in the early stage of infection. Zhao et al. (2017) confirmed the influence of some PR proteins in clubroot disease resistance through the use of *A. thaliana* mutants.

Some authors moved from the *A. thaliana*-*P. brassicae* model and turned to host of agronomical interest. For example Chen et al. (2016) and Jia et al. (2017) investigated the transcriptional response of resistant and susceptible

CHAPTER II. Tripartite interaction in rhizomania

Brassica rapa lines to *P. brassicae* inoculation during the early stages of infection (after 0-96 hours) and after 30 days, respectively. Chen et al. (2016) observed the differential regulation of genes related to disease resistance between two near-isogenic *B. rapa* lines, one resistant to club root and the other susceptible. Genes encoding for PR proteins, including chitinases, and NB-LRR resistance proteins as well as genes involved in MAPK cascades, Ca^{++} influx, respiratory burst oxidase homologs (Rboh) and SA response were up-regulated in the resistant line. Some WRKY TFs were differentially regulated but their implication in resistant phenotype remain unclear. Conversely, genes involved in JA/ET biosynthesis and signaling as well as in cell division, cell walls modeling and expansion were down-regulated in the resistant line. Most of these observations were confirmed by Jia et al. (2017) in a well-established infection, after 30 days. They showed that genes known to be involved in plant-pathogen interaction and plant hormone signal transduction were up-regulated in a resistant line in comparison with a susceptible one (e.g. PR genes, Ca^{++} influx, Rboh, cell wall thickening, SA homeostasis, chitin metabolism, glucosinolates biosynthesis). The susceptible phenotype was unsurprisingly associated with induction of genes involved in cell division and expansion as well as energy production and conversion. Of interest, up-regulation of genes associated with auxin and CK signaling pathways was confirmed in this species and nodulin-related genes were induced as well (Jia et al., 2017). These observations globally confirmed results from another project using a susceptible broccoli genotype and its wild resistant relative (Zhang et al., 2016). They are also mainly supported by recent results obtained with *B. napus* resistant lines. It is noteworthy that both SA signaling and ROS response were putatively involved in resistance and that underlying mechanisms could differ in *B. napus* lines carrying distinct resistance genes (Shah et al., 2020). The involvement of SA hormone in the induction of a better tolerance to *P. brassicae* was also confirmed by SA exogenous application on broccoli (Lovelock et al., 2013). Besides hormones and defense induction, sugar transport reprogramming would also be key for the establishment of susceptible interaction between *P. brassicae* and host plants (Malinowski et al., 2019). Notably, the importance of SWEET transporters in the compatible Chinese cabbage-*P. brassicae* interaction was recently reported (Li et al., 2018).

As mentioned above contrasted molecular responses to *P. brassicae* infection have been observed in distinct root tissues as well as in root cells containing *P. brassicae* plasmodia in comparison to uninfected cells of inoculated roots (Schuller et al., 2014). Supporting this observation, Ciaghi et al. (2019) showed that transcriptomics differed in root galls and symptomless parts of a single kohlrabi root system infected by *P. brassicae*. In comparison to root galls, an up-regulation of genes linked to disease resistance, cell wall synthesis/reinforcement, auxin and CK metabolism and to SA-signaling was observed in symptomless roots. The biosynthesis of secondary metabolites through phenylpropanoid and flavonoid pathways were also induced in symptomless parts of inoculated roots. This response could be linked to the absence of galls. A general down-regulation of genes

involved in cell wall degradation/modification as well as of some brassinosteroid-synthesis and some JA-synthesis genes was also recorded in symptomless root parts. This kind of response is surprisingly similar to the response observed in resistant hosts (Ciaghi et al., 2019). The regulation of cell wall related genes were further studied in kohlrabi-*P. brassicae* interaction (Badstöber et al., 2020). Globally transcripts for proteins associated with synthesis of cell-wall components such as callose, cellulose, hemicellulose, lignin and pectin were less expressed during gall formation whereas genes coding for enzymes involved in cell wall degradation/modification and cell expansion were up-regulated.

Potato (*Solanum tuberosum*) response to *Spongospora subterranea* infection was also investigated using RNA-sequencing using one susceptible and one tolerant cultivars infected or not with virus-free *S. subterranea* resting spores (Lekota et al., 2019). Even though the global response of both cultivars was similar, a higher number of defense-related genes were observed in the tolerant cultivar. In particular, up-regulation of the SA pathway is suspected to be associated with the tolerant phenotype.

b. Transcriptomics oriented toward plasmodiophorid's genes expression

The publication of annotated genomic sequences of *P. brassicae* facilitated the implementation of transcriptomic studies focusing on protist gene expression, where previous studies were limited to plant genes. Using RNA-seq, Bi et al. (2019) reported a specific expression modulation of GPCR signaling genes in *P. brassicae* during spore germination and root infection, suggesting that this versatile signal transduction pathway has an important role in *P. brassicae* life cycle. Rolfe et al. (2016) found 617 differentially expressed genes (DEGs) in *P. brassicae* interaction with *B. napus* during a six-weeks course (one sample/week) infection, among which 41 genes encoding putative secreted protein encompassing peptidases, hydrolases, proteinase inhibitors and a gene encoding a SABATH-type methyltransferase, PbBSMT (see next section). The modulation of *P. brassicae* gene expression was also monitored between two time points at 16 dpi (plasmodial stage) and 26 dpi (resting spore formation) during compatible interaction with *A. thaliana*. In general, genes linked to cytoskeletal functions, cell differentiation and membrane activities were down-regulated during switch between plasmodial growth and spore formation. General metabolism related genes showed the opposite regulation (Rolfe et al., 2016). This study also evidenced some DEGs annotated as Carbohydrate-Active enZymes (CAZy) and putatively involved in spore formation and germination. Likewise, Schwelm et al. (2015) reported the high expression of genes encoding enzymes potentially involved in the weakening of cell wall during the germinating of resting spores. Genes predicted to be involved in starch, trehalose and pyruvate metabolism as well as in citrate and pentose phosphate pathways were also particularly expressed at this life stage. In later stages, when secondary

CHAPTER II. Tripartite interaction in rhizomania

infection and cortex invasion occur, many genes participating to lipid metabolism are drastically impacted (Bi et al., 2016). Finally, sequences resembling genes involved in cancer-related pathway in KEGG, referred as proto-oncogenes, were detected in *P. brassicae* genome. Some of them would be induced in growing plasmodia where active nuclear divisions occur suggesting a key role of tumorigenic-related signal transduction pathway in plasmodiophorid developmental biology (Bi et al., 2018).

c. Identification of pathogenicity/ virulence genes in *P. brassicae*

Transcriptome studies brought clues that *P. brassicae* is able to hijack plant defenses in the early infection stages (Schwelm et al., 2015b; Rolfe et al., 2016). To achieved this, the protist would need to produced virulence factors, probably small secreted proteins, able to influence the plant defense signaling.

Research of plasmodiophorid pathogenicity/virulence genes such as effectors is an old challenge (Pérez-López et al., 2018). Findings in this area were promoted by the use of comparative genomic approaches, unlocked thanks to the increasing availability of phytopathogen genomes. To find virulence proteins candidates in phytopathogen genomes, research naturally turned to proteins which could be secreted and transferred to host cells. Those proteins seem to be abundant in *P. brassicae* genome (>550 predicted) and could act as effectors in the host plants (Schwelm et al., 2015b; Rolfe et al., 2016). Impressively, not less than 119 predicted secreted proteins from *P. brassicae* pathotype 3 have no homologs in the pathotype 6. These two pathotypes differ in their host range, Pb3 being more virulent on *B. napus* than on other Brassicaceae and Pb6 infecting only vegetable Brassica crops. This suggest that secretome could influence host specificity (Rolfe et al., 2016). Among *P. brassicae* secreted proteins, an enrichment has been noted in protease and protease inhibitor, cysteine-rich proteins, proteins with chitin binding domains and proteins rich in ankyrin repeats domains (Schwelm et al., 2015b).

Some of these pathogenicity/virulence genes have been characterized and were shown to be secreted by the pathogen to manipulate phytohormones signaling. For example, a methyltransferase active on SA which present homology to plant benzoic acid/ SA methyltransferase enable the protist to manipulate SA signaling (Ludwig-Müller et al., 2015). Another example is the auxin-responsive Gretchen Hagen 3 (GH3) protein PbGH3, involved in plant defense hormone homeostasis (Schwelm et al., 2015b). Two isopentenyltransferase genes involved in CK biosynthesis have also been found expressed in *P. brassicae* genome (Schwelm et al., 2015b) and are thought to produce minute amounts of CK able to increase CK responses in host defective for CK synthesis (Malinowski et al., 2016). Effect, however, would be marginal. *PbBSMT* was found differentially expressed during the

infection (Ludwig-Müller et al., 2015; Rolfe et al., 2016). Its role in suppressing plant local defenses by converting SA in inactive Me-SA (the systemic signal of SAR) was further investigated. In *A. thaliana*, PbBSMT is more effective than the plant BSMT1 itself to converting SA. Plants constitutively overexpressing PbBSMT were more susceptible to *P. brassicae* and to phytopathogenic bacteria *Pseudomonas syringae* pv. *tomato*. Moreover the non-host interaction with *Albugo candida* was partially compromised (Djavaheri et al., 2018a). Transient expression of *PBBSMT* also induced SAR in distal leaves of tobacco, increasing resistance against *Pseudomonas syringae* pv. *tabaci* (Rolfe et al., 2016). More generally, it was proposed that the methylation of SA is a common strategy to attenuate plant defense response in roots infected by intracellular parasites (Bulman et al., 2018). Recently, another effector from *P. brassicae*, PBZF1 was characterized. PBZF1 is expressed in resting spores and in primary infection steps and interacts with the plant SnRK1 to impair its biological functions. SnRK1 mediates broad-spectrum disease resistance by complex mechanisms involving energy signaling and carbon metabolism (Chen et al., 2021). Immunophilins have also been uncovered in *P. brassicae* and could have a role in virulence. In particular, a cyclophilin (PbCYP3) was able to complement *Magnaporthe oryzae* gene-inactivated Δ Cyp1 strain virulence in rice (Singh et al., 2018). Yu et al. (2019) suggested that secreted E3 ubiquitin ligases could also have a key role in clubroot development, probably by acting as pathogen effectors. Lastly, a serine protease gene, PRO1 would have a role in pathogenesis, stimulating resting spores germination through its proteolytic activity on root exudates (Feng et al., 2010).

Other remarkable features of *P. brassicae* genomes are the reduced number of genes encoding plant cell wall degrading enzymes and of protein associated with inorganic nitrogen and sulfur assimilation, with amino acids/thiamine biosynthesis and with toxin production (Spanu, 2012; Schwelm et al., 2015b; Rolfe et al., 2016). Few predicted genes associated with toxin production (polyketide synthases) are found in biotrophic pathogen. Only one was found in *P. brassicae* (Pb3). The reduced number of cell wall degrading enzymes support the theory that root hair infection occur through mechanical forces with aid of the “Rohr” and the “Stachel” rather than through plant cell wall degradation (Schwelm et al., 2015a). Some *P. brassicae* genes have similarities with prokaryote genes. For example, Rolfe et al. (2016) discovered a putative novel transporter type containing substrate binding motifs previously reported only in prokaryotic ABC transporters. Similarly, Schwelm et al. 2015 pointed out genes similar to bacterial chitinases, unknown in eukaryotes. Chitinases with lysosomes and fatty acid degradation, would have a major role in resting spore germination degrading spore walls (Schwelm et al., 2015a).

CHAPTER III.

The sugar beet and plant defenses

Polymyxa betae, described in Chapter I, and BNYVV, described in chapter II infect the sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) and several wild relatives from *Beta* genus. The first section of this Chapter aims to introduce the reader to the taxonomy of the *Beta* genus as well as to the agronomical and societal importance of sugar beet production in Europe. The second section of this chapter is dedicated to a general description of the plant immune system, important for the comprehension of plant-parasite interactions developed in Chapter V and in the general discussion.

1. *The sugar beet*

Cultivated beets (*Beta vulgaris* L. subsp. *vulgaris*), including sugar beet, are plants in the family Amaranthaceae native to the Mediterranean coast (Francis, 2006). They are now classified as subspecies, along with the wild sea beet (*Beta vulgaris* L. subsp. *maritima*), of *Beta vulgaris* in the *Beta* section. The other species and subspecies included in the *Beta* section are not cultivated (see Table III-1). They are often called wild beets. As early as five centuries before Christ, it was cropped as a leafy vegetable. Today, it is cultivated for sugar production (the sugar beet), as a leaf or root vegetable (e.g. swiss chard and table root, respectively) and as forage (e.g. mangel beets) (Cooke and Scott, 1993; Draycott, 2008).

Sugar beet is a biennial plant: vegetative growth takes place in the first year during which the plant accumulates sucrose in its taproot for the reproduction that takes place in the second year. Sugar beet cultivation takes advantage of high sugar storage during the vegetative growth, trying to avoid the reproductive phase that sometimes occurs already during the first year. Unlike sugar cane, another plant used for sugar production, which is cultivated in tropical and sub-tropical zones, the sugar beet is more adapted to temperate climate. It is therefore important in Northern countries (Draycott, 2008). Sugar beet production in Europe is estimated to be 184 and 194 million tons in 2018 and 2019, respectively (FAOSTAT, 2021). Europe is the world's largest sugar beet producer with about half of the world's production (European Commission, 2021). However, it is worth mentioning that the sugar beet accounts for only 20% of the global sugar production, with the remainder being processed from sugar cane, which is more productive and less expensive to process (European Commission, 2021). Despite this drawback, there is a strategic interest in maintaining this crop in Europe. Sugar beet is a key element in crop rotation. Its use at the head of crop rotation has a positive impact on the cultivation of cereals in the second year. It also has a significant socio-economic impact in Northern countries, where sugar beet production generates more than 23.000 jobs, mainly in rural areas, in addition to 360.000 jobs in the supply chain (CEFS, 2021). Moreover, sugar beet production plays an important role in food security in Europe which is the second largest consumer of sugar in the world (CIBE, 2021). Lastly, sugarcane requires high water consumption, limiting the possibility of expansion needed to cover global sugar demand (Biancardi and Lewellen, 2016). However, given the strong competition with sugarcane in the global sugar market, production losses due to biotic and abiotic stresses must be particularly avoided. Of these, rhizomania is probably the most significant (Biancardi and Lewellen, 2016).

As stated above, subspecies *vulgaris* and *maritima* have a common ancestor. Despite this, cultivated beets appear to have lost useful features during domestication. Selection during domestication was performed to increase traits useful to farmers, processors and consumers. However, it could also be responsible for the unwanted loss of traits potentially useful for

the future of the crop (Biancardi and Lewellen, 2016). Knowing that the activation of plant defenses would inevitably have a fitness cost, this theory is highly likely for resistance genes, leading to the resurgence of plant diseases. Crosses with wild beets outside the *Beta* section are difficult. Breeders have therefore used the *maritima* subsp. to recover resistance genes useful for managing major beet diseases, including rhizomania. However, with the development of biotechnology, the huge reservoir of genetic variability in wild relatives, even outside the *Beta* section, could be useful in finding new useful traits.

Table III-1 Taxonomy of the cultivated beets and wild relatives as proposed by Kadereit et al. (2006) (adapted from Biancardi and Lewellen 2016)

Genus <i>Beta</i>
Section I <i>Beta</i>
<i>Beta vulgaris</i>
subsp. <i>vulgaris</i> (cultivated forms)
subsp. <i>maritima</i>
subsp. <i>adanensis</i>
<i>Beta macrocarpa</i>
Section II <i>Corollinae</i>
<i>Beta corolliflora</i>
<i>Beta lomatogona</i>
<i>Beta trigyna</i>
<i>Beta nana</i>
Genus <i>Patellifolia</i>
<i>Patellifolia procumbens</i>
<i>Patellifolia patellaris</i>
<i>Patellifolia webbiana</i>

2. *Plant immunity*

a. *Nonself recognition in plants*

Plants are in constant contact with pathogenic, commensal and beneficial microorganisms. While pathogens can deprive plants of nutrients, affect growth, disturb physiological processes, and produce compounds directed against the plants (toxins, enzymes, virulence proteins) (Dou and Zhou, 2012), association with beneficial microbes can be of great benefit to plants. For example, association of plants with mycorrhizas is known to increase plant fitness by improving nutrition and response to biotic and abiotic stresses (Pozo and Azcón-Aguilar, 2007). This is especially true underground, where plant roots are in contact with numerous microbes forming complex communities in the rhizosphere (Coninck et al., 2015). The challenge for the plants is to defend themselves against pathogenic microbes while promoting association with beneficial organisms. Therefore, they have evolved complex systems to perceive and integrate multiple immunogenic signals related to biotic and abiotic stress, physiological status, etc. (Zhou and Zhang, 2020).

In order to perceive immunogenic signals derived from microorganisms, plants are equipped with numerous “interconnected” receptors, forming sophisticated networks (Kourelis and Hoorn, 2018; Van de Weyer et al., 2019). On the cell surface are found transmembrane receptors associated or not with cytoplasmic protein kinases: the receptor(-like) kinases (RKs or RLKs), and the receptor-like proteins (RLPs), respectively. These proteins are called pattern recognition receptors (PRRs) and are often associated with other RLKs, or co-receptor proteins forming multi-protein immune complexes on the cell surface (Kawai and Akira, 2010; Tang et al., 2017). At the cytoplasmic level, plants also possess a myriad of immune receptors known as nucleotide-binding, leucine-rich repeat receptors (NLRs or NB-LRRs) (Jones et al., 2016).

The nature of immunogenic signals is diverse (Zhou and Zhang, 2020). Plants are able to recognize molecular patterns associated with their pathogens or pathogen-associated molecular patterns (PAMPs). Since plants also recognize commensal and beneficial microorganisms, the concept of PAMPs can be extended to microbe-associated molecular patterns (MAMPs). The release of PAMPs/MAMPs in the apoplast is often facilitated by the action of plant lytic enzymes as it is the case for flagellin epitope flg22 and peptidoglycan (Liu et al., 2014b; Buscaill et al., 2019). In turn, the action of pathogen enzymes, toxins and other virulence factors in the apoplast can also induce cell wall degradation and the escape of plant cell components (e.g. ATP, nicotinamide adenine dinucleotide). Plants can detect the products resulting from this damage, called damage-associated molecular patterns (DAMPs) and activate their defenses (Gust et al., 2017). Sometimes, the combination of plant and pathogen derivatives is

responsible for a stronger activation of plant defenses. For example, the COS-OGA elicitor is formed by the interaction of cationic chitosan oligomers (COS), typically derived from fungal cell wall degradation, with anionic pectin oligomers (OGA), by-products of plant cell wall degradation, and induces plant immunity in several pathosystems, involving potato, tomato, grapevine, etc. (van Aubel et al., 2016; Clinckemaigne et al., 2017). Plants have also recently been shown to have evolved the ability to monitor cell wall integrity (Bacete et al., 2018) and to modulate the activation of defense by the production of signaling peptides, called phytoalexins which are also sensed by PRRs or PRR-associated receptors (Gust et al., 2017). For example, the *Arabidopsis* Rapid Alkalinization Factors (RALFs), are involved in the regulation of plant defenses and various other biological processes (Stegmann et al., 2017). The nature of cytoplasmic signals is often related to effector proteins, which are small proteins secreted by pathogens in order to manipulate the host immunity, hormone homeostasis/signaling and hijack other specific host physiological processes (Dou and Zhou, 2012). Plants can directly perceive effectors themselves, either alone or in combination with host components. Most often, they sense effector-induced changes or alterations in the host target. The integration of different danger signals associated with pathogen infection allow the plant to induce the most convenient defense output (Zhou and Zhang, 2020). For example, it was recently shown the combined signals triggered by MAMPs and DAMPs are needed to activate immune responses in *A. thaliana* roots, thus avoiding the activation of defense after recognition of MAMPs derived from commensal bacteria (Zhou et al., 2020).

b. The so-called zigzag model

Jones and Dangl (2006) established a theoretical model, called “zigzag” model to illustrate the sequential coevolution of plant immune system with (un)successful pathogens. Plant defense against pathogen attack can be viewed as three successive barriers. The first is physical and consists of a cell wall, a cuticle that can be reinforced in case of aggression (e.g. by the deposition of callose, lignin, waxy and phenolic compounds). In case of host-pathogen interaction, this first barrier is often bypassed by microorganisms. The second one is triggered by the recognition of infection by the pathogen through the perception of PAMPs/DAMPs (Jones and Dangl, 2006; Boller and Felix, 2009). Recognition of these general elicitors leads to the induction of a non-specific basal defense response called PAMPs triggered immunity (PTI). This response is characterized by the production of PR proteins and antimicrobial compounds as well as the induction of systemic defenses such as SAR. In compatible interactions, successful pathogens are able to overcome the second defense barrier through effector secretion, leading to a compatible interaction called effector-triggered susceptibility (ETS). In response, some plants have evolved to be able to specifically recognize certain pathogenic effectors produced by their aggressors and induce a stronger defense response: this is the effector triggered immunity (ETI). In some cases, a strong hypersensitive response (HR) is then induced, leading

CHAPTER III. The sugar beet and plant defenses

to programmed cell death and conferring a total resistance. During the evolution of plants and pathogens, several cycles of ETS-ETI may have occurred (Jones and Dangl, 2006). This coevolution leads to the establishment of a gene-for-gene interaction in which a specific pathogen effector, encoded by an “avirulence gene” (*Avr*) is specifically recognized by a plant resistance protein, encoded by a resistance gene (*R*), leading to induction of resistance and ultimately to an incompatible interaction. Although this model has weaknesses (Pritchard and Birch, 2014), as all theoretical models, it retains great value in conceptualizing plant-pathogen interactions.

c. Commensal organisms can also trigger and suppress plant immunity

As noted above, plants, and in particular roots, come into contact with a wide range of microorganisms. For decades, most studies on plant immune system were logically focused on plant-pathogen interactions. However, recent research reported that, like pathogens, commensal microbes in the phytobiome are able to circumvent or suppress the plant immune responses (Teixeira et al., 2019). In fact, there are many similarities between plant interactions with commensal and pathogenic microorganisms. For example, plants produce MAMPS receptors derived from commensal microbes and induce the MAMPS-triggered immunity (MTI) that can then be suppressed by commensal microbes and pathogens. Teixeira et al. (2019) proposed that plants use their immune system to shape their associated microbiome and that the microbiome can in turn extend plant immunity. The microbiome could act as a supplementary layer of interactions with pathogenic microorganisms through direct microbe-microbe interactions and via stimulation/repression of plant defenses.

d. Activation of defenses

When a danger is perceived by PRRs, a cascade of reactions occurs in the plant cell, leading to the activation of defense response as well as the production of systemic signals. Defense signaling includes the phosphorylation of receptor-like cytoplasmic kinases (RLCKs), the activation of NADPH oxidase respiratory burst oxidase homolog D (RbohD) which stimulates ROS production, increase in intracellular Ca^{++} concentration (influx), activation of calcium-dependent kinases and cascades of mitogen-activated protein kinases (MAPKs) and G proteins (Tang et al., 2017; Zhou and Zhang, 2020). Similarly, NLRs also associate with RLCKs. Interaction with their targets triggers conformation changes and reaction cascades, ultimately leading to the activation of the immune response, HR and/or SA biosynthesis. It was proposed that, in some cases, NLRs signaling involves the enhanced disease susceptibility 1 (EDS1) protein as a downstream component of signaling. A membrane pore-forming activity responsible for cell death and/or calcium influx would be involved. Some NLRs were shown

to localize to the nucleus, suggesting a direct activity in the transcriptional regulation of defenses. However, the exact mechanisms are unclear (Zhou and Zhang, 2020). Activated defenses include ROS burst, HR, callose deposition, activation of defense genes such as genes involved in PR proteins production (Table III-2) and other defense compounds. Activation of secondary metabolites biosynthesis pathways was often observed such as phenylpropanoids, flavonoids, alkaloids and terpenoids. (van Loon et al., 2006; Pauwels et al., 2009; Adedeji and Babalola, 2020). Plants are also capable of producing systemic signals to potentiate or activate a defense response in distal tissues.

Table III-2. The 17 recognized families of pathogenesis-related (PR) proteins (van Loon et al., 2006). Families highlighted in light grey are reported as SA responsive, whereas families highlighted in dark grey are reported as JA/ET (Glazebrook, 2005; Bari and Jones, 2009; Ali et al., 2018).

Family	Type member	Properties
PR-1	Tobacco PR-1a	Unknown, SAR marker
PR-2	Tobacco PR-2	β -1,3-glucanase
PR-3	Tobacco P, Q	Chitinase (type I, II, IV, V, VI, VII)
PR-4	Tobacco 'R'	Barwin domain Chitinases (type I, II)
PR-5	Tobacco S	Thaumatococcus-like
PR-6	Tomato inhibitor I	Proteinase inhibitor
PR-7	Tomato P69	Endoproteinase
PR-8	Cucumber chitinase	Chitinase (type III)
PR-9	Tobacco "lignin-forming peroxidase"	Peroxidase
PR-10	Parsley "PR1"	Ribonuclease-like
PR-11	Tobacco "Class V" chitinase	Chitinase (type I)
PR-12	Radish Rs-AFP3	Defensin
PR-13	Arabidopsis THI2.1	Thionin
PR-14	Barley LTP4	Lipid-transfer protein
PR-15	Barley OxOa	Oxalate oxidase
PR-16	Barley OxOLP	Oxalate oxidase-like
PR-17	Tobacco PRp27	Unknown

e. Systemic defenses

Two major systemic defense pathways have long been recognized in plants: the SAR and the induced systemic resistance (ISR). SAR is activated in the plant after induction of local defenses by biotic and abiotic stresses and is mediated by a major defense-related phytohormone: the salicylic acid (SA). This hormone activates Nonexpressor of Pathogenesis-Related Genes 1 (NPR1) which migrates to the nucleus and activates the production of PR

CHAPTER III. The sugar beet and plant defenses

proteins by recruiting TGA transcription factors (Conrath, 2006; van Loon et al., 2006; Backer et al., 2019). Two other proteins in the same family, NPR3 and NPR4 are also *bone fide* SA receptors but act as negative regulators of SAR (Backer et al., 2019). The inactive oligomers of NPR1 are found in the cytoplasm of unchallenged plant cells. They are monomerized following redox changes associated with the oxidative burst induced as a result of the HR activated by pathogen recognition. The monomers are then localized in the nucleus and activate the SAR response, such as the expression of *PR* genes (Mou et al., 2003; Conrath, 2009). *PR* proteins are encoded by genes that are inducible by the recognition of pathogens but also of other microorganisms or effects derived from abiotic stresses. Some of them have shown antimicrobial function, such as endoglucanases and chitinases which could be involve in the degradation of fungal cell wall (Ali et al., 2018). A summary is presented in Table III-2. Another systemic defense pathway, ISR, was shown to be induced when plant roots interact with beneficial bacteria, called plant growth promoting rhizobacteria (PGPR). This pathway is thought to rely on two phytohormones: jasmonic acid (JA) and ethylene (ET) (Pieterse et al., 1998; Choudhary et al., 2007). ISR is also mediated by NPR1 but is not characterized by the immediate activation of *PR* genes. Instead, this pathway is characterized by the systemic “priming” of plant defenses, which are then induced more rapidly and strongly upon pathogen infection (van Wees et al., 1999). Notably, negative cross-talk between SA and JA/ET pathways was demonstrated in plants. The SAR/ISR model was useful in describing plant defenses but does not explain every specific plant-microorganism interaction. While the involvement of SA and JA/ET pathways in plant defenses is well established in many pathosystems, it should be noted that other systemic signals may be involved and form a complex signaling network involving cross-talk between various pathways. Other phytohormones putatively involved in plant defense signaling includes abscisic acid, auxins, cytokinins, gibberellins, brassinosteroids and signal peptides (Pieterse et al., 2012; Berens et al., 2017). For convenience, plant defenses were primarily studied on selected pathosystems involving foliar diseases on model plants. However, less is known about root defenses against soil-borne pathogens. Roots interact with a plethora of non-pathogenic and beneficial organisms and must distinguish friends from foes. While many similarities were found between above- and below-ground defenses, we should keep in mind that some differences were also evidenced (Coninck et al., 2015).

OBJECTIVES

The rhizomania of sugar beet remains a significant threat for sugar beet production (McGrann et al., 2009), despite the wide use of resistant varieties (Panella and Biancardi, 2016) due to the appearance of resistance breaking BNYVV isolates (Biancardi and Tamada, 2016). Current knowledge about the molecular interactions *Polymyxa betae*, the sugar beet and BNYVV is limited. Reverse genetic approaches have been successfully used to decipher the function of BNYVV genomic components in plant-virus interactions (Gilmer, 2016). For example, the role of P25 (Chiba et al., 2008; Peltier et al., 2011; Thiel et al., 2012; Liebe et al., 2020) and RNA5 (Link et al., 2005; Covelli et al., 2009; Tamada et al., 2021) in pathogenicity was demonstrated in various models. Similarly, viral components involved in transmission by *P. betae* (CP-RT and P31) were highlighted (Tamada and Abe, 1989; Tamada and Kusume, 1991; Adams et al., 2001). Plant root response to BNYVV infection was also monitored in various studies, including transcriptomics (Schmidlin et al., 2008; Fan et al., 2014, 2015a; Liu et al., 2017, 2020a; Fernando Gil et al., 2020), proteomics (Larson et al., 2008; Webb et al., 2014, 2015) and metabolomics (Webb et al., 2020). In particular, the main rhizomania symptom, i.e. lateral root proliferation, was shown to involve the regulation of auxin signaling pathway (Schmidlin et al., 2008; Peltier et al., 2011; Fernando Gil et al., 2018, 2020). A first glimpse on *Polymyxa* spp.-plants interactions was obtained by McGrann et al. (2007, 2009). They found that early host and non-host responses to *Polymyxa* spp. were similar. Desoignies et al. (2014) suggested that, after a fine molecular interplay, *P. betae* is able to hijack sugar beet. Moreover, the interactions of *Polymyxa* spp. and f. sp. with viruses seem to result from specific molecular mechanisms, also involving the host plant (Vaïanopoulos et al., 2007; Dieryck et al., 2011). Few studies are addressing the tripartite interactions between plants, *Polymyxa* spp. and transmitted viruses. In particular, very few information is available on the effects of BNYVV transmission from the *P. betae* “point of view”. This gap probably reflects the difficulty to simultaneously handle *P. betae* and the BNYVV under fully controlled soil-free assay conditions (Kanyuka et al., 2003) and the lack of genomic information on *Polymyxa* spp. (Schwelm et al., 2015a, 2018). More generally, few phylogenomic studies encompassing rhizarian protists did not include *P. betae* sequences (Sierra et al., 2016; Cavalier-Smith et al., 2018). Broadly speaking, more protist genomes are needed to fully embrace the microbial eukaryotic diversity present in all ecosystems on earth (Sibbald and Archibald, 2017).

CHAPTER III. The sugar beet and plant defenses

The main objective of this thesis was to decipher the molecular events involved in BNYVV-sugar beet-*P. betae* tripartite interaction.

Three successive specific objectives have been defined in order to overcome the limitations highlighted above and achieve our main goal:

- 1) The first objective was to sequence and annotate the first *P. betae* reference genome. To achieve this, a cutting-edge metagenomics pipeline was used to assemble the *P. betae* genome from shotgun DNA libraries containing sequences from the host and other microorganisms. The *P. betae* genome is presented in chapter IV.
- 2) Our second objective was to develop a method aimed to study the specific interaction between BNYVV, *P. betae* and the sugar beet under controlled conditions, using exclusively genetically characterized organisms. A protocol enabling the soil-free transmission of BNYVV to sugar beet by *P. betae* zoospores was optimized, using the fully sequenced *B. vulgaris* and *P. betae* accessions as well as infectious cDNA clones of BNYVV genomic RNAs. This protocol is described in Chapter V.
- 3) The last objective was to highlight some of the critical interactions between the three rhizomania protagonists. A transcriptomic approach, using RNA-sequencing, was applied to study the tripartite interactions in the rhizomania pathosystem. Results are discussed in Chapter V.

The implication of the genomic and transcriptomic results obtained in this thesis is then integrated in a general discussion (Chapter VI) with regard to our current knowledge of rhizomania pathosystem. Finally, the main conclusions of this work are summarized in Chapter VII. Besides transcriptomics experiments such as the one achieved in this thesis, the tools developed here open up new research avenues for the study of the interactions of *Polymyxa* spp. with plants and viruses. Notably new perspectives are brought for deciphering the molecular keys allowing specific viral transmission. These aspects are also discussed in Chapter VII.

RESULTS

CHAPTER IV.

The *Polymyxa betae* genome

Forewords

This Chapter is structured around the first specific objective of the present work: to sequence and annotate the first *P. betae* reference genome. In that purpose, a bioinformatic pipeline allowing the extraction of high-quality draft eukaryotic rhizarian genomes from metagenomic data was optimized and used to obtain draft genomes from two *P. betae* isolates. *P. betae* drafts genomes were then valued in a comparative genomics investigation of Plasmodiophorids.

The sequencing of intracellular parasite such as plasmodiophorids is complicated by their obligate parasite nature, impeding the establishment of axenic structures. We therefore elaborated a protocol to purify sporosori of the *P. betae* A26-41 isolate and extract good quality DNA from these tick-walled survival structures. This allowed us to perform an Illumina run with paired short reads (2x250 pb) as well as to attempt a long-read sequencing with Oxford Nanopore MinION technology. Unfortunately, we couldn't obtain long reads and decided to pursue only with Illumina reads. Even after extensive purification steps, there was still "contaminating DNA sequence" from other microorganisms in our dataset. Consequently, we applied metagenomic methods to recover the first *P. betae* draft genome. This genome was published in "Microbiology Resource Announcements" (American Society for Microbiology) and the related publication is the subject of the first part of this chapter. The paucity in public databases of rhizarian genomic sequences and the limited resemblance with the other available plasmodiophorid didn't enabled us to verify the validity of our data and the isolation of non-degraded DNA from partially purified sporosori was very laborious. However, following the publication of the first *P. betae* draft genome, we developed a collaboration with two international partners to release the draft genome of a second isolate. During this collaboration, we improved the metagenomic pipeline to reassemble and clean the genome from the A26-41 isolate and acquire the draft genome of a second *P. betae* (RES F41) from a highly contaminated sample composed of *P. betae* zoospore holobionts. Then, both draft genomes were annotated. The close resemblance between both isolates validated our method to recover eukaryotic genomes from metagenomic data. It also validated the accuracy of the *P. betae* genome. The object of the second part of this Chapter was accepted in "Genomics" (Elsevier).

First Draft Genome Sequence of a *Polymyxa* Genus Member, *Polymyxa betae*, the Protist Vector of Rhizomania

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1. ABSTRACT

Polymyxa betae belongs to the Plasmodiophorida (Phytomyxea, Rhizaria). Here, we report the first draft genome sequence of a member of the *Polymyxa* genus, which includes two obligate root endoparasite species, vectors of important soilborne plant viruses. The genome assembly was represented by 1,001 contigs, with a cumulated length of 27,085,946 bp.

2. ANNOUNCEMENT

P. betae and *P. graminis* belong to the *Plasmodiophorida*, a protist order which includes obligate root endoparasites of plants (Neuhauser et al., 2010, 2014). Although *Polymyxa* sp. infections are symptomless, these species are considered a real threat because of their vectorial capacity for viruses (Barr, 1979; Kanyuka et al., 2003; Tamada and Kondo, 2013). Molecular studies of these organisms are difficult due to their biotrophic nature. Recently, they have been boosted with the publication of *Plasmodiophora brassicae* and *Spongospora subterranea* genomes and transcriptomes (Schwelm et al., 2015b, 2015a; Rolfe et al., 2016; Ciaghi et al., 2018). Here, we present the first full draft genome sequence of *P. betae*, the vector of *Beet necrotic yellow vein virus*, the agent of rhizomania, *Beet soilborne mosaic virus*, *Beet soilborne virus*, and *Beet virus Q*. Three-week-old *Beta vulgaris* of the fully sequenced monogerm DH line KWS2320 (Dohm et al., 2014) were inoculated with zoospores of a *P. betae* single sporosorus (aggregation of resting spores) strain, and grown for 14 weeks in an automated immersion system (Legrève et al., 1998). Heavily infested roots were harvested, surface disinfected with 1 N NaOH and 0.1% SDS, and the sporosori were extracted in a Virtis blender. After sequential filtration steps (500, 200, and 100 µm), sporosori were disinfected with 70% ethyl alcohol (EtOH) and 0.5% antiseptic liquid (Dettol), rinsed three times in sterile distilled water, resuspended in 100 µg/ml cycloheximide (Carl-Roth), 50

µg/ml tetracycline (Sigma-Aldrich), and 100 µg/ml gentamicin sulfate (Sigma-Aldrich) and kept at 4°C. Sporosori were then purified by two 3-h-long centrifugations at 4,500 g on a 0 to 30% continuous gradient of HS-40 colloidal silica (Ludox; Sigma-Aldrich), treated with 50 U/ml RQ1 DNase (Promega) at 37°C for 1 h, and rinsed five times. The resulting material was ground in liquid nitrogen with mortar and pestle, and DNA was extracted following a cetyltrimethylammonium bromide (CTAB) extraction procedure (Brandfass and Karlovsky, 2008). One library was prepared with the TruSeq Nano DNA sample preparation kit and paired-end sequenced using an Illumina MiSeq instrument and the reagent kit version 3, with a sequencing length of 2 250 bp, yielding 12,292 Mb. After adapter trimming with Trimmomatic version 0.32 (Bolger et al., 2014), 49,092,018 reads were quality trimmed and assembled using CLC Genomics Workbench version 9.5.2, resulting in 12,450 contigs (1 Kbp). Contigs were binned using MetaBAT version 0.32.4 (Kang et al., 2015), resulting in 47 bins, which were assessed for genome completeness and contamination and assigned bin higher-level taxonomy with CheckM version 1.0.11 (Parks et al., 2015). Bacterial metagenome-assembled genomes (MAGs) and putative viral (phage) genomes were discarded following further BLAST annotation against the NCBI nr database. Raw reads originating from *B. vulgaris* KWS2320 leaf DNA (SRA accession numbers SRR869626 and SRR869627) were mapped to the resulting contigs with CLC Genomics Workbench version 9.5.2 to further remove plant DNA contamination. The remaining contigs were visualized with ICoVeR (Broeksema et al., 2017), and based on the GC content, coding density, contig coverage, and the tetranucleotide composition, a subset of 1,001 contigs was selected. This final potentially free-of-contamination assembly had a total length of 27,085,946 bp, a coverage of 225, and an N50 value of 61,339 bp (L50, 106). The GC content is 45.1%, a percentage similar to that of *S. subterranea* (45.7%) but lower than that of *P. brassicae* (58.5%) (Schwelm et al., 2015b; Ciaghi et al., 2018). Genome completeness was assessed using CEGMA version 2.5 (Parra et al., 2007). Of the 248 CEGMA eukaryotic core genes, 91.3% were completely (89.1%) or partially (2%) recovered in the assembly. The k-mer genome size estimation using the 17-mer coverage distribution inferred with Jellyfish version 2.0 (Marçais and Kingsford, 2011) led to a complete genome size of 29,353,583 bp.

Data availability.

This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number RBZT00000000 and BioProject number PRJNA494670. Raw reads are available under SRA accession number SRR8105394. The version described in this paper is the first version, RBZT01000000.

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Metagenomics Approach for *Polymyxa betae* Genome Assembly Enables Comparative Analysis Towards Deciphering the Intracellular Parasitic Lifestyle of the Plasmodiophorids

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1. ABSTRACT

Genomic knowledge of the tree of life is biased to specific groups of organisms. For example, only six full genomes are currently available in the rhizaria clade. Here, we have applied metagenomic techniques enabling the assembly of the genome of *Polymyxa betae* (Rhizaria, Plasmodiophorida) RES F41 isolate from unpurified zoospore holobiont and comparison with the A26-41 isolate. Furthermore, the first *P. betae* mitochondrial genome was assembled. The two *P. betae* nuclear genomes were highly similar, each with just ~10.2k predicted protein coding genes, ~3% of which were unique to each isolate. Extending genomic comparisons revealed a greater overlap with *Spongospora subterranea* than with *Plasmodiophora brassicae*, including orthologs of the mammalian cation channel sperm-associated proteins, raising some intriguing questions about zoospore physiology. This work validates our metagenomics pipeline for eukaryote genome assembly from unpurified samples and enriches plasmodiophorid genomics; providing the first full annotation of the *P. betae* genome.

2. INTRODUCTION

The Plasmodiophorida (plasmodiophorids), is an eukaryotic order of mainly strict plant endoparasites, previously considered as fungi and now classified in the Phytomyxea embedded within the Cercozoa in the Rhizaria protist supergroup (Adl et al., 2012; Neuhauser et al., 2014; Hittorf et al., 2020). They were discovered early in the 19th century with the description of *Plasmodiophora brassicae* in 1877 by Woronin (Woronin, 1877) and, in spite of this early scientific interest, this group remains poorly studied. Research on their interactions with plants and on virus transmission under controlled conditions is complicated by their strict biotrophic endoparasitic habit and continues mainly in the context of agricultural plant hosts.

Four of the known plasmodiophorids species are considered to be of economic significance in agriculture. *Plasmodiophora brassicae* is responsible for clubroot disease in Brassica crops (e.g. canola, cabbage). Infected plants develop root galls and are stunted, leading to significant economic losses (Schwelm et al., 2015a). *Spongospora subterranea* infects potatoes causing powdery scab, characterized by small circular lesions on the tuber filled with a powdery mass of spores. *S. subterranea* also causes galls on roots and stolons and transmits the *Potato mop-top virus* (PMTV), a damaging virus in potato crops (Merz and Falloon, 2009; Torrance et al., 2011). *Polymyxa graminis* symptomlessly invades roots of numerous Poaceae species but becomes damaging as a virus vector, being capable of transmitting at least 14 plant viruses (Benyvirus, Bymovirus, Furovirus, Pecluvirus) to major crops worldwide such as rice, wheat, maize, barley, rye, oat, triticale, sugarcane and peanut (Dieryck et al., 2011; Smith and Rush, 2016). *P. betae* has been reported in all sugar beet (*Beta vulgaris* subsp.

vulgaris) production regions. Even though, like *P. graminis*, it is widely accepted that *P. betae* is not pathogenic to its host, in sugar beet crops it is responsible for the transmission of the *Beet necrotic yellow vein virus* (BNYVV). This benyvirus is the causal agent of rhizomania syndrome, a devastating disease in susceptible sugar beet. *P. betae* also transmit another benyvirus, the *Beet soil-borne mosaic virus* (BSBMV) and two pomoviruses, the *Beet soil-borne virus* (BSBV) and the *Beet virus Q* (BVQ) which are often associated with the rhizomania syndrome (Meunier et al., 2003; Galein et al., 2018). *P. brassicae* and *S. subterranea* are both associated with cell proliferation and the formation of hypertrophic cells in the host plants (Graaf et al., 2007; Ludwig-Müller et al., 2009) whereas roots of plants infected by non-viruliferous *Polymyxa* spp. generally appear normal. *P. betae* and *S. subterranea* share the ability to transmit plant viruses to their hosts while no virus transmission has yet been reported for *P. brassicae* (Schwelm et al., 2018).

Until 2000, most research on plasmodiophorids consisted of ultrastructural and karyological observations as well as rDNA-based and immuno-based detection and co-occurrence with viruses (Legrève et al., 2000, 2002; Bulman and Neuhauser, 2017). In the early 2000s, Mutasa-Gottgens et al. (2000) generated 11 *P. betae* Expressed Sequence Tags (EST) with phagemid library of cDNA clones from infected sugar beet roots. A few years later, Bulman and colleagues used a suppression subtractive hybridization strategy to identify 76 new *P. brassicae* genes (Bulman et al., 2006, 2007). The same strategy was applied for *P. graminis*, generating four EST (Šubr et al., 2002) and for *P. betae*, generating 76 *P. betae* EST as well as 120 sugar beet ESTs up-regulated during the different stages of the infection process (Desoignies et al., 2014). The rapid evolution of sequencing technologies brought new perspectives in plasmodiophorids research starting with the release of the *Arabidopsis thaliana* annotated genome, which, as a susceptible host plant, promoted *P. brassicae*-plant interactions studies (Devos et al., 2006; Siemens et al., 2006, 2009; Malinowski et al., 2012; Ludwig-Müller et al., 2015). For example, Siemens et al. (2006) used a transcriptomics approach to study regulation of defense genes in *A. thaliana* during compatible interaction with *P. brassicae*. Cao et al. (2008) studied reactive oxygen species metabolism in *Brassica napus* infected with *P. brassicae*. Their findings suggest a fine-grained molecular interplay between plasmodiophorids and their host, probably by means of direct effector secretion from intracellular plasmodia to their host's cytoplasm but also by means of membrane proteins such as receptors and transporters. Subsequently, Burki et al. (2010) used 454 pyrosequencing technology to sequence cDNA from five rhizarian species including *P. brassicae* and *S. subterranea*. In the last five years, the release of genomes of *P. brassicae* in 2015 (Schwelm et al., 2015b, 2015a) *S. subterranea* in 2018 (Ciaghi et al., 2018) and *P. betae* in 2019 (Decroës et al., 2019) facilitated transcriptomic studies oriented toward plant-plasmodiophorids interactions, especially *P. brassicae* (Bi et al., 2016, 2019; Rolfe et al., 2016; Ciaghi et al., 2018; Shah et al., 2020). Several *P. brassicae* isolates have now been (re)sequenced (Bi et al., 2016; Rolfe et al., 2016; Daval et al., 2019; Sedaghatkish et al., 2019;

CHAPTER IV. The *Polymyxa betae* genome

Stjelja et al., 2019). Before 2015, the lack of enough plasmodiophorid genomic information naturally led scientists to focus on the plant side of the interaction (Neuhauser et al., 2010).

The sequencing effort granted to the Rhizaria supergroup remains small by comparison to other eukaryotic groups (Burki and Keeling, 2014; Sibbald and Archibald, 2017). In addition to plasmodiophorids only three genomes from other rhizaria protists are available: the Chlorarachnea *Bigelowiella natans* (Curtis et al., 2012), the Foraminifera *Reticulomyxa filosa* (Glöckner et al., 2014) and the Imbricatea *Paulinella micropora* (accession no.: PRJDB3528). More protist genomes are needed for comparative analyses that fully encompass the eukaryotic diversity and the microbial biosphere (Sibbald and Archibald, 2017). This would be expected to provide new clues on evolution and adaptation of eukaryotes, and to improve knowledge of the functional repertoire of eukaryotic genomes. The great majority of sequenced eukaryotic genomes belong to animals, plants and fungi, representing only two of the eight main eukaryotic groups summarized by Burki et al. (2020).

Although methods for purification of plasmodiophorid resting spores have been developed, the sequencing and assembly of plasmodiophorid genomes remain challenging because, whatever the technical approach chosen, the purified samples are never free of host and other contaminating microorganisms (Buhariwalla et al., 1995; Wakeham and White, 1996; Delfosse et al., 2000; Walsh et al., 1996; Šubr et al., 2002; Qu and Christ, 2006a). This is especially true for *Polymyxa* spp. which, compared to *P. brassicae* and *S. subterranea*, do not produce structures similar to galls filled with numerous resting spores which help to bulk protist DNA for extraction. Moreover, isolation and purification of *Polymyxa* spp. sporosori have proven to be difficult due to the variation of their size and shape. Additional confounders include the possibility that some “contaminant” microorganisms might be able to develop within hatched individual spores in sporosori (Chen et al., 1998) or that DNA from the host could pass to the plasmodia (Buhariwalla et al., 1995). Despite the delays caused by these challenges, a first draft genome of *P. betae* was recently recovered through a metagenomic approach using DNA obtained from sporosori of the monosporosorus *P. betae* A26-41 isolate grown in sugar beet in a soil-free hydroponic system (Decroës et al., 2019).

The objectives of this new study were to validate the metagenomics approach for recovering a new *P. betae* genome from a geographically distinct isolate (RES F41; DNA sequence generated from zoospores contaminated with bacteria), and to carry out some comparative analysis with *P. betae* isolate A26-41 and existing genomes from other plasmodiophorids (*P. brassicae* and *S. subterranea*). To achieve this, a bioinformatic pipeline including Metaspades, Metawrap and EukCC softwares was applied to assemble and isolate two *P. betae* genomes from the RES F41 unpurified zoospore holobiont and the A26-41 partially purified

sporosori sample. The new genomes were used to carry out some comparative sequence analysis. This study is the first to provide a full nuclear genome annotation for *P. betae*, and to also present the first *P. betae* mitochondrial genome.

3. MATERIALS AND METHODS

a. Construction of two *Polymyxa betae* draft Genomes

***P. betae* isolates origin and DNA extraction**

Two *P. betae* isolates were used as starting material: the Belgian isolate A26-41 (Legrève et al., 1998) and the UK isolate RES F41 (Mutasa et al., 1993). The methodology followed to obtain high-quality DNA extract from each isolate is described in the respective papers and represented in (Supplementary Figure S1).

Libraries preparation and sequencing

The methodology used to prepare library and sequence A26-41 isolate is described in Decroës et al. (2019). For the RES F41 isolate, paired-end libraries were prepared from fragmented dA-tailed DNA. After addition of dT-tailed DNA adaptors, the ligated products were purified and whole-genome sequencing was performed using the Illumina paired-end HiSeq 2000 platform with a sequencing length of 2×150 bp (Novogene, Beijing, China).

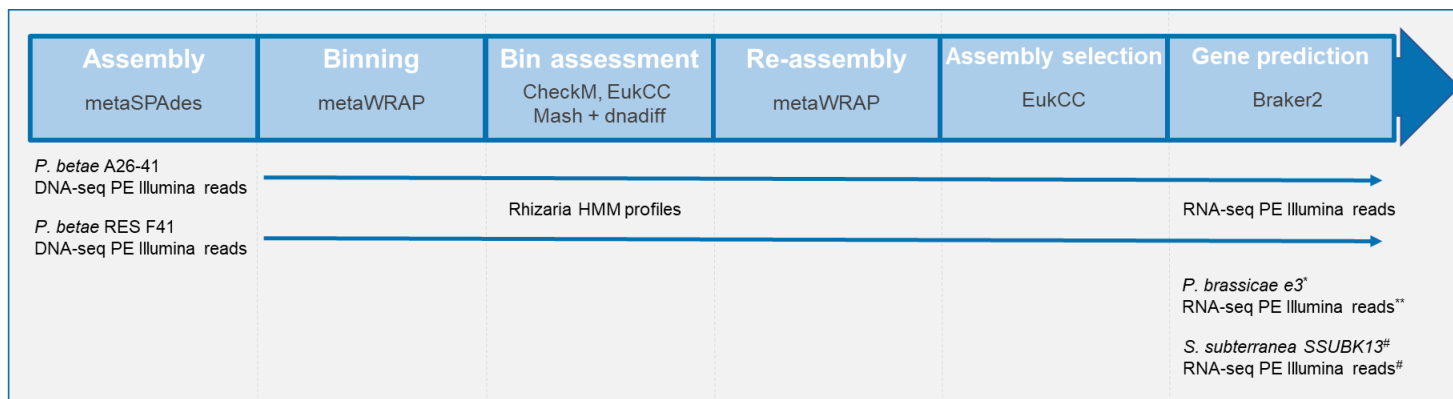
Genome assembly and cleaning

The obligate nature of *P. betae* excludes the option of a large-scale axenic culture (Desoignies and Legrève, 2011). Contaminating DNA from bacteria, protozoa, algae, fungi is unavoidable even after extensive purification. Both sample sequence datasets were independently processed using the same bioinformatic workflow (see Figure IV-1) aiming to assemble reads and filter *P. betae* contigs from contaminating DNA sequences. Reads were assembled with the metagenome assembler metaSPAdes v3.12.0 (Nurk et al., 2017) and assembled contigs were subsequently binned using the metaWRAP software (Uritskiy et al., 2018). MetaWRAP produces consensus bins based on three tools, namely MetaBAT 2.0, MaxBins 2.0 and CONCOCT, which are then assessed for completeness and contamination using CheckM (Parks et al., 2015). However, as CheckM is optimized for bacterial genomes, the bins were assessed using a bespoke version of EukCC (Saary et al., 2019), a tool designed for microbial eukaryote genome quality estimation. Briefly, a bespoke single-copy marker gene reference set was constructed from five available Rhizaria genomes (GenBank accession

CHAPTER IV. The *Polymyxa betae* genome

no. GCA_000320545.1, GCA_001049375.1, GCA_003833335.1, GCA_000512085.1, GCA_900404475.1), enabling the identification of 636 profile HMMs from PANTHER (v14.1), which were utilized by EukCC to estimate the completeness and contamination of the bins. Mitochondrial genome was re-assembled for both *P. betae* isolates with the assemblers MITObim 1.8 using MIRA 4.0.2 (Hahn et al., 2013) and NOVOPlasty (Dierckxsens et al., 2017). For comprehensiveness, bins were compared to a set of complete reference genomes of bacterial and unicellular eukaryotic genomes in RefSeq as of January 2018 and bespoke references from other Rhizaria species: *B. natans* (GenBank accession no. GCA_000320545.1), *P. brassicae* (GenBank accession no. GCA_003833335.1), *R. filosa* (GenBank accession no. GCA_000512085.1), *S. subterranea* (GenBank accession no. GCA_900404475.1). This was performed using a two-stage process, as described in (Almeida et al., 2019). First, Mash was used to find the best matching reference genome, followed by a whole-genome alignment between the query metagenome-assembled genome (MAG) and reference genome using dnadiff (part of the MUMmer package), to evaluate the alignment fraction and average nucleotide identity (ANI). Bins from each isolate with the highest completeness/lowest contamination score based on the bespoke EukCC reference set were determined to represent *P. betae*. These were then reassembled using the *reassemble bins* module within metaWRAP, in order to produce the best quality assembly. Reassembled bins were compared against the original bins using the bespoke EukCC again, and the best assembly for each isolate (completeness, contamination, genome size and N50) was selected as the *P. betae* draft genome. Reads were mapped back to corresponding assemblies using Bowtie2 v2.3.4 (Langmead and Salzberg, 2012) and per-contig average coverage was computed. Overall ANI value between both draft genomes, fragmented with a window size of 1000 base pairs (bp) and a step size of 200 bp, were computed with the “ani.rb” ANI calculator (Rodriguez-R and Konstantinidis, 2014, 2016). Thresholds to consider alignments for ANI computation were a minimum length of 700 bp and identity of 0.7. Moreover, both draft genomes were compared using ProgressiveMauve v2.4.0 (Darling et al., 2010) with default parameters. Low complexity sequences from both *P. betae* genomes were masked prior to any further analysis. *De novo* identification of novel repeat families was performed with RepeatModeler v-open-1.0.11 and both genomes were then softmasked using RepeatMasker with rmbblastn v2.2.27+. RepBase-20170127 and Dfam_Consensus-20170127 databases were combined to the *de novo* identified repeats.

The remaining (non-*P. betae*) bins initially generated by metaWRAP for each isolate represented bacterium found in association with *P. betae*. These bins were assigned a quality score (QS) calculated as “completeness” - (5* “contamination”). Bins with a QS > 50 are considered to be medium/high quality, and were assigned a taxonomic classification based on the Genome Taxonomy Database (GTDB) using GTDB-Tk (Chaumeil et al., 2020).



Ciaghi *et al.*, 2018

* Stjelja *et al.*, 2019

** Schwelm *et al.*, 2015a

Figure IV-1 Schematic representation of the workflow of metagenomics analysis. The software(s) used in each step is (are) indicated in the boxes. Input data are given below the boxes.

b. *Polymyxa betae* RNA preparation, sequencing and data processing

Three-week-old sugar beet seedlings monogerm DH line KWS2320 were inoculated with a zoospore suspension of the isolate A26-41 and grown as previously described (Legrève et al., 1998). Heavily infected roots were harvested after four weeks, surface disinfected with 1N NaOH and 0.1% SDS and rinsed three times with sterile water. Total RNA was then isolated using Trizol™ (Invitrogen, Carlsbad, CA) and DNase treated with RQ1 DNase (Promega, Madison, Wi). Roots were first examined under bright field microscopy to confirm that all *P. betae* life stages, i.e. undifferentiated plasmodia, sporangia, sporogenous plasmodia, sporosori, were present. mRNA was purified from total RNA through Poly(A)-tail selection then fragmented, retro-transcribed with random primers and one library was prepared using the TruSeq stranded mRNA kit (Illumina, San Diego, CA). The library was paired-end sequenced using an Illumina NextSeq instrument and the “NextSeq High kit”, with a sequencing length of 2x150 bp. Raw reads were trimmed for adapters and low-quality sequences with bbdduk.sh (Bushnell, 2014) and quality checked with FastQC v.0.72 (Andrews, 2010). They were then mapped against the sugar beet draft genome KDHBv-1.0 (Dohm et al., 2014) with Bowtie2 v2.3.4 (Langmead and Salzberg, 2012) as a first filtering step to remove plant sequences. Unaligned read pairs were then mapped against the *P. betae* draft genomes with HISAT2 v2.1.0 (Kim et al., 2015).

c. Genome Annotation

Gene predictions and annotation

The resulting HISAT2-created BAM-files and the softmasked *P. betae* draft genomes were used as input for gene prediction with the BRAKER2 v2.4.1 pipeline (Hoff et al., 2019; Brûna et al., 2021). This takes advantage of GeneMark-ET v.4.48_3.60 (Ter-Hovhannisyan et al., 2008; Lomsadze et al., 2014) and AUGUSTUS v3.3.3 (Stanke et al., 2008) for RNA-seq-based genome annotation. The ProtHint pipeline v2.1.1 from Georgia Institute of Technology (Brûna et al., 2020) was also used to generate genome-wide footprints of homologous proteins to assist gene prediction with BRAKER2. In our case, the reference protein set constituted 1,383,384 amino acid (aa) sequences originated from UniProtKB/TrEMBL (all rhizarian proteins, 20-01-2020) + UniProtKB/Swiss-Prot (all sequences, 20-01-2020) + Stramenopiles, Alveolata and Rhizaria orthologs retrieved from the “protist” clusters node of OrthoDB protein database (Kriventseva et al., 2019) if the corresponding clusters were represented in at least 2 Stramenopiles, Alveolata or Rhizaria species. Gene prediction was performed for each *P. betae* draft genome, with and without using protein hints. Both sets of predicted genes (with and without protein hints), were compared with rhizarian proteins present in UniProtKB/TrEMBL using OrthoVenn2 (Xu et al., 2019). This, along with

visual inspection of gene models and aligned RNA-seq reads using Integrated Genomic Viewer v9.1.2 (Robinson et al., 2011), allowed us to identify the gene set (generated with the protein hints) for further analysis. Protein products of genes predicted by BRAKER2 without protein hints, which were absent from the dataset predicted with protein hints, were then compared to NCBI nr database through BLASTP searches (Camacho et al., 2009). Sequences showing >30% identity with deposited proteins from other Stramenopiles, Alveolata or Rhizaria species were manually added to the gene set predicted with protein hints. Predicted proteins were scanned for matches against the InterPro v78.0 (Mitchell et al., 2019) protein signatures database using the InterProScan v5.42-78.0 tool (Jones et al., 2014). EggNOG-mapper v2 (Huerta-Cepas et al., 2017) with the EggNOG v5.0 clusters and phylogenies (Huerta-Cepas et al., 2019) was also used to assign predicted proteins to clusters of orthologous groups (COG) categories. The dbCAN2 meta server (Zhang et al., 2018) allowed the annotation of carbohydrate active enzymes (CAZymes) with DIAMOND (E-Value < 10^{-102}), HMMER (E-Value < 10^{-15} , coverage > 0.35) and Hotpep (Frequency > 2.6, Hits > 6) using CAZy, dbCAN and PPR database, respectively. The KEGG release 93.0 was searched for orthologies by HMMER/HMMSEARCH using KofamKOALA (E-Value < 10^{-2}) (Aramaki et al., 2020). Gene prediction and annotation from *P. brassicae* and *S. subterranea* genomes (Ciaghi et al., 2018; Stjelja et al., 2019) and transcriptomes (Schwelm et al., 2015b) were also performed for the purpose of proteome comparisons.

The *P. betae* draft genomes were analyzed for non-coding RNA (ncRNA) using cmsearch against the Rfam database v14.4 (Kalvari et al., 2018). tRNA matches were further categorized using tRNAscan-SE (Lowe and Eddy, 1997).

Proteins predicted from both draft genomes, i.e. from isolates A26-41 and RES F41, were compared with each other using BLASTP and clustered with UniProtKB/TrEMBL rhizarian proteins using OrthoVenn2. Since both draft genomes were resolved from contaminated DNA datasets, predicted proteins not found on the other draft genome or in rhizarian proteins were then compared to the NCBI nr database with BLASTP in order to evaluate if they had originated from foreign DNA. Contigs containing only predictions found on one draft genome, and matching bacterial proteins or bacterial RNA models were manually removed as bacterial contamination.

Overall amino acid identity (AAI) between both *P. betae* predicted proteomes was computed with the “aai.rb” AAI calculator (Rodriguez-R and Konstantinidis, 2014, 2016). Thresholds to consider alignments for AAI computation were a minimum alignment of 50 aa and a minimum identity of 0.2.

The mitochondrial genome was annotated using AGORA (Jung et al., 2018), GeSeq (Tillich et al., 2017), MITOS2 (Bernt et al., 2013; Donath et al., 2019)

and tRNAscan-SE softwares with the support of additional blast and Rfam searches. The synteny figure (Figure IV-2) was produced with the SimpleSynteny web-based tool (Veltri et al., 2016).

Secretome, membranome and putative effector selection

Secreted and membrane protein predictions were investigated following a computational pipeline developed for fungi, and adapted from Vivek-Ananth et al. (2018). First, all predicted proteins were searched for signal peptide (SP) with SignalP v5.0b (Armenteros et al., 2019), in “Eukarya” organism group and Phobius (Käll et al., 2007), for Glycosylphosphatidylinositol (GPI) anchor using big-PI with the “Protozoa” learning set (Eisenhaber et al., 1999) and for transmembrane domains (TM) using Phobius and TMHMM v2.0 (Krogh et al., 2001). Proteins with a SP and/or a GPI and/or a TM were selected through an inclusive rule and retained for the second step. Endoplasmic reticulum (ER) retrieval signal was then searched using PS SCAN (Gattiker et al., 2002) for the PROSITE (Sigrist et al., 2013) pattern PS00014. Proteins with an ER signal were discarded. Next, proteins were separated into two groups, one containing proteins with transmembrane domains and/or GPI anchors, and one containing proteins without clues of membrane localization. TMHMM tends to predict TM domain in the first 60 N-terminal amino acids (aa) when SP are present. However, the presence of a SP is a suggestion of extracellular localization rather than membrane localization. Therefore, TM predicted by TMHMM in the first 60 aa were not considered at this step. The last step was the subcellular localization of proteins classified in these two groups using three tools: TargetP v2.0 (Emanuelsson et al., 2000) with the “Non-plant” organism group, WoLF PSORT v0.0.1.1 (Horton et al., 2007) with the “Animal” organism type, and ProtComp v9.0 (Animal & Fungi) (Klee and Ellis, 2005). Proteins were considered as secreted or transmembrane if they are predicted as such by at least two tools. Candidate secreted proteins were eventually screened for effector using the EffectorP 2.0 machine learning classifier (Sperschneider et al., 2018).

d. Comparison of plasmodiophorid predicted proteomes

Predicted proteins from *P. betae* isolates A26-41 and RES F41, *P. brassicae* isolate e3, and *S. subterranea* isolate SSUBK13 were clustered and compared with OrthoVenn2 (Xu et al., 2019) with E-value and inflation value parameters set to $1e^{-2}$ and 1.5, respectively. This clustering analysis was performed with *S. subterranea* data kindly provided by the *S. subterranea* genome paper authors (Ciaghi et al., 2018) and the *P. brassicae* proteome UP000039324 retrieved from the UniProtKB/TrEMBL database.

COG categories were parsed from the EggNOG mapping results obtained for each isolate as well as for *B. natans* (Bigna1), *Phytophthora infestans* (ASM14294v1) and *Plasmodium falciparum* 3D7 (ASM276v2) retrieved from

EnsemblProtists. Comparisons of COG categories abundances in full proteomes, secretomes and membranomes were then performed and visualized with R software. Gene ontology (GO) terms were assigned to the predicted proteins according to the InterProScan analysis results and parsed to produce customized GO-annotation files for each isolate. For *B. natans*, *P. infestans* and *P. falciparum*, GO terms were retrieved from EnsemblProtists with Biomart.

Different custom protein subsets were then generated for each isolate: membrane proteins, secreted proteins, proteins containing ankyrin repeats according to Pfam searches (Anks), and proteins with a hit in PHI-base (Pathogen Host Interactions). Comparisons of GO terms abundances in secretomes and membranomes were then performed with WEGO v2.0 (Ye et al., 2006, 2018) and visualized with R software. Significant differences between protein frequencies were assessed using Chi-square tests. Singular Enrichment Analyses (SEA) were performed on those subsets using the agriGO v2.0 (Tian et al., 2017) analysis toolkit with customized GO-annotated reference sets for each isolate as background. A hypergeometric statistical test followed by the Hochberg adjustment method was used to compute adjusted P-values, only GO terms with an adjusted P-value < 0.05 were considered.

Predicted proteins were also scanned for Pfam domain signatures (Finn et al., 2014) with hmmscan v.3.2.1. Best non-overlapping Pfam matches were parsed from hmmscan results for each protein dataset with an E-value of 10^{-4} . Pfam enrichment analysis was calculated as described by Chandran et al. (2009). Briefly, a hypergeometric distribution was used to compute P-values which were then adjusted for multiple hypothesis testing as explained by Benjamini and Hochberg (1995) with an allowed false discovery rate of less than 10%. Enrichment analyses were performed for each species and species combination, with all domains identified from all compared species as background. Moreover, enrichments were also achieved from protein subsets corresponding to orthologs and co-orthologs identified from each species combination with the OrthoVenn2. Only matches with an adjusted P-value < 0.05 and mapped on at least three proteins were kept.

Sequence manipulation and phylogeny

Alignment of sequences was achieved with MAFFT (Kuraku et al., 2013; Katoh et al., 2019) and visualized either in Mview (Brown et al., 1998) or in MEGAX software (Kumar et al., 2018). A maximum-likelihood (ML) concatenation-based species tree was inferred from MAFFT alignments of 31 single copy genes in IQ-TREE (Nguyen et al., 2015) in combination with ModelFinder (Kalyaanamoorthy et al., 2017) and ultra-fast bootstrap with 1000 replicates (Hoang et al., 2018). The tree was rooted using *Plasmodium falciparum* and visualized using ITOL v.5 (Letunic and Bork, 2019). Dotplots from mitogenomes were obtained with LAST v1170.

4. RESULTS

a. Assembly and annotation of two *Polymyxa betae* draft genomes

The sequencing run for the A26-41 and RES F41 isolates yielded 12.3 and 7.1 Gbps accounting for 24,584,570 and 23,751,328 paired reads after adaptor and quality trimming, respectively. After metaSPAdes assembly, 235,649 and 1,115,426 contigs were generated, respectively. Following binning with metaWRAP, and evaluation of the resulting bins using the bespoke EukCC as described, one bin from each isolate was selected as most likely to be *P. betae*, based on a genome completeness of more than 92% and contamination of less than 3% (Supplementary table S1). Some medium to high quality bacterial genomes were also recovered and assigned to a taxonomic classification. These are shown in Supplementary table S2, with bins common between the two isolates highlighted. The contents of both predicted *P. betae* bins were reassembled to achieve the best quality assembly for each draft genome. The resulting draft genomes for A26-41 and RES F41 encompassed 1039 and 1476 contigs, respectively. The A26-41 average contig read coverage was 266-fold whereas the average RES-F41 contig coverage was 37-fold. The absence of significant *Beta vulgaris* (sugar beet host) DNA contamination was confirmed by mapping raw reads from the fully sequenced monogerm DH line KWS2320 (SRA accession no. SRR869626 and SRR869627) to both *P. betae* draft genomes. The GC-content (45.0%) is comparable to *S. subterranea* (45.7%) but lower than *P. brassicae* (58.5-59.4%). Total assembly length (25.4 Mb for RES F41 and 26.5 Mb for A26-41) is comparable to the assemblies of other available full genomes in plasmodiophorids (general genome statistics are detailed in Supplementary Table S3). Similarity between both *P. betae* draft genomes was evaluated through ProgressiveMauve full length genome alignment (Supplementary Figure S2). From Mauve alignment, 49 A26-41 contigs did not find correspondence in RES F41 whereas 234 RES F41 contigs were absent from A26-41. A total of 26717 SNP was identified between the isolates as well as 3566 and 3695 gaps in A26-41 and RES F41 respectively. To get a better idea of full-length similarity between isolates, ANI value was computed. The “ani.rb” tool generated 127606 and 120483 fragments and discarded 936345 bp (3.5 %) and 1320853 bp (5.2 %) from the A26-41 and the RES F41 isolates, respectively. From the aligned portions, the average nucleotide identity was 99.87% (Supplementary Figure S3). These statistics demonstrate that the two draft genomes, despite being from different isolates, had a high degree of similarity. One contig, containing *P. betae* ribosomal DNA genes (18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, and 28S ribosomal RNA gene) was not properly binned along with the other A26-41 contigs, possibly due to extremely high average read coverage (17237X). It was manually added to the A26-41 draft genome.

We analyzed both draft genomes (including the extra contig identified for A26-41) with cmsearch as described to determine the non-coding RNA (ncRNA) content (Supplementary Table S12). For both draft genomes we were able to recover all components of the spliceosome (U1-U6) and the ribosome (both the large ribosomal subunit (LSU) and the small ribosomal subunit (SSU)), as well as signal recognition particles, ribonucleoproteins, a self-splicing ribozyme, and small nucleolar RNAs common to both genomes. We used tRNAscan-SE to further categorize the tRNA genes present (Supplementary Table S12). The results show that both draft genomes include tRNA genes for all 20 amino acids, as well as an unusual stop codon (TCA) found in the mitochondria of some green algae. This coverage of both rRNA and tRNA genes is consistent with the MIMAG criteria for a "high-quality" genome.

MITObim reassembly resulted in the first sequence of *P. betae* mitochondrial genome from isolate A26-41. Reassembly was also performed with the NOVOPlasty software with highly similar results, confirming the accuracy of reassembly. The total length of the MITObim assembly is 33,862 bp, shorter than the mitogenomes of *S. subterranea* (37,699 bp; Gutiérrez *et al.*, 2014) and, from *P. brassicae* (102,962 - 114,663; Daval *et al.*, 2019 - Stjelja *et al.*, 2019). Its GC content is 29.0 %, slightly higher than the percentage reported for *S. subterranea* (26.8%) and *P. brassicae* (26.2%). The mitogenome annotation using AGORA, GeSeq, MITOS2 and tRNAscan-SE softwares as well as additional blast and Rfam searches identified 30 protein-coding genes and 27 structural RNAs on the *P. betae* mitochondrion. Sixteen proteins involved in the mitochondrial respiratory chain were found among protein-coding genes, namely ATP1,6,9, COX1-3, COB, NAD1-7, NAD4l, NAD9. Twelve ribosomal proteins were also identified: RPL6,14,16, RPS3,4,7,8,11,12,13,14,19. Two ORF encoding hypothetical proteins were also detected. The structural RNAs that were found are rRNS, rRNL, rRN5 and 24 tRNA (Supplementary Table S12). A comparison with *P. brassicae* and *S. subterranea* mitochondrial genomes revealed that the three plasmodiophorid mitogenomes show a near perfect synteny (Figure IV-2). However, *P. betae* and *S. subterranea* mitochondrial genomes are closer in length and in gene content. This difference is explained by the high intron content, containing additional ORFs, and larger intergenic spaces found within the *P. brassicae* mitochondrial genome.

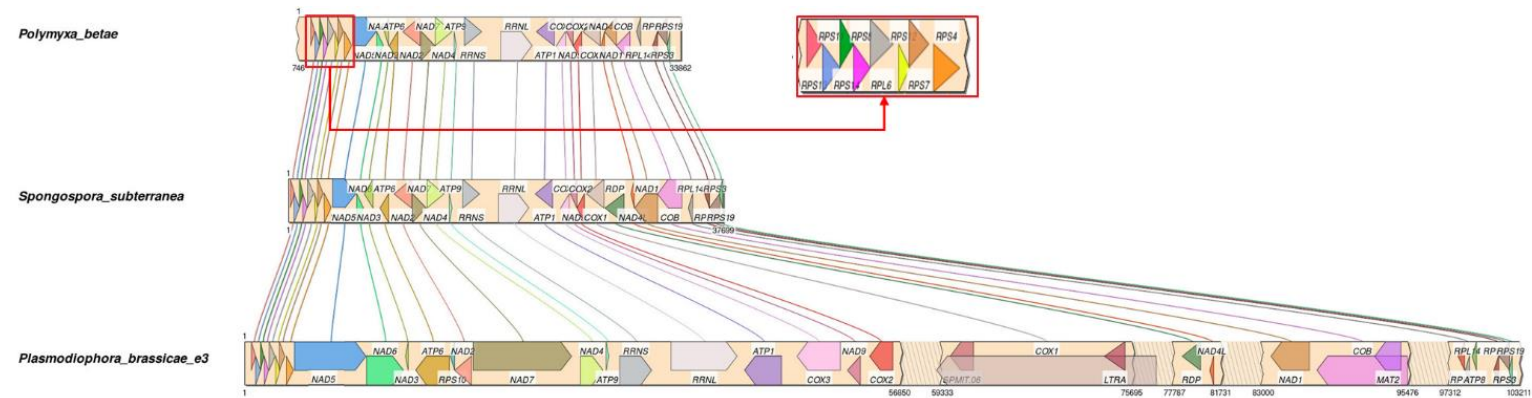


Figure IV-2. Syntenic comparison between the mitochondrial genomes of *P. betae*, *P. brassicae* and *S. subterranea*. Homologous genes share the same colors and are connected by curved lines. Gene names are indicated. To make the comparison easier, the *P. brassicae* genome (LS992577) was opened at position 43000, then both fragments were flipped and merged

Next, we investigated the proteome encoded by each *P. betae* nuclear genome, aided by gene annotations predicted using sequence data (31,798,532 paired reads) from poly(A)-RNA purified from sugar beet roots infected with high levels of *P. betae*. After filtering steps, ~16% of the RNAs-seq reads mapped against both *P. betae* draft genomes (this low rate was expected due to the presence of sugar beet RNA). The RNA-seq alignments on both draft genomes were used along with protein hints to assist gene prediction using the BRAKER2 pipeline. In total 10224 protein-coding genes accounting for 10799 protein-coding mRNAs were predicted for the A26-41 isolate while 10222 protein-coding genes accounting for 10808 protein-coding mRNAs were predicted for the RES-F41 isolate. These two mRNA datasets were then compared to each other using BLASTP and showed that 3.04% (328 out of 10799) of predicted proteins in isolate A26-41 found no match in the predicted protein set of isolate RES F41. Similarly, 3.22% (348 out of 10808) of isolate RES F41 predicted proteins found no match in the predicted protein set of isolate A26-41. Overall amino acid identity between the aligned portion of both *P. betae* predicted proteomes computed with the “aai.rb” AAI calculator was again very high, reaching 99.18%.

The InterProScan analysis of the A26-41 predicted proteins found at least one annotation in 9379 predicted proteins (86.8 %) from which 4749 (44.0%) could be associated with at least one GO term. For the RES F41 predicted proteins, annotations were found in 9363 predicted proteins (86.6%) from which 4736 (43.8%) could be associated with at least one GO term. EggNOG mapping found seed orthologs for a total of 5633 predictions (52.2%) from A26-41 among which 4247 (39.3%) were assigned to a known Cluster of Orthologous Group (COG) category (other than “S”). Similarly, 5591 predictions (51.7%) from RES F41 were mapped to a seed ortholog from which 4220 (39.0%) were assigned to a known COG category. Functional profiles generated from EggNOG COG categories are presented in Figure IV-3. Interestingly, these functional profiles are remarkably similar across the compared plasmodiophorids, but distinct from those of *B. natans*, *Plasmodium falciparum* and *P. infestans*, the three other species from Stramenopiles, Alveolata and Rhizaria included in the comparison. The major difference being the distribution of poorly characterized proteins. A large proportion of predicted rhizaria proteins (including *B. natans*) were unmapped, indicating that they are unrepresented in EggNOG, whereas more poorly characterized proteins from *P. falciparum* and *P. infestans* were matched but unassigned to a category or assigned to the “unknown function” category, probably due to the inclusion of these two species in EggNOG v5.0. Functional profiles have also been generated from selected GO terms derived from the InterProScan analysis (Supplementary Figure S4). A comparison of genome metrics for A26-41, RES 41 and other plasmodiophorids is presented at Table IV-1.

CHAPTER IV. The *Polymyxa betae* genome

Table IV-1 Comparison of *P. betae* (Pbet) draft genomes A26-41 and RES F41 with other fully sequenced plasmodiophorids: *P. brassicae* (Pbra) e3, eH, ZJ-1 and *S. subterranea* (Ssub) SSUBK13.

Features	Pbet A26- 41 (this study)	Pbet RES F41 (this study)	Ssub SSUBK13 (Ciaghi et al., 2018)	Pbra Pb3 (Rolfe et al., 2016)	Pbra e3 (Sch welm et al., 2015 b)	Pbra e3 (Stjelja et al., 2019)	Pbra eH (Daval et al., 2019)	Pbra ZJ-1 (Bi et al., 2019)
N° of contigs/ scaffolds	1040	1476	2340	107	165	20	136	667
N50 (Kb)	49	57	28	722.7	473	1300	741	510
Assembly length (Mb)	26.5	25.4	28.1	24.2	24.0	25.1	24.6	24.1
GC content (%)	45.0	45.0	45.7	59.0	58.5	59.0	59.3	59.4
Protein- coding Genes	10224	10222	9742 (this study)	10851	9730	9231	12590	10951
CDSs percentage	52.4	54.3	47.9 (this study)	ND	56.8	ND	ND	60.2
Repeats percentage	7.9	5.7	10.7 (this study)	<2%	5.4	11.5	ND	5.9

CAZyme annotation (see methods) allowed the identification of 177 and 175 carbohydrate-utilizing protein candidates from the A26-41 and RES F41 isolates respectively. To be stringent, we restricted the results to CAZymes predicted with at least two methods on dbCAN2 meta server which represent 64 and 62 proteins, respectively. Identification of isolate A26-41 enzymes involved in known biosynthetic pathways with KofamKOALA allowed us to assign 3001 predicted proteins to a KEGG Ortholog (KO). From these, 1204 were associated with enzyme codes (EC). Similarly, 3010 RES F41 proteins were assigned to a KO from which 1204 were also linked to an EC.

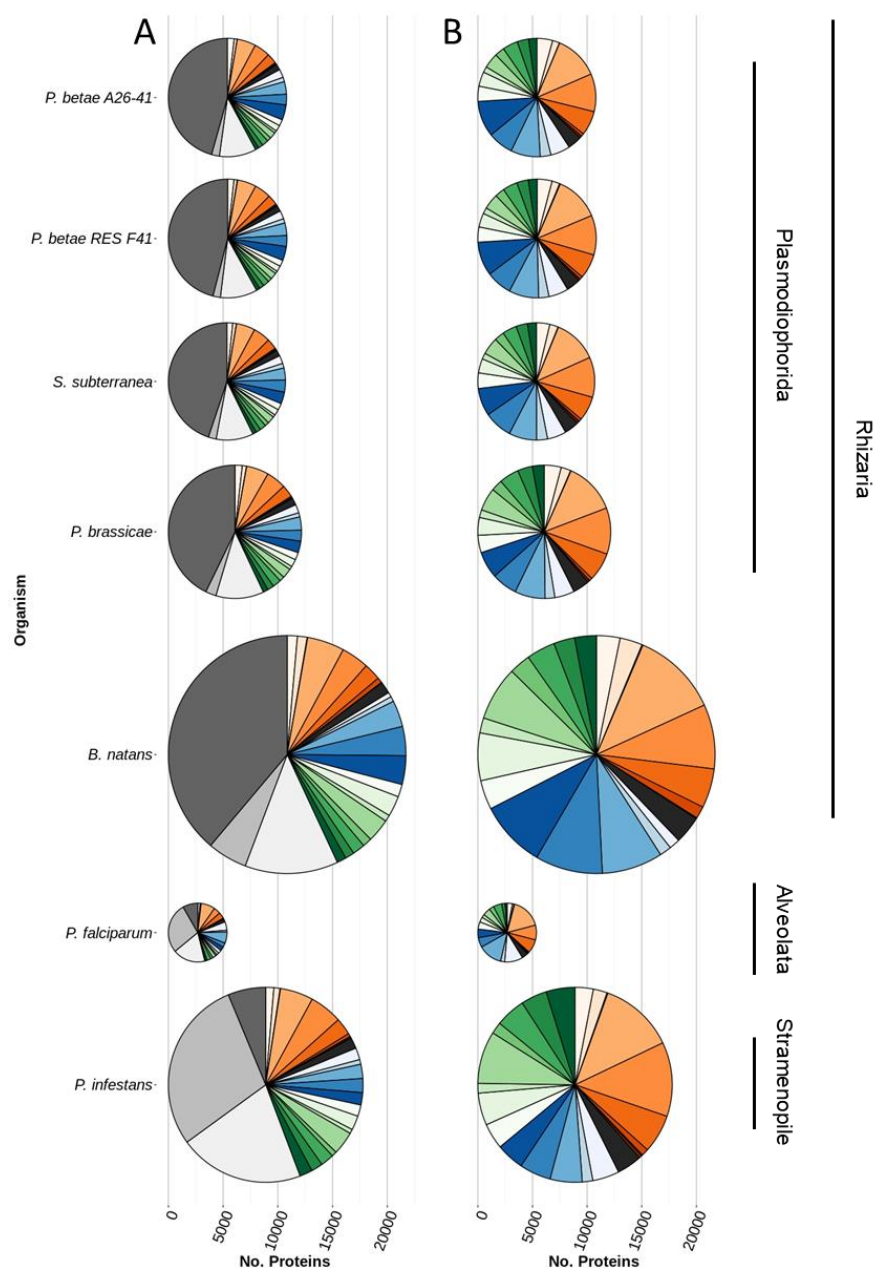


Figure IV-3. Comparison of COG categories predicted for *P. betae* (Pbet) draft genomes A26-41 and RES F41, *P. brassicae* (Pbra) e3 and *S. subterranea* (Ssub) SSUBK13 with COG from *B. natans* (Bnat), *Plasmodium falciparum* (Pfal) et *P. infestans* (Pinf). A: COG category profiles with poorly characterized proteins. B: COG category profiles without poorly characterized proteins. The diameter of pie charts is proportional to the number of proteins in full proteomes. Legend is reported below.

CHAPTER IV. The *Polymyxa betae* genome

CELLULAR PROCESSES AND SIGNALING

-  [D] Cell cycle control, cell division, chromosome partitioning
-  [M] Cell wall/membrane/envelope biogenesis
-  [N] Cell motility
-  [O] Post-translational modification, protein turnover, and chaperones
-  [T] Signal transduction mechanisms
-  [U] Intracellular trafficking, secretion, and vesicular transport
-  [V] Defense mechanisms
-  [W] Extracellular structures
-  [Y] Nuclear structure
-  [Z] Cytoskeleton

INFORMATION STORAGE AND PROCESSING

-  [A] RNA processing and modification
-  [B] Chromatin structure and dynamics
-  [J] Translation, ribosomal structure and biogenesis
-  [K] Transcription
-  [L] Replication, recombination and repair

METABOLISM

-  [C] Energy production and conversion
-  [E] Amino acid transport and metabolism
-  [F] Nucleotide transport and metabolism
-  [G] Carbohydrate transport and metabolism
-  [H] Coenzyme transport and metabolism
-  [I] Lipid transport and metabolism
-  [P] Inorganic ion transport and metabolism
-  [Q] Secondary metabolites biosynthesis, transport, and catabolism

POORLY CHARACTERIZED

-  [S] Function unknown
-  [UA] Unassigned = No function assigned
-  [UM] Unmapped = No ortholog found

b. Protein clustering with *Spongospora subterranea* and *Plasmodiophora brassicae*

A protein clustering analysis was performed on proteins predicted from both *P. betae* nuclear genomes as well as from *P. brassicae* (GenBank accession no. GCA_900303365.2) and *S. subterranea* (GenBank accession no. GCA_900404475.1). For a true comparison, the protein sets for *P. brassicae* and *S. subterranea* were generated with the same bioinformatic pipeline (BRAKER2 v2.1.4) as used for *P. betae* (further analysis using “published” gene models for *P. brassicae* and *S. subterranea* are presented in Supplementary notes, but show near identical results). Figure IV-4 shows there was a high similarity between both *P. betae* isolates, with 9270 shared clusters containing 9718 and 9673 proteins from A26-41 and RES F41 respectively. Moreover, a closer relationship between both *P. betae* and *S. subterranea* (5400 shared clusters) than between both *P. betae* and *P. brassicae* (5300 shared clusters) is indicated. Results from separate analyses for each *P. betae* isolate are available in Supplementary Figure S5. Figure IV-4 also shows a closer relationship between *P. brassicae* and *S. subterranea* than between *P. betae* and *P. brassicae*, further illustrated by the maximum likelihood phylogenetic tree (Figure IV-4C). It should be noted that 767, 870, 3178 and 2564 proteins have been classified as singletons (only clusters are shown in Figure IV-4) in *P. betae* A26-41, RES F41, *P. brassicae* and *S. subterranea*, respectively. Taking singletons and species-specific clusters into account, 42.5 %, 45 %, and 37.6 % of predicted proteins are specific to *P. betae*, *P. brassicae* and *S. subterranea*, respectively. Lastly, 4585 protein clusters are shared by the four organisms, and therefore taken to represent the plasmodiophorids core proteome.

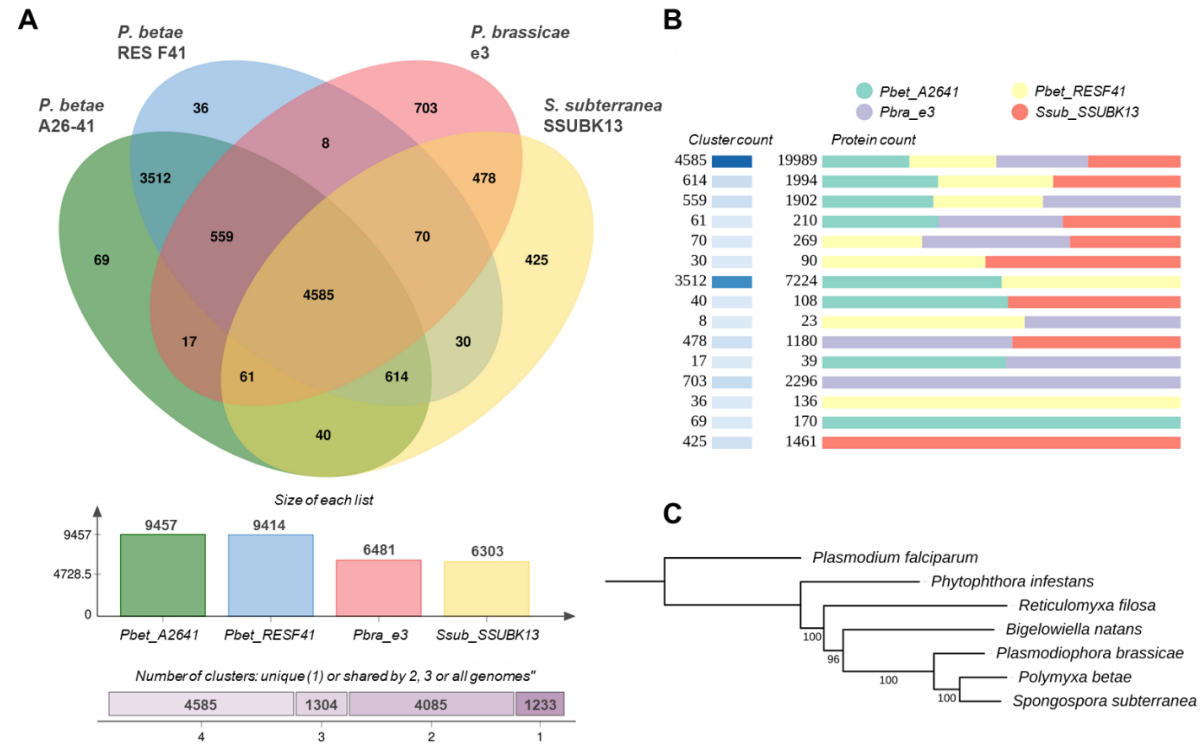


Figure IV-4. Protein clustering from *P. betae* (Pbet) draft genomes A26-41 and RES F41, *P. brassicae* (Pbra) e3 and *S. subterranea* (Ssub) SSUBK13 with OrthoVenn2. A: Venn diagram of ortholog clusters. B: cluster and protein counts. C: Maximum likelihood phylogenetic tree of the fully sequenced rhizarias computed with the A26-41 *P. betae* isolate with 31 single copy gene encoded aa sequences. Bootstrap support (in %) is shown on branches.

c. A pipeline for secretome and membrane protein predictions

Proteins predicted from both *P. betae* isolate draft genomes as well as from *P. brassicae* and *S. subterranea* genomes were screened for secreted and membrane proteins using a four-step pipeline adapted from Vivek-Ananth et al. (2018). As presented in Table IV-2, numbers of remaining proteins at each step are very similar in both *P. betae* draft genomes and closer to *S. subterranea* than to *P. brassicae*. The effectorP 2.0 machine learning classifier was used to predict effector candidates among the secretomes determined from the four genomes. Respectively 104 (15.9%), 104 (15.5%), 94 (17.9%) and 112 (13.4%) of these proteins from *P. betae* A26-41, *P. betae* RES F41, *S. subterranea* and *P. brassicae* are predicted to be effectors. No RXLR effector-like proteins were detected.

Table IV-2. Secretome and membranome prediction pipeline results for *P. betae* (Pbet) draft genomes A26-41 and RES F41, *P. brassicae* (Pbra) e3 and *S. subterranea* (Ssub) SSUBK13. Step 1: proteins containing a signal peptide and/or a GPI anchor and/or a transmembrane domain. Step 2: step 1 proteins containing an ER retrieval signal are discarded. Step 3: Membr. = step 2 proteins containing a GPI anchor and/or a transmembrane domain, Secreted = step 2 proteins lacking GPI anchor and transmembrane domain. Step 4: step3 proteins filtered according to their predicted localization. For details, see methods.

	Pbet A26-41		Pbet RES F41		Pbra e3		Ssub SSUBK13	
Proteome	10799		10808		12163		10700	
Step 1	3423		3420		4332		3098	
Step 2	3413		3409		4319		3092	
	Secreted	Membr.	Secreted	Membr.	Secreted	Membr.	Secreted	Membr.
Step 3	991	2422	1010	2399	1283	3036	796	2296
Step 4	655	1525	670	1514	834	1942	526	1489

Level 2 GO annotation of secretomes and membranomes predicted from the four data sets is presented in Figure IV-5. Globally, the same tendencies are visible in each plasmodiophorid. Prevailing molecular functions predicted in the plasmodiophorid secreted proteins are linked to binding and catalytic activities and intervention in metabolic and cellular processes. Other GO terms are poorly represented in all predicted secretomes with fewer than ten proteins mapped even for GO related to cellular components, and of those that fall in this category, some are annotated as part of the cell, organelles or protein complexes. Prevailing molecular functions predicted in the membranomes are linked to binding, catalytic and transporter activities and overrepresented biological processes include cellular and metabolic processes as well as localization. Notably, membrane proteins involved in

CHAPTER IV. The *Polymyxa betae* genome

response to stimulus, signaling and regulation of biological process have been revealed.

A clustering analysis was performed with OrthoVenn2 and revealed that most secreted proteins are not shared in plasmodiophorids (Supplementary Figure S6). Functional profiles generated from EggNOG COG categories showed that the majority of plasmodiophorid secreted proteins remain poorly characterized (Supplementary Figure S7).

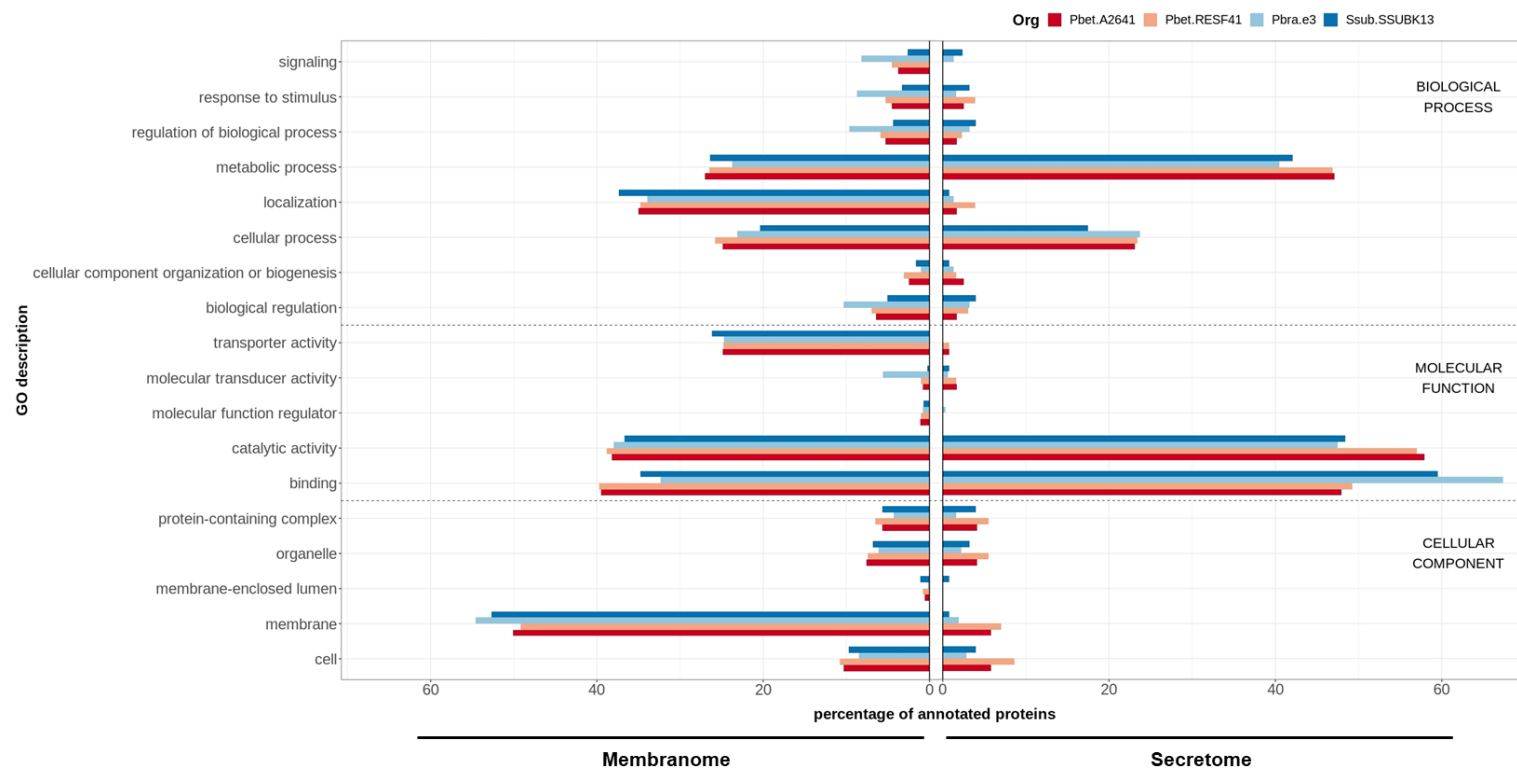


Figure IV-5. Assignments of proteins predicted as secreted (right) and as membrane proteins (left) to selected general GO terms (level 2). Results for *P. betae* (Pbet) drafts genomes A26-41 and RES F41, *P. brassicae* (Pbra) e3 and *S. subterranea* (Ssub) SSUBK13 are presented as percentages of annotated proteins in membranome or secretome.

d. Comparison of *Polymyxa betae* with gall-forming plasmodiophorids

GO terms and Pfam domains overrepresented in protein clusters absent from *P. betae* but shared in *P. brassicae* and *S. subterranea* were examined to detect potential molecular functions, biological processes or domains associated with the gall-forming phenotype (Supplementary Tables S5 and S10). Among GO biological processes enriched in overlapping clusters shared only by *P. brassicae* and *S. subterranea*, we noted “cell differentiation” and “histidyl-tRNA aminoacylation”, “histone acetylation”, “methylation”, as well as “dicarboxylic acid metabolic process”. Moreover, the GO molecular functions “metalloprotease activity”, “kinase regulator activity” and “FMN binding” were also found remarkably enriched. Lastly, GO terms linked to endosome cellular trafficking are also overrepresented in *P. brassicae* and *S. subterranea* overlapping clusters.

Each predicted proteome and secretome was also searched for enriched Pfam domains (Supplementary Tables S6 and S7). Substantially more proteins bearing ankyrin repeats (Anks) were discovered in the gall-makers *S. subterranea* (245) and *P. brassicae* (243) than in *P. betae* (54 in A26-41 and 56 in RES F41), even though based on our clustering and phylogenetic analysis (Figure IV-4), *S. subterranea* seems globally closer to *P. betae*. To better characterize proteins containing ankyrin repeats for potential involvement in interactions with the plant host, GO terms were investigated from secreted Anks. Only the ubiquitous “protein binding activity” term was associated with these proteins in both *P. betae* and *S. subterranea*. Enrichment tests were also carried out for subsets representing Anks from each plasmodiophorid proteome (not only secretomes). The sole enriched GO term found was again “protein binding activity” despite the presence of a few other domains. Anks were then searched for pathogenicity relevant characteristics including for the presence of a high confidence BLASTP hits in PHI database, for the classification or not as a CAZyme and for their predicted secreted and effector nature. Despite the lower number of Anks in *P. betae*, proportionally less *P. betae* Anks (<10%) are considered as secreted than *S. subterranea* Anks (11.8%) and *P. brassicae* Anks (30.5%). Only one *P. betae* Ank from the A26-41 isolate is an effector candidate compared with two in *P. brassicae* and six in *S. subterranea*. However, proportionally more correspondence in the PHI database was found among *P. betae* Anks (16.1% -18.5 %) than in *S. subterranea* (13.9%) and *P. brassicae* Anks (11.1%). Altogether, this suggests that the spectrum of Anks is different between the compared plasmodiophorids and from references available in databases.

The respective secretomes and membranomes of all the plasmodiophorids were also searched for enriched GO terms (Supplementary Tables S8 and S9). A weaker representation of carbohydrate metabolism related proteins, in particular “carbohydrate binding” was found in both *P. betae* computational secretomes than in both gall-makers secretomes. In the

computational membranome, proportionally more proteins involved in and transferase activities and carbohydrate binding are found in *P. betae* than in *P. brassicae* and *S. subterranea* membranomes.

e. Plant defense related genes

We also searched the plasmodiophorid genomes for genes encoding plant defense related proteins. Three *P. betae* secreted proteins containing a Barwin domain were recovered in both *P. betae* isolates and have no orthologs in *P. brassicae* and *S. subterranea* secretomes. The proteins share the same general structure: a signal peptide followed by a Barwin domain (barley wound-induced, RlpA-like domain superfamily) or PR-4 in the PR protein nomenclature. The C-terminal end is characterized by the presence of a long intrinsic disorder region. As shown in the Supplementary Figure S8, the N-terminal region of those three proteins, after signal peptide cleavage, is conserved over a ~120 bp region corresponding to the PR-4-like domain. Conversely, they are greatly dissimilar through the C-terminal region. Another PR-like protein from the Thaumatin family (PR5-like) was also recovered in both *P. betae* and *P. brassicae* predicted secretomes. Proteins belonging to the CAP superfamily with BLASTP hits to plant PR-1 proteins were also found in the four secretomes.

Lastly, we identified other proteins which could interfere with plant defense. One SOBER1-like immunity suppressor protein was found in *P. betae*, as well as in both of the other compared plasmodiophorids. Three isochorismatases were also found in both *P. betae* proteomes against four in *P. brassicae* and one in *S. subterranea*. These also lack signal peptide and are thus not predicted as secreted through the “classical” secretion pathway. Nevertheless, these three proteins are homologs of experimentally characterized fungal isochorismatases which are known to be secreted through unconventional pathways.

f. Sperm-specific voltage-gated calcium channel (CatSper)

The clustering and comparative analysis revealed the presence of six orthologs of the mammalian cation channel sperm-associated proteins (CatSper complex) in *P. betae* and *S. subterranea* proteomes and not in *P. brassicae* proteome (Supplementary Tables S5). These include proteins annotated as CatSper1,2,4, CatSper β , CatSper δ/ϵ and CatSper γ by InterProScan. Since there is no InterPro entry for CatSper3, we identified CatSper3 homologs in *P. betae* and *S. subterranea* proteomes through a BLASTP search. The CatSper ζ subunit, specific to mammals, raised no BLASTP hits in any plasmodiophorid. The recently identified CatSper associated protein EF-hand calcium-binding domain-containing protein 9 (EFCAB9) also found homologous sequences in both *P. betae* and *S. subterranea* but not in *P. brassicae*. Furthermore, we searched the InterPro database for CatSper protein families and used identified sequences as

CHAPTER IV. The *Polymyxa betae* genome

BLASTP queries against NCBI nr. and as tBLASTn queries against the *P. brassicae* genome sequence (Stjelja et al., 2019). This confirmed the absence of the CatSper complex from *P. brassicae* but also from sequences of any other species included in the Stramenopiles, Alveolata and Rhizaria group except the stramenopile *Hondaea fermentalgiana*, which also contains the CatSper α 1,2,3,4 and CatSper β , δ/ϵ , γ InterPro families. CatSper protein sequences are 100% conserved between both *P. betae* isolates except CatSper γ where one conserved aa substitution is present and EFCAB9 in which a 2 aa deletion is present in the gene model for isolate *P. betae* A26-41. *P. betae* and *S. subterranea* CatSper1, 2, 3 and 4 share 51.73%, 63.11%, 57.35% and 50.65% identity, respectively. Identities for CatSper β , δ , γ are a bit lower at 37.08%, 39.84% and 38.29% (38.43%), respectively. The *S. subterranea* EFCAB9 homolog was also found in both *P. betae* isolates (76.19% identity). In *P. betae* the EFCAB9 protein is fused in the N-terminal portion with a sequence stretching across a 394-396 aa region containing a mitogen-activated protein kinase (MAPK) domain. The CatSper genes are located on different contigs in both the *P. betae* and the *S. subterranea* draft genomes. More generally, the “calcium activated cation channel activity” molecular function was found overrepresented in *P. betae* and *S. subterranea* membranomes but not in *P. brassicae*.

g. GPCR signal transduction pathway

Comparative analyses revealed a much lower level of annotations associated with signal transduction, especially with the “G protein-coupled receptor signaling pathway” (GPCR), in *P. betae* and *S. subterranea* proteomes (with 17 and 14 proteins, respectively) than in *P. brassicae* proteome (82 proteins) (Supplementary Table S4). Significantly enriched Pfam domains were searched from each plasmodiophorid proteome against the entire plasmodiophorids (Supplementary Tables S7 and S8). Several enriched Pfam domains potentially linked to signaling process were identified: “GPCR proteolysis site”, “7 transmembrane sweet-taste receptor of 3 GPCR”, “Leucine rich repeat”, “NHL repeat”, “receptor family ligand binding region”, “Regulator of G protein signaling domain”. Membranome GO enrichments also confirmed the overrepresentation of signal transduction related terms. In particular, *P. brassicae* computational membranome contains 50 predicted proteins belonging to the “G protein-coupled receptor signaling pathway” against six in *P. betae* and none in the *S. subterranea* membranome. This is partly due to the high number of proteins (26) annotated as “G-protein coupled GABA receptor activity” against none in *S. subterranea* and *P. betae* membranomes.

5. DISCUSSION

a. An efficient method to sequence and compare obligate endoparasites eukaryotic genomes

The results presented in this study were generated with DNA extracted from two distinct *P. betae* life stages, sporosori and zoospores, and from two geographically distinct isolates (Belgium and UK). Despite these differences, and the fact that both draft genomes were assembled from samples including distinct levels of contaminating plant and microbial DNA sequences, the alignment of the “cleaned” draft genomes showed a high degree of coverage and identity. Moreover, predicted protein comparisons from both also showed outstanding similarity. These results endorse the use of the bioinformatic pipeline (Figure IV-1) to extract accurate eukaryotic assembled genomes from datasets highly contaminated with prokaryotic sequences. Interestingly, this strategy showed effectiveness with both datasets despite distinct *P. betae* mean coverages and the fact that the RES F41 isolate dataset was substantially more contaminated than the A26-41 isolate dataset (1,115,426 vs. 235,649 putative prokaryotic contigs, respectively). While it would have been advantageous to generate long reads, this was unfortunately not an option in this study. Despite repeated attempts to produce enough good quality DNA from sporosori for MinION nanopore sequencing, our tests remained unsuccessful. Using such long reads to perform hybrid assemblies is possible with metaSPAdes and would have been useful for reducing the numbers of contigs and gaps in the final assemblies. However, the fact that two different samples provided comparable assemblies with short reads assures the reliability of our results.

Pfam domain signatures were searched against the *P. betae* A26-41 and RES F41 predicted proteins as well as *P. brassicae* and *S. subterranea* *de novo* and published sequence sets (Supplementary Table S11). It is noteworthy that *de novo* gene prediction from published *S. subterranea* and *P. brassicae* genomes noticeably increased the number of Pfam domains returned by hmmscan. This could indicate either more sensitivity, or more redundancy through gene prediction with the BRAKER2 pipeline. Similarly, *P. betae* protein clustering was carried out with *de novo* and published sequence sets. Again, the number of *P. betae* proteins clustered with proteins from *P. brassicae* and *S. subterranea* was higher if all compared gene models had been predicted with the BRAKER2 pipeline. These results indicate a potential bias in comparative analyses depending on the gene prediction method used. However, our analyses show that use of *de novo* gene predictions in place of published gene data is suitable for such comparative analyses. Indeed, it may be a better approach in that it helps to normalize the gene predictions across the different data sets, and gave us the confidence that we were comparing like with like.

CHAPTER IV. The *Polymyxa betae* genome

Successful plant pathogens have the ability to manipulate the defence response of their host through the secretion of effector proteins (Dodds and Rathjen, 2010). Plasmodiophorids have the particular character to develop plasmodia inside plant root cells, in close contact with their host cytoplasm. Thus, we might speculate that membrane and secreted proteins will constitute, through a long co-evolution, a significant proportion of the proteome coordinating the interaction between *P. betae* and its host. Previously, various methods have been used to predict secreted proteins in plasmodiophorid species including, using only SignalP to detect signal peptide (Ciaghi et al., 2018), following a two-step method relying on SignalP and TMHMM to eliminate proteins with a TM domain (Schwelm et al., 2015b), or using a four-step pipeline involving SignalP, TMHMM in combination with PredGPI to keep only proteins lacking GPI, and defined as secreted by TargetP (Rolfe et al., 2016). In this study, a four-step pipeline (adapted from (Vivek-Ananth et al., 2018)) was used to concomitantly predict secreted and membrane proteins. The results of the secretome and membranome GO annotation classification support the predictions generated from this pipeline, as applied to plasmodiophorid species. In fact, the number of *P. brassicae* secreted proteins that were predicted with this pipeline (834) is greater than in previous studies (553-741) (Schwelm et al., 2015b; Rolfe et al., 2016; Daval et al., 2019) while the methodology is at least as stringent. More recently, two studies focused on *P. brassicae* effectors prediction, using RNA-seq data and reducing the scope to small secreted proteins highly expressed in primary infection of *Brassica napus* (Chen et al., 2019) and secondary infection of *A. thaliana* (Pérez-López et al., 2020), resulting in the prediction of 33 (<300aa) and 32 (<400aa) effectors, respectively. Comparison of the expression of predicted *P. betae* secreted proteins in zoospores, sporosori and at different stages of the infection cycle would be useful to generate a shortlist for experimental functional analysis. We should also mention that we can't rule out the possibility that some of the predicted secreted proteins are delivered in vacuoles rather in the host cytoplasm.

More shared protein clusters were found between *P. betae* isolates than had been previously reported for *P. brassicae* (Daval et al., 2019). While the protein clustering results also suggest a similarity in proteomes across plasmodiophorids, there were a significant number of differences which should be further investigated to better understand their biology. Globally, the clustering results and phylogenomics support the idea that *P. betae* is more phylogenetically related to *S. subterranea* than to *P. brassicae* and that *S. subterranea* should be classified between *P. betae* and *P. brassicae*. This contrasts with some previous studies based only on ribosomal DNA sequence information which grouped *P. brassicae* and *S. subterranea* in a poorly supported clade distinct from *P. betae* and *P. graminis* (Ward and Adams, 1998; Bulman et al., 2001; Down et al., 2002) but agrees with a recent study revising the taxonomy of Phytomyxea (Hittorf et al., 2020). The analysis of the mitochondrial genomes further supports this hypothesis, even if the three mitogenomes showed a nearly perfect synteny. In comparison with the *P. brassicae* mitochondrial genome, *P. betae* and *S. subterranea*

mitogenomes are both approximately three times smaller with a reduced number of introns and intergenic sequences.

b. Host-plasmodiophorids interactions

In this comparative study, we noticed a lower enrichment in proteins bearing ankyrin-repeat domains (Anks) in the predicted proteomes and secretomes of the asymptomatic *P. betae* than in both gall-makers, *P. brassicae* and *S. subterranea*. We also found that functional prediction of Anks through homology researches raised poor results, especially for secreted Anks. Ankyrin repeats consist of 30-34 aa repeats widely spread in eukaryote proteins (Bork, 1993) and involved in various interactions, primarily with proteins (Li et al., 2006) but also with lipids and sugars (Islam et al., 2018). The versatile interaction potential of Anks suggests that they are involved in a diverse range of functions enumerated in Voronin and Kiseleva (2008) and Islam et al. (2018). Interestingly, some Anks are considered as virulence genes in bacteria, being used to hijack host cellular mechanisms (Al-Khodori et al., 2010). In particular, Anks genes could be key in establishing the intracellular parasitic lifestyle of some bacteria such as *Wolbachia* (Habyarimana et al., 2008; Gomez-Valero et al., 2011; Singhal and Mohanty, 2019) as well as the symbiotic interaction between *Shewanella* sp. and sponges (Anoop and Agostinho, 2019). More generally, Anks abundance seems to be correlated with the microbe symbiotic lifestyle, being particularly enriched in the reduced genomes of some obligate intracellular bacteria in comparison to free living bacteria (Jernigan and Bordenstein, 2014, 2015). Plasmodiophorids also have compact genomes (Rolfe et al., 2016). Moreover, Schwelm et al. (2015b) observed that *P. brassicae* effectors highly expressed in plasmodia contained ankyrin repeats. The expression of Anks putative effectors was further confirmed during primary and secondary infection with *P. brassicae* (Chen et al., 2019; Pérez-López et al., 2020) and Voronin and Kiseleva (2008) suggested that Anks genes acquisition could be a pathway used for Protozoa evolution and adaptation. Collectively, current evidence indicates that it is possible that Anks could have played an important role during the co-evolution of plasmodiophorids with their hosts.

Genes harboring similarities with plant defense genes were also uncovered. Three PR-4-like proteins containing a signal peptide were detected in *P. betae* computational secretomes but not in other plasmodiophorids. Schwelm et al. (2015b) already identified a PR-1-like secretory protein in *P. brassicae* with no homologs in the non-plant pathogenic Rhizarias as well as PR-5-like proteins in *P. brassicae* secretome. Likewise, PR-1-like and PR-5-like proteins were also uncovered in the *P. betae* secretome. Interestingly, a *P. betae* PR-1-like (Pbef2) was previously identified by suppressive subtractive hybridization in the *P. betae*-*B. vulgaris* pathosystem (Desoignies et al., 2014). In plants, PR genes are generally induced in response to an infection or an attack and are part of the plant defense response (van Loon et al., 2006; Sels et al., 2008). However, PR protein recognition was also shown to regulate plant defense response. For instance, PR4-LRR1

CHAPTER IV. The *Polymyxa betae* genome

interaction in pepper resulted in suppression of cell death and defense responses (Hwang et al., 2014). Therefore, the possible implication of secreted PR-like effectors in plasmodiophorids pathogenicity is worth further investigation. Three isochorismatases-like proteins were also predicted in *P. betae* as well as in other plasmodiophorids. Secreted isochorismatases effectors encoded by the root fungal and oomycete pathogens *Verticillium dahliae* and *Phytophthora sojae* have previously been experimentally shown to suppress salicylate pathway through hydrolyzation of its isochorismate precursor, notably in potato (Liu et al., 2014a; Zhu et al., 2017). As for plasmodiophorid isochorismatases, these are lacking a signal peptide, suggesting they would be secreted through an unconventional pathway. Moreover, it was previously reported that the manipulation of the salicylic acid pathway using a secreted effector is involved for *P. brassicae* virulence (Ludwig-Müller et al., 2015; Bulman et al., 2018; Djavaheri et al., 2018b). Further research is needed to determine if secreted isochorismatases are involved in the molecular interaction between the plasmodiophorids and their respective hosts.

On the other hand, the *P. brassicae* genes encoding PbBSMT, a methyltransferase acting on plant salicylic acid (Ludwig-Müller et al., 2015) and the auxin-responsive Gretchen Hagen 3 (PbGH3), a protein involved in plant defense hormone homeostasis (Schwelm et al., 2015b), were not identified in either *P. betae* or *S. subterranea*, suggesting that plasmodiophorids have adopted different strategies to facilitate the infection and their development within host roots/underground stems. In a similar vein, we report that GPCR pathway genes are by far less represented in both *P. betae* and *S. subterranea* than in *P. brassicae* e3. Bi et al. (2019) highlighted the importance of GPCR signaling pathway for *P. brassicae* development, pathogenicity and induction of clubroot symptoms. They hypothesized that it could be involved in the recognition of extracellular environment signals (e.g. root exudates) and intracellular signals in the host cells (Pérez-López et al., 2018; Bi et al., 2019).

Our results showed that homologs of CatSper complex principal (α 1-4) and auxiliary subunits ($\beta\delta\gamma$) were detected in *P. betae* and *S. subterranea* proteomes but not in *P. brassicae*, and to the best of our knowledge, this is peculiar within the protist world (Cai et al., 2014). In mammals, CatSper are sperm-specific Ca^{++} ion channel complex proteins initially known for their role in sperm hyperactivation, motility, fertility and chemotaxis to the egg (Ren et al., 2001; Chung et al., 2017; Sun et al., 2017). This complex requires the presence of the four subunits to work, controlling the cytoplasmic Ca^{++} concentrations in a pH-sensitive manner. Globally, an intracellular alkalization and high level of Ca^{++} inside the cell favors sperm activation (Sun et al., 2017). The remarkable conservation of the full CatSper complex as well as the presence of other key elements of Ca^{++} signaling machinery lead us to hypothesize that the Ca^{++} signaling could have a critical role in *P. betae* and *S. subterranea* life cycles, putatively through zoospore motility regulation and chemotaxis (Cai et al., 2014; Medina and Buchler, 2020) where the CatSper complex would conceivably play an

important role. These results could also explain a previous field observation that the infection potential of *P. betae* is positively correlated with Ca^{++} content and pH in soils (Legrève and Maraite, 1991) and it should be noted that liming to neutralize soil acidity was historically used to tackle clubroot of crucifers caused by *P. brassicae* (Campbell and Greathead, 1989; Murakami et al., 2002) and is still widely practiced as a soil conditioner in agriculture, including for sugar beet crops (Draycot, 1993). Similarly, alkalization of the soil by liming was reported to increase powdery scab incidence (Hughes, 1980). Our results are in accordance with the hypothesis that Ca^{++} signaling machineries appeared from early eukaryotic lineages, before initial divergence, rather than from a subsequent horizontal gene transfer event as previously suggested (Cai et al., 2014). It is interesting to note that no CatSper subunit signature was found in *P. brassicae*, *B. natans* or *R. filosa*. If evolutionary origin is common, it would mean that all three organisms have lost the four α as well as the three auxiliary subunits during evolution as has been demonstrated for numerous metazoa (Cai and Clapham, 2008). More generally, we should keep in mind that calcium channels underwent rapid evolution leading to an impressive diversity (Verkhatsky and Parpura, 2014). Further research should clearly be conducted to better characterize these proteins.

6. CONCLUSIONS

In the last decade, most plasmodiophorid research focused on *P. brassicae*. Taken together, our results suggest that even though *P. betae*, *P. brassicae* and *S. subterranea* are classified in the *Plasmodiophorida* and share a similar underground intracellular lifestyle, they probably have different strategies, relying on distinct key molecular mechanisms, to invade hosts root cells and multiply. For example, we pointed out striking differences such as a lower enrichment of proteins with ankyrin-repeat domains, the detection of CatSper complex proteins, different enrichment of GPCR genes for signaling pathways that could be relevant for development and pathogenicity. This emphasizes the need to enlarge plasmodiophorids research focus to more species. Extending the comparison to new plasmodiophorid genome sequences such as *P. graminis* could be of great help. In any case, the methods and analytical strategy we have developed for eukaryote genome assembly from unpurified samples will no doubt make some contribution towards current efforts to sequence representatives of all living organisms as in the newly established Earth BioGenome Project (<https://www.earthbiogenome.org/>).

CHAPTER IV. The *Polymyxa betae* genome

Availability of data and materials

Illumina raw reads are deposited at European Nucleotide Archive (ENA) under accession no. ERR5922443 for *P. betae* isolate RES-F41 DNA-seq data and ERR5905993 for *P. betae* isolate A26-41 RNA-seq data in the Bioproject PRJEB43927. *P. betae* isolate A26-41 DNA-seq data are available under accession no. SRR8105394. Both Genome assemblies and the mitochondrial genome of *P. betae* isolate A26-41 are available in the PRJEB43927.

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CHAPTER V.

Molecular interactions in the *Beta vulgaris*-*Polymyxa betae*-BNYVV tripartite interaction

Sequencing and assembly of a *P. betae* reference genome described in previous chapter is a major step forward in the study of molecular interactions between *P. betae*, host plants and transmitted viruses. A second requirement to study the tripartite interaction in rhizomania was to be able to bring genetically characterized sugar beet, *P. betae* and BNYVV isolate together in controlled conditions. A new protocol was therefore optimized to achieve sugar beet infection with standardized zoospores inoculum carrying or not the BNYVV. This protocol was implemented in a soil free hydroponic system in order to avoid interferences from soil biotic and abiotic properties. Then, this new method was used to perform a transcriptomic study of the interaction between the sugar beet, the BNYVV and *P. betae* using an RNA-seq approach. The fully sequenced monosporous *P. betae* isolate A26-41 obtained during the present project (Chapter IV) and the completely sequenced double haploid monogerm line KWS2320 were valorized in this study, facilitating bioinformatic data analyses. Our method also took advantage of using a genetically uniform starting BNYVV inoculum from cDNA clones. The total control of the pathosystems allowed us to raise interesting results about the tripartite interaction in rhizomania which are reported and discussed in this Chapter. The use of cDNA clones with our protocol could be advantageous in studies combining reverse genetics and transcriptomics to further evaluate the role of each viral features in the pathosystem.

This chapter consists of a paper in preparation to be submitted in the journal "viruses" (mdpi).

Rhizomania: hide and seek of *Polymyxa betae* and the *Beet necrotic yellow vein virus* with *Beta vulgaris*

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1. ABSTRACT

The *Beet necrotic yellow vein virus* (BNYVV) is the causal agent of rhizomania, a major disease of sugar beet. In this pathosystem, the bipartite interactions between sugar beet and BNYVV have been extensively studied. In comparison, little is known about their molecular interaction with *Polymyxa betae*, the soil-borne plasmodiophorid vector of the disease, due the difficulty of obtaining purified viruliferous culture of this protist. Here, we optimized, the transmission of BNYVV to sugar beet by *P. betae* zoospores using genetically characterized organisms and performed a transcriptomic analysis of aviruliferous and viruliferous protist infection on sugar beet to highlight molecular interaction. Our study demonstrates that BNYVV speeds up the first stages of *P. betae* life cycle on sugar beet, as shown by the faster increase of relative protist DNA level and by the earlier appearance of sporangia and sporosori in plants inoculated with viruliferous *P. betae* zoospores than in plants inoculated with *P. betae* alone. The transcriptomic analysis revealed that, when inoculated alone, *P. betae* induced limited gene expression changes in sugar beet: most differentially expressed genes are down regulated, including resistance gene analogs and cell wall peroxidases. Remarkably, several enzymes involved in stress regulation such as the glutathione-S-transferases, were significantly induced. In the presence of BNYVV, a clear activation of plant defenses and the modulation of genes involved in plant cell wall metabolism were observed. Additionally, the analysis of *P. betae* transcriptome revealed the induction of genes suspected to be involved in the switch to the survival stage of the life cycle of the vector. Altogether, BNYVV promotes the early rapid invasion of plant roots by *P. betae* and the appearance of thick-walled resting spores. In return, *P. betae* alleviates plant defense response.

2. INTRODUCTION

Rhizomania is a major disease of sugar beet, caused by the type member of the genus *Benyvirus*, in the family *Benyviridae* (Gilmer and Ratti, 2017), the *Beet necrotic yellow vein virus* (BNYVV), a multipartite single-stranded RNA virus. BNYVV is transmitted by the plasmodiophorid protist *Polymyxa betae* Keskin (Tamada and Asher, 2016b; Decroës et al., 2019), an obligate intracellular parasite of *Beta vulgaris* roots, surviving as resting spores in soils for decades. *P. betae* is as well a vector of other beet RNA viruses: *Beet soil-borne mosaic virus* (BSBMV) (Heidel et al., 1997; Lee et al., 2001), *Beet soil-borne virus* (BSBV) (Mahillon et al., 2021) and probably also *Beet virus Q* (BVQ) (Crutzen et al., 2009b). Other species of the Plasmodiophorida order can transmit plant viruses: *Spongospora subterranea* is the vector of *Potato mop-top virus* (Jones and Harrison, 1969; Reavy et al., 1998) and *P. graminis* is the vector of some *Beny-* (Bagayoko et al., 2021), *Bymo-* (Vaïanopoulos et al., 2006), *Furo-* (Vaïanopoulos et al., 2005) and *Pecluviruses* (Dieryck et al., 2009) associated to major diseases mainly on cereals (Dieryck et al., 2011). On the other hand, no virus transmission has been reported by *Plasmodiophora brassicae*, the causal agent of clubroot of crucifers and type member of the plasmodiophorids. At the physiological level, *P. brassicae* and *S. subterranea* induce the formation of galls in their host roots, whereas the infection by virus-free *Polymyxa* spp. remains asymptomatic. However, the induction of lateral root (PO:0020121), proliferation is observed following transmission of BNYVV and would be influenced by the P25 virulence factor encoded on BNYVV RNA3 (Schmidlin et al., 2008; Peltier et al., 2011). Both phenomena, i.e. galls formation and lateral roots proliferation, involve the auxin biosynthesis and signaling pathway as well as an interplay with plant defense resulting in up-regulation of defense related Transcription Factors (TF), Pathogenesis-Related (PR) proteins and secondary phenolic compounds (Malinowski et al., 2019; Fernando Gil et al., 2020). Currently, the control of plant viruses transmitted by plasmodiophorid vectors cannot be achieved by application of chemicals, due to ecological concerns (Bragard et al., 2013). Therefore, plant breeding leading to the introgression of two resistance genes, namely *Rz1* and *Rz2*, appeared as the only way to reduce viral disease incidence (Panella and Biancardi, 2016; Elmore et al., 2018).

There are only a few transcriptome profiling studies of the interactions between *Polymyxa* spp. and their hosts. McGrann et al. (2007, 2009) found a similar basal response in host and non-host interactions of *P. betae* and *P. graminis* with sugar beet and barley, suggesting that resistance to *Polymyxa* spp. is not governed by pathogen triggered immunity. Genes encoding for PR1a, PR5, cysteine proteases, a chitinase, a glutathione S-transferase (GST), lipid transfer proteins, thionins, XTH and extensin-like proteins were found among induced transcripts in early infection (McGrann et al., 2009). Two *B. vulgaris* xyloglucan endo-transglycosylases (*XTH*) were reported to be involved in repairing cell wall damages caused by *P. betae* infection

(Mutasa-Gottgens et al., 2000; Dimmer et al., 2004). Later, Desoignies et al. (2014) recorded 120 sugar beet ESTs up-regulated during the life cycle *P. betae*. The transcriptomic profiling of eleven sugar beet genes revealed an active defense response, that began after one day and peaked after five day, during plasmodial growth. After 15 days, when zoosporangia and sporosori are formed, the plant response seemed quieter, as if *P. betae* was “immunitary hidden”.

Until now, much effort in molecular plasmodiophorid-host interaction studies focused on *P. brassicae* and *Arabidopsis thaliana* system model. While there are some reports of *A. thaliana* infection by *P. betae*, protist levels in this host were much lower than in *B. vulgaris* with a divergent phenotype, likely revealing a distinct interaction (Desoignies et al., 2011; Smith et al., 2011). To our knowledge, no study reporting *S. subterranea* infection in *A. thaliana* has been published. The interaction between *P. brassicae* and *A. thaliana* involves the regulation of plant auxins and cytokinins homeostasis (Siemens et al., 2006; Schuller et al., 2014; Malinowski et al., 2016), as confirmed using *A. thaliana* defective mutants (Grsic-Rausch et al., 2000; Siemens et al., 2006; Jahn et al., 2013). The involvement of these phytohormones in a compatible interaction with cultivated *Brassica* species was also reported (Zhang et al., 2016; Jia et al., 2017; Shah et al., 2020). Different studies also converged to the observation that SA pathway is down regulated, whereas JA pathway is up-regulated during compatible interactions of *P. brassicae* with susceptible *A. thaliana* (Siemens et al., 2006; Agarwal et al., 2011; Schuller et al., 2014) and Brassicaceae accessions (Chen et al., 2016a; Jia et al., 2017). The opposite effect was observed during incompatible interactions of *P. brassicae* with tolerant *A. thaliana* (Jubault et al., 2013) and Brassicaceae accessions (Chen et al., 2016a; Jia et al., 2017). Similarly, Lekota et al. (2019) suggested that the activation of the SA pathway genes is involved in tolerance to *S. subterranea*. The role of the SA pathway and PR proteins in plant protection against *P. brassicae* was confirmed using *A. thaliana* PR protein gene mutants (Zhao et al., 2017) and exogenous SA treatments (Lovelock et al., 2013). Ciaghi et al. (2019) showed in a single *Brassica oleracea* susceptible plant infected by *P. brassicae* that transcriptomic responses differed in root galls and symptomless root portions. The response of symptomless was indeed similar to the response observed in resistant hosts. The regulation of cell wall-related genes were further studied in root galls by Badstöber et al. (2020). They reported that genes involved in cell wall synthesis were less expressed during gall formation, whereas genes coding for enzymes involved in cell wall degradation/modification and cell expansion were up-regulated.

Transcriptomic studies orientated toward *P. betae* genes expression have long remained scarce. Mutasa-Gottgens et al. (2000) reported 11 *P. betae* Expressed Sequence Tags (EST) with a glutathione S-transferase highly expressed in *P. betae* during interaction with sugar beet. Later, 76 *P. betae* EST were identified suggesting the involvement of protist profilin and Von Willebrand factor domain-containing proteins in the early phase of infection (Desoignies et al., 2014). With the recent publications of complete genomes

CHAPTER V. Interactions between *B. vulgaris*, *P. betae* and BNYVV

of *P. brassicae* (Schwelm et al., 2015b; Bi et al., 2016; Rolfe et al., 2016; Daval et al., 2019; Sedaghatkish et al., 2019; Stjelja et al., 2019), *S. subterranea* (Ciaghi et al., 2018) and *P. betae* (Decroës et al., 2019), one would expect an increased interest in plasmodiophorid transcriptomes. In this respect, Rolfe et al. (2016) identified 617 *P. brassicae* genes for which the expression was modulated during the life cycle of the protist in *Brassica napus*. From these, 41 genes encoding likely secreted protein such as peptidases, hydrolases, proteinase inhibitors and a gene encoding a SABATH-type methyltransferase were identified. Genes linked to cytoskeletal, cell differentiation and membrane activities were down regulated during the transition between plasmodial growth and spore formation. General metabolism-related genes showed the opposite regulation. Some Carbohydrate-Active enZymes (CAZy) putatively involved in spore formation and germination were also expressed (Schwelm et al., 2015b; Rolfe et al., 2016). The transcriptomic analysis of *P. brassicae* enzymes participating to lipid metabolism suggested that a dynamic conversion of lipids occurs in different development stages of the protist (Bi et al., 2016).

Despite the threat that rhizomania represents for the sugar beet production, until now, no study reported a full omic analysis of the tripartite interaction occurring between *B. vulgaris*, *P. betae* and BNYVV. This probably reflects the difficulty to simultaneously handle *P. betae* and BNYVV under fully controlled soil-free assay conditions (Kanyuka et al., 2003). We therefore elaborated a protocol to study interactions in the rhizomania pathosystem, using genetically characterized organisms, in order to perform a RNA-seq analysis of the interaction between the three protagonists. Results are discussed in the light of the previous transcriptomic analysis of the plant-BNYVV and plant-*P. brassicae* interactions.

3. MATERIALS AND METHODS

a. Biological material and bioassays

Biological material and inoculum production

Three accessions of *Beta* were used: *Beta macrocarpa*, the double haploid monogerm line KWS2320 of *B. vulgaris* that was fully sequenced by Dohm et al. (2014) and *B. vulgaris* var. Cadyx. Seeds were pregerminated on moistened germination paper (22°C, in the dark) and transplanted in tubes filled with sterilized quartz sand. Tubes with *B. macrocarpa* and *B. vulgaris* KWS2320 were then placed in individual flow through collecting pots, daily amended with half-strength Hoagland solution (Hoagland and Arnon, 1950) and maintained in a growth cabinet at 20-25°C (night-day) with a 14h photoperiod. Plants of *Chenopodium quinoa* were grown in individual pots filled with compost and placed in a greenhouse in the same conditions.

The *P. betae* A26-41 monosporosorus-derived isolate, sequenced by Decroës et al. (2019) was multiplied in roots of *B. vulgaris* var. Cadyx plants grown in an automatic immersion system as described by Legrève et al., (1998).

BNYVV infectious clones pB15, pB214, pB35 and p45, containing respectively the cDNAs for type B BNYVV RNAs 1, 2, 3 and 4 (Ziegler-Graff et al., 1988; Quillet et al., 1989) were used in bioassays. The initial BNYVV RNA inoculum was produced by *in vitro* transcription and inoculated on *C. quinoa* leaves, as described by Crutzen et al. (2009). After appearance of symptoms (1 week), infected leaves were harvested and ground in phosphate buffer (KH₂PO₄ 50 mM, pH 7). The resulting sap was used for mechanical inoculation on *B. macrocarpa* leaves using carborundum as abrasive. Leaves from *B. macrocarpa* plants showing systemic symptoms (14-21 days after inoculation) were then ground in four volume (w/v) of root inoculation buffer (KH₂PO₄ 50 mM, carborundum 30g/L, Na₂SO₃ 1g/L, pH 7) and mechanically inoculated onto the root systems of individual two-weeks-old *B. macrocarpa* seedlings using a protocol adapted from Koenig and Stein (1990). Briefly, roots were individually vortexed 2 X 30 sec in inoculation sap with 30 sec on ice in between. The conservation of the sap infectivity during the whole inoculation process was confirmed by mechanical inoculation of *C. quinoa* leaves. Mock-inoculated plants were obtained following the same procedure with a sap produced from healthy *B. macrocarpa* leaves. Plants were transplanted in tubes and grown under controlled conditions (see above).

Two weeks later, plants were inoculated with one milliliter of a suspension containing 10⁴ virus-free *P. betae* zoospores. Virus-free plants were inoculated in the same way with *P. betae* and other plants were mock-inoculated using a suspension produced from healthy sugar beet roots. After

three weeks growth, the *B. macrocarpa* were carefully extracted from the quartz sand, 30-50 mg of roots were sampled to check their inoculation status (presence or absence of *P. betae* and BNYVV) by RT-PCR (see below) and the plants were transplanted. After one more week, plants infected with both *P. betae* and BNYVV, plants with *P. betae* alone and mock-inoculated plants were used to release zoospores as previously described (Desoignies et al., 2011). Ten thousand BNYVV-transmitting and virus-free zoospores were inoculated to three-week-old *B. vulgaris* KWS2320 roots. Negative controls were mock inoculated with zoospore-free solutions produced with mock inoculated *B. macrocarpa* plants following exactly the same procedure to maintain uniform conditions between treatments. The general scheme of viruliferous inoculum production is represented in Supplementary Figure S1.

Experimental design

Three bioassays comprising three treatments - mock-inoculated sugar beet (C-), sugar beet infected with *P. betae* alone (PB) and sugar beet infected with *P. betae* and the BNYVV (PBBN) - were conducted. Each bioassay was performed in two successive phases, represented in Supplementary Figure S1. In the first phase, *P. betae* zoospores inoculum carrying or without BNYVV were produced on *B. macrocarpa* plantlets (19, 15 and 33 plants in bioassays 1, 2 and 3, respectively). This plant species was used for its ability to be systemically infected and to multiply BNYVV. In the second phase, susceptible *B. vulgaris* plantlets from the KWS2320 were inoculated with *P. betae* zoospores, carrying or not the virus, produced in the first phase. In both first bioassays, 30 plants were inoculated with viruliferous zoospores, 20 plants with virus-free zoospores and 10 plants were mock-inoculated with a "fake" zoospore suspension produced from healthy *B. macrocarpa*. In the third bioassay, 80 plants (20/time points, see below) were inoculated with viruliferous zoospores, 48 plants (12/time points) with virus-free zoospores and 24 plants (6/time points) were mock-inoculated. For the first two bioassays, the entire root systems of sugar beet plants were collected at 28 days post-inoculation (28 dpi), briefly washed in autoclaved water, quickly snap-frozen, ground in liquid nitrogen using a mortar and pestle and stored at -80°C. In the third bioassay, roots were collected after 24 hours, 7 days, 14 days and 28 days. At 14- and 28-days post-inoculation (dpi), two small portions (5 mm of taproot and all lateral roots attached) of each root system were excised for microscope observation. The rest was snap-frozen and ground in liquid nitrogen with a mortar and pestle and stored at -80°C, as for the complete root systems collected at 1 and 7 dpi.

Nucleic acids extractions and RT-PCR

For every sample, 30-50 mg of root powder were homogenized using FastPrep (MP Biomedicals, Santa Ana, CA) for 40 s at 6 m s⁻¹ and used for total RNA extraction using Trizol reagent (Invitrogen, Waltham, MA)

according to manufacturer's instructions. RNA samples from *B. vulgaris* were then treated with RQ1 RNase-free DNase (Promega, Madison, WI), further purified with a phenol:chlorophorm:isoamyl alcohol 124:25:1 solution, and kept in RNase-free water at -80°C until needed. In the third bioassay, 50 mg of root powder was also used for DNA extraction at each time point following a cetyltrimethylammonium bromide (CTAB) extraction procedure (Brandfass and Karlovsky, 2008) after homogenization in FastPrep for 40 s at 6 m s⁻¹. The presence of *P. betae* and BNYVV in inoculated plants was then verified using the multiplex reverse transcriptase polymerase chain reaction (RT-PCR) (Meunier et al., 2003) and BNYVV infection was confirmed using simplex RT-PCR targeting each of the four BNYVV RNAs in the first two assays and RNA2 in the third one (primers list is available in Supplementary Table S1). All RT-PCR were performed using M-MLV reverse transcriptase as described in Desoignies et al. (2014) and GoTaq G2 DNA polymerase (Promega, Madison, WI) using manufacturer instructions. The amplification reaction for simplex RT-PCR comprised a first denaturation step at 95 °C for 3 min; then 35 cycles of 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C and a final elongation step of 7 min at 72°C.

b. RNA-sequencing

RNA quantity and purity were evaluated using a NanoDrop™ 1000 spectrophotometer and quality was visually evaluated after electrophoresis on a denaturant RNA gel (formaldehyde 0.65 %, agarose 1.2 %). Five (C- & PB) or six (PBBN) biological replicates were selected for RNA sequencing from the first bioassay (28 dpi) based on RNA quantity and integrity as well as on the detection of required inoculum components (*P. betae*, BNYVV RNA 1, 2, 3, 4). For each sample, 800 ng RNA in 25 µl was sent to Fasteris SA (Geneva, Switzerland) which performed additional quantity and integrity assessments, polyA selection and library preparation with the TruSeq Stranded mRNA Library Prep kit (Illumina, San Diego, CA) and paired end sequencing (2x100bp) on a Novaseq6000 platform (Illumina, San Diego, CA). Data analysis was performed in-house.

c. Bioinformatics

Fastq files containing paired raw reads were received from Fasteris. Initial quality check was performed with FastQC v.0.72 (Andrews, 2010). Illumina adapters (ILLUMINACLIP: TruSeq3) and quality trimming (sliding window 4bp; average quality score > 20) was achieved using Trimmomatic v0.36.5 (Bolger et al., 2014). Reads shorter than 20 nucleotides were removed. Poly-G tails contaminations with a minimum length of 10, commonly seen in NovaSeq/NextSeq data, were trimmed with Fastp v.0.19.5 (Chen et al., 2018). FastQC check was repeated to confirm improvement.

RNA-seq data were aligned to *P. betae* reference genome (accession number ERZ3364346) using STAR v.2.7.3a in two-pass mode (Dobin et al.,

2013) with the following non-default parameters: intron sizes were set to 15 minimum and 500 maximum; alignments with a ratio of mismatches to read length of maximum 0.04 were considered ; minimum overhang for novel spliced alignments and annotated spliced alignments were set to 8 and 1, respectively ; maximum number of loci a read is allowed to map was set to 20 ; maximum allowed gap between two mates was 2000. Unmapped reads were then aligned to the *Beta vulgaris* reference genome (Dohm et al., 2014; RefSeq assembly accession: GCF_000511025.2), with the same settings except that intron sizes were adjusted to a minimum of 20 and a maximum of 5000 and that allowed gap between two mates was adjusted to a maximum of 5000. Since current *P. betae* genome annotation greatly rely on *in silico* gene predictions with limited transcriptomic support, *P. betae* transcripts were assembled using StringTie with default settings except for the strand information specified to “reverse (RF)” (Pertea et al., 2015). All assembled transcripts were then merged with StringTie merge mode and aggregated with the available annotation (Chapter IV) using GffCompare (Pertea and Pertea, 2020), with default settings. Read counts on both genomes’ features were then computed with Htseq-count v.0.9.1 with the “union” mode and the strandedness option set to “reverse” (Anders et al., 2015). Open reading frames (ORFs) were predicted on *P. betae* assembled transcripts using TransDecoder v3.0.1 (Haas and Papanicolaou, 2016). The single best ORF on each transcript was retained prioritizing homology searches (BlastP against swissprot ; hmmscan against Pfam) and ORF length. Translated peptides sequences were then annotated with the Trinotate v3.2.2 (Bryant et al., 2017). Differential gene expression analysis was performed using the R software with edgeR v3.28.1 and limma v3.42.2 R/Bioconductor packages as described in Law et al. (2018). Notably, lowly expressed gene filtering and normalization with the trimmed mean of M-values method were achieved using “filterByExpr” and “calcNormFactors” functions in edgeR, respectively. Linear models (Smyth, 2004) combined with abundance dependent and sample-level weights (Law et al., 2014; Liu et al., 2015b) were fitted to the data using the limma package with the “voomWithQualityWeights” function. The choice of this strategy was based on the fact that multidimensional scaling (MDS) plots showed some variability between replicates (Liu et al., 2015a). Differentially expressed genes (DEGs) of *B. vulgaris* were determined from P-values computed from empirical Bayes moderated t-statistics using the “treat” method (McCarthy and Smyth, 2009) with a fold change (FC) of 1.2. DEGs of *P. betae* were determined using an adjusted P-value cutoff of 0.05 without FC requirement. Plant genes were further annotated with Mercator4 and DEGs were classified in functional context (e.g. pathways) within MapMan4 (Schwacke et al., 2019) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) *B. vulgaris* (Kanehisa et al., 2017) frameworks. Resistance genes analogs (RGAs) were classified according to Dohm et al. (2014). Genes encoding Pathogenesis-Related-like (PR-like) proteins were searched in *B. vulgaris* refseq-rna using tBLASTn (E-value < 1e-06) with PR family’s founder proteins coding sequence as queries and manually refined based on reference *B. vulgaris* annotation. The expression of selected genes groups

was represented on heatmaps with the pheatmap v1.0.12 R package. Other data were visualized with the ggplot2 v3.3.2 R package.

d. *Polymyxa betae* microscopic observation

Root samples from the third bioassay were used to assess *P. betae* structures abundances at 14 and 28 days after inoculation. To this end, ten pieces of roots (~5 mm long) were randomly selected from two portions of each root system. These portions were located at mid-point of the taproot (hereafter called the “middle” part) and at one centimeter below the collar (hereafter called the “upper” part). The abundances of *P. betae* plasmodia, sporangia and sporosori were visually assessed in each root portion after cotton blue lactophenol staining as described in Legrève et al. (1998) but using a customized semi-quantitative 0-5 scale, where 0 = no infection, 1 = <10% cells infected by a particular development stage, 2 = 10% - 30% cells infected, 3 = 30% - 50% cells infected, 4 = 50% - 70% cells infected, 5 = >70% cells infected. Then, an infection index was calculated for each plant in the upper and middle part of the roots using the following formulae:

$$\sum_{i=0}^5 (n_i * i) / \sum_{i=0}^5 n_i$$

where n_i is the number of rootlets at infection level i (from 0 to 5).

Four weeks after inoculation, five root pieces were randomly selected from the middle part of the root system and vacuum infiltrated with the LD540 lipophilic dye (Spandl et al., 2009) as described in Jurkiewicz et al. (2018). Roots were immediately imaged in confocal microscopy with a pinhole of 1 airy unit (excitation 488 nm, Zeiss LSM 710; Zeiss, Thornwood, NY, USA). Each image was captured using the same settings in order to allow comparison of LD derived fluorescence in the presence or the absence of BNYVV. LD fluorescence was quantified in ten optical fields distributed in five root pieces by sample using the ZEN 2 (blue edition) image analysis software (Carl Zeiss AG, Oberkochen, Germany).

e. (RT-)qPCR analysis

In two bioassays, RT-qPCR analyses were carried out to quantify the proportion of *P. betae* Internal Transcribed Spacer 1 (ITS1) transcripts in comparison to the expression of *B. vulgaris* 18S gene after 28 days (primers are listed in Supplementary Table S2). A qPCR targeting DNA template of the same genes was achieved in a third bioassay at four-time points (1, 7, 14, 21 days after inoculation). The relative gene expression quantification of 12 genes of interest and two reference genes was also achieved in this third assay. Moreover, the relative gene expression quantification of 10 genes of interest and two reference genes was also achieved on RNA extract used in the RNA-seq analysis in order to confirm RNA-seq results. The list of targeted genes and primers is available in Supplementary Table S3. When needed, cDNA was synthesized from 2-3 µg of Dnase treated RNA, using M-

CHAPTER V. Interactions between *B. vulgaris*, *P. betae* and BNYVV

MLV reverse transcriptase (Promega) and anchored oligo(dT)18 primers or the corresponding reverse primer for *ITS* and *28S* amplifications. qPCR assays were carried out using Takyon No ROX SYBR 2X MasterMix blue dTTP (Eurogentec, Seraing, Belgium) on a CFX96™ Real-Time System (BIO-RAD, Hercules, CA). For each sample, a reaction was set up with 10 µL of MasterMix, 0.5 µL of each primer (20 µM), 7 µL DEPC-treated water and 2 µL cDNA using the following protocol: denaturation step at 95 °C for 7 min; then 40 cycles of 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C; and a final melting curve (55–95 °C with a heating rate of 0.5 °C s⁻¹). Each qPCR assay was performed in two replicates. Reaction efficiency in each plate was monitored through the establishment of standard curves using five serial dilution of target cDNAs in order to guarantee the linearity of amplification over the samples C_q range. Only qPCR with efficiency comprised 90-105% were considered. Data analysis was performed using CFX Manager software (Bio-Rad) and the R software using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001) and taking into account of the MIQE recommendations (Bustin et al., 2009).

4. RESULTS AND DISCUSSION

a. New protocol to study the tripartite interaction in rhizomania pathosystem

In the field, plant infection by BNYVV strictly requires *P. betae*, as there is no other known natural mode of transmission (Tamada, 2016). In order to draw a realistic picture of rhizomania, an inoculation protocol was therefore optimized to mimic natural BNYVV transmission by zoospores under controlled conditions. In our experimental set-up, *B. macrocarpa* was used as an intermediate host for BNYVV mechanical inoculation, multiplication and loading in *P. betae* zoospores for transmission to *B. vulgaris*. Following mechanical inoculation, BNYVV infection rates on *B. macrocarpa* roots ranged from 52 to 100 %, whereas *P. betae* incidence reached 100 % in three independent assays (Supplementary Table S4). Most of *B. macrocarpa* plants inoculated with the BNYVV were stunted and showed strong symptoms on leaves. Moreover, a significant reduction of 70 % in the root dry weight was observed on plants infected by both *P. betae* and the BNYVV after six weeks (Supplementary Figures S2 & S3). *B. macrocarpa* plants on which both *P. betae* and BNYVV were detected and showing symptoms (6-10 plants, depending on bioassay) were used to produce BNYVV carrying-zoospores and perform transmission on *B. vulgaris*. This resulted in viral transmission rates ranging from 53 to 90 % in the three assays, whereas *P. betae* infection rates reached 100 %, both in plants inoculated with *P. betae* with or without the virus (Supplementary Table S5). The absence of BNYVV and *P. betae* was confirmed in mock inoculated plants. Foliar symptoms were observed on *B. vulgaris* after inoculation through virus-transmitting zoospores only in one plant from one of the three bioassays. After 28 days, when roots were entirely harvested for the RNA-seq analysis, no massive lateral root proliferation were observed yet, even though weak root proliferation was observed in some samples infected with the BNYVV. All plants inoculated with *P. betae* showed the characteristic light brown discoloration of the roots, associated with the presence of numerous protist structures in root cells. Surprisingly, the overwhelming majority of studies on the rhizomania have focused on BNYVV without considering the vector, therefore skipping an essential component of the disease. For example, previous transcriptomic studies on the interaction of BNYVV with *Nicotiana benthamiana* (Fan et al., 2014; Liu et al., 2020a), *Beta macrocarpa* (Fan et al., 2015a) and *B. vulgaris* (Fernando Gil et al., 2020) were all performed based on mechanically-inoculated plants. Compared to a natural infection by *P. betae*, wounding associated with mechanical viral inoculation likely induces a stress response from the plant (Koenig et al., 1991). Furthermore, plant response to biotic and abiotic stresses is known to be the result of complex, interleaved signaling networks (Rejeb et al., 2014). Consequently, the respective effects of mechanical injury and of BNYVV inoculation on plants cannot be considered additive and comparison of infected plants with mock-inoculated plants is not

sufficient to selectively mask the plant response to mechanical wounding. To date, most studies that include both *P. betae* and BNYVV have used infested soils as inoculum (eg. Larson et al., 2008; Schmidlin et al., 2008; Webb et al., 2014, 2015, 2020) and are therefore subjected to interference derived from the inoculum genetic heterogeneity as well as other soil biotic and abiotic characteristics (Schmidlin et al., 2008). The same issue was reported in studies on interactions between *P. graminis*, plants and viruses (Dieryck et al., 2011). The protocol described here allows to reproduce viral transmission by zoospores as it occurs in the field but in a soil-free assay, including exclusively genetically characterized BNYVV and *P. betae* isolates as well as a completely sequenced sugar beet accession. A similar strategy was recently applied to evaluate the role of BNYVV RNA5 presence under natural inoculation conditions (Tamada et al., 2021).

b. Overview and validation of RNA-seq data

Roots of *B. vulgaris* showing a strong RT-PCR detection of *P. betae* alone (five plants) or of the four BNYVV RNA genomic components (six plants) at 28 dpi were selected from one bioassay for RNA sequencing. Five mock-inoculated plants were also included. Between 37.0 and 59.6 million of good quality read pairs were obtained in each of the 16 sequenced libraries (Supplementary Table S6). The percentages of mapped reads on the *P. betae* reference genome ranged from 14.4 to 34.3%. They ranged from 61.4 (in the presence of *P. betae*) to 98.1% (on mock-inoculated plants) on *B. vulgaris* genome. In comparison to mock-inoculated plants, the differential expression analysis revealed twice as many genes influenced by inoculation of viruliferous *P. betae* (2192) than with *P. betae* alone (1002, mostly down-regulated). Numbers of up- and down-regulated genes for each comparison are summarized in a Venn diagram in Supplementary Figure S4. For a large majority of genes, the co-inoculation of *P. betae* zoospores and BNYVV did not trigger opposite regulations on the plant compared to *P. betae* alone. These results probably reflect the long co-evolution and interdependency between the BNYVV and its vector and the asymptomatic phenotype associated to *P. betae* infection. The expression changes of ten target genes in each inoculation condition (PB or PBBN vs C-) measured by RNA-seq and RT-qPCR were highly correlated ($R^2 = 0.91$), confirming reliability of RNA-seq data and analysis (Supplementary Figure S5).

KEGG categorization of potential DEGs functions revealed that more pathways were significantly enriched in the presence of the virus (PBBN) than with *P. betae* alone (PB), including pathways related to signaling, zeatin biosynthesis as well as secondary metabolite synthesis (Table V-1). Glutathione metabolism was the most enriched pathway in plants infected with *P. betae* alone and, in a lesser extent, with the virus. Seven of the enriched pathways in the presence of BNYVV (P-val < 0.01, shaded in Table V-1) were already reported in a previous RNA-seq study using BNYVV without its vector on *B. vulgaris* (Fernando Gil et al., 2020), further confirming the reliability of the RNA-seq data generated in the present work.

Table V-1. Most enriched KEGG pathways in the up-regulated and/or down-regulated DEGs from *P. betae* alone (PB) and *P. betae* + BNYVV (PBBN) infected plants in comparison to mock-inoculated plants (C-). Pathways are classified from the most significant to the less significant firstly in the PBBN vs C- comparison and secondly in the PB vs C- comparison. Only P-values below 0.01 are shown. Shaded pathways have already been reported in the study of Fernando Gil et al. (2020), using full-length infectious cDNA clones of BNYVV.

KEGG Pathway	PB vs C-		PBBN vs C-	
	<i>P-val Up</i>	<i>P-val Down</i>	<i>P-val Up</i>	<i>P-val Down</i>
Glutathione metabolism	5,61E-16	-	3,65E-08	-
Metabolic pathways	9,19E-05	-	4,77E-09	-
Phenylpropanoid biosynthesis	-	-	1,14E-05	-
Biosynthesis of secondary metabolites	-	-	1,22E-05	-
Nitrogen metabolism	-	1,95E-05	-	1,57E-05
MAPK signaling pathway - plant	-	-	3,47E-05	-
Flavonoid biosynthesis	-	-	3,71E-05	-
Cyanoamino acid metabolism	-	-	4,20E-05	-
Zeatin biosynthesis	-	-	7,87E-05	-
Isoquinoline alkaloid biosynthesis	-	-	1,38E-04	-
Tyrosine metabolism	-	-	1,32E-03	-
Amino sugar and nucleotide sugar metabolism	-	-	2,10E-03	-
Carotenoid biosynthesis	-	-	-	6,35E-03
Pentose and glucuronate interconversions	-	-	7,96E-03	-
Circadian rhythm - plant	-	-	8,62E-03	-
Proteasome	3,01E-04	-	-	-
Ascorbate and aldarate metabolism	7,08E-04	-	-	-
Ribosome	2,69E-03	-	-	-

Since available annotation of *P. betae* draft genome greatly rests on *in silico* predictions, an assembly of transcriptome sequences was performed in this study and integrated with the initial annotation. As a result, 20,297 transcripts were reported belonging to 11,872 genes. Protein-coding genes were then annotated and their expression levels (TPM) and regulation were monitored in the presence or absence of BNYVV. Unfortunately, only 165 DEGs (93 up- and 72 down-regulated, visible in Supplementary Table S7) were found without applying a fold change threshold, probably due to high variability between replicates. The high sample dispersion found by multidimensional scaling analysis of gene expression data illustrates this high variability (Supplementary Figure S6). To carefully exploit these results, we have chosen to focus only on significantly regulated genes, showing log fold changes superior to one (64 genes).

c. The BNYVV hastens *P. betae* life cycle in sugar beet roots

In rhizosphere, free *P. betae* zoospores enters sugar beet roots by infecting epidermal cells, preferably through roots hairs. Consequently, when the taproot starts enlarging, new infections occurs essentially in thin lateral roots, bearing root hairs. The main symptom induced by BNYVV is precisely the proliferation of those lateral roots, which is likely an advantage for *P. betae* in later infection stages.

During our experiments, no pronounced lateral root proliferation was observed, yet. However, significantly higher relative *P. betae* DNA quantities were measured in plants infected with BNYVV than in plants inoculated with aviruliferous *P. betae*, at one, seven and 14 dpi (Figure V-1A). At 28 dpi, a higher relative quantity of *P. betae* DNA was measured in plants inoculated with *P. betae* alone than in the presence of BNYVV. On the one hand, this higher relative quantity at 28 dpi was confirmed by comparing the percentages of RNA-seq reads mapped to the *P. betae* reference genome (Figure V-1B) and by the higher relative level of *P. betae ITS1* transcripts measured by RT-qPCR in two independent bioassays (Figure V-1C). On the other hand, a greater number of sporangia and sporosori was observed after 14 days in the PBBN infected roots compared to PB infected roots in both the upper part and the middle part of the roots. The same observation was made in the upper part of the roots at 28 dpi (Figure V-1D). *P. betae* life cycle was therefore to be accelerated in roots infected by the virus. Once entering in the survival stage (sporosori), the multiplication of *P. betae* slowed down while root growth continued, thus explaining the lower *P. betae* relative quantity measured. This effect could also explain the observation of similar sporosori relative quantities with and without BNYVV in the middle part of the roots at the same time point. We should note that the plant tissues taken at 14 and 28 dpi for visual observation differ between the two time points, as root length increased and new lateral roots were probably formed between the two sampling times. On a molecular level, strikingly, *P. betae* genes encoding homologs to Yippee-like protein, involved in conidiation in the rice blast pathogen *Magnaporthe oryzae* (Han et al., 2018), and to the conidiation-specific protein 10, a stress-responsive gene expressed during conidiation in the ascomycota *Neurospora crassa* (Shrode et al., 2001) were up-regulated in the presence of BNYVV. These findings endorse the hypothesis that *P. betae* enters earlier into the resting spore stage in roots co-inoculated with the virus. Similarly, the up-regulation of *P. betae* genes involved in cell signaling (serine/threonine protein kinase activity), regulation of translation (Pumilio homolog 12), proteolytic activity (cathepsin-like, two metalloproteases) and other hydrolytic activities (lysosomal acid phosphatase, β -lactamase) could also be involved in the switch from the sporangial stage to the sporogenic stage of the life cycle of the protist (Supplementary Table S7).

In addition to the induction of lateral root proliferation, our results suggest that the presence of BNYVV would also benefit to *P. betae* during early

infection and root colonization. Similarly, Gerik and Duffus (1988) also reported greater infection levels on sugar beets inoculated with BNYYV-carrying *P. betae* isolates when compared with the same isolates without the virus, and this for five out of the six monosporosorus isolates tested.

CHAPTER V. Interactions between *B. vulgaris*, *P. betae* and BNYVV

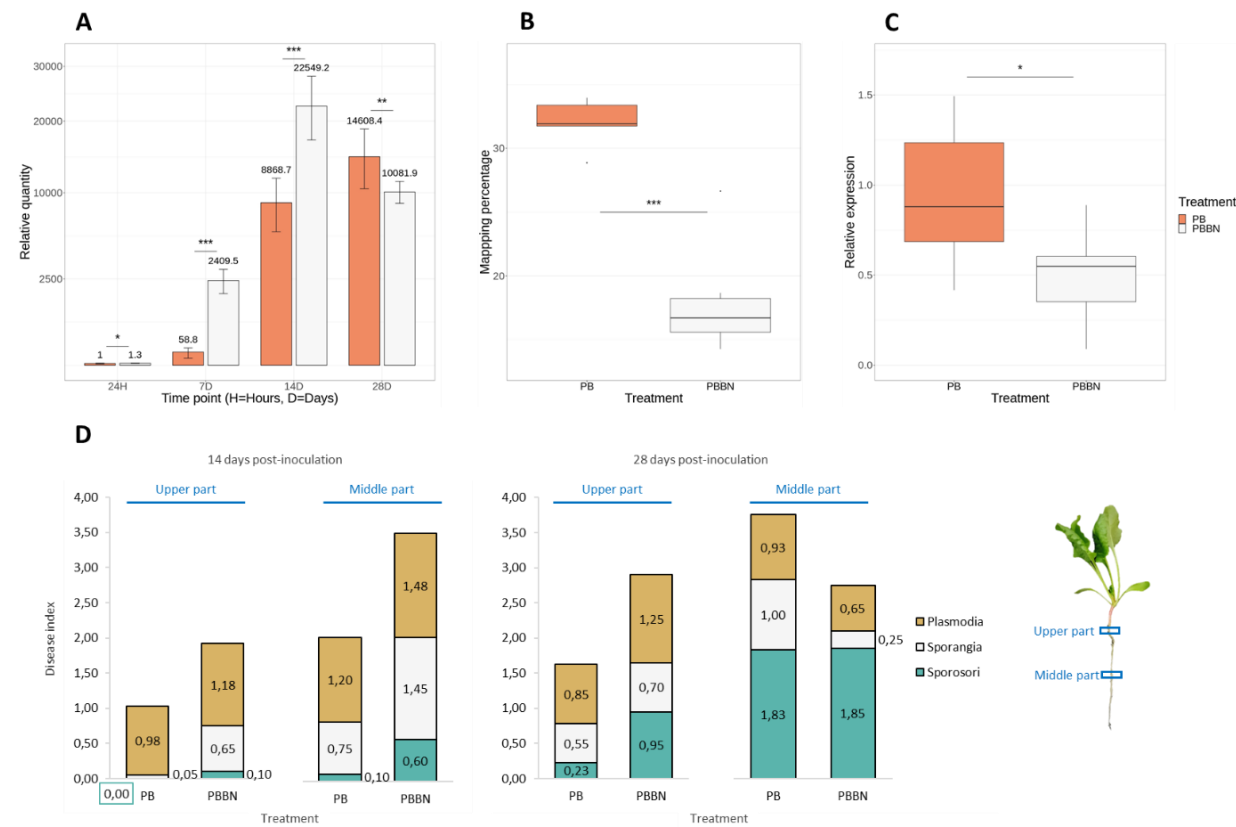


Figure V-1. Legend on the next page.

Figure V-1. Relative quantification of *P. betae* in sugar beet roots in three successive bioassays (A-C) and visual assessment of *P. betae* life cycle in sugar beet roots (D). A: Bar chart of relative quantity of *P. betae* DNA normalized to *B. vulgaris* DNA obtained by qPCR (n=8). B: Boxplot representation of percentages of mapped reads on *P. betae* A26-41 reference genome (n=5-6). C: Boxplot representation of the relative quantity of *P. betae* ITS1 rRNA expression obtained by RT-qPCR (n=6-8). D: Stacked bar chart of the visual assessments of three *P. betae* structures (i.e. sporosori, sporangia and undifferentiated plasmodia) at two different time points (14, 28 dpi) and in two parts of the root system (top and middle) (scale from 0 to 5 with 1 = <10% cells infected by a particular development stage, 2 = 10% - 30% cells infected, 3 = 30% - 50% cells infected, 4 = 50% - 70% cells infected, 5 = >70% cells infected). C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV. Significantly different levels were determined by t-test (* for $P \leq 0.05$ and * for $P \leq 0.001$).**

d. Regulation of cell wall-related genes in response to *P. betae* and BNYVV infection

It is known that BNYVV influences sugar beet genes associated with cell wall organization during auxin-triggered lateral roots development (Fernando Gil et al., 2018, 2020). Cell wall remodeling is also suspected to be involved in *P. betae*-sugar beet interaction (Desoignies et al., 2014). Heat maps of the major cell wall DEGs were therefore prepared to investigate these changes in tripartite interaction (Figure V-2). Cell wall synthesis related transcriptome was mainly repressed in *P. betae* infected roots compared to mock-inoculated roots (Figure V-2A). Transcripts related to proteins involved in cellulose synthase complex (CSC) (i.e. CSC-interactive protein and the catalytic component Cesa), in hemicellulose synthesis (two glucuronosyltransferase and one xyloglucan galacturonosyltransferase) and in pectin synthesis (xylogalacturonan xylosyltransferase) were significantly down-regulated in comparison to mock-inoculated plants. Genes coding for enzymes involved in hemicellulose modification/degradation (endo-beta-1,4-mannanase/xylanase, xylan O-acetyltransferase) and in pectin modification degradation, including pectin methylesterases (PME) and a beta-galactosidase (BGAL), were also significantly repressed in the presence of *P. betae* alone (Figure V-2B). The repression of cell wall synthesis related genes was less marked in plants infected with BNYVV and several genes encoding enzymes involved in cell wall modification were induced, especially with regard to pectin modification/degradation. Three pectin acetyltransferases, two PMEs and one polygalacturonase (QRT2) encoding genes were significantly up-regulated. The expression levels of cell wall protein genes were also impacted by the inoculation of PB and BNYVV (Figure V-2C). With *P. betae* alone, *glycoprotein* and *extensin* (EXT) genes were mostly repressed, whereas *expansin* (EXP) genes were mainly up-regulated in the presence of BNYVV.

The lateral roots proliferation induced by BNYVV is initiated by the conversion of root cells into meristematic cells and involves the regulation of

genes associated with cell wall organization (Fernando Gil et al., 2018, 2020). Regulated genes includes *PMEs*, *polygalacturonases* and *EXPs* with BNYVV alone [Fernando Gil et al. (2020): Supplementary Table S3] and could influence the cell wall biomechanical properties such as rigidity (Wormit and Usadel, 2018). In particular, *EXPs* were previously found to be up-regulated after BNYVV infection with or without the vector (Schmidlin et al., 2008; Fan et al., 2015a; Fernando Gil et al., 2018) as well as in BNYVV p25 protein-expressing transgenic *A. thaliana* roots (Peltier et al., 2011) and are associated to cell wall loosening during root hairs and lateral roots formation (Cosgrove, 2015). It was also previously proposed that cell wall alterations, involving PME and EXP induction, in *A. thaliana* are involved in *P. brassicae* gall formation (Devos et al., 2006; Agarwal et al., 2011; Irani et al., 2018). Cell-to-cell movement of *P. betae* is thought to happen thanks to the release of zoospores inside the roots and/or through myxamoeboid movement of the protist (Mithen and Magrath, 1992; Barr and Asher, 1996; Tamada, 2016). Both mechanisms are thought to rely on cell-wall degrading enzymes and could benefit from reduction of plant cell wall integrity. Here, cell wall peroxidases (PR-9 family), known to be involved in cell wall lignification (van Loon et al., 2006) were repressed in both inoculation conditions, especially with *P. betae* alone (**Table V-2**). Moreover, EXT and cell wall glycoprotein encoding genes were down-regulated following infection with *P. betae* alone. Notably, three *EXT-1-like* were found in the TOP20 of the most down-regulated genes with *P. betae* alone in comparison to mock-inoculated plants (Supplementary Table S8). EXT were previously shown to be involved in cell wall strengthening and root defenses, notably against pathogenic oomycetes (Castilleux et al., 2020). These repressions could therefore be key for *P. betae* root colonization. Taken together, these results highlight the probable importance of cell wall remodeling enzymes/proteins in the *B. vulgaris*-*P. betae* interaction. The effect of BNYVV on cell wall organization, including the likely loosening of the cell walls, observed here and reported elsewhere (Fernando Gil et al., 2018, 2020) may have a beneficial effect on *P. betae*, allowing its faster early development in the roots as suggested for *P. brassicae* which does not transmit viruses but induces the formation of root galls (Devos et al., 2006; Malinowski et al., 2019). This hypothesis has to be further investigated, especially in early infection.

Even though we didn't observed transcriptional reprogramming of genes involved in RNA silencing at 28 dpi, we cannot rule out the possibility that *P. betae* would also benefit from the viral ability to suppress host RNA silencing, mediated by the RNA2-encoded P14 (Chiba et al., 2013), the noncoding RNA3 (Flobinus et al., 2016, 2018) and RNA4-encoded p31 (Rahim et al., 2007). The RNA-dependent RNA-polymerase 6 silencing pathway, thought to be targeted by P14 activity, was indeed previously reported to participate in defense against bacterial and fungal pathogens in rice (Wagh et al., 2016) and the up-regulation of an *RDR6* homolog in sugar beet during *P. betae* early infection was reported by Desoignies et al. (2014).

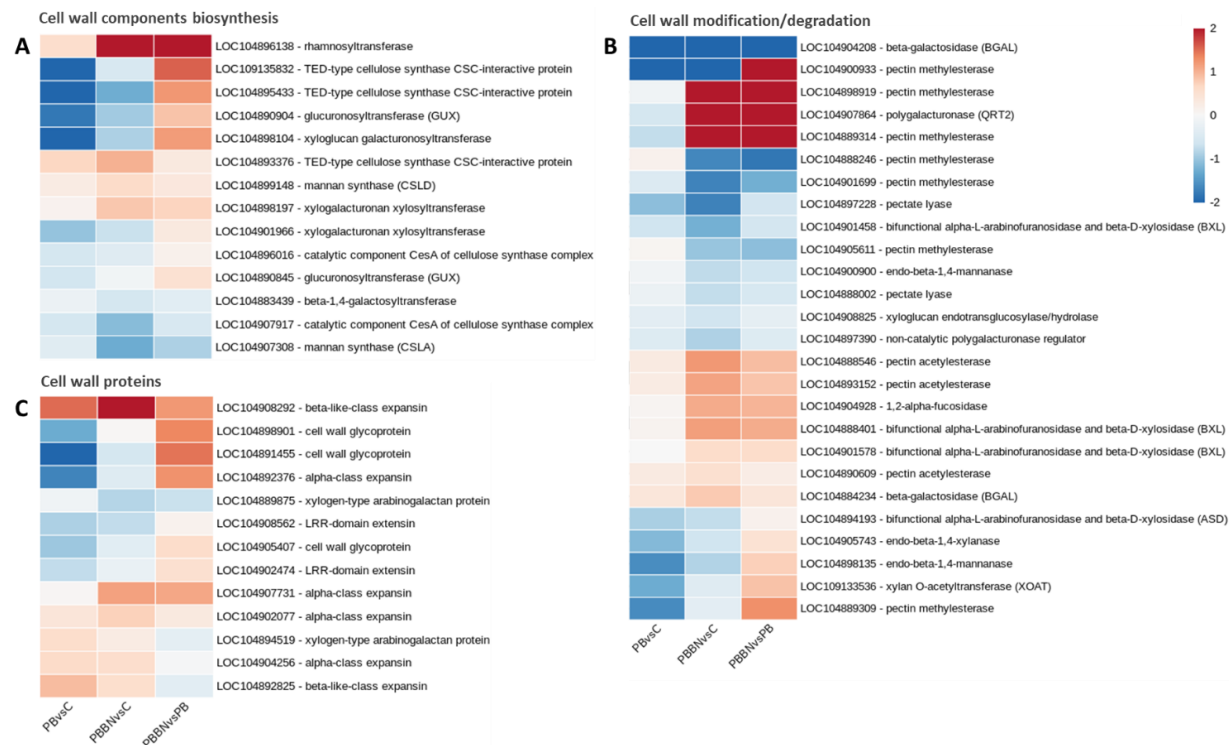


Figure V-2. Cell walls related genes expression in sugar beet in response to *P. betae* +/- BNYVV inoculation. The heatmaps of DEGs related to cell wall components biosynthesis (A), cell wall proteins (B) and cell wall modification and degradation (C) are shown. Up- and down-regulation are represented in red and blue, respectively. The scale is based on log₂ fold changes. C = mock-inoculated plants. PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV. Genes were classified according to Mercator/MapMan4. Annotations comprised the NCBI Gene symbol and the MapMan4 annotation.

The hormonal modulation underpinning changes in the expression of cell wall-related gene was also investigated. DEGs involved in auxin/cytokinin balance were evidenced in our study (Supplementary Figure S7). As reported by Fernando Gil et al., (2020), some genes involved in auxin synthesis from L-tryptophan (*YUCCA*, *tyrosine decarboxylase 1*, *aldehyde dehydrogenase*) were up-regulated in the presence of BNYVV but also with *P. betae* alone, except for *tyrosine decarboxylase 1*. Consistent with the study of Schmidlin et al. (2008), our results confirmed the up-regulation of auxin binding protein genes (*ABP*). Genes putatively involved in auxin transport (*PIN* and an *auxin transport regulator kinase* [*PINOID*]) were also up-regulated, only in the presence of BNYVV. Conversely, three auxin efflux transporters genes from PIN-like family (*PILS*) were repressed, especially in the presence of BNYVV. The influence of inoculation on auxin-related genes was expected since lateral root proliferation induced by BNYVV was reported to be controlled by auxin signaling due to the interaction of the viral P25 virulence factor with the auxin/indole acetic acid protein (AUX/IAA) BvAUX28 (Fernando Gil et al., 2018). Interestingly, overexpression of *PINOID* was found to trigger lateral root formation in *A. thaliana* mutants. On the other hand, *PILS* action was reported to decrease auxin signaling in *A. thaliana* roots. *pils* knockout mutants displayed a higher density of lateral roots, while overexpression of *PILS* in root hairs led to a reduction in root hair growth (Barbez et al., 2012). Even if the proliferation of lateral roots was not clearly visible yet at time of analysis, it's tempting to link these regulations with the initiation of lateral root proliferation by BNYVV. The "classical" cytokinin signaling pathway was repressed in the presence of both *P. betae* and BNYVV. As a matter of fact, *histidine-kinase receptors* (*AHK*) genes were not regulated and *AHP6*, encoding repressor of AHK signaling, was induced. However, the cytokinin signaling pathway activator gene (*CKI1*) was strongly induced in both inoculation conditions. In *A. thaliana*, CKI1 activates similar cytokinin responses than AHK but in a cytokinin-independent manner and is regulated by light perception (Deng et al., 2010; Dobisova et al., 2017). The development of *P. brassicae* root galls is controlled by auxin and cytokinin pathways (Malinowski et al., 2019). Similarly, these pathway seems key in *P. betae*-BNYVV-sugar beet interaction. The rôle of cytokinin pathway is unclear and deserves further study.

e. Effect of *P. betae* and BNYVV on defense-related genes expression

In the present study, we found that more than 30% poly(A)-RNA extracted from sugar beet roots infected with *P. betae* alone originated from the latter. Despite this huge proportion, no symptoms were visible on plants one month after inoculation. Desoignies et al. (2014) reported that the sugar beet defense response to an established *P. betae* infection (when all life stages are present) was very weak, in comparison to earlier time points (McGrann et al., 2007; Desoignies et al., 2014). Conversely, several studies found a clear induction of sugar beet defenses after inoculation with BNYVV (e.g.

Larson et al., 2008; Schmidlin et al., 2008; Webb et al., 2015, 2015; Fernando Gil et al., 2020). To gain insights into the differences between defense response to *P. betae* alone or carrying BNYVV, the expression of plant PR and PR-like proteins genes was systematically monitored (Table V-2). A stronger increase in expression of PR and PR-like encoding genes was observed in the presence of BNYVV (17 and 71 down- and up-regulated DEGs, respectively) compared to *P. betae* alone (15 and 25 down- and up-regulated DEGs, respectively), including three *PR-1-type* represented in the TOP20 of the most up-regulated genes in PBBN plants (Supplementary Table S9). Although induction of plant PR protein by viral infection has been known for decades (Bol et al., 1990) and already reported for the BNYVV (Fan et al., 2015a; Fernando Gil et al., 2020), our analysis reveals that this up-regulation could affect *P. betae*. In particular, several genes encoding putative endo-1,3-beta-glucosidases (PR-2) and chitinases (PR-3,4,8,11) were strongly up-regulated. Such induction was previously proposed to be involved in resistance against *P. betae* (Burketová et al., 2003) and *P. brassicae* (Zhang et al., 2016).

Similarly, KEGG pathways associated to biosynthesis of secondary metabolite were only significantly enriched among DEGs found in the presence of BNYVV. The tyrosine pathway, potentially acting upstream to flavonoid and phenylpropanoid pathways was induced. Phenylpropanoid, flavonoid and dopamine pathways were also globally stimulated, as represented on KEGG maps in Supplementary Figures S8 & S9. Accordingly, all genes belonging to LNK family were significantly down-regulated, only in the presence of the BNYVV. LNK 1 and 2 are known to be co-repressors of the phenylpropanoid pathway in *A. thaliana* (Zhou et al., 2017). Their repression in sugar beet probably coincides with the activation of phenylpropanoid pathway. Flavonoid metabolism genes were further classified using MapMan4 (Supplementary Figure S10). Most of them were up-regulated, mainly in the presence of BNYVV, including two *chalcone synthases*, a *chalcone isomerase*, a *shikimate O-hydroxycinnamoyltransferase* and five *aureusidin synthases*. The activation of the flavonoid pathway and the accumulation of flavonoids was already documented in the rhizomania pathosystem (Fernando Gil et al., 2020; Webb et al., 2020) and during gall formation by *P. brassicae* on *A. thaliana* (Päsold et al., 2010). As observed here with *P. betae* and the BNYVV, biosynthetic pathways downstream of the shikimic acid were previously reported to be up-regulated by *P. brassicae*, including *chalcone synthases*. These observations could be related to the accumulation of antioxidants and antimicrobials (Dao et al., 2011; Irani et al., 2018). Overexpression of *aureusidin synthases* is probably involved a plant reaction against BNYVV, as aurones have been shown to inhibit viral replication (Haudecoeur et al., 2011). Other sugar beet genes encoding antimicrobial proteins were influenced, such as a polygalacturonase-inhibiting protein (PGIP), up-regulated solely in PBBN, suggesting that the plant is trying to counteract cell wall degrading enzymes (Kalunke et al., 2015). Two antiviral protein alpha genes were down-regulated in PB and PBBN. Their down-regulation could benefit BNYVV.

Table V-2. DEGs belonging to pathogenesis-related proteins encoding genes (PR and PR-like) in response to *P. betae* +/- BNYVV inoculation (list of genes detailed in Supplementary Table S10). Significant up- (up) and down-regulation (down) in comparison to mock-inoculated plants are summarized. PR and PR-like were identified through tBLASTn searches on *B. vulgaris* refseq_rna (E-value < e^{-6}). C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV.

PR families		PBvsC			PBBNvsC		
Acronym	Total number	down	up	Total	down	up	Total
PR-1	15	1	2	3	1	7	8
PR-2	36	0	0	0	0	7	7
PR-3	10	0	0	0	0	5	5
PR-4	2	0	0	0	0	1	1
PR-5	20	1	1	2	2	6	8
PR-6	2	0	1	1	1	1	2
PR-7	22	1	0	1	2	1	3
PR-8	9	0	1	1	0	3	3
PR-9	83	10	7	17	7	13	20
PR-10	8	0	3	3	0	4	4
PR-11	3	0	0	0	0	2	2
PR-12	2	0	0	0	0	1	1
PR-13	0	0	0	0	0	0	0
PR-14	4	0	0	0	1	0	1
PR-15-16	52	2	9	11	3	19	22
PR-17	3	0	1	1	0	1	1
Total	271	15	25	40	17	71	88

f. Differentially expressed genes involved in pathogen detection and plant defense signaling

Resistance Genes Analogues (RGAs) and transcriptions factors were systematically monitored to further evaluate the regulation of genes putatively involved in pathogen recognition and signaling (Table V-3 & Supplementary Figure S11). The expression of 644 RGAs genes were recorded in this study. When *P. betae* was inoculated alone, only four of these were significantly up-regulated compared to 58 down-regulated. However, 30 RGAs were significantly induced (against 49 repressed) when *P. betae* was associated with BNYVV, including genes encoding eight membrane Receptor-Like Kinases (RLK, LRR-TM-STK), 14 cytosolic serine-threonine kinases (Pto, cytosolic STK), four Receptor-Like Proteins (RLP, LRR-TM) and one CNL (CC-NBS-LRR). The *brassinosteroid insensitive 1 – associated receptor kinase* (*BAK1*) was significantly up-regulated solely in the presence of BNYVV. Interestingly, some genes belonging to the

WAK/WAKL protein kinase family were repressed in the presence of *P. betae* alone. WAKs are thought to bind to pectin and could possibly have a role of cell wall “sensors” (Kohorn and Kohorn, 2012). Genes encoding damage elicitor peptide (proPEP) and one transcription factor involved in symbiosis signaling (ERN1) were also strongly down-regulated in plants infected with *P. betae* alone. The global repression of RGAs with *P. betae* alone suggests that the protist would be able to hijack host cell metabolism and that defense signaling pathways repression likely plays a central role. In previous work, we reported some *P. betae* predicted proteins putatively involved in plant defense interference such as ankyrin containing proteins, PR-4 homologs and isochorismatases (Chapter IV). Here, *P. betae* genes encoding one ankyrin rich protein as well as proteases and other hydrolases were up-regulated in the presence of BNYVV (Supplementary Table S7). These proteins could be involved in *P. betae*-*B. vulgaris* interaction through the catabolism of host compounds (Jashni et al., 2015). Their precise localization and action should be further studied.

Table V-3. DEGs belonging to resistance genes analogues (RGAs) in response to *P. betae* +/- BNYVV inoculation. Significant up- (up) and down-regulation (down) in comparison to mock-inoculated plants are summarized. RGAs were identified and classified by Dohm et al. (2014) according to the presence of domains known to be involved in resistance to pathogens: coiled-coil (CC), leucine zipper (LZ), nucleotide-binding site (NBS), leucine rich repeat (LRR), serine-threonine kinase (STK), Toll/Interleukin-1 receptor (TIR) and transmembrane (TM) domains (list of genes and acronyms are detailed in Supplementary Table S11). C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV.

RGA group		PBvsC			PBBNvsC		
Acronym	Nbr gene	down	up	Total	down	up	Total
CNL	27	4	0	4	4	1	5
TNL	1	0	0	0	0	0	0
RLK	128	5	0	5	10	8	18
RLP	45	2	0	2	6	4	10
Pto	335	24	4	28	13	14	27
Ntir	2	1	0	1	0	0	0
Nltir	8	2	0	2	1	0	1
Nlcc	39	8	0	8	7	1	8
Ncc	15	1	0	1	2	1	3
CN	21	5	0	5	3	0	3
NA	23	6	0	6	3	1	4
Total	644	58	4	62	49	30	79

The presence of *P. betae* and BNYVV also influenced the expression of genes encoding transcription factors (TF) (Supplementary Table S12). A summary of up- and down-regulated TF families known to intervene with

CHAPTER V. Interactions between *B. vulgaris*, *P. betae* and BNYVV

plant defenses is presented in Table V-4. Consistent with the results for RGAs and PR genes, TF expression was more strongly affected in plants infected with the virus than with *P. betae* alone. TF from the AP2/ERF, WRKY and bHLH families were particularly influenced when the BNYVV was transmitted. Notably, only down-regulated WRKY were found with *P. betae* alone. TF from the MYB superfamily members also showed distinct regulation patterns depending on the infection with *P. betae* and BNYVV.

Table V-4. DEGs belonging to defense related TF families in response to *P. betae* +/- BNYVV inoculation. Significant up-regulation (up) and down-regulation (down) in comparison to mock-inoculated plants are summarized. TF were classified according to MapMan4. C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV.

Transcription factor	PBvsC			PBBNvsC		
	down	up	Total	down	up	Total
<i>MYB</i>	2	1	3	3	6	9
<i>MYB-related</i>	0	2	2	5	1	6
<i>G2-like GARP</i>	0	1	1	3	0	3
<i>bZIP</i>	2	1	3	4	2	6
<i>TGA</i>	0	0	0	1	0	1
<i>BOP</i>	0	0	0	1	0	1
<i>ERF</i>	1	1	2	0	8	8
<i>DREB</i>	1	0	1	3	2	5
<i>AP2</i>	0	0	0	1	3	4
<i>NAC</i>	2	1	3	5	5	10
<i>WRKY</i>	4	0	4	2	9	11
<i>bHLH</i>	4	1	5	10	5	15
<i>TIFY</i>	0	0	0	1	2	3
<i>TPL/TPR</i>	0	0	0	1	0	1
<i>AFP/NINJA</i>	0	0	0	1	0	1
Total	16	8	24	41	43	84

The hormonal modulation underlying the activation of plant defenses remain ambiguous. Jasmonates synthesis and signal transduction pathways were poorly influenced by inoculation. Salicylic acid (SA) signal transduction and ethylene biosynthesis pathway and response seem to be induced in the presence of BNYVV (Supplementary Figure S7). This is consistent with the activation of multiple *PR-1* and *endochitinase* genes. Notably, four plant *SAMT*, coding for enzymes involved in SA modification to its inactive methyl salicylate form (MeSA), were repressed in the presence of BNYVV and, to a lesser extent with *P. betae* alone. The activation of a *SA-binding protein 2* (*SABP2*) gene, responsible of the conversion MeSA into SA (Forouhar et al., 2005), was also observed in the presence of the virus. It was demonstrated that a secreted methyltransferase produced by *P. brassicae* (*PbBSMT*) contributes to the susceptibility of *A. thaliana* to this plasmodiophorid through metabolism of SA in its methylated volatile form (Ludwig-Müller et al., 2015; Bulman et al., 2018). However, no homolog of *PbBSMT* were found in the *P. betae* draft genome (Decroës et al. 2021). The reduction of SA methylation could induce a progressive local accumulation of SA which would in turn activate defenses in plant roots. Accordingly, the *TGA4* gene, thought to encode a repressor of SA defenses responses (Foley and Singh, 2004), was also repressed. The hypothesis has to be further investigated.

Altogether, it seems that a stronger activation of plant defenses occurred in the presence of BNYVV compared to *P. betae*. Once a certain threshold is reached, the stress induced by plant defense reaction may have a detrimental effect on *P. betae*, forcing the protist to switch to its resting spore lifestage. Supporting this hypothesis, we observed that the induction of plant defenses was maximal after 14 days, at the same time as the switch to the survival stage of *P. betae* that occurred mainly in BNYVV infected plants (see Chapter V-4i). This agrees with early observations that BNYVV pre-infection inhibited subsequent development of *P. betae* in sugar beet (Schlösser, 1990; Prillwitz and Schlösser, 1992). However, it should be noted that faster root invasion by the protist in the presence of BNYVV could also result in a lack of available free cells (not already containing *P. betae* structures) or resources to support further *P. betae* multiplication, which therefore moves to the survival stage.

g. *P. betae* induced genes related to oxidative stress management

As illustrate by KEGG enrichments, *P. betae* inoculation strongly impacted the glutathione metabolism with and without BNYVV. Therefore, DEGs involved in redox regulation in plants were categorized using MapMan4 and visualized on heatmaps (Figure V-3).

The protist infection induced significant up-regulation genes coding for 17 or 15 glutathione S-transferase enzymes (GSTs), in the presence or absence of BNYVV, respectively. Plant GSTs are known to be expressed under (a)biotic stress (Gullner et al., 2018) and GSTs up-regulation has been evidenced in *A. thaliana* upon infection by *P. brassicae* (Irani et al., 2018), suggesting an important role for these enzymes in plant-plasmodiophorid interactions. Interestingly, such massive regulation was not reported in previous RNA-seq studies on BNYVV-plant interactions without the vector (Fan et al., 2014, 2015a; Fernando Gil et al., 2020) but was observed by Schmidlin et al. (2008) on *B. vulgaris* infected with the BNYVV and *P. betae*. This effect on plants could therefore be attributed to *P. betae* and illustrates the importance of considering the vector when studying rhizomania pathosystem. In the current study, two putative GSTs were found in the 20 most expressed *P. betae* genes, further indicating that GST activity is crucial for the protist. The implication of GSTs in *P. betae*-*B. vulgaris* and *P. brassicae*-*B. napus* interactions was already suggested by Mutasa-Gottgens et al. (2000) and Fei et al., (2015), respectively.

Other plant genes related to redox homeostasis encoding glutaredoxins and ascorbate peroxidases were significantly up-regulated by *P. betae* alone or with BNYVV compared to mock-inoculated plants. *Thioredoxin* genes were significantly up-regulated only in plant inoculated with *P. betae* alone. One *NADPH-oxidase* (*Rboh*) gene, involved in H₂O₂ production, was significantly down-regulated by both agents, especially with *P. betae* alone. Interestingly,

P. betae genes possibly linked to oxidative stress response (coding for one catalase, involved in H₂O₂ decomposition, two HSP20 and one AhpC/TSA domain-containing proteins) were up-regulated when BNYVV was transmitted to the plants by *P. betae*. Recently, two effectors of *P. brassicae* were also shown to trigger cell death in association with H₂O₂ accumulation (Chen et al., 2019). As previously proposed, the alleviation of redox-stress-induced apoptosis could be beneficial for the BNYVV (Schmidlin et al., 2008). We therefore postulate that the control of redox homeostasis is essential in *P. betae* - *B. vulgaris* interaction and that the BNYVV could also benefit from this regulation.

CHAPTER V. Interactions between *B. vulgaris*, *P. betae* and BNYVV

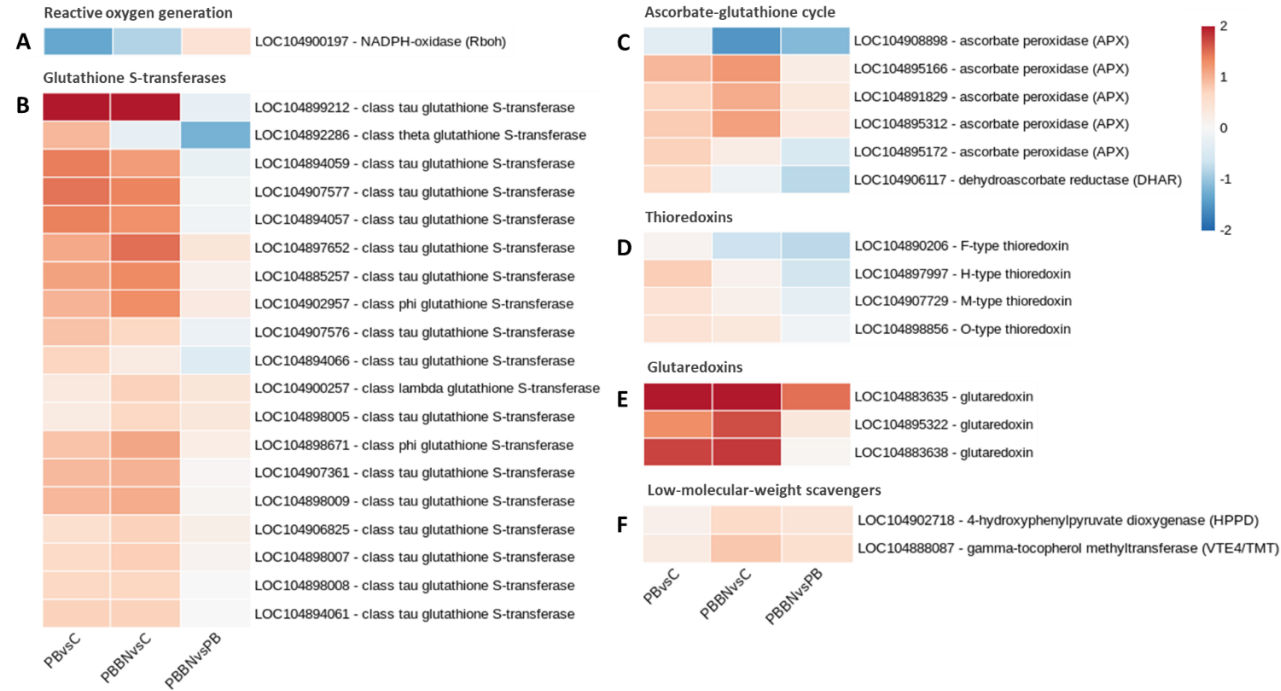


Figure V-3. Redox homeostasis in response to *P. betae* +/- BNYVV inoculation. The heatmaps of DEGs related to reactive oxygen generation (A), glutathione S-transferase activities (B), ascorbate-glutathione cycle (C), thioredoxins (D), glutaredoxins (E) and low-molecular-weight scavengers (F) are shown. Up- and down-regulation are represented in red and blue, respectively. The scale is based on $\log_2$ fold changes. C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV. Genes were classified according to Mercator/MapMan4. Annotations comprised the NCBI Gene symbol and the MapMan4 annotation.

h. Establishment of new physiological sinks in sugar beet roots ?

The infection of *B. vulgaris* by *P. betae* results in the development of plasmodia, sporangia and eventually thick-walled resting spores within host root cells. BNYVV infection leads to an abnormal production of lateral roots and a reduced sugar content in the taproot. Therefore, transcriptomic changes related to a re-organization of carbohydrates metabolism were expected. The set-up of physiological sink tissues was already reported upon attack of host plants by *P. brassicae* and is important for root cells proliferation during gall development (Siemens et al., 2011; Malinowski et al., 2019). In contrast, *P. betae* does not cause galls and our study reveals that no meaningful reprogramming of genes related to carbohydrate metabolism was observed upon infection with *P. betae* alone. On the other hand, clues of glucose shortage appeared in the presence of BNYVV (Supplementary Figure S12). In particular, genes for key enzymes of the glyoxylate cycle, isocitrate lyase (ICL) and malate synthase (MS), were also strongly up-regulated in the presence of *P. betae* and BNYVV. Only MS was significantly affected by virus-free *P. betae*. The activation of glyoxylate cycle generally occurs when complex carbon sources are lacking and allows use of acetate originated from fatty acids (FA) beta-oxidation to produce glucose (Lorenz and Fink, 2002; Fan et al., 2015b). One *SWEET* transporter gene and one sucrose transport protein (*SUT/SUC*) gene were also significantly activated in the presence of the virus. Two genes encoding a cell wall invertase (CWIN) and a vacuolar invertase (VIN) were slightly induced only in the presence of BNYVV in comparison to mock-inoculated plants. Such regulation was previously reported as enhancing sugar supply from the phloem during the establishment of sink tissues (Tauzin and Giardina, 2014) and could be linked to lateral roots proliferation (Sergeeva et al., 2006). This effect may also contribute to the reduced sugar content in diseased *B. vulgaris* taproots (Mcgrann et al., 2009; Nieberl et al., 2017).

Lipid metabolism was also suspected to be influenced by infection. A recent metabolome profiling study showing a modulation of *B. vulgaris* root lipid composition following BNYVV and *P. betae* inoculation (Webb et al., 2020). Moreover the induction of several plant FA synthesis and elongation-related genes was also reported in *A. thaliana* roots infected with *P. brassicae* (Irani et al., 2018). Accordingly, our results reveal that four and seven genes involved in fatty acid (FA) synthesis were slightly induced in plants infected with *P. betae* alone and in the presence of BNYVV, respectively, whereas none was significantly repressed (Supplementary Figure S13). Conversely, most DEGs involved in complex lipid synthesis such as glycerolipids and phytosterols synthesis were repressed, especially when BNYVV was present. Five phospholipase-related genes were significantly down-regulated in the presence of *P. betae* alone, whereas five were down-regulated (*PI-PLC* and *PLD-epsilon* in common) and two were up-regulated in plants inoculated with virus-transmitting zoospores. One lipid droplets (LD) biogenesis factor gene (*SEIPIN*) was also strongly repressed, only in the

presence of BNYVV. The repression of this gene can be linked to the fact that the mean fluorescence of plant root cells (free of *P. betae* structures) stained the LD specific LD540 lipophilic fluorescent dye appeared higher in mock-inoculated plants than in plants infected with *P. betae* and BNYVV (Figure V-4A). Meanwhile, a considerable number of LD were observed by confocal microscopy in all *P. betae* structures, especially sporangia (Figure V-4B) and the up-regulation of some *P. betae* genes putatively involved in lipid metabolism (predicted to code for two lipases and two apolipoprotein domain-containing proteins) was evidenced in the presence of BNYVV (Supplementary Table S7). The LD540 fluorescent dye appeared highly efficient to specifically label *P. betae* lipid droplets and could be tested with other plasmodiophorids. Interestingly, LD organization seems to differ between *P. betae* life stages, being denser in sporangia and sporosori (Figure V-4B: e-h) than in earlier life stages (Figure V-4B: a-c). The high content in LD is possibly linked to energy storage in *P. betae*, that could be mobilized for membrane extension or in case of energy/carbohydrates shortage, as proposed for *P. brassicae* (Bi et al., 2016; Irani et al., 2019). In the latter, transcriptomic changes associated with lipid metabolism were also observed during resting spore formation (Schwelm et al., 2015b; Bi et al., 2016). Here, the regulation of lipid metabolism-related genes in the plant and in *P. betae* coincides with a faster *P. betae* cycle, suggesting that similar mechanisms could occur in both plasmodiophorids. Whether *P. betae* is directly able to use host derived FA or mainly produce its own from nutrients absorbed from host cell remains a mystery. Unlike *P. brassicae* which probably depends on the plant for FA synthesis since its genome lacks fatty acid synthase genes (Schwelm et al., 2015b), five putative fatty acid synthase genes were found in the *P. betae* transcriptome. These genes are putatively involved in the lipid metabolic pathways.

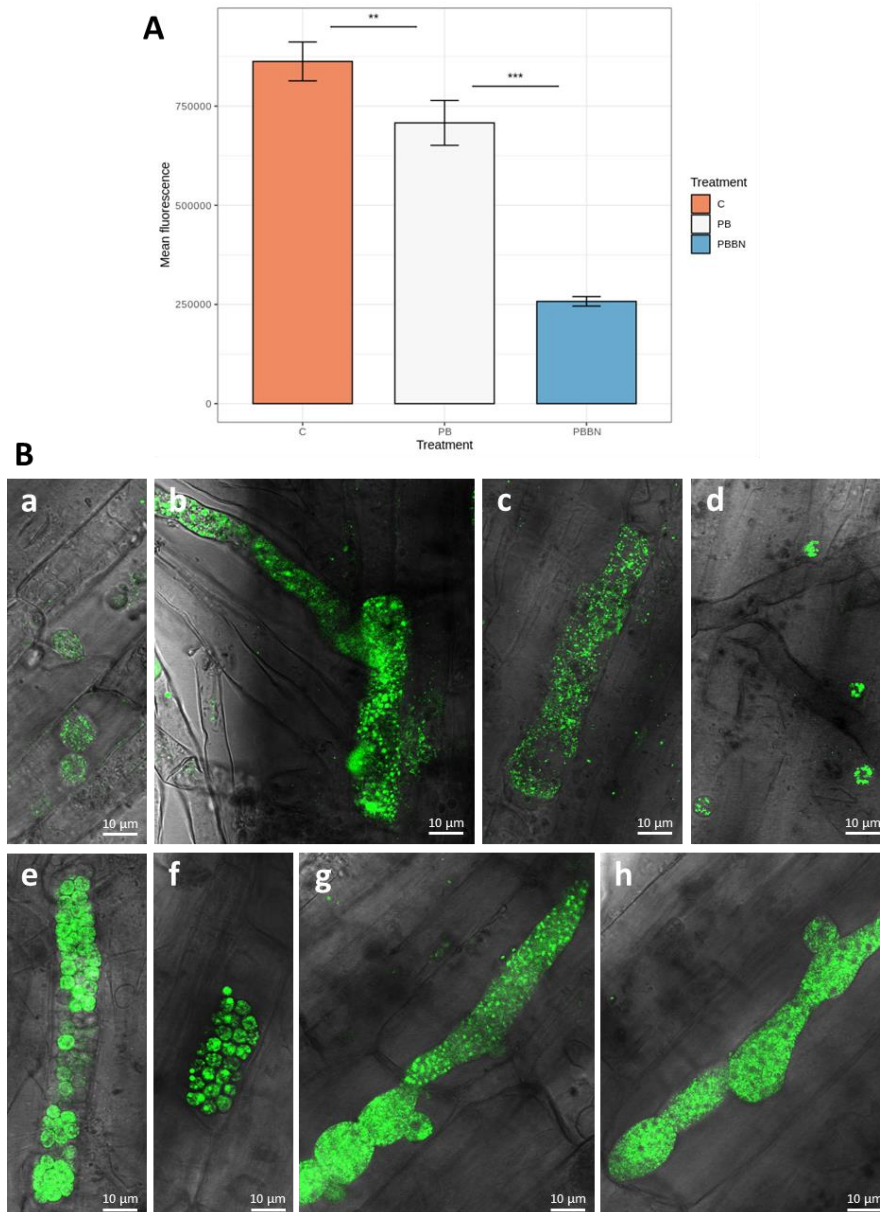


Figure V-4. Barplot of the mean fluorescence derived from LD in *B. vulgaris* (A) and representative images of LD content observed in various *P. betae* life stages, without BNYYV (B). Roots were stained *in vivo* with the lipid droplet-specific dye LD540 and observed with a LSM710 confocal microscope. Data in A represent the means $\pm$ SD (n=4) and significantly different levels were determined by t-test (** for $P \leq 0.01$ and *** for $P \leq 0.001$). C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYYV. *P. betae* structures visible in B are meronts (a) plasmodia (b, c), encysted secondary zoospores (d), sporosori (e, f) and zoosporangia (g, h).

i. Expression dynamics of selected sugar beet genes at four-time points after the infection by PB and PBBN

The expression dynamics of selected genes involved in oxidative stress regulation and plant defenses was monitored 1, 7, 14 and 28 days after the inoculation with PB with and without BNYVV in an independent assay (Figure V-5). Results showed a dynamic plant response depending on genes and treatments (presence or not of *P. betae* +/- BNYVV). Only a down-regulation of one of these genes (one peroxidase: *BvPER44*) was observed after 24 hours but a significant up-regulation of most of the tested genes was initiated at 7 days, which peaked after 14 days in the presence of BNYVV and decreased later. Results obtained by qRT-PCR in this assay followed a similar trend than the results obtained in the RNA-seq analysis (e.g. *BvPR1a*, *BvGSTU*, *BvTLP1*, *BvSE2*, *BvRGA3*) with some exceptions (e.g. *BvAPX3*, *BvGRXS9*, *BvPER44*), possibly associated with environmental variations between both assays. Interestingly, the expression of *Rboh* and oxidative stress response genes were systematically lower in plants inoculated with *P. betae* alone than with the virus-transmitting zoospores. The same trend was observed with the selected PR (*BvPR1a*, *BvTLP1*, *BvSE2*). This confirms that the presence of BNYVV is associated with a stronger activation of plant defenses, even in time points preceding the RNA-seq analysis.

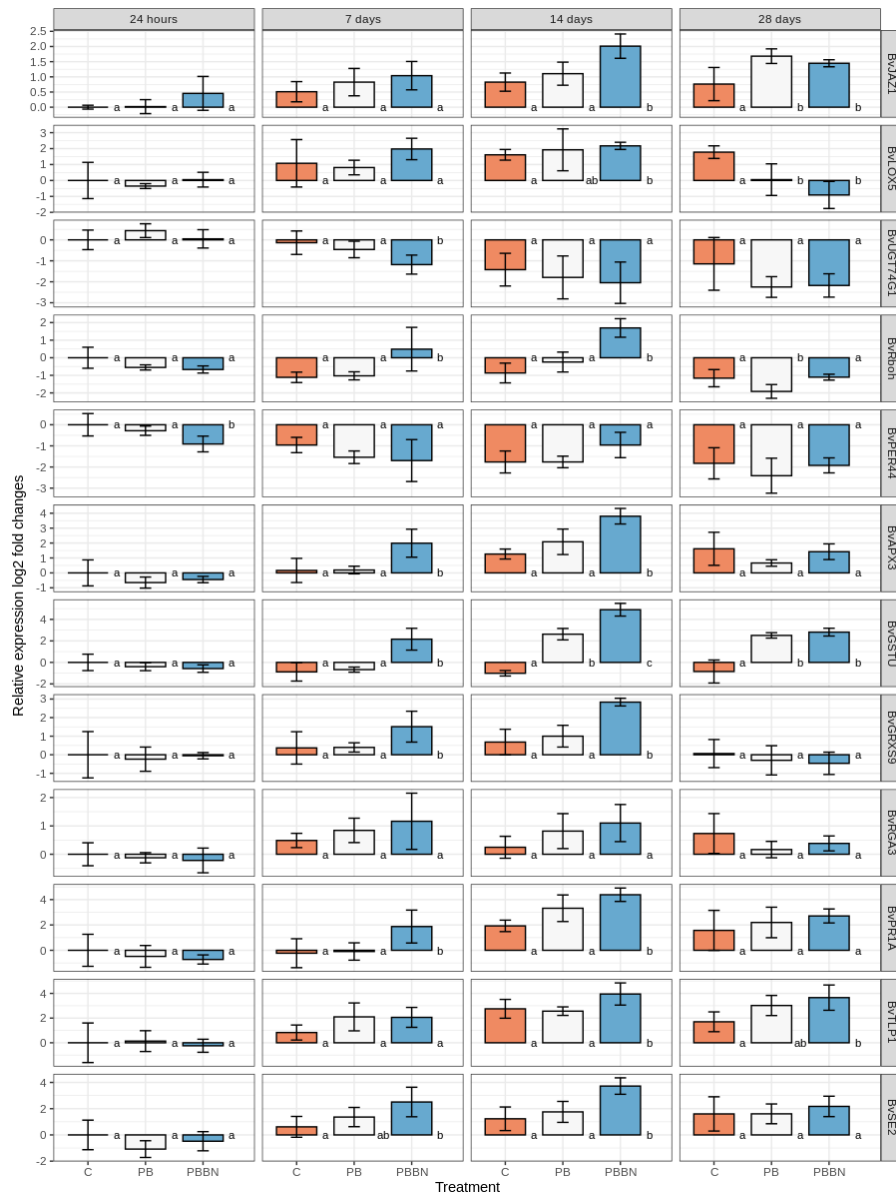


Figure V-5. RT-qPCR expression quantification of five genes related to oxidative stress regulation (*BvRboh*, *BvPER44*, *BvAPX3*, *BvGSTU8*, *BvGRXS9*) and seven plant defenses genes (*BvRGA3*, *BvPR1A*, *BvTLP1*, *BvSE2*, *BvJAZ1*, *BvLOX5*, *BvUGT74G1*) at 1, 7, 14 and dpi. Details about targeted gene are available in Supplementary table S3. Data represent the means $\pm$ SD and significantly different levels for each gene at each time point are indicated by different lower-case letters. C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV.

5. CONCLUSIONS

In conclusion, a soil-free BNYVV transmission protocol was successfully developed and implemented using infectious clones of the virus and completely sequenced *P. betae* isolate and *B. vulgaris* line. This method could definitively be useful for further research on rhizomania. At a biological level, this study shows that the BNYVV hastened the early invasion of *B. vulgaris* roots by *P. betae*, between 1 and 14 dpi. Then the protist entered into the resting spore stage earlier in the presence of BNYVV than when it was alone. The susceptible interaction of non-viruliferous *P. betae* with its host resulted in reduced transcriptomic changes in the plant in comparison with the changes previously reported in *P. brassicae*-plant transcriptome analyses. Notably, *P. betae* alleviates the defense response as shown by the down-regulation of defense related genes belonging to RGAs, TF and cell wall peroxidase families and the up-regulation of genes involved in redox homeostasis such as GSTs. The lack of plant defense reaction may explain why *P. betae* does not induce obvious symptoms. In that view, the interaction between the plant and the protist can be described as commensalism. However, the effect of BNYVV transmission by *P. betae* on the expression of plant genes related to defense signaling pathways as well as to PR and phenolic compounds synthesis was somewhat stronger than the one of *P. betae* alone. The induction of plant defenses by BNYVV could result in an increased stress for *P. betae*, eventually leading to the induction of the resting spore stage. Nevertheless, we cannot rule out the possibility that the switch to the survival stage could be induced by the lack of available space or resources in the BNYVV infected roots, such as FA and carbohydrates. Moreover, the virus also impacted genes involved in plant cell walls synthesis/modification which could be profitable for *P. betae* and may explain the faster early multiplication in the roots. This faster development could also be due to the effect of BNYVV suppressors of RNA silencing on the plant, which could also benefit the protist.

The up- and down-regulation of a great numbers of genes were highlighted in this study revealing that a lot of pathways are involved in the interaction between *P. betae* and the sugar beet or between *P. betae*, BNYVV and the sugar beet. At this stage, the interpretations drawn based on our transcriptomic approach were made at a single time point in a single compatible plant-virus-vector interaction. The expression changes of plant genes measured by RT-qPCR at three additional time points revealed that gene expression in the rhizomania pathosystem is derived from a highly dynamic process. Further transcriptomic investigations covering the entire infection process, along with proteomic and/or metabolomic research will be useful for deepening interaction studies.

Availability of data and materials

Data will be deposited at European Nucleotide Archive (ENA)

Funding

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GENERAL DISCUSSION, CONCLUSIONS AND PERSPECTIVES

CHAPTER VI.

General discussion

Most research on rhizomania focused on plant-BNYVV interactions without considering *P. betae*. The scarce studies specifically oriented toward BNYVV-*P. betae* interaction comprehension mostly aimed to find viral genomic components involved in transmission. The main goal of this project was to investigate the molecular interactions between the three actors of rhizomania, as a first step to better characterize their biology. To achieve this goal, a toolbox consisting of the first *P. betae* genome as well as to a protocol allowing controlled BNYVV transmission through *P. betae* zoospore to sugar beet was developed. In Chapter IV, we reported the sequencing, assembling and annotation of the first two draft genomes of *P. betae*. We showed that these genomes were very similar, despite the fact that DNA was extracted from distinct life stages of *P. betae* isolates from different geographical areas. Predicted proteins were then further analyzed in order to establish computational membranomes and secretomes. These first annotated genomes were then evaluated in a comparative study with the plasmodiophorids *Plasmodiophora brassicae* and *Spongospora subterranea*, the closest relative species already sequenced. Afterward we took advantage of the fully sequenced A26-41 monosporosorus *P. betae* isolate, BNYVV cDNA clones and the fully sequenced KWS2320 *Beta vulgaris* doubled haploid line to perform a transcriptomic analysis of the tripartite interactions in the rhizomania pathosystem. This work was performed in a soil-free transmission system, previously optimized in the lab. The results are presented and discussed in Chapter V.

Here, we integrate genomic and transcriptomics results together with current knowledge on rhizomania and highlight the contribution of the present work to the advancement of knowledge in the research on rhizomania. In particular, we formulate new hypotheses on the interactions of *P. betae* with sugar beet immune system. We also try to clarify the status of *P. betae* in sugar beet: is it an ancient pathogen? Afterward, we discuss the advantages and disadvantages of the BNYVV-*P. betae* interaction in sugar beet and try to answer the question whether the two organisms are involved in a mutually beneficial interaction. Finally, we state the main conclusions of this work. The toolbox developed in this thesis provides new research perspectives on the interactions of *Polymyxa* spp. with plants and viruses. It also extends the genomic knowledge about rhizarian protists and open the way for *P. graminis* genome sequencing.

1. *Defense response of Beta vulgaris to Polymyxa betae infection*

a. Main results

The present work aimed at studying the transcriptomic response of the sugar beet to *P. betae*. This analysis was performed 28 days after inoculation (dpi) with zoospores to ensure that sugar beet roots are heavily infected with the protist. As illustrated by the visual observations presented in Chapter IV, the rate of infection was high and the proportion of root cells containing sporosori and, to a lesser extent, sporangia observed in plants inoculated with *P. betae* was higher at 28 dpi than at 14 dpi. Upon infection, the cycle of *P. betae*, detailed in Chapter I, starts by an active growing phase where multinucleate plasmodia invade the host cell. These plasmodia differentiate to sporangia (sporangial phase, involved in dispersion) or in sporosori (sporogenic phase, involved in survival). Usually the sporangial phase occurs during the active colonization of the roots and the sporogenic phase is observed latter. However, the determinants driving the appearance of each stage are still unknown and both phases greatly overlap in infected roots (Braselton, 1995; Tamada and Asher, 2016b). In our experiments, at 28 dpi, the cycle of *P. betae* was thus globally at an advanced stage during which the induction of sporogenic phase was already initiated. At that time, the influence of *P. betae* on the sugar beet transcriptome was relatively limited, mainly involving down regulations of genes. Despite *P. betae* transcripts representing about 30% of the RNA extracted from infected roots, the presence of *P. betae* alone didn't trigger a much of defense reaction in sugar beet at 28 dpi, as illustrated by the overall down-regulation of Resistance Genes Analogues (RGAs), defense-related transcription factors from several families (WRKY, bHLH, ...) and by the weak impact on genes encoding most PR protein families. On the other hand, plant genes involved in REDOX homeostasis were up-regulated, such as genes encoding glutathione-S-transferases (GSTs), ascorbate peroxidases, thioredoxins and glutaredoxins suggesting that the plant is trying to mitigate oxidative stress. Moreover, genes involved in cellulose, hemicellulose and pectin synthesis as well as cell wall peroxidases were repressed by infection, as were some genes encoding cell wall glycoproteins and extensins (EXTs). Finally, the systemic acquired resistance (SAR) signal, methyl salicylate (MeSA), seems impaired in a well-established *P. betae* infection in sugar beet.

b. The control of oxidative stress in sugar beet-*Polymyxa betae* compatible interaction

As discussed in Chapter II, very few works have been addressing the molecular interactions between *P. betae* and plants. An previous work performed with *P. betae* A26-41 isolate, the same as the one we used in this work, on *B. vulgaris* subsp. *vulgaris* var. Cadyx revealed interesting results

(Desoignies, 2012). In that study, the authors hypothesized that the induction of reactive oxygen species (ROS) production (and thus oxidative stress) occurs early after infection by *P. betae*, which in turn induces the systemic defenses (i.e. SAR) in infected plants. The activation of SAR would be responsible for the better protection of the sugar beet against a foliar fungal pathogen, *Cercospora beticola*, causing the Cercospora Leaf Spot disease (CLS).

The analyzes of the Expressed Sequence Tags (EST) produced during early infection of sugar beet by *P. betae* led Desoignies (2012) to propose that the plant is reacting to oxidative stress deriving from ROS production occurring soon after infection, notably by the induction of a thioredoxin gene. Our results also highlighted the up-regulation thioredoxin (*TRX*) genes supporting the results of Desoignies (2012). Moreover, genes related to the glutathione cycle encompassing ascorbate peroxidases (*APXs*), glutaredoxins (*GRXs*), and a high number of *GSTs* were highlighted. *GSTs* are involved in the detoxification of toxic substances, the attenuation of oxidative stress and are known to participate to plant-pathogen interactions (Gullner et al., 2018). The regulation of *GSTs* was also shown to be involved in the interaction of plants with non-pathogenic, symbiotic bacteria and fungi living in the rhizosphere (Gullner et al., 2018). At 28 dpi, similar levels of induction of *APXs*, *GRXs*, *GSTs* and *TRXs* were observed when *P. betae* was inoculated alone or co-inoculated with BNYVV, whereas a clearly stronger defense reaction occurred in the presence BNYVV, as shown by the greater induction of *PR* genes. Moreover, Fernando Gil et al. (2020) didn't report such regulation of *GSTs* when analyzing the transcriptome of sugar beet infected with the BNYVV without *P. betae*. Therefore, the regulation of genes involved in redox homeostasis such as *GSTs* appears, at this stage, more likely related to the presence of *P. betae* than to the level of biotic stress experienced by the sugar beet roots. As proposed by Desoignies (2012), it could be a reaction of the sugar beet to the oxidative stress related to excessive ROS accumulation induced by *P. betae* infection. Another hypothesis would be that *P. betae* is able to manipulate the sugar beet to avoid the induction of oxidative stress. Scavenging of ROS and host cytoskeleton targeting is indeed an essential mechanism to maintain symbiosis balance (Kogel et al., 2006). *P. betae* is able to release zoospores inside the roots of sugar beets in order to colonize neighboring cells. Alternatively, a cell-to-cell myxamoeboid movement of the protist could also be possible as observed in both *P. brassicae* and *H. nasturtii* (Mithen and Magrath, 1992; Claxton et al., 1996), probably in the vicinity of plasmodesmata. Both phenomena are thought to rely on cell-wall degrading enzymes such as polygalacturonases and pectate lyases. Derivatives of cell wall degradation, or Damage-Associated Molecular Patterns (DAMPs), are known to trigger the production of ROS, the activation MAPKs, the deposition of callose, the accumulation of phytoalexins, and the expression of defense-related genes (Hou et al., 2019). Hence, it could be important for the protist to "prepare the ground" for further colonization of the roots. In this respect, it seems useful for *P. betae* to avoid the propagation of oxidative stress in roots cells adjacent to infected cells. Accordingly, the expression a

NADPH-oxidase gene (*Rboh*), involved in H₂O₂ production, was significantly reduced by the infection. Similarly, the *proPEP* gene, involved in the amplification of defense signaling, was also decreased. Moreover, a malondialdehyde (MDA) dosage was performed at 28 days after the infection of 3-weeks-old sugar beets with *P. betae* zoospores carrying or not the BNYVV. Reduced MDA levels were obtained in roots infected with *P. betae* (both with and without the BNYVV) in comparison to mock-inoculated plants (data not shown). This would indicate a reduced peroxidation of membranes, probably associated with a lower ROS production or better ROS-scavenging ability in the presence of the protist. One of the first genes discovered in *P. betae* was a *GST*. The constitutive expression of this *GST* during infection was reported, suggesting the importance of oxidative stress control of the protist (Mutasa-Gottgens et al., 2000; Kingsnorth et al., 2003b, 2003a). Similarly, we observed the high expression of two *P. betae* *GST* in our assays, being in the TOP20 of the most expressed genes. This reinforces the idea that *P. betae* is trying to avoid deleterious effects from oxidative stress and/or toxic compounds.

c. *Polymyxa betae* interaction with plant immunity

Overall, a weak defense reaction of sugar beet against *P. betae* alone was observed in roots heavily colonized by sporangial and sporogenic phases in our study. This confirmed the observations of Desoignies et al. (2014), which showed that plant defenses were more strongly activated during early infection and plasmodial growth than once sporosori were produced. The dynamic monitoring of the expression of genes involved in oxidative stress homeostasis and plant defenses (Chapter V) confirmed that a stronger defense induction probably occurred earlier, when the sporogenic phase was barely observed.

The plant defenses against a pathogen attack could basically be considered as three successive barriers. The first one is physical, consisting in the cell wall, cuticle which could be strengthened in case of aggression (e.g. by deposition of callose, lignin, waxy and phenolic compounds). *Polymyxa* spp. zoospores are able to cross this first line of defense due to their unique infectious process relying on a needle-like structure, the “Stachel”, to quickly break plant cell walls and inject their cytoplasm. It was demonstrated that *P. betae* zoospores are attracted and encysted in a similar way in susceptible and resistant *Beta* species even though sporosori production was compromised in resistant species (Barr et al., 1995). Similarly, the molecular detection of *P. betae* and *P. graminis* in quite a large diversity of plants inoculated in laboratory condition was possible, including plants for which no significant colonization could be observed by microscopic observations (Legrève et al., 2005b). The outcome of the plant-*Polymyxa* interaction (compatible or not) should therefore be determined after the initial penetration through the cell wall. The second line of plant defense is triggered by the recognition of pathogen infection and results in the induction of a non-specific defense response called PAMPs triggered immunity (PTI).

This response is characterized by the production of PR proteins and antimicrobial compounds as well as the induction of systemic defenses such as SAR. The early transcriptomic reactions (i.e. during zoospore attachment and root cell penetration) that arose in host and non-host interaction of sugar beet and barley with *P. betae* and *P. graminis* were previously monitored (McGrann et al., 2007, 2009). It was found that both *P. betae* and *P. graminis* induced the same basal response in barley, comparable to the defense induced by PAMPs (McGrann et al., 2009). Genes encoding PR proteins (PR-1a, PR-5, cysteine proteases, a chitinase, lipid transfer proteins, thionins) as well as genes encoding xyloglucan endotransglycosylases (XTH), GST and extensin-like proteins were found among induced transcripts (McGrann et al., 2009). Even though an active non-host reaction was observed in sugar beet inoculated with *P. graminis*, the host and non-host responses were also overlapping (McGrann et al., 2007). These studies strongly suggest that the PTI is activated at the beginning of both host and non-host interactions with *Polymyxa* spp. but is not sufficient to limit the protist development. Some of the defense-related transcriptomic changes (e.g. up-regulation of genes encoding thionins, lipid transfer protein and PR-10 proteins) observed during early plasmodial growth and root colonization by Desoignies et al. (2014) and later in this study (e.g. genes encoding PR-1, PR-5, a chitinase and PR-10 proteins) could be related to this basal response, continuously induced in new root cells by infections with secondary zoospores. Large numbers of secondary zoospores are indeed known to be produced as soon as 5-7 days after initial infection (Dahm and Buchenauer, 1993). The establishment or not of *Polymyxa* spp. biotrophy would be initiated in a second time, after successful cell penetration, regardless of the PTI induction. Supporting this hypothesis, we observed the high expression of two *P. betae* GSTs and of a gene coding for ubiquitin-like modifier-activating enzyme, which could be used by the protist to mitigate the effects of the PTI. These enzymes could participate to the protection of the protist against plant-antimicrobial peptides, toxic metabolites and oxidative stress, as shown for GSTs produced by some bacteria and fungi (Gullner et al., 2018). Successful pathogens are able to overcome the second defense barrier by the secretion of effectors, leading to a compatible interaction called effector triggered susceptibility (ETS). The effectors are small proteins directly injected in the host cell with the aim to manipulate the host defense and hijack host metabolism. For an intracellular parasite such as *P. betae*, it would be surprising if the secretion of such molecules were not involved in compatible interactions (discussed below). Some plants have evolved to be able to specifically recognize the effectors produced by their aggressors with cytoplasmic receptors (NLRs) and induce a third line of defense: it is the effector triggered immunity (ETI). In the case of an intracellular parasite such as *Polymyxa*, any protein anchored in the plasmalemma with an extracellular domain or secreted outside the plasmodium is possibly in direct contact with the host cytoplasm (at least in the early life stages) and could be recognized by the plant, enlarging the pool of possible plant targets. Hence, the outcome of compatible or non-compatible interaction could be determined by this third barrier (ETS or ETI).

A rapid hypersensitive response could restrict further parasitic development in resistant interaction (McGrann et al., 2009).

d. The *Polymyxa betae* strategy for bypassing plant defenses

When challenged by a pathogen, plant defense signaling pathways are activated in which the production of phytohormones such as Salicylic Acid (SA), Jasmonic Acid (JA) and Ethylene (ET) play key roles (De Vos et al., 2005; Bari and Jones, 2009). The resistance against biotrophic pathogens, is classically mediated by the activation of SA pathway that is often associated with the local and systemic up-regulation of *PR-1*, *PR-2* and *PR-5* signature genes. Conversely, resistance against necrotrophic pathogens relies on the JA/ET pathway that often locally induces *PR-3*, *PR-4* and *PR-12* signature genes (Glazebrook, 2005; Bari and Jones, 2009; Ali et al., 2018). At the time point chosen in our study, when sporosori are actively formed in some root parts, we didn't observe the up-regulation of any PR associated with the JA pathway in plants inoculated with *P. betae* alone in comparison to non-inoculated plants. Only two *PR-1* and one *PR-5* were induced compared to one *PR-1* and one *PR-5* repressed. This is actually a weak reaction in comparison to the response observed in the presence of BNYVV or, more generally, in plant-pathogen interactions (Ali et al., 2018). These up-regulations could be the persistence of an earlier SA response and/or the PTI response of a reduced number of newly infected cells, as zoospores were still produced at analysis time point. Interestingly, the presence of numerous nonviruliferous *P. betae* structures in infected roots didn't result in significant regulation of genes from most PR proteins families. In particular, no β -1,3-glucanase (*PR-2*) and only one chitinase (*PR-3,4,8,11*) were induced. These proteins are known as fungal cell wall degrading enzymes and could be directed against the cell walls of forming sporosori. Moreover, ten peroxidases (*PR-9*), involved in lignification of plant cell walls were down-regulated, along with multiple cell wall synthesis genes. These regulations could be attributed to a specific interaction in which the sugar beet response is probably influenced by *P. betae* effectors. The protist seems to try to avoid the reinforcement of root cell walls to ease its cell-to-cell movement and to avoid excessive ROS accumulation (as discussed above).

The down-regulation of several *RGAs* in sugar beets inoculated with *P. betae* alone supports the hypothesis that the protist tries to avoid plant immunity. Among down-regulated *RGAs* were found genes encoding cell surface receptors (receptor-like kinases [RLK], receptor-like proteins [RLP]) and cytosolic receptors (CC-NBS-LRR, cytosolic serine-threonine kinases [STK] and NBS-LRR variants). All these receptors are organized in complex signaling networks responsible for the integration of multiple signals in the plant cell and activate the appropriate defense output. The origins of these signals are diverse, encompassing cell development, biotic and abiotic stresses (Zhou and Zhang, 2020). In particular, it was shown in *A. thaliana* that the *Cantharanthus roseus* receptor-like kinase-1-like (CrRLK1L) family

RK FERONIA (FER) and Lorelei-like glycosylphosphatidylinositol-anchored proteins (LLGs) co-receptors (Xiao et al., 2019) are involved in cell-wall-integrity sensing (Zhou and Zhang, 2020). The process is mediated by *Arabidopsis* RAPID ALKALINIZATION FACTORS (RALFs) and leucine-rich extensins and involves the interaction with PRR receptors and PRR co-receptors (Dünser et al., 2019; Zhou and Zhang, 2020). In our study, we observed the down-regulation of various leucine-rich extensins genes, *CrRLK1L* and *RLKs/RLPs*. As discussed above, the cell-to-cell movement of *P. betae* inevitably induces the perturbation of cell-wall integrity. Moreover, the extension of plasmodia into the plant cell could also affect mechanical constraints to the cell wall. The repression of the genes mentioned above would likely reduce the capacity of the plant to sense that perturbation, allowing the colonization by *P. betae*. A theoretical model of cell wall integrity sensing complex in sugar beet is presented in Figure VI-1. Our study also revealed the repression of more than 40 cytosolic receptors-like proteins following inoculation with *P. betae* alone. Considering the intracellular lifestyle of the protist, it is likely that these repressions are involved in the compatible sugar beet-*P. betae* interaction. For example, at least two CC-NBS-LRR homologous to the Recognition of *Peronospora parasitica* 13-like (*RPP13*-like) gene family (typical R genes) were found significantly repressed. *RPP13* was previously reported to be involved in the resistance against other biotrophic pathogens such as *Peronospora parasitica* in *A. thaliana* (Bittner-Eddy et al., 2000) and *Blumeria graminis* f. sp. *tritici* in wheat (Liu et al., 2020b).

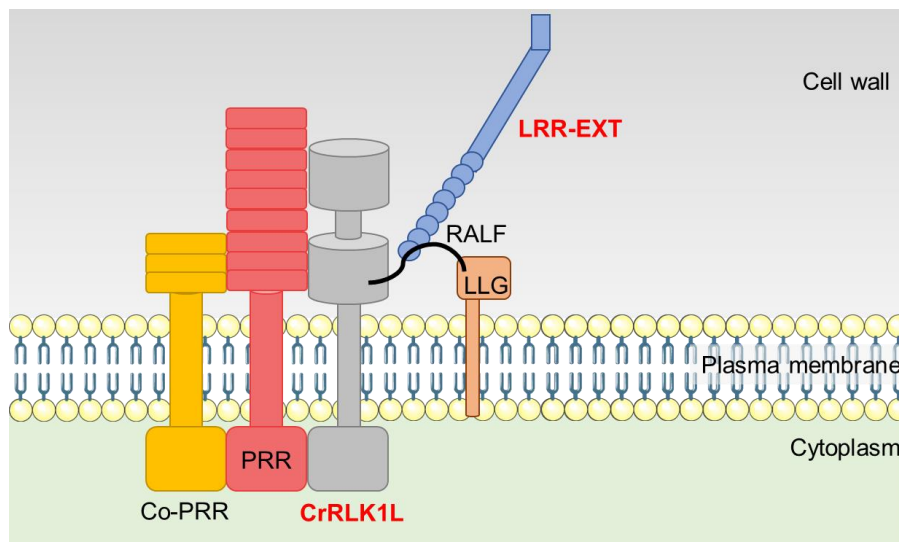


Figure VI-1. Theoretical model of cell-wall-integrity sensing in sugar beet and effect of *P. betae* infection. Names in red are proteins for which encoding genes were found down-regulated by the infection with *P. betae* alone. Figure drawn based on Zhou and Zhang (2000).

As stated above, the PTI seems to be induced during early infection of the sugar beet by *P. betae* but a reduced response signature to the major defense hormone SA was recorded at the time of our RNA-seq analysis. As opposed to the proposition made by Desoignies (2012) we didn't find elements demonstrating the efficient activation of systemic defenses, such as SAR, in sugar beet upon infection by nonviruliferous *P. betae*. In our experimental conditions, we were not able to confirm that root infection by *P. betae* has a protective effect against a foliar pathogenic fungus, *Cercospora beticola* (data not shown). Moreover, the expression of genes related to SA biosynthetic pathway was not influenced by the inoculation in our assays at 28 dpi. However, the down-regulation of genes encoding putative SA methyltransferases (*SAMT*) was observed. These enzymes are able to methylate SA in MeSA, a mobile inactive form. MeSA can quickly move in the plant and be converted back to SA in distant tissues, mediating SAR signal (Park et al., 2007). The repression of this signal could be associated with the absence of activation of SAR in distant root tissues, which thus remains highly susceptible to infection by secondary zoospores. Increased SA levels at local level could be controlled by the glycosylation of the hormone. SA glucose conjugates are an inactive form of SA formed the transfer of glucose from UDP-glucose to SA (George Thompson et al., 2017). Interestingly, increased levels of salicylic acid glycosides were measured by Webb et al. (2020) in plants inoculated with *P. betae* and the BNYVV in comparison to healthy plants.

e. How *Polymyxa betae* could manipulate host defenses?

For a plant pathogen, the secretion of effectors is recognized as a key element in successful infection especially by suppressing plant defense responses (Toruño et al., 2016). It is highly probable that this is also the case for a parasite with an intracellular lifestyle such as *P. betae*. After *in silico* screening steps (described in Chapter IV) we found between 655 and 670 putatively secreted proteins in *P. betae* genomes, among which 104 are predicted to be effectors. However, this tool was optimized for the prediction of fungal and oomycete effectors. These effectors often share some characteristics such as a small size as well as the enrichment in some amino acids (e.g. cysteine) and other features that enable their stability in the apoplast and delivery into plant cells. In the plasmodial stage, the plasmodium membrane can be considered as the exclusive interface between *Polymyxa* cytoplasm and the “exterior”, being the host cytoplasm. Therefore, any molecule secreted outside the plasmodium at this stage will be directly in contact with the host cell and is a candidate effector. We therefore searched the genome of *P. betae* for proteins that match classical features of secreted proteins: the presence of N-terminal signal peptides and the absence of extra transmembrane regions and considered all putatively secreted proteins as potential effectors.

Interestingly, our comparative analysis (Chapter IV) revealed that the “Papain family cysteine protease” Pfam domain as well as “hydrolase

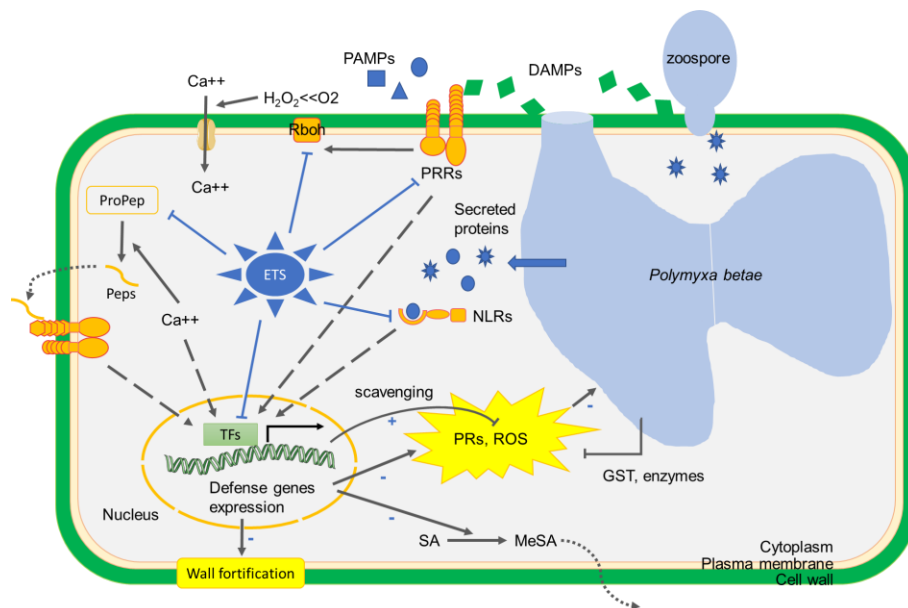
activity” and “peptidase activity” GO terms were enriched in secretomes of all sequenced plasmodiophorids. Protease inhibitors were also found. This result suggests that secretion of enzymes with a proteolytic activity is essential for the establishment of intracellular lifestyle of plasmodiophorids. These enzymes are potentially involved in the protection of the protist against host products but could also interfere with host metabolism, as shown for other plant pathogens (Shindo and Hoorn, 2008). For example, protease inhibitors could target host proteases. The Pfam domains “Cysteine-rich secretory protein superfamily”, “chitin recognition protein” and “polysaccharide deacetylases” were also enriched in all plasmodiophorids secretomes. The secretion of small cysteine-rich proteins effectors by the pathogenic fungus such as *Fusarium graminearum* (Lu and Edwards, 2016), the soil-borne fungi *Verticillium dahliae* (Wang et al., 2020) and the oomycete *Phytophthora cactorum* (Chen et al., 2016b) is known to be involved in specific plant-pathogens interactions. Moreover, one polysaccharide deacetylase of biotrophic fungal pathogen *Puccinia striiformis* f. sp. *tritici* was reported to suppress chitin-induced wheat defense (Xu et al., 2020). Similar results were obtained with *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum* and it was proposed that chitin deacetylation is a major virulence strategy for soil-borne fungal pathogens (Gao et al., 2019). Moreover, Desoignies et al. (2014) and Bulman et al. (2006) also reported the up-regulation of genes coding for polysaccharide deacetylases in *P. betae* and *P. brassicae*, respectively. These authors hypothesized that this enzyme could be responsible for parietal degradations during the protist cell-to-cell movement. Secreted proteins bearing these domains could therefore act as plasmodiophorid effectors in their respective hosts. Besides protein predicted to be secreted by the “conventional pathway”, some effectors could be secreted through an “unconventional pathway”. For example, the genome of *P. betae* encodes putative effectors encoding homologues to known isochorismatases. Secreted isochorismatases effectors are used by other root pathogens *Verticillium dahliae* and *Phytophthora sojae* to suppress salicylate pathway through hydrolyzation of the isochorismate precursor of SA, notably in potato (Liu et al., 2014a; Zhu et al., 2017). Another biotrophic pathogen, *U. maydis*, secretes a chorismate mutase (Cmu1) into the host cytoplasm to prevent SA synthesis. Interestingly, Cmu1 could move to manipulate adjacent cell in order to prepare further *Ustilago maydis* infection (Djamei et al., 2011). The interaction with SA pathway is key in plasmodiophorids infection. It was indeed previously reported that the manipulation of the salicylic acid pathway using a secreted SA methyltransferase, PbBSMT, is necessary for *P. brassicae* virulence (Ludwig-Müller et al., 2015; Bulman et al., 2018; Djavaheri et al., 2018b). It has also been established that the activation of SA pathway is involved in host resistance/tolerance against *P. brassicae* (Agarwal et al., 2011; Lovelock et al., 2013; Ji et al., 2021) and *S. subterranea* (Lekota et al., 2019).

As mentioned above, a lot of genes encoding secreted proteins predicted in *P. betae*, *P. brassicae* and *S. subterranea* genomes are not shared by the three species. This is for example the case of *PbBSMT* and for the recently

characterized *PBZF1*, a *P. brassicae* effector involved in the control of host disease resistance (Chen et al., 2021). Neither *PbBSMT* nor *PBZF1* homologous genes were detected in *P. betae* genome (BLASTP E-value < e^{-6}). However, in this work, PR-1-like, PR-4-like, PR-5-like homologues were found in both *P. betae* isolates predicted secretomes. In particular PR-4-like have no orthologs in *P. brassicae* and *S. subterranea* secretomes. As discussed in Chapter IV, PR proteins recognition by plants was shown to regulate plant defenses. For instance, PR4-LRR1 interaction in pepper resulted in suppression of cell death and defense responses (Hwang et al., 2014). The secretion of pathogen-derived PR proteins could therefore be used as a strategy to suppress host defenses. As observed in our comparative study and already observed by Schwelm et al. (2015b), the plasmodiophorid genomes and secretomes are enriched in genes encoding proteins which harbors ankyrin-repeat domains (Anks). The high expression of ankyrin-rich putative effectors was further observed during primary and secondary infection with *P. brassicae* (Chen et al., 2019; Pérez-López et al., 2020). Interestingly, some ankyrin-rich proteins were shown to be involved in the establishment of intracellular parasitic lifestyle of some bacteria, as discussed in chapter IV. Here a lower enrichment in *Anks* was observed in *P. betae* secretome than in *P. brassicae* and *S. subterranea* secretome. However, these proteins could still be important for *P. betae* infection. To conclude, we highlighted candidate genes putatively involved in *P. betae*-sugar beet interaction. However, our results are only based on informatic predictions and would deserve experimental functional characterization.

f. Summarized scheme

A recapitulated scheme of *P. betae*-plant interactions is presented in Figure VI-2. Early events after infection, such as cell wall and/or membrane destabilization and recognition of DAMPS/PAMPS by the sugar beet could trigger a PTI. These early events explain the activation of PR genes and other stress or defense related genes. However, the reaction is not sufficient to abort *P. betae* invasion, due to its own protection mechanisms. In a second phase, when zoosporangial plasmodia are maturing, *P. betae* would secrete effectors to manipulate host response. At a local level, the protist would potentiate discrete cell-to-cell movement through cell walls by hiding from the immune system and altering wall reinforcement. At a systemic level, the suppression of SAR systemic signal would avoid the potentiation of non-infected part of the root system against new infections by swimming secondary zoospores released in the soil water.



2. *Is Polymyxa betae an ancient pathogen?*

As described in Chapter I, *P. betae* is not widely considered as a pathogen since its infection is generally asymptomatic. However, it should be noted that there are a few studies that report deleterious effect induced *P. betae* on its host. Barr (1979) reported some cases, in which *P. betae* appeared to be a harmful pathogen on *S. oleracea* and *C. album*. Similarly, Gerik and Duffus (1988) observed a reduction of top plant weight in sugar beet, two months after infection with six nonviruliferous North American *P. betae* isolates. However, a negative impact attributed to nonviruliferous *P. betae* infection on sugar beet yields in the field was not reported. When *P. betae* transmits BNYVV, it can reasonably be considered as part of rhizomania pathobiome. Rhizomania is associated with the appearance of obvious symptoms, such as the proliferation of lateral roots (PO:0020121), and yield lost. The induction of lateral roots production (cell proliferation and enlargement) in the presence of BNYVV is expected to be associated with an increase of carbohydrate consumption in affected roots. Our results confirmed the hypothesis that the infection with BNYVV-transmitting *P. betae* induces additional energy sinks in sugar beet roots, whereas the impact of *P. betae* seems limited. Moreover, the absence of clear symptoms in the absence of the virus may indicate that the sugar beet benefits from the interaction, even though the nature of this benefit remains a mystery. The absence of adverse effects on sugar beet leads us to propose that the relationship may be considered as commensal. The commensal interactions can be evolved from a pathogenic interaction. Therefore, one can wonder if it is the case for *P. betae*.

Putatively secreted proteins are encoded by *P. betae* genome and could be considered as potential effectors (see above). If some of them share characteristics with some known fungal and oomycetes effectors, a larger proportion was not shared among plasmodiophorids and were poorly annotated by homology searches against EggNOG and InterPro databases. This reflects the unique evolutionary status of plasmodiophorids but also gives some novel insights into *P. betae* lifestyle. The absence of homology with known proteins suggests that these proteins i) are not essential for the protist primary metabolism, ii) that these underwent high selective pressure. This is typically the case for effectors involved in a highly specialized interaction, such as the one observed in host-biotrophic pathogen (Ökmen and Doehlemann, 2014). As stated in the general introduction, three special forms of *P. betae* were described based only specialized host ranges despite the absence of a clear genetic variability. These specific interactions reinforce the hypothesis that *P. betae* underwent a high selective pressure (i.e. plant defenses) during a long coevolution with its hosts. Brader et al. (2017) postulate that pathogenicity is the consequence of highly specialized interactions between the microorganism and the host in a specific environment. It is also thought that a pathogenic interaction could evolve to a commensal interaction or even to a mutualistic interaction and *vice versa*

(Kemen and Jones, 2012; Brader et al., 2017). However, it should be noted that both beneficial microorganisms and pathogens can secrete effectors to manipulate host immunity and establish a successful relationship and that a clear distinction between mutualistic and pathogenic endophytes on the basis of genomic differences is difficult (Brader et al., 2017).

Some indications are also given by the status of other plasmodiophorid species. *P. brassicae* is commonly recognized as a plant pathogen, inducing strong symptoms in compatible interaction (root galls) (Schwelm et al., 2018). *S. subterranea* has an intermediary status, causing obvious symptoms on susceptible potato cultivars and other Solanaceae but remaining nearly asymptomatic on other hosts (Falloon et al., 2016). Even on potato, various symptoms (i.e. galls on stolon and roots and/or powdery scab lesions) can occur depending on the cultivar, suggesting that different phenomena are responsible for symptom appearance. On tomato, galls occur rarely, whereas zoosporangia develop well in root epidermis. In our comparative genomic study, we showed a closer relationship between *P. betae* and *S. subterranea* than between *P. betae* and *P. brassicae*. Moreover, as stated above, some deleterious effects on the host were scarcely reported and are probably related to very specific situations (e.g. specific isolates, hosts, infection pressure, life stage, etc.). Regarding these elements, we can postulate that *P. betae* is an ancient pathogen which has evolved toward a nonpathogenic interaction with sugar beet. Supporting the idea of an ancient pathogen, some other poorly known plasmodiophorids are also considered as harmful plant pathogens such as *Hillenburgia nasturtii*, formerly *Spongospora subterranea* f. sp. *nasturtii* (Claxton et al., 1996; Hittorf et al., 2020), *Sorosphaerula viticola* (Neuhauser et al., 2009) and *Lignera junci* (Grau and Leonard, 1978).

3. *The interaction between BNYVV and Polymyxa betae*

a. Main results

In the first section of this discussion, we discussed the fine interplay between *P. betae* and the sugar beet without the BNYVV. Here, we interested in what's happening when the virus enters the game.

In Chapter IV, we reported the genomic sequence of the *P. betae* monosporosorus isolate A26-41. A protocol was then optimized with this isolate to achieve the controlled zoospore transmission of BNYVV, transcribed from infectious cDNA clones, to *B. vulgaris* KWS2320, a completely sequenced double haploid monogerm line. A higher multiplication of *P. betae* was observed in plants co-infected with BNYVV than in plants inoculated with aviruliferous *P. betae*, at one, seven and 14 dpi. At 28 dpi this trend was reversed. Moreover, globally more zoosporangia and sporosori were observed in roots inoculated with the BNYVV at 14 and 28 dpi than in roots inoculated with *P. betae* alone. These results reveal that *P. betae* colonization of sugar beet root was accelerated by the presence of BNYVV and that the protist enters earlier in its survival life stage. At the transcriptomic level (28 dpi), we observed a larger plant reaction to the inoculation with viruliferous zoospores than with *P. betae* alone even though both inoculi did not trigger significant opposite effects on root transcriptome. Transcriptomic results showed a modulation of the expression of genes involved in carbohydrate and lipid metabolism in inoculated plant compared to mock-inoculated plants, suggesting that further physiological sinks are induced in the sugar beet roots, mainly in the presence of BNYVV. We also noticed the up-regulation of genes encoding enzymes/proteins involved in cell-walls modification and degradation (e.g. pectin methylesterases, polygalacturonases, expansins), which indicate a probable influence of the virus on the cell wall biomechanical properties such as rigidity. Moreover, a larger impact on plant defenses was observed in the presence of BNYVV than with *P. betae* alone. In particular, the induction of nearly all PR families and of genes involved in secondary metabolic pathways (e.g. phenylpropanoids, flavonoids) was observed in the plant in the presence of BNYVV. A greater impact was also found on TF and RGA encoding genes. The Figure VI-3 illustrates the greater impact of BNYVV infection on plant defenses than *P. betae* alone. Detailed results are reported in Chapter V. Altogether, the infection of *P. betae* with BNYVV caused a greater activation of genes related to defense signaling and to the production of plant defense compounds compared to *P. betae* alone.

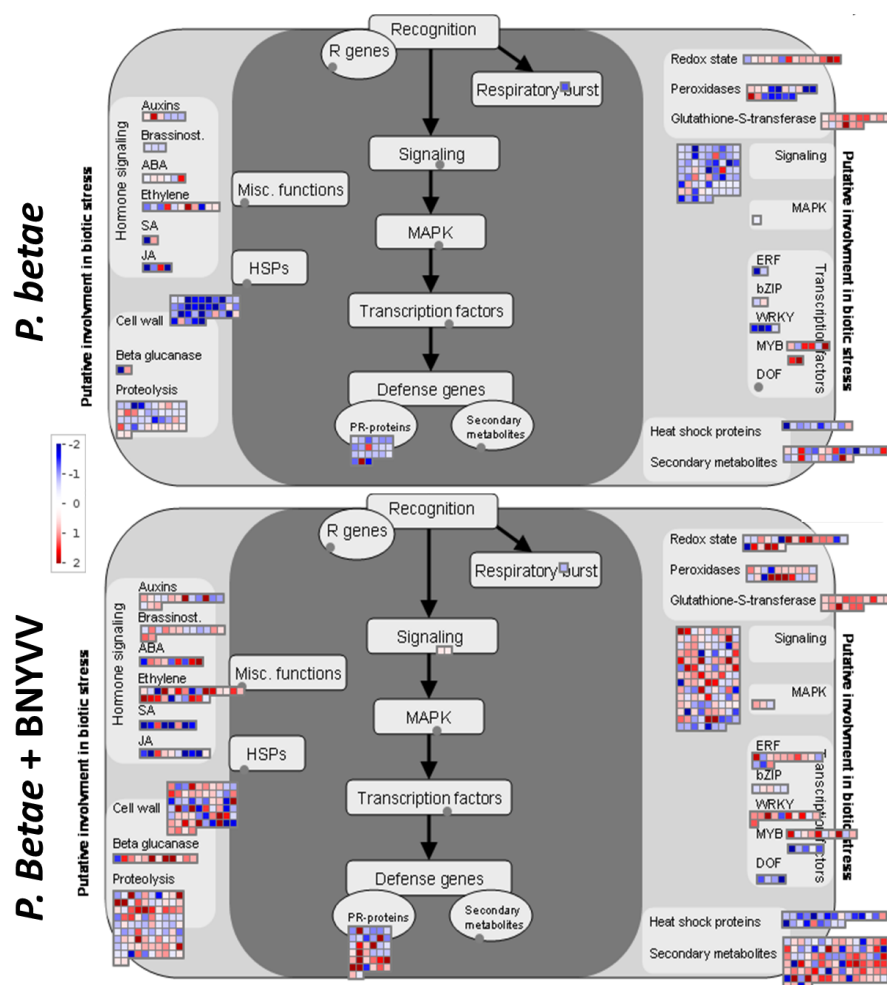


Figure VI-3. Overview of the transcriptional changes of defense-related genes in *P. betae* or *P. betae* + BNYVV sugar beet plants at 28 dpi. DEGs are assigned to different categories according to Mercator/MapMan. Genes in red are up-regulated and in blue are down-regulated. The regulation of genes is based on log2 fold change.

b. *Polymyxa betae* benefits from the presence of BNYVV

The free zoospores of *P. betae* are able to directly infect epidermal root cells but preferentially invade young host roots through root hairs. Secondary zoospores produced in epidermal cells, could allow cortical infections. Once the taproot of sugar beet starts to enlarge, new *P. betae* infection is thought to mainly occurs in lateral roots (LR). The BNYVV induces an excessive LR proliferation giving a bearded aspect to taproot. The proliferation of LR is thought to be beneficial to the protist, enlarging the contact surface of the

CHAPTER VI. General discussion

root with the soil and giving a new “substrate” to invade during the entire sugar beet lifetime. This could result in greater soil infestation at the end of growing season. However, at the time of our analysis, the lateral root proliferation was not obvious, yet. As soon as 24 hours after infection and more markedly after seven and 14 days, a much larger protist relative DNA quantity was measured in roots infected with BNYVV than in roots infected with *P. betae* alone. This indicates that BNYVV gives another advantage to *P. betae*, at early infection and root colonization stages. Interestingly, Gerik and Duffus (1988) also observed a positive impact of BNYVV on *P. betae* in a controlled transmission experiment, using dried infested *B. macrocarpa* roots as inoculum. They reported greater infection levels on sugar beets inoculated with BNYVV-carrying *P. betae* isolates when compared with the same isolates without the virus, and this for five out of the six monosporosorus isolates tested. Therefore, the positive effect of the virus on the sugar beet colonization observed in our study is confirmed with a different inoculum and different *P. betae* isolates. However, the pre-infection of sugar beet with the BNYVV (without *P. betae*) was also previously shown to reduce the subsequent development of *P. betae* (Schlösser, 1990). This could be due to the different timing of inoculation as well as the method of inoculation. Fernando Gil et al. (2020) mechanically inoculated sugar beet plants with BNYVV and BSBMV without *P. betae*. They observed the repression of genes belonging to the ethylene signal transduction pathway and hypothesized that it could be related to the susceptibility of sugar beet to benyvirus infection. The inverse situation was observed in our transcriptomic data. Therefore, it is possible that distinct defense mechanisms occurred in both studies. Several explanations can explain this difference. Firstly, we used a B-type BNYVV while they used a A-type one. Secondly, they performed the transcriptomic analysis at a late time point (44 dpi). Thirdly, we used different sugar beet genotypes. Lastly, BNYVV was mechanically inoculated in the absence of *P. betae*. If all the explanations are probable, the most obvious difference is, in our opinion, the last one. Discrepancies between these results highlight that natural BNYVV transmission through *P. betae* zoospores (primary or secondary) has a greater influence on the pathosystem and stress the importance to mimic natural conditions to study the plant-virus-vector interactions.

Several hypotheses can be advanced to explain the advantage given by the BNYVV to *P. betae*. A first hypothesis is that BNYVV-induced changes in cell wall properties could facilitate the root colonization by *P. betae*. As discussed in Chapter V, the BNYVV has an effect on the auxin signaling pathway, linked to its capacity to induce lateral root proliferation (Peltier et al., 2011; Fernando Gil et al., 2018, 2020). Even if a massive rootlet proliferation was not observed at the time of analysis, we recorded the significant regulation of various genes encoding enzymes involved in cell wall modification/degradation and expansins, indicating that the virus already induced changes in infected roots. As stated above, cell-to-cell movement of *P. betae* implies degradation of the root cell walls. The potential influence of BNYVV on the cell wall biomechanical properties such as rigidity may therefore benefit *P. betae*. Interestingly, we also noticed the up-regulation of

pectin acetyltransferase genes. One pectin acetyltransferase was recently found to be a virulence factor of the pathogenic oomycete *Peronophythora litchii* and its heterologous expression in *N. benthamiana* could promote the infection of *Phytophthora capsici* (Kong et al., 2019). It was also demonstrated that the minor shifts in cellulose and pectin cell wall composition of *A. thaliana* influence the plant resistance to the obligate biotrophic fungal *Golovinomyces cichoracearum* (Chiniquy et al., 2019). These two studies illustrate that small changes in cell wall composition and fungal resistance are intimately connected.

Another hypothesis to explain the faster development of *P. betae* in the presence of BNYVV is that the protist could profit from the RNA silencing suppression by BNYVV. The suppression of RNA silencing by the BNYVV is mediated by the RNA2-encoded P14 (Chiba et al., 2013), the RNA4-encoded P31 (Rahim et al., 2007), and the noncoding RNA3 (Flobinus et al., 2016, 2018). The *Burdock mottle virus* (BdMoV) and the *Rice stripe necrosis virus* (RSNV), two other benyviruses have only two genomic RNAs to complete their life cycle. Thus, they encode homologs of the BNYVV P14 but not from the other known silencing suppressors. We could therefore reasonably expect that P14 is the centerpiece of BNYVV RNA silencing suppression. Flobinus et al. (2016) identified the RNA-dependent RNA polymerase 6 (RDR6) pathway as a plant target of P14. Interestingly, the up-regulation of the sugar beet *RDR6* was reported by Desoignies et al. (2014) in early infection by *P. betae* alone. Furthermore, the rice RDR6-mediated RNA silencing pathway was shown to participate in defense against viruses but also against *Xanthomonas oryzae* pv. *oryzae* or *Magnaporthe oryzae* (Wagh et al., 2016). Taken together, it seems that the sugar beet RDR6-mediated silencing pathway could be involved in defense against *P. betae* and that the protist could take advantage of the P14 silencing suppressor. Supporting this hypothesis, it was recently reported that a biotrophic plant pathogen of wheat, *Puccinia graminis* f. sp. *tritici*, secretes a suppressor of RNA silencing in order to regulate the abundance of plant small RNAs, including miRNAs and their associated targets. Expression of this fungal silencing suppressor was shown to alter plant defenses and to enhance the susceptibility to multiple pathogens (Yin et al., 2019).

Although both hypotheses considered above are possible, we can't rule out the possibility that changes induced in carbohydrates and lipids metabolism occurring in the presence of BNYVV could promote *P. betae* development. The presence of BNYVV induced one *SWEET* transporter and one sucrose transport protein (*SUT/SUC*) gene as well as two genes encoding a cell wall invertases. Moreover, we observed the up-regulation of gene responsible for the synthesis of fatty acids along with a down-regulation of complex lipids and lipid droplet synthesis in the presence of the virus. Since *P. betae* entirely depends on its host to get building blocks, its development is probably influenced by the availability of assimilable sugars and/or lipids. The establishment of energy sinks and possibly the relocation of carbohydrates to actively growing LR could match with the location of the protist which could thus get more "fuel" for its own development.

CHAPTER VI. General discussion

Finally, it is interesting to compare the effect of *P. betae* and BNYVV on the plant with the effect of *P. brassicae*. Both BNYVV and *P. brassicae* induce localized root cells proliferations in the form of excessive LR production of root galls, respectively. These symptoms derive from profound changes in auxin/cytokinin balance in both cases and involve the formation of new physiological sinks, probably due to the action of sugar transporters (SWEET, SUT/SUC) and sucrose invertases. Similarly, the activation of genes involved in FA synthesis, the modification of cell walls (e.g. *PMEs*, *XTHs*, *EXPs*), defenses (e.g. *endo-1,3-beta-glucosidases*, *chitinases*, phenylpropanoids and flavonoids pathways), detoxification (e.g. glutathione). Altogether, the plant reaction to *P. brassicae* exhibited more similarities with the plant reaction to *P. betae* with BNYVV than to *P. betae* alone. The transformations caused to roots are key in the interactions of these pathogens with their respective hosts. In some ways, the association of *P. betae* with BNYVV seems to give back virulence factors possibly lost during its co-evolution with specific hosts.

c. Advantages for the BNYVV

P. betae is the only known vector of BNYVV. The virus epidemiology is therefore inextricably bound to the protist life cycle. It has long been known that BNYVV benefits from zoospore motility to spread from plant to plant and from long-lasting sporosori to remain in soils in the absence of host plant. As discussed above and in Chapter V, an earlier switch of *P. betae* to the survival stage was observed in the presence of BNYVV. This switch could either be due to the activation of plant defenses or to the lack of nutrients and/or of free cell roots due to the fact that a large number of root cells are already infected by *P. betae*. On one hand, the activation of genes from most PR families (including *chitinases* and *endo-1,3-beta-glucosidases*) and of genes involved in the synthesis of phenolic compounds could directly target *P. betae*. The stress induced by plant defenses to *P. betae* could trigger the switch to the survival stage, as supported by the up-regulation of a *P. betae* gene encoding a homolog of the conidiation-specific protein 10 from *Neurospora crassa* (Shrode et al., 2001). This hypothesis is corroborated by the observation that plant defenses were maximal after 14 days, at the same time as the switch to the survival stage of *P. betae*, observed mainly in BNYVV infected plants. On the other hand, a faster invasion of the young plant's root system, available at the time of inoculation, could lead to a lack free cells and resources for *P. betae*. Consequently, the protist would be unable to continue its development and would "naturally" switch to the survival stage. This phenomenon was often observed in the lab when inoculating young plantlets with large inoculum quantities. Nevertheless, if this early switch may seem paradoxical, it could advantage the BNYVV. Indeed, the better resting spores containing the virus are produced, the sooner the BNYVV ensures its subsistence. Both the vector and the protist increase, in this way, their chances of survival.

At the beginning of this chapter, we stated that pathways linked to the oxidative stress control and detoxification are induced by *P. betae* alone or in the presence of BNYVV. It was previously reported that a reduction of oxidative stress and to the related induction of HR could be beneficial for the BNYVV (Schmidlin et al., 2008). The virus could therefore take advantage of the regulation of oxidative stress in the presence of *P. betae*. However, it should be mentioned that effective BNYVV infection and multiplication can be obtained in sugar beet roots in the absence of *P. betae*, by mechanical inoculation. The impact of oxidative stress on BNYVV infection and the putative effect of *P. betae* should therefore be clarified. Nevertheless, considering all the elements mentioned above, we can postulate that *P. betae* and BNYVV are involved in a mutually beneficial interaction.

d. *Polymyxa betae* is closer to *Spongospora subterranea*, also a virus-vector, than to *Plasmodiophora brassicae*

The comparison of plasmodiophorids single copy genes and mitochondrial genomes clearly indicate that *P. betae* and *S. subterranea* are more closely related than both are with *P. brassicae* and that *S. subterranea* should be classified between the other two. As stated above, symptom induction by *S. subterranea* could be considered as intermediary, causing galls and growth reduction in some hosts but having nearly no harmful impact on others. Moreover, among the three entirely sequenced plasmodiophorids, both *P. betae* and *S. subterranea* are able to transmit viruses, whereas viral transmission was never observed in *P. brassicae*, despite the latter was largely more studied.

It was previously reported that *Spongospora subterranea* genome is enriched in transposable elements and virus-related genes (Bulman et al., 2011; Schwelm et al., 2015b; Ciaghi et al., 2018). Similarly, our clustering analyses of *P. brassicae*, *S. subterranea* and *P. betae* proteins and subsequent domain enrichment analysis revealed that PFAM domains related to transposable elements were enriched in clusters of protein shared between *P. betae* and *S. subterranea* but not *P. brassicae*. In particular, we found the following PFAM enriched domains: “Tc5 transposase DNA-binding domain”, “DDE superfamily endonuclease”, “Retrotransposon gag protein”, “MULE transposase domain”, “GAG-pre-integrase domain”, “Integrase core domain”, “gag-polypeptide of LTR copia-type”. Terminal-Repeat Retrotransposons with GAG Domain were proposed to be involved in the molecular diversities of plant genomes (Chaparro et al., 2015). The contribution of transposable elements (TE) to pathogenic fungal lifestyle was also proposed (Muszewska et al., 2019). TEs could be involved in fungal adaptation to new ecological niches, in host switching and host specialization (Ma et al., 2010; Yoshida et al., 2016; Muszewska et al., 2019). They may thus have influenced host-parasite interaction in plasmodiophorids. We discussed in the introduction of this thesis the possibility that *P. betae* could act as host of the BNYVV (and not only as vector). This question is controversial. It is tempting to think that enrichment

CHAPTER VI. General discussion

of both *P. betae* and *S. subterranea* in retrotransposon-related domains in comparison to *P. brassicae* is somewhat linked with their viral transmission ability. The first two could be more favorable ground for the viral infection. This remains highly hypothetical yet worth being mentioned here.

CHAPTER VII.

Conclusion and perspectives

1. Main conclusions

In the first part of this project were obtained the two first genomic sequences of *P. betae*. The sequencing of plasmodiophorids has long been impeded by the impossibility to establish axenic cultures of these protists as well as the difficulty to separate and purify these organisms from their host holobiont. For that reason, up-to-date metagenomics tools were successfully implemented to assemble the *P. betae* genome from shotgun DNA libraries containing sequences from the host and other microorganisms. A comparative genomics analysis of *P. betae*, *P. brassicae* and *S. subterranea* revealed that these three plasmodiophorids probably rely on distinct molecular interactions, to invade hosts root cells and multiply. For example, genes involved in the GPCR signaling pathway, that could be relevant for development and pathogenicity of *P. brassicae*, were far less enriched in *P. betae* and *S. subterranea*. Distinct spectrum of proteins containing ankyrin-repeat domains were also observed in the computational proteome and secretome of the three plasmodiophorids. Of interest, we also reported the detection of CatSper complex proteins, raising intriguing questions about zoospore physiology. The experimental verification of hypothesis drawn from our computational predictions will be the next challenge to further characterized plasmodiophorids. In any case, our data and approaches will make some contribution to address the bias of available genomic knowledge in the eukaryotic tree of life and should be of general interest for biologists, especially for evolutionary biologists.

Secondly, a protocol enabling the soil-free transmission of BNYVV to sugar beet by *P. betae* zoospores was optimized under controlled conditions, using the fully sequenced *B. vulgaris* and *P. betae* accessions as well as infectious cDNA clones of BNYVV. A similar protocol was implemented by Tamada et al. (2021) to infect sugar beets with *P. betae* zoospores carrying various BNYVV isolates. The advantage of the approach proposed here is the use of genetically characterized *P. betae* and sugar beet accessions and BNYVV cDNA clones. A second advantage is the use of standardized zoospores inoculi. This tool could also be of interest to private companies involved in beet improvement as it could, for example, be adapted to search for new rhizomania resistance genes.

Lastly, our method was used in a transcriptomic study of the tripartite interactions in rhizomania. The BNYVV was shown to favorize the early multiplication of *P. betae* during infection of *B. vulgaris* roots by *P. betae*. Then a more rapid onset of the protist survival stage was observed, as evidenced by the earlier production of sporosori in the presence of BNYVV than with *P. betae* alone. In a well-established infection, when all *P. betae*

life stages are observed in roots, *P. betae* had a surprisingly moderated impact on sugar beet defense activation. For example, most PR family's genes were not up-regulated by the infection. It seems that *P. betae* can manipulate the sugar beet to escape plant immunity, as shown by the down-regulation of genes involved in pathogen perception and signaling and the up-regulation of genes involved in detoxification and redox homeostasis. Genes involved in plant cell wall reinforcement were also repressed. These regulations are probably partly responsible for the lack of symptom induction and thus for the likely commensal interaction between nonviruliferous *P. betae* and the sugar beet. Conversely, a clear defense reaction was observed when BNYVV was inoculated with *P. betae*, including the up-regulation of PR genes, phenylpropanoids and flavonoids pathways. *P. betae* could eventually suffer from the plant defense reaction to BNYVV, leading to the induction of the resting spore stage. Another possibility is that the faster root invasion by the protist in the presence of BNYVV resulted in a lack of available free cells (not already containing *P. betae* structures) or resources in the BNYVV infected roots, such as FA and carbohydrates. Viral inoculation also resulted in the induction of genes involved in plant cell walls modification. Such regulation is supposed to have a "negative" impact on cell wall integrity which could be profitable for *P. betae* and explain the faster multiplication of the protist in the roots observed in the first days after inoculation. Another possibility would be that the protist benefits from the viral RNA silencing suppressors. Our transcriptomic study raised interesting results and many hypotheses could be drawn. However further investigations are definitely needed to confirm or refute the assumption made in this work.

2. More phytomyxids genomes

The sequencing of microscopic protists genomes such as plasmodiophorids is restrained by the difficulty to culture these organisms. It's especially the case for obligate endoparasites which are intimately tied to their host and to all the other microorganisms living in close association with their host (the holobiont). To overcome this challenge, we proposed to use a metagenomic pipeline taking advantage of advanced tools to recover *P. betae* genomes from DNA-seq datasets contaminated with host and other microorganisms. This method allowed us to recover very similar genomes from two different samples: one from partially purified sporosori (+58% of the sequences originated from *P. betae*) and one from highly contaminated zoospore holobiont (<7% of the sequences originated from *P. betae*). This new genomic resource in the poorly known Rhizaria supergroup is very valuable to the scientific community, as shown by our comparative study. Our method could be applied to recover other genomes from non-culturable microscopic eukaryotes. The progress of long reads technologies and single-cell genomics will allow to further refine our method and assemblies. These new approaches have already been applied to sequence other protists such as *P. brassicae* and marine diplomonads (Gawryluk et al., 2016; Stjelja et al.,

2019). Fortunately, the pipeline used in this work can easily be adapted to complement Illumina's short reads with long-reads from MinION and PacBio. It will definitely be useful to obtain more plasmodiophorid genomes, including *P. graminis*. Extending comparative genomics to other phytomyxids able to parasitize plants, algae, diatoms and oomycetes will be of great help to gain insights into host specialization in this poorly known clade.

3. Future studies are needed to complement transcriptomic approach

In this thesis, we used a single time point RNA-seq study to gain insights in BNYVV-*P. betae*- sugar beet interactions and discussed these interactions in terms of plant defenses. Plant defenses activation is a complex mechanism involving multiple interconnected signaling pathways eventually resulting in a fine-tuned plant response (Pieterse et al., 2012; Tsuda and Somssich, 2015; Ruan et al., 2019). Transcriptomic reprogramming following stress detection is highly context dependent (i.e. culture condition, transcriptome evaluation time point, plant species, the nature of the stress). If some pathways, including JA and SA and oxidative stress are recurrent, various downstream defense responses involving different secondary metabolite classes (e.g. phenylpropanoids, flavonoids alkaloids, terpenoids, glucosinolates, anthocyanins, isoprenoids) are observed even in the same plant species (Pauwels et al., 2009). For example, a high-resolution transcriptomic study on responses dynamics of *Arabidopsis thaliana* challenged with MeJA demonstrates that responses change almost hourly over a 16H period (Hickman et al., 2017). Some clusters of “co-expressed genes” showed brief transcriptomic changes followed by a return to basal levels. Other are more sustainably induced or repressed (Hickman et al., 2017). Our results should be therefore considered as a snapshot of a well-established infection and the hypothesis proposed in this Chapter should therefore be confirmed at various time points with complementary techniques (i.e. transcriptomics, proteomics, metabolomics).

Ciaghi et al., (2019) showed that opposite transcriptomic changes occurred in kohlrabi root galls containing structures of *P. brassicae*, and in symptomless portions of the same infected root system (portions exempt of *P. brassicae*). A differential transcriptomic study of micro dissected *A. thaliana* cells infected with *P. brassicae* at different life stages also revealed distinct reactions (Schuller et al., 2014). In our study, we homogenized entire root systems. If a differential host response occurs in different parts of the root, it could have masked some of the changes induced by *P. betae*. Unfortunately, *P. betae* doesn't produce localized symptoms which can be targeted for RNA extraction (i.e. galls). In the future, single cell RNA-sequencing could bring a better resolution to elucidate interaction of specific *P. betae* life stages with the plant, in the presence or absence of the virus.

We postulate that cell walls modifications induced by the BNYVV and related hormonal changes could be positive for *P. betae* early development in coinfecting roots, allowing faster multiplication and root invasion by the protist. These modifications are likely linked with the initiation of root proliferation. Another hypothesis was that *P. betae* could benefit from BNYVV abilities to suppress host gene silencing machinery. Beet soil-borne mosaic virus (BSBMV) is also transmitted by *P. betae* but doesn't induce typical rhizomania root symptoms such as root proliferation. However, this virus is able to suppress plant RNA silencing. Beet soil-borne virus BSBV and Beet virus Q (BVQ) are also transmitted by *P. betae* but apparently do not produce symptoms and, as far as we know, do not encode an RNA silencing suppressor. The simultaneous monitoring of *P. betae* multiplication and plant transcriptomics in sugar beet roots infected with the BNYVV, BSBMV, BSBV and BVQ could further challenge this hypothesis.

4. Identification of *Polymyxa betae* effectors

Plant pathogens secrete proteins in order to manipulate the plant's immune system. The identification of these proteins, called effectors, is key to figure out how pathogens and plants are interacting and to identify novel genes involved in resistance. The annotation of the draft *P. betae* genomes obtained during this project enabled the prediction of putative effectors. Some proteins predicted as secreted harbor features shared by effectors encoded by other plant pathogens. Others are poorly or not annotated. This is not surprising since effectors are known to be fast-evolving during host specialization and also in view of the paucity of public databases used for annotation in rhizarian-related sequences. To further characterize *P. betae* effectors we suggest to firstly reduce the list of potential effectors, then to experimentally verify that they could effectively be secreted. To reduce the candidate list, it would be interesting to compare *P. betae* transcriptome in samples enriched with different life stages, i.e. secondary zoospores, early infection, maturing plasmodia, resting spores and germinating resting spores, and in different hosts more or less susceptible. A similar approach was recently adopted with *P. brassicae* (Pérez-López et al., 2020). This would help to select effectors which are differentially expressed in key life stages of *P. betae*. Then, the functionality of putative effectors N-terminal signal peptides could be verified using a yeast invertase assay. This way, we could further reduce the candidate effector list to proteins experimentally verified as secreted (Yu et al., 2019). Afterwards, it would be useful to confirm that candidate effectors could interact with plant defenses. The heterologous expression in a model plant, such as *N. benthamiana* or *A. thaliana* could help to determine their subcellular localization, to assess their ability to induce or to suppress programmed cell death and to identify potential plant targets (Chen et al., 2019; Hossain et al., 2021). Finally, it will be necessary to verify the effective interaction of candidate effectors with their potential plant targets. This could be achieved using yeast two-hybrid

assay, glutathione S-transferase pull-down assay, and bimolecular fluorescence complementation.

5. Clarify the mechanisms of virus transmission by *Polymyxa* spp.

The CP-RT is essential for the transmission of BNYVV by *P. betae* (Tamada et al., 1996b). This type of protein was shown to be protruding from BNYVV (Haeblerlé et al., 1994) and PMTV (Cowan et al., 1997) virions extremities (see Figure II-3, p52). It was proposed to have a similar role in BSBV and BVQ (Barbarossa et al., 1992; Crutzen, 2010; Mahillon, 2020). The RT domain of CP-RT contains two transmembrane domains probably allowing the anchorage of virions in host and/or vector membrane. It seems to be a common feature of plasmodiophorid-transmitted viruses (Adams et al., 2001). However, the mechanism by which the virions are moved from the host to the *P. betae* plasmodia is still unknown. Since plasmodiophorid-transmitted viruses are found inside zoospores, the transmission requires the internalization of the virus, probably involving pinocytosis. The fact that the viral transmission is specific to the vector (as illustrated for PCV and *P. graminis* f. sp. *tropicalis*, f. sp. *subtropicalis* and f. sp. *temperata*) suggests that a molecular key might govern the pinocytosis process. Moreover, the co-transmission of up to five RNA from multipartite viruses by *P. betae* zoospores also suggests that a specific mechanism of acquisition may occur. The process of pinocytosis is likely to involve the interaction of a viral element with a membrane protein of *P. betae*. In Chapter IV, we've predicted the membrane proteins from *P. betae*. Complementary *in silico* analyses could be performed to select candidate proteins with extracellular domains likely involved in protein-protein interactions. In a complementary way, the interactome (comprising plant and protist proteins) of CP-RT could also be experimentally determined by co-immunoprecipitation of Flag tagged RT protein coupled to MS/MS identification at various life stages of *P. betae*. Once the scope is reduced, a yeast two-hybrid assay could be implemented to determine the interaction between the CP-RT and candidate *P. betae* membrane proteins. Candidate interacting proteins genes should then be sequenced in various *P. betae* and *P. graminis* special forms and conserved domains could be searched using bioinformatic tools (e.g. the MEME suite). If successful, the approach described here might bring useful insights into the molecular key used by viruses for specific plasmodiophorid transmission.

At the end of this study, it is still not known if *P. betae* is a propagative host for the BNYVV or acts simply as a carrier. Even if that question seems simple, the intracellular nature of both *P. betae* and the BNYVV hampered attempts to answer this critical question because of the difficulty of discriminating viral multiplication in *P. betae* thalli and in the host cells. Our controlled soil free transmission protocol could be useful to further study the interactions between *P. betae* and the BNYVV in infected sugar beet roots.

CHAPTER VII. Conclusions

As a matter of fact, cDNA clones of the BNYVV could be easily engineered, for example to add tags allowing the subcellular localization of viral proteins (e.g. tag histidine [Lai et al., 2015]). A dynamic multiplex *in situ* localization of BNYVV RNAs with cDNA probes (Dieryck et al., 2011) and proteins in sugar beet roots after zoospore transmission could help to find out when and how the BNYVV is released by *P. betae*. Alternatively, pre-infection of *B. macrocarpa* roots with BNYVV followed by *P. betae* inoculation could be achieved to study the acquisition of the virus. Such approaches associated with dsRNA localization with the Anti-dsRNA monoclonal antibody J2 could also be useful to determine if a multiplication of the BNYVV occurs in *P. betae* thalli. J2 antibodies were already successfully used to localize BNYVV dsRNA in endoplasmic reticulum of *C. quinoa* protoplasts (Pakdel et al., 2015). The colocalization of dsRNA with BNYVV RNA's and proteins specific signals in *P. betae* thalli would strongly suggest that the BNYVV is effectively replicated and translated in the protist.

REFERENCES

- Abe, H. (1987). Studies on the ecology and control of *Polymyxa betae* Keskin, as a fungal vector of the causal virus (beet necrotic yellow vein virus) of rhizomania disease of sugar beet. *Rep. Hokkaido Prefect. Agric. Exp. Stn.* 60, 1–99.
- Abe, H., and Tamada, T. (1986). Association of Beet Necrotic Yellow Vein Virus with Isolates of *Polymyxa betae* Keskin. *Jpn. J. Phytopathol.* 52, 235–247. doi:10.3186/jjphytopath.52.235.
- Abe, H., and Ui, T. (1986). Host range of *Polymyxa betae* Keskin strains in rhizomania-infested soils of sugar beet fields in Japan. *Ann. Phytopathol. Soc. Jpn.* 52, 394–403.
- Acosta-Leal, R., Bryan, B. K., and Rush, C. M. (2009). Generation and dispersion of resistance breaking variants of beet necrotic yellow vein virus in the field. *Proc. 35th ASSBT.*
- Acosta-Leal, R., Bryan, B. K., and Rush, C. M. (2010a). Host effect on the genetic diversification of beet necrotic yellow vein virus single-plant populations. *Phytopathology* 100, 1204–1212. doi:10.1094/PHYTO-04-10-0103.
- Acosta-Leal, R., Bryan, B. K., Smith, J. T., and Rush, C. M. (2010b). Breakdown of Host Resistance by Independent Evolutionary Lineages of Beet necrotic yellow vein virus Involves a Parallel C/U Mutation in Its p25 Gene. *Phytopathology®* 100, 127–133. doi:10.1094/PHYTO-100-2-0127.
- Acosta-Leal, R., Fawley, M. W., and Rush, C. M. (2008). Changes in the intrasolate genetic structure of Beet necrotic yellow vein virus populations associated with plant resistance breakdown. *Virology* 376, 60–68. doi:10.1016/j.virol.2008.03.008.
- Acosta-Leal, R., and Rush, C. M. (2007). Mutations associated with resistance-breaking isolates of Beet necrotic yellow vein virus and their allelic discrimination using TaqMan technology. *Phytopathology* 97, 325–330. doi:10.1094/PHYTO-97-3-0325.
- Adams, M. J., Antoniow, J. F., and Mullins, J. G. L. (2001). Plant virus transmission by plasmodiophorid fungi is associated with distinctive transmembrane regions of virus-encoded proteins. *Arch. Virol.* 146, 1139–1153. doi:10.1007/s007050170111.
- Adams, M. J., Jones, P., and Swaby, A. G. (1987). The effect of cultivar used as host for *Polymyxa graminis* on the multiplication and transmission of barley yellow mosaic virus (BaYMV). *Ann. Appl. Biol.* 110, 321–327. doi:10.1111/j.1744-7348.1987.tb03262.x.
- Adams, M. J., and Swaby, A. G. (1988). Factors affecting the production and motility of zoospores of *Polymyxa graminis* and their transmission of barley yellow mosaic virus (BaYMV). *Ann. Appl. Biol.* 112, 69–78. doi:https://doi.org/10.1111/j.1744-7348.1988.tb02042.x.
- Adams, M. J., Swaby, A. G., and Jones, P. (1988). Confirmation of the transmission of barley yellow mosaic virus (BaYMV) by the fungus *Polymyxa graminis*. *Ann. Appl. Biol.* 112, 133–141. doi:10.1111/j.1744-7348.1988.tb02048.x.
- Adams, M. J., and Ward, E. (1999). Characterisation of *Polymyxa* transmitting rice stripe necrosis virus in Colombia. *Proc. Fourth Symp. Int. Work. Group Plant Viruses Fungal Vectors Asilomar Conf. Cent. Monterey Calif. USA 5-8 Oct. 1999*, 77–80.
- Adediji, A. A., and Babalola, O. O. (2020). Secondary metabolites as plant defensive strategy: a large role for small molecules in the near root region. *Planta* 252, 61. doi:10.1007/s00425-020-03468-1.

REFERENCES

- Adl, S. M., Bass, D., Lane, C. E., Lukeš, J., Schoch, C. L., Smirnov, A., et al. (2019). Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. *J. Eukaryot. Microbiol.* 66, 4–119. doi:10.1111/jeu.12691.
- Adl, S. M., Simpson, A. G. B., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., et al. (2005). The New Higher Level Classification of Eukaryotes with Emphasis on the Taxonomy of Protists. *J. Eukaryot. Microbiol.* 52, 399–451. doi:10.1111/j.1550-7408.2005.00053.x.
- Adl, S. M., Simpson, Alastair. G., Lane, C. E., Lukeš, J., Bass, D., Bowser, S. S., et al. (2012). The revised classification of eukaryotes. *J. Eukaryot. Microbiol.* 59, 429–493. doi:10.1111/j.1550-7408.2012.00644.x.
- Agarwal, A., Kaul, V., Faggian, R., Rookes, J. E., Ludwig-Müller, J., and Cahill, D. M. (2011). Analysis of global host gene expression during the primary phase of the *Arabidopsis thaliana*–*Plasmodiophora brassicae* interaction. *Funct. Plant Biol.* 38, 462–478. doi:10.1071/FP11026.
- AHDB (2021). An introduction to clubroot in oilseed rape | AHDB. Available at: <https://ahdb.org.uk/clubroot> [Accessed February 1, 2021].
- Aist, J. R., and Williams, P. H. (1971). The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. *Can. J. Bot.* 49, 2023–2034. doi:10.1139/b71-284.
- Alghishi, P., and D'Ambra, V. (1966). RICERCHE SULLA RIZOMANIA DELLA BIETOLA. *Riv. Patol. Veg.* 2, 3–41.
- Ali, S., Ganai, B. A., Kamili, A. N., Bhat, A. A., Mir, Z. A., Bhat, J. A., et al. (2018). Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiol. Res.* 212–213, 29–37. doi:10.1016/j.micres.2018.04.008.
- Al-Khodor, S., Price, C. T., Kalia, A., and Abu Kwaik, Y. (2010). Functional diversity of ankyrin repeats in microbial proteins. *Trends Microbiol.* 18, 132–139. doi:10.1016/j.tim.2009.11.004.
- Almeida, A., Mitchell, A. L., Boland, M., Forster, S. C., Gloor, G. B., Tarkowska, A., et al. (2019). A new genomic blueprint of the human gut microbiota. *Nature* 568, 499–504. doi:10.1038/s41586-019-0965-1.
- Anders, S., Pyl, P. T., and Huber, W. (2015). HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* 31, 166–169. doi:10.1093/bioinformatics/btu638.
- Andika, I. B., Kondo, H., Nishiguchi, M., and Tamada, T. (2012). The cysteine-rich proteins of beet necrotic yellow vein virus and tobacco rattle virus contribute to efficient suppression of silencing in roots. *J. Gen. Virol.* 93, 1841–1850. doi:10.1099/vir.0.043513-0.
- Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data*. Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom Available at: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- Anoop, A., and Agostinho, A. (2019). Whole-Genome Comparisons Among the Genus *Shewanella* Reveal the Enrichment of Genes Encoding Ankyrin-Repeats Containing Proteins in Sponge-Associated Bacteria. *Front. Microbiol.* 10. doi:10.3389/fmicb.2019.00005.

- Aramaki, T., Blanc-Mathieu, R., Endo, H., Ohkubo, K., Kanehisa, M., Goto, S., et al. (2020). KofamKOALA: KEGG Ortholog assignment based on profile HMM and adaptive score threshold. *Bioinformatics* 36, 2251–2252. doi:10.1093/bioinformatics/btz859.
- Armenteros, J. J. A., Tsirigos, K. D., Sønderby, C. K., Petersen, T. N., Winther, O., Brunak, S., et al. (2019). SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat. Biotechnol.* 37, 420. doi:10.1038/s41587-019-0036-z.
- Asher, M. J. C., and Blunt, S. J. (1987). The ecological requirements of *Polymyxa betae*. *Proc. 50th Congr. IIRB*, 45–55.
- Asher, M. J. C., Grimmer, M. K., and Mutasa-Goettgens, E. S. (2009). Selection and characterization of resistance to *Polymyxa betae*, vector of Beet necrotic yellow vein virus, derived from wild sea beet. *Plant Pathol.* 58, 250–260. doi:https://doi.org/10.1111/j.1365-3059.2008.01978.x.
- Bacete, L., Mérida, H., Miedes, E., and Molina, A. (2018). Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. *Plant J.* 93, 614–636. doi:10.1111/tpj.13807.
- Backer, R., Naidoo, S., and van den Berg, N. (2019). The NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1) and Related Family: Mechanistic Insights in Plant Disease Resistance. *Front. Plant Sci.* 10, 102. doi:10.3389/fpls.2019.00102.
- Badstöber, J., Ciaghi, S., and Neuhauser, S. (2020). Dynamic cell wall modifications in brassicas during clubroot disease. *bioRxiv*, 2020.03.02.972901. doi:10.1101/2020.03.02.972901.
- Bagayoko, I., Celli, M. G., Romay, G., Poulicard, N., Pinel-Galzi, A., Julian, C., et al. (2021). Genetic Diversity of Rice stripe necrosis virus and New Insights into Evolution of the Genus Benyvirus. *Viruses* 13, 737. doi:10.3390/v13050737.
- Balendres, M. A., Nichols, D. S., Tegg, R. S., and Wilson, C. R. (2016). Metabolomes of Potato Root Exudates: Compounds That Stimulate Resting Spore Germination of the Soil-Borne Pathogen *Spongospora subterranea*. *J. Agric. Food Chem.* 64, 7466–7474. doi:10.1021/acs.jafc.6b03904.
- Balendres, M. A., Tegg, R. S., and Wilson, C. R. (2017). Resting Spore Dormancy and Infectivity Characteristics of the Potato Powdery Scab Pathogen *Spongospora subterranea*. *J. Phytopathol.* 165, 323–330. doi:10.1111/jph.12565.
- Balotf, S., Tegg, R. S., Nichols, D. S., and Wilson, C. R. (2021a). Spore Germination of the Obligate Biotroph *Spongospora subterranea*: Transcriptome Analysis Reveals Germination Associated Genes. *Front. Microbiol.* 12, 691877. doi:10.3389/fmicb.2021.691877.
- Balotf, S., Wilson, R., Tegg, R. S., Nichols, D. S., and Wilson, C. R. (2021b). Quantitative proteomics provides an insight into germination-related proteins in the obligate biotrophic plant pathogen *Spongospora subterranea*. *Environ. Microbiol. Rep.* 13, 521–532. doi:10.1111/1758-2229.12955.
- Barbarossa, L., Vetten, H. J., Kaufmann, A., Lesemann, D.-E., and Koenig, R. (1992). Monoclonal antibodies to beet soil-borne virus. *Ann. Appl. Biol.* 121, 143–150. doi:10.1111/j.1744-7348.1992.tb03994.x.
- Barbez, E., Kubeš, M., Rolčík, J., Béziat, C., Pěnčík, A., Wang, B., et al. (2012). A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature* 485, 119–122. doi:10.1038/nature11001.

REFERENCES

- Bari, R., and Jones, J. D. G. (2009). Role of plant hormones in plant defence responses. *Plant Mol. Biol.* 69, 473–488. doi:10.1007/s11103-008-9435-0.
- Barr, D. J. S. (1979). Morphology and host range of *Polymyxa graminis*, *Polymyxa betae*, and *Ligniera pilorum* from Ontario and some other areas. *Can. J. Plant Pathol.* 1, 85–94. doi:10.1080/07060667909501468.
- Barr, D. J. S. (1987). Zoosporic plant parasites as fungal vectors of viruses: taxonomy and life cycles of species involved. in Cooper, J. I., and M, J. C. Asher (eds). (Lavenham: The Lavenham Press Ltd), 123–137.
- Barr, D. J. S., and Allan, P. M. E. (1982). Zoospore ultrastructure of *Polymyxa graminis* (Plasmodiophoromycetes). *Can. J. Bot.* 60, 2496–2504.
- Barr, K. J., and Asher, M. J. C. (1996). Studies on the life-cycle of *Polymyxa betae* in sugar beet roots. *Mycol. Res.* 100, 203–208. doi:10.1016/S0953-7562(96)80123-7.
- Barr, K. J., Asher, M. J. C., and Lewis, B. G. (1995). Resistance to *Polymyxa betae* in wild Beta species. *Plant Pathol.* 44, 301–307. doi:10.1111/j.1365-3059.1995.tb02781.x.
- Bass, D., van der Gast, C., Thomson, S., Neuhauser, S., Hilton, S., and Bending, G. D. (2018). Plant Rhizosphere Selection of Plasmodiophorid Lineages from Bulk Soil: The Importance of “Hidden” Diversity. *Front. Microbiol.* 9. doi:10.3389/fmicb.2018.00168.
- Beemster, A. B. R., and de Heij, A. (1987). A method for detecting *Polymyxa betae* and beet necrotic yellow vein virus in soil using sugar-beet as a bait plant. *Neth. J. Plant Pathol.* 93, 91–93. doi:10.1007/BF01998095.
- Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300.
- Berens, M. L., Berry, H. M., Mine, A., Argueso, C. T., and Tsuda, K. (2017). Evolution of Hormone Signaling Networks in Plant Defense. *Annu. Rev. Phytopathol.* 55, 401–425. doi:10.1146/annurev-phyto-080516-035544.
- Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritsch, G., et al. (2013). MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol.* 69, 313–319. doi:10.1016/j.ympev.2012.08.023.
- Bi, K., Chen, T., He, Z., Gao, Z., Zhao, Y., Fu, Y., et al. (2018). Proto-oncogenes in a eukaryotic unicellular organism play essential roles in plasmodial growth in host cells. *BMC Genomics* 19, 881. doi:10.1186/s12864-018-5307-4.
- Bi, K., Chen, T., He, Z., Gao, Z., Zhao, Y., Liu, H., et al. (2019). Comparative genomics reveals the unique evolutionary status of *Plasmodiophora brassicae* and the essential role of GPCR signaling pathways. *Phytopathol. Res.* 1, 12. doi:10.1186/s42483-019-0018-6.
- Bi, K., He, Z., Gao, Z., Zhao, Y., Fu, Y., Cheng, J., et al. (2016). Integrated omics study of lipid droplets from *Plasmodiophora brassicae*. *Sci. Rep.* 6. doi:10.1038/srep36965.
- Biaggi, M. D., Stevanato, P., Trebbi, D., Saccomani, M., and Biancardi, E. (2011). Sugar Beet Resistance to Rhizomania: State of the Art and Perspectives. *Sugar Tech* 12, 238–242. doi:10.1007/s12355-010-0047-z.
- Biancardi, E., and Lewellen, R. T. (2016). “Introduction,” in *Rhizomania*, 3–28. doi:10.1007/978-3-319-30678-0_1.

- Biancardi, E., Lewellen, R. T., Biaggi, M. D., Erichsen, A. W., and Stevanato, P. (2002). The origin of rhizomania resistance in sugar beet. *Euphytica* 127, 383–397. doi:10.1023/A:1020310718166.
- Biancardi, E., and Tamada, T. eds. (2016). *Rhizomania*. Springer International Publishing doi:10.1007/978-3-319-30678-0.
- Bittara, F. G., Thompson, A. L., Gudmestad, N. C., and Secor, G. A. (2016). Field Evaluation of Potato Genotypes for Resistance to Powdery Scab on Tubers and Root Gall Formation Caused by *Spongospora subterranea*. *Am. J. Potato Res.* 93, 497–508. doi:10.1007/s12230-016-9526-4.
- Bittner-Eddy, P. D., Crute, I. R., Holub, E. B., and Beynon, J. L. (2000). RPP13 is a simple locus in *Arabidopsis thaliana* for alleles that specify downy mildew resistance to different avirulence determinants in *Peronospora parasitica*. *Plant J.* 21, 177–188. doi:10.1046/j.1365-3113x.2000.00664.x.
- Bleykasten, C., Gilmer, D., Guilley, H., Richards, K. E., and Jonard, G. (1996). Beet necrotic yellow vein virus 42 kDa triple gene block protein binds nucleic acid in vitro. *J. Gen. Virol.* 77, 889–897. doi:10.1099/0022-1317-77-5-889.
- Bleykasten-Grosshans, C., Guilley, H., Bouzoubaa, S., Richards, K. E., and Jonard, G. (1997). Independent expression of the first two triple gene block proteins of beet necrotic yellow vein virus complements virus defective in the corresponding gene but expression of the third protein inhibits viral cell-to-cell movement. *Mol. Plant. Microbe Interact.* 10, 240–246. doi:10.1094/MPMI.1997.10.2.240.
- Blunt, S. J., Asher, M. J. C., and Gilligan, C. A. (1991). Infection of sugar beet by *Polymyxa betae* in relation to soil temperature. *Plant Pathol.* 40, 257–267. doi:10.1111/j.1365-3059.1991.tb02375.x.
- Bol, J. F., Linthorst, H. J. M., and Cornelissen, B. J. C. (1990). Plant Pathogenesis-Related Proteins Induced by Virus Infection. *Annu. Rev. Phytopathol.* 28, 113–138. doi:10.1146/annurev.py.28.090190.000553.
- Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinforma. Oxf. Engl.* 30, 2114–2120. doi:10.1093/bioinformatics/btu170.
- Boller, T., and Felix, G. (2009). A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annu. Rev. Plant Biol.* 60, 379–406. doi:10.1146/annurev.arplant.57.032905.105346.
- Bork, P. (1993). Hundreds of ankyrin-like repeats in functionally diverse proteins: Mobile modules that cross phyla horizontally? *Proteins Struct. Funct. Bioinforma.* 17, 363–374. doi:10.1002/prot.340170405.
- Bork, P., Bowler, C., Vargas, C. de, Gorsky, G., Karsenti, E., and Wincker, P. (2015). Tara Oceans studies plankton at planetary scale. *Science* 348, 873–873. doi:10.1126/science.aac5605.
- Bornemann, K., Hanse, B., Varrelmann, M., and Stevens, M. (2015). Occurrence of resistance-breaking strains of Beet necrotic yellow vein virus in sugar beet in northwestern Europe and identification of a new variant of the viral pathogenicity factor P25. *Plant Pathol.* 64, 25–34. doi:10.1111/ppa.12249.
- Bornemann, K., and Varrelmann, M. (2011). Analysis of the resistance-breaking ability of different beet necrotic yellow vein virus isolates loaded into a single polymyxa

REFERENCES

- betae population in soil. *Phytopathology* 101, 718–724. doi:10.1094/PHYTO-06-10-0157.
- Bornemann, K., and Varrelmann, M. (2013). Effect of sugar beet genotype on the Beet necrotic yellow vein virus P25 pathogenicity factor and evidence for a fitness penalty in resistance-breaking strains. *Mol. Plant Pathol.* 14, 356–364. doi:10.1111/mpp.12012.
- Bouzoubaa, S., Guilley, H., Jonard, G., Richards, K., and Putz, C. (1985). Nucleotide Sequence Analysis of RNA-3 and RNA-4 of Beet Necrotic Yellow Vein Virus, Isolates F2 and G1. *J. Gen. Virol.* 66, 1553–1564. doi:10.1099/0022-1317-66-7-1553.
- Bouzoubaa, S., Quillet, L., and Guilley, H. (1987). Nucleotide sequence of beet necrotic yellow vein virus RNA-1. *J. Gen. Virol.* 68, 615–626. doi:10.1099/0022-1317-68-3-615.
- Bouzoubaa, S., and Scheidecker, D. (1990). Infection of protoplasts with Beet necrotic yellow vein virus RNA. *Proc. First Symp. Int. Work. Group Plant Viruses Fungal Vectors* 1, 16–20.
- Bouzoubaa, S., Ziegler, V., and Beck, D. (1986). Nucleotide sequence of beet necrotic yellow vein virus RNA-2. *J. Gen. Virol.* 67, 1689–1700. doi:10.1099/0022-1317-67-8-1689.
- Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L.-J., et al. (2017). Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annu. Rev. Phytopathol.* 55, 61–83. doi:10.1146/annurev-phyto-080516-035641.
- Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J. J., MacFarlane, S., Peters, D., et al. (2013). Status and Prospects of Plant Virus Control Through Interference with Vector Transmission. *Annu. Rev. Phytopathol.* 51, 177–201. doi:10.1146/annurev-phyto-082712-102346.
- Brandfass, C., and Karlovsky, P. (2008). Upscaled CTAB-based DNA extraction and real-time PCR assays for *Fusarium culmorum* and *F. graminearum* DNA in plant material with reduced sampling error. *Int. J. Mol. Sci.* 9, 2306–2321. doi:10.3390/ijms9112306.
- Braselton, J. P. (1984). Karyotypic analysis of *Polymyxa graminis* (Plasmodiophoromycetes) based on serial sections of synaptonemal complexes. *Can. J. Bot.* 62, 2414–2416. doi:10.1139/b84-329.
- Braselton, J. P. (1995). Current Status of the Plasmodiophorids. *Crit. Rev. Microbiol.* 21, 263–275. doi:10.3109/10408419509113543.
- Braselton, J. P., Miller, C. E., and Pechak, D. G. (1975). The Ultrastructure of Cruciform Nuclear Division in *Sorosphaera veronicae* (Plasmodiophoromycete). *Am. J. Bot.* 62, 349–358. doi:10.2307/2442088.
- Broeksema, B., Calusinska, M., McGee, F., Winter, K., Bongiovanni, F., Goux, X., et al. (2017). ICoVeR – an interactive visualization tool for verification and refinement of metagenomic bins. *BMC Bioinformatics* 18, 233. doi:10.1186/s12859-017-1653-5.
- Brown, C. R., Vandemark, G., Johnson, D., Cummings, T., Batchelor, D., Miller, J., et al. (2007). Resistance to powdery scab in potato. *Potato Prog.* 7, 1.
- Brown, N. P., Leroy, C., and Sander, C. (1998). MView: a web-compatible database search or multiple alignment viewer. *Bioinforma. Oxf. Engl.* 14, 380–381. doi:10.1093/bioinformatics/14.4.380.

- Brůna, T., Hoff, K. J., Lomsadze, A., Stanke, M., and Borodovsky, M. (2021). BRAKER2: automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database. *NAR Genomics Bioinforma.* 3. doi:10.1093/nargab/lqaa108.
- Brůna, T., Lomsadze, A., and Borodovsky, M. (2020). GeneMark-EP and -EP+: automatic eukaryotic gene prediction supported by spliced aligned proteins. *bioRxiv*, 2019.12.31.891218. doi:10.1101/2019.12.31.891218.
- Bryant, D. M., Johnson, K., DiTommaso, T., Tickle, T., Couger, M. B., Payzin-Dogru, D., et al. (2017). A Tissue-Mapped Axolotl De Novo Transcriptome Enables Identification of Limb Regeneration Factors. *Cell Rep.* 18, 762–776. doi:10.1016/j.celrep.2016.12.063.
- Buczacki, S. T., and Moxham, S. E. (1983). Structure of the resting spore wall of *Plasmodiophora brassicae* revealed by electron microscopy and chemical digestion. *Trans. Br. Mycol. Soc.* 81, 221–231. doi:10.1016/S0007-1536(83)80073-4.
- Buhariwalla, H., Greaves, S., Magrath, R., and Mithen, R. (1995). Development of specific PCR primers for the amplification of polymorphic DNA from the obligate root pathogen *Plasmodiophora brassicae*. *Physiol. Mol. Plant Pathol.* 47, 83–94. doi:10.1006/pmpp.1995.1044.
- Bulman, S., Candy, J. M., Fiers, M., Lister, R., Conner, A. J., and Eady, C. C. (2011). Genomics of Biotrophic, Plant-infecting Plasmodiophorids Using In Vitro Dual Cultures. *Protist* 162, 449–461. doi:10.1016/j.protis.2010.09.004.
- Bulman, S., and Neuhauser, S. (2017). “Phytomyxea,” in *Handbook of the Protists*, eds. J. M. Archibald, A. G. B. Simpson, and C. H. Slamovits (Cham: Springer International Publishing), 783–803. doi:10.1007/978-3-319-28149-0_24.
- Bulman, S. R., Kühn, S. F., Marshall, J. W., and Schnepf, E. (2001). A Phylogenetic Analysis of the SSU rRNA from Members of the Plasmodiophorida and Phagomyxida. *Protist* 152, 43–51. doi:10.1078/1434-4610-00042.
- Bulman, S., Richter, F., Marschollek, S., Benade, F., Jülke, S., and Ludwig-Müller, J. (2018). *Arabidopsis thaliana* expressing PbBSMT, a gene encoding a SABATH-type methyltransferase from the plant pathogenic protist *Plasmodiophora brassicae*, show leaf chlorosis and altered host susceptibility. *Plant Biol. Stuttg. Ger.* doi:10.1111/plb.12728.
- Bulman, S., Ridgway, H. J., Eady, C., and Conner, A. J. (2007). Intron-rich gene structure in the intracellular plant parasite *Plasmodiophora brassicae*. *Protist* 158, 423–433. doi:10.1016/j.protis.2007.04.005.
- Bulman, S., Siemens, J., Ridgway, H. J., Eady, C., and Conner, A. J. (2006). Identification of genes from the obligate intracellular plant pathogen, *Plasmodiophora brassicae*. *FEMS Microbiol. Lett.* 264, 198–204. doi:https://doi.org/10.1111/j.1574-6968.2006.00466.x.
- Burketová, L., Štillerová, K., and Feltlová, M. (2003). Immunohistological localization of chitinase and β -1,3-glucanase in rhizomania-diseased and benzothiadiazole treated sugar beet roots. *Physiol. Mol. Plant Pathol.* 63, 47–54. doi:10.1016/j.pmpp.2003.08.002.
- Burki, F., and Keeling, P. J. (2014). Rhizaria. *Curr. Biol.* 24, R103–R107. doi:10.1016/j.cub.2013.12.025.

REFERENCES

- Burki, F., Kudryavtsev, A., Matz, M. V., Aglyamova, G. V., Bulman, S., Fiers, M., et al. (2010). Evolution of Rhizaria: new insights from phylogenomic analysis of uncultivated protists. *BMC Evol. Biol.* 10, 377. doi:10.1186/1471-2148-10-377.
- Burki, F., Roger, A. J., Brown, M. W., and Simpson, A. G. B. (2020). The New Tree of Eukaryotes. *Trends Ecol. Evol.* 35, 43–55. doi:10.1016/j.tree.2019.08.008.
- Buscaill, P., Chandrasekar, B., Sanguankiatichai, N., Kourelis, J., Kaschani, F., Thomas, E. L., et al. (2019). Glycosidase and glycan polymorphism control hydrolytic release of immunogenic flagellin peptides. *Science* 364, eaav0748. doi:10.1126/science.aav0748.
- Bushnell, B. (2014). BBTools software package. Available at: <http://sourceforge.net/projects/bbmap/>.
- Bustin, S. A., Benes, V., Garson, J. A., Hellems, J., Huggett, J., Kubista, M., et al. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clin. Chem.* 55, 611–622. doi:10.1373/clinchem.2008.112797.
- Cai, X., and Clapham, D. E. (2008). Evolutionary Genomics Reveals Lineage-Specific Gene Loss and Rapid Evolution of a Sperm-Specific Ion Channel Complex: CatSper and CatSperβ. *PLOS ONE* 3, e3569. doi:10.1371/journal.pone.0003569.
- Cai, X., Wang, X., and Clapham, D. E. (2014). Early Evolution of the Eukaryotic Ca²⁺ Signaling Machinery: Conservation of the CatSper Channel Complex. *Mol. Biol. Evol.* 31, 2735–2740. doi:10.1093/molbev/msu218.
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., et al. (2009). BLAST+: architecture and applications. *BMC Bioinformatics* 10, 421. doi:10.1186/1471-2105-10-421.
- Campbell, R. N. (University of C., and Greathead, A. S. (1989). “Control of clubroot of crucifers by liming,” in *Soilborne Plant Pathogens: Management of Diseases with Macro- and Microelements*. (St. Paul, Minnesota: APS Press), 90–101. Available at: <https://agris.fao.org/agris-search/search.do?recordID=US9037509> [Accessed September 9, 2020].
- Canova, A. (1959). On the pathology of sugar beet. *Inf. Fitopatol.* 9, 390–396.
- Canova, A. (1966). Ricerche sulle malattie da virus delle Graminacee. III. Polymyxa graminis Led. vettore del virus del mosaico del frumento. *Phytopathol. Mediterr.* 5, 53–58.
- Canova, A., Giunchedi, L., and Biancardi, E. (2016). “History and current status,” in *Rhizomania*, 29–51. doi:10.1007/978-3-319-30678-0_2.
- Cao, T., Srivastava, S., Rahman, M. H., Kav, N. N. V., Hotte, N., Deyholos, M. K., et al. (2008). Proteome-level changes in the roots of Brassica napus as a result of Plasmodiophora brassicae infection. *Plant Sci.* 174, 97–115. doi:10.1016/j.plantsci.2007.10.002.
- Capistrano-Gossman, G. G., Ries, D., Holtgräwe, D., Minoche, A., Kraft, T., Frerichmann, S. L. M., et al. (2017). Crop wild relative populations of Beta vulgaris allow direct mapping of agronomically important genes. *Nat. Commun.* 8, 15708. doi:10.1038/ncomms15708.
- Castilleux, R., Plancot, B., Gügi, B., Attard, A., Loutelier-Bourhis, C., Lefranc, B., et al. (2020). Extensin arabinosylation is involved in root response to elicitors and limits oomycete colonization. *Ann. Bot.* 125, 751–763. doi:10.1093/aob/mcz068.

- Cavalier-Smith, T., Chao, E. E., and Lewis, R. (2018). Multigene phylogeny and cell evolution of chromist infrakingdom Rhizaria: contrasting cell organisation of sister phyla Cercozoa and Retaria. *Protoplasma* 255, 1517–1574. doi:10.1007/s00709-018-1241-1.
- CEFS (2021). The EU Sugar Sector. *Eur. Assoc. Sugar Manuf.* Available at: <https://cefs.org/sugar/> [Accessed September 17, 2021].
- Chandran, D., Tai, Y. C., Hather, G., Dewdney, J., Denoux, C., Burgess, D. G., et al. (2009). Temporal global expression data reveal known and novel salicylate-impacted processes and regulators mediating powdery mildew growth and reproduction on *Arabidopsis*. *Plant Physiol.* 149, 1435–1451. doi:10.1104/pp.108.132985.
- Chaparro, C., Gayraud, T., de Souza, R. F., Domingues, D. S., Akaffou, S., Laforga Vanzela, A. L., et al. (2015). Terminal-Repeat Retrotransposons with GAG Domain in Plant Genomes: A New Testimony on the Complex World of Transposable Elements. *Genome Biol. Evol.* 7, 493–504. doi:10.1093/gbe/evv001.
- Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P., and Parks, D. H. (2020). GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* 36, 1925–1927. doi:10.1093/bioinformatics/btz848.
- Chen, J., Pang, W., Chen, B., Zhang, C., and Piao, Z. (2016a). Transcriptome Analysis of *Brassica rapa* Near-Isogenic Lines Carrying Clubroot-Resistant and -Susceptible Alleles in Response to *Plasmodiophora brassicae* during Early Infection. *Front. Plant Sci.* 6. doi:10.3389/fpls.2015.01183.
- Chen, J., Swaby, A. G., Adams, M. J., and Yili, R. (1991). Barley mild mosaic virus inside its fungal vector, *Polymyxa graminis*. *Ann. Appl. Biol.* 118, 615–621. doi:10.1111/j.1744-7348.1991.tb05351.x.
- Chen, J., Wang, Z., Hong, J., Collier, C. R., and Adams, M. J. (1998). Ultrastructural studies of resting spore development in *Polymyxa graminis*. *Mycol. Res.* 102, 687–691. doi:10.1017/S0953756297005406.
- Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34, i884–i890. doi:10.1093/bioinformatics/bty560.
- Chen, W., Li, Y., Yan, R., Ren, L., Liu, F., Zeng, L., et al. (2021). SnRK1.1-mediated resistance of *Arabidopsis thaliana* to clubroot disease is inhibited by the novel *Plasmodiophora brassicae* effector PBZF1. *Mol. Plant Pathol.* 22, 1057–1069. doi:10.1111/mpp.13095.
- Chen, W., Li, Y., Yan, R., Xu, L., Ren, L., Liu, F., et al. (2019). Identification and Characterization of *Plasmodiophora brassicae* Primary Infection Effector Candidates that Suppress or Induce Cell Death in Host and Nonhost Plants. *Phytopathology*® 109, 1689–1697. doi:10.1094/PHYTO-02-19-0039-R.
- Chen, X.-R., Li, Y.-P., Li, Q.-Y., Xing, Y.-P., Liu, B.-B., Tong, Y.-H., et al. (2016b). SCR96, a small cysteine-rich secretory protein of *Phytophthora cactorum*, can trigger cell death in the Solanaceae and is important for pathogenicity and oxidative stress tolerance. *Mol. Plant Pathol.* 17, 577–587. doi:10.1111/mpp.12303.
- Chiba, S., Hleibieh, K., Delbianco, A., Klein, E., Ratti, C., Ziegler-Graff, V., et al. (2013). The Benyvirus RNA silencing suppressor is essential for long-distance movement, requires both zinc-finger and nols basic residues but not a nucleolar localization for its silencing-suppression activity. *Mol. Plant. Microbe Interact.* 26, 168–181. doi:10.1094/MPMI-06-12-0142-R.

REFERENCES

- Chiba, S., Kondo, H., Miyanishi, M., Andika, I. B., Han, C., and Tamada, T. (2011). The evolutionary history of Beet necrotic yellow vein virus deduced from genetic variation, geographical origin and spread, and the breaking of host resistance. *Mol. Plant-Microbe Interact. MPMI* 24, 207–218. doi:10.1094/MPMI-10-10-0241.
- Chiba, S., Miyanishi, M., Andika, I. B., Kondo, H., and Tamada, T. (2008). Identification of amino acids of the beet necrotic yellow vein virus p25 protein required for induction of the resistance response in leaves of *Beta vulgaris* plants. *J. Gen. Virol.* 89, 1314–1323. doi:10.1099/vir.0.83624-0.
- Chiba, S., Miyanishi, M., Kondo, H., Tamada, T., Rush, C. M., and Merz, U. (2003). Single amino acid changes in the P25 protein gene of Beet necrotic yellow vein virus are involved in resistance responses in *Beta vulgaris* ssp. *maritima*. *5th Symp. Int. Work. Group Plant Viruses Fungal Vectors*, 134.
- Chiniquy, D., Underwood, W., Corwin, J., Ryan, A., Szemenyei, H., Lim, C. C., et al. (2019). PMR5, an acetylation protein at the intersection of pectin biosynthesis and defense against fungal pathogens. *Plant J. Cell Mol. Biol.* 100, 1022–1035. doi:10.1111/tpj.14497.
- Choudhary, D. K., Prakash, A., and Johri, B. N. (2007). Induced systemic resistance (ISR) in plants: mechanism of action. *Indian J. Microbiol.* 47, 289–297. doi:10.1007/s12088-007-0054-2.
- Chung, J.-J., Miki, K., Kim, D., Shim, S.-H., Shi, H. F., Hwang, J. Y., et al. (2017). CatSper ζ regulates the structural continuity of sperm Ca²⁺ signaling domains and is required for normal fertility. *eLife* 6, e23082. doi:10.7554/eLife.23082.
- Ciafardini, G., Arena, L. M., Mares, D., and Bruni, A. (1995). Cell wall ultrastructure in resting-spores of *Polymyxa betae* Keskin (Plasmodiophoraceae). *Phyton - Ann. Rei Bot.* 35, 153–163.
- Ciafardini, G., and Marotta, B. (1988). Scanning electron microscopy of the sporosorus in *Polymyxa betae* (Plasmodiophoromycetes). *Can. J. Bot.* 66, 2518–2522. doi:10.1139/b88-342.
- Ciafardini, G., and Marotta, B. (1989). Localization of fragile areas in walls of resting spores of *Polymyxa betae*. *Can. J. Bot.* 67, 3123–3126. doi:10.1139/b89-391.
- Ciaghi, S., Neuhauser, S., and Schwelm, A. (2018). Draft Genome Resource for the Potato Powdery Scab Pathogen *Spongospora subterranea*. *Mol. Plant-Microbe Interactions*® 31, 1227–1229. doi:10.1094/MPMI-06-18-0163-A.
- Ciaghi, S., Schwelm, A., and Neuhauser, S. (2019). Transcriptomic response in symptomless roots of clubroot infected kohlrabi (*Brassica oleracea* var. *gongylodes*) mirrors resistant plants. *BMC Plant Biol.* 19, 288. doi:10.1186/s12870-019-1902-z.
- CIBE (2021). Key figures. *Int. Confed. Eur. Beet Grow.* Available at: <https://www.cibe-europe.eu/KeyFigures> [Accessed September 17, 2021].
- Claxton, J. R., Potter, U. J., Blakesley, D., and Clarkson, J. M. (1996). An ultrastructural study of the interaction between *Spongospora subterranea* f. sp. *nasturtii* and watercress roots. *Mycol. Res.* 100, 1431–1439. doi:10.1016/S0953-7562(96)80074-8.
- Clay, C. M., and Walsh, J. A. (1996). Watercress yellowspot virus (WYSV): cytopathic alterations suggest it is a member of the Tombusviridae. in *Proc 3rd Symp Int Work Group Plant Viruses Fungal Vectors, Dundee, Scotland* (Citeseer).

- Clinckemaillie, A., Decroës, A., Aubel, G. van, Santos, S. C. dos, Renard, M. E., Cutsem, P. V., et al. (2017). The novel elicitor COS-OGA enhances potato resistance to late blight. *Plant Pathol.* 66, 818–825. doi:10.1111/ppa.12641.
- Coninck, B. D., Timmermans, P., Vos, C., Cammue, B. P. A., and Kazan, K. (2015). What lies beneath: belowground defense strategies in plants. *Trends Plant Sci.* 20, 91–101. doi:10.1016/j.tplants.2014.09.007.
- Conrath, U. (2006). Systemic Acquired Resistance. *Plant Signal. Behav.* 1, 179–184. doi:10.4161/psb.1.4.3221.
- Conrath, U. (2009). "Chapter 9 Priming of Induced Plant Defense Responses," in *Advances in Botanical Research* Advances in Botanical Research. (Academic Press), 361–395. doi:10.1016/S0065-2296(09)51009-9.
- Cooke, D. A., and Scott, J. E. (1993). *The Sugar Beet Crop*. Springer Netherlands doi:10.1007/978-94-009-0373-9.
- Cosgrove, D. J. (2015). Plant expansins: diversity and interactions with plant cell walls. *Curr. Opin. Plant Biol.* 25, 162–172. doi:10.1016/j.pbi.2015.05.014.
- Coutts, R. H. A., Linton, D. J., and Bolwell, G. P. (1994). Patterns of protein synthesis in infected and stressed sugar beet (*Beta vulgaris* L.). *J. Phytopathol.* 142, 274–282. doi:10.1111/j.1439-0434.1994.tb00023.x.
- Covelli, L., Klein, E., and Gilmer, D. (2009). The first 17 amino acids of the beet necrotic yellow vein virus RNA-5-encoded p26 protein are sufficient to activate transcription in a yeast one-hybrid system. *Arch. Virol.* 154, 347–351. doi:10.1007/s00705-008-0306-4.
- Cowan, G. H., Torrance, L., and Reavy, B. (1997). Detection of potato mop-top virus capsid readthrough protein in virus particles. *J. Gen. Virol.* 78, 1779–1783. doi:10.1099/0022-1317-78-7-1779.
- Cox, B. A., Luo, H., and Jones, R. a. C. (2014). Polymyxa graminis Isolates from Australia: Identification in Wheat Roots and Soil, Molecular Characterization, and Wide Genetic Diversity. *Plant Dis.* 98, 1567–1575. doi:10.1094/PDIS-02-14-0128-RE.
- Crutzen, F. (2010). Pomoviruses associated with the rhizomania syndrome : a reverse genetic approach of the genome organization and multiplication requirements in planta.
- Crutzen, F., Kreit, M., and Bragard, C. (2009a). The beet virus Q coat protein readthrough domain is longer than previously reported, with two transmembrane domains. *J. Gen. Virol.* 90, 754–758. doi:10.1099/vir.0.008029-0.
- Crutzen, F., Mehrvar, M., Gilmer, D., and Bragard, C. (2009b). A full-length infectious clone of beet soil-borne virus indicates the dispensability of the RNA-2 for virus survival in planta and symptom expression on *Chenopodium quinoa* leaves. *J. Gen. Virol.* 90, 3051–3056. doi:10.1099/vir.0.014548-0.
- Curtis, B. A., Tanifuji, G., Burki, F., Gruber, A., Irimia, M., Maruyama, S., et al. (2012). Algal genomes reveal evolutionary mosaicism and the fate of nucleomorphs. *Nature* 492, 59–65. doi:10.1038/nature11681.
- Dahm, H. D., and Buchenauer, H. (1993). Studies on the Biology of Polymyxa betae, the Vector of Beet Necrotic Yellow Vein Virus. *J. Phytopathol.* 139, 329–338. doi:10.1111/j.1439-0434.1993.tb01434.x.
- D'Alonzo, M., Delbianco, A., Lanzoni, C., Autonell, C. R., Gilmer, D., and Ratti, C. (2012). Beet soil-borne mosaic virus RNA-4 encodes a 32kDa protein involved in

REFERENCES

- symptom expression and in virus transmission through *Polymyxa betae*. *Virology* 423, 187–194. doi:10.1016/j.virol.2011.12.007.
- D'Ambra, V. (1967). Piante ospiti di *Polymyxa betae* Keskin. *Atti Ist Ven Sc Lett Ed Arti* 125, 177–182.
- D'Ambra, V., and Mutto, S. (1975). ULTRAISTRUTTURA DI POLYMYXA BETAE KESKIN. PLASMODIO, SPORANGIO E CISTOSORO. *Riv. Patol. Veg.* 11, 115–124.
- D'Ambra, V., and Mutto, S. (1977). The ultrastructure of *Polymyxa betae* zoospore exit-tube differentiation. *Can. J. Bot.* 55, 831–839. doi:10.1139/b77-098.
- Dao, T. T. H., Linthorst, H. J. M., and Verpoorte, R. (2011). Chalcone synthase and its functions in plant resistance. *Phytochem. Rev.* 10, 397. doi:10.1007/s11101-011-9211-7.
- D'Arcy, C. J., Eastburn, D. M., and Schumann, G. L. (2001). Illustrated Glossary of Plant Pathology. *Plant Health Instr.* Available at: <https://www.apsnet.org/edcenter/resources/illglossary/Pages/default.aspx> [Accessed January 29, 2021].
- Darling, A. E., Mau, B., and Perna, N. T. (2010). progressiveMauve: Multiple Genome Alignment with Gene Gain, Loss and Rearrangement. *PLOS ONE* 5, e11147. doi:10.1371/journal.pone.0011147.
- Daval, S., Belcour, A., Gazengel, K., Legrand, L., Gouzy, J., Cottret, L., et al. (2019). Computational analysis of the *Plasmodiophora brassicae* genome: mitochondrial sequence description and metabolic pathway database design. *Genomics* 111, 1629–1640. doi:10.1016/j.ygeno.2018.11.013.
- De Vos, M., Van Oosten, V. R., Van Poecke, R. M. P., Van Pelt, J. A., Pozo, M. J., Mueller, M. J., et al. (2005). Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. *Mol. Plant-Microbe Interact. MPMI* 18, 923–937. doi:10.1094/MPMI-18-0923.
- Decroës, A., Calusinska, M., Delfosse, P., Bragard, C., and Legrève, A. (2019). First Draft Genome Sequence of a *Polymyxa* Genus Member, *Polymyxa betae*, the Protist Vector of Rhizomania. *Microbiol. Resour. Announc.* 8. doi:10.1128/MRA.01509-18.
- Decroës, A., Clausse, M., Galein, V., Legreve, A., Wauters, A., and Bragard, C. (2015). NEW BNYYV P25 VARIANTS IN BELGIUM. *Commun. Agric. Appl. Biol. Sci.* 80, 407–409.
- Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M.-A., Jaques Miret, J. A., Fejer Justesen, A., et al. (2020). Pest categorisation of beet necrotic yellow vein virus. *EFSA J.* 18. doi:10.2903/j.efsa.2020.6360.
- Delfosse, P., Reddy, A. S., Legrève, A., Devi, K. T., Abdurahman, M. D., Maraite, H., et al. (2000). Serological Methods for Detection of *Polymyxa graminis*, an Obligate Root Parasite and Vector of Plant Viruses. *Phytopathology* 90, 537–545. doi:10.1094/PHYTO.2000.90.5.537.
- Delfosse, P., Reddy, A. S., Thirumala Devi, K., Legrève, A., Risopoulos, J., Doucet, D., et al. (2002). Dynamics of *Polymyxa graminis* and Indian peanut clump virus (IPCV) infection on various monocotyledonous crops and groundnut during the rainy season. *Plant Pathol.* 51, 546–560. doi:10.1046/j.1365-3059.2002.00752.x.
- Deng, Y., Dong, H., Mu, J., Ren, B., Zheng, B., Ji, Z., et al. (2010). *Arabidopsis* Histidine Kinase CK1 ϵ Acts Upstream of HISTIDINE PHOSPHOTRANSFER PROTEINS to

- Regulate Female Gametophyte Development and Vegetative Growth. *Plant Cell* 22, 1232–1248. doi:10.1105/tpc.108.065128.
- Desoignies, N. (2012). *Polymyxa betae* - *Beta vulgaris*: understanding the molecular interactions through transcriptome and plant defense analysis. Available at: <https://dial.uclouvain.be/pr/boreal/object/boreal:117081> [Accessed August 18, 2021].
- Desoignies, N., Carbonell, J., Moreau, J.-S., Conesa, A., Dopazo, J., and Legrève, A. (2014). Molecular interactions between sugar beet and *Polymyxa betae* during its life cycle. *Ann. Appl. Biol.* 164, 244–256. doi:10.1111/aab.12095.
- Desoignies, N., and Legrève, A. (2011). In Vitro Dual Culture of *Polymyxa betae* in *Agrobacterium rhizogenes* Transformed Sugar Beet Hairy Roots in Liquid Media. *J. Eukaryot. Microbiol.* 58, 424–425. doi:10.1111/j.1550-7408.2011.00563.x.
- Desoignies, N., Schramme, F., Ongena, M., and Legrève, A. (2013). Systemic resistance induced by *Bacillus* lipopeptides in *Beta vulgaris* reduces infection by the rhizomania disease vector *Polymyxa betae*. *Mol. Plant Pathol.* 14, 416–421. doi:10.1111/mpp.12008.
- Desoignies, N., Stocco, C., Bragard, C., and Legrève, A. (2011). A new phenotype of *Polymyxa betae* in *Arabidopsis thaliana*. *Eur. J. Plant Pathol.* 131, 27–38. doi:10.1007/s10658-011-9783-5.
- Devos, S., Laukens, K., Deckers, P., Van Der Straeten, D., Beeckman, T., Inzé, D., et al. (2006). A Hormone and Proteome Approach to Picturing the Initial Metabolic Events During *Plasmodiophora brassicae* Infection on *Arabidopsis*. *Mol. Plant-Microbe Interactions*® 19, 1431–1443. doi:10.1094/MPMI-19-1431.
- Diao, A., Chen, J., Gitton, F., Antoniw, J. F., Mullins, J., Hall, A. M., et al. (1999). Sequences of European Wheat Mosaic Virus and Oat Golden Stripe Virus and Genome Analysis of the Genus Furovirus. *Virology* 261, 331–339. doi:10.1006/viro.1999.9880.
- Dierckxsens, N., Mardulyn, P., and Smits, G. (2017). NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Res.* 45, e18. doi:10.1093/nar/gkw955.
- Dieryck, B., Otto, G., Doucet, D., Legrève, A., Delfosse, P., and Bragard, C. (2009). Seed, soil and vegetative transmission contribute to the spread of pecluviruses in Western Africa and the Indian sub-continent. *Virus Res.* 141, 184–189. doi:10.1016/j.virusres.2008.08.017.
- Dieryck, B., Weyns, J., Doucet, D., Bragard, C., and Legrève, A. (2011). Acquisition and Transmission of Peanut clump virus by *Polymyxa graminis* on Cereal Species. *Phytopathology* 101, 1149–1158. doi:10.1094/PHYTO-12-10-0335.
- Dilweg, I. W., Gultyaev, A. P., and Olsthoorn, R. C. (2019). Structural features of an Xrn1-resistant plant virus RNA. *RNA Biol.* 16, 838–845. doi:10.1080/15476286.2019.1592070.
- Dimmer, E., Roden, L., Cai, D., Kingsnorth, C., and Mutasa-Göttgens, E. (2004). Transgenic analysis of sugar beet xyloglucan endo-transglucosylase/hydrolase Bv-XTH1 and Bv-XTH2 promoters reveals overlapping tissue-specific and wound-inducible expression profiles. *Plant Biotechnol. J.* 2, 127–139. doi:https://doi.org/10.1046/j.1467-7652.2004.00056.x.
- Dirven, J. A. A. M., and Peters, D. (1995). Characteristics of Serially Produced Zoospore Suspensions of *Polymyxa betae* for Transmission of Beet Necrotic Yellow Vein Virus. *J. Phytopathol.* 143, 537–541. doi:10.1111/j.1439-0434.1995.tb00659.x.

REFERENCES

- Dixon, G. R. (2009). The Occurrence and Economic Impact of <Emphasis Type="Italic">Plasmodiophora brassicae</Emphasis> and Clubroot Disease. *J. Plant Growth Regul.* 28, 194–202. doi:10.1007/s00344-009-9090-y.
- Dixon, G. R. (2014). Clubroot (*Plasmodiophora brassicae* Woronin) – an agricultural and biological challenge worldwide. *Can. J. Plant Pathol.* 36, 5–18. doi:10.1080/07060661.2013.875487.
- Djamei, A., Schipper, K., Rabe, F., Ghosh, A., Vincon, V., Kahnt, J., et al. (2011). Metabolic priming by a secreted fungal effector. *Nature* 478, 395–398. doi:10.1038/nature10454.
- Djavaheri, M., Ma, L., Klessig, D. F., Mithöfer, A., Gropp, G., and Borhan, H. (2018a). Mimicking the Host Regulation of Salicylic Acid: A Virulence Strategy by the Clubroot Pathogen *Plasmodiophora brassicae*. *Mol. Plant-Microbe Interactions®* 32, 296–305. doi:10.1094/MPMI-07-18-0192-R.
- Djavaheri, M., Ma, L., Klessig, D. F., Mithöfer, A., Gropp, G., and Borhan, H. (2018b). Mimicking the Host Regulation of Salicylic Acid: A Virulence Strategy by the Clubroot Pathogen *Plasmodiophora brassicae*. *Mol. Plant-Microbe Interactions®* 32, 296–305. doi:10.1094/MPMI-07-18-0192-R.
- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., et al. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21. doi:10.1093/bioinformatics/bts635.
- Dobisova, T., Hrdinova, V., Cuesta, C., Michlickova, S., Urbankova, I., Hejatkova, R., et al. (2017). Light Controls Cytokinin Signaling via Transcriptional Regulation of Constitutively Active Sensor Histidine Kinase CK11. *Plant Physiol.* 174, 387–404. doi:10.1104/pp.16.01964.
- Dodds, P. N., and Rathjen, J. P. (2010). Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat. Rev. Genet.* 11, 539–548. doi:10.1038/nrg2812.
- Dohm, J. C., Minoche, A. E., Holtgräwe, D., Capella-Gutiérrez, S., Zakrzewski, F., Tafer, H., et al. (2014). The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*). *Nature* 505, 546–549. doi:10.1038/nature12817.
- Donath, A., Jühling, F., Al-Arab, M., Bernhart, S. H., Reinhardt, F., Stadler, P. F., et al. (2019). Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. *Nucleic Acids Res.* 47, 10543–10552. doi:10.1093/nar/gkz833.
- Dou, D., and Zhou, J.-M. (2012). Phytopathogen Effectors Subverting Host Immunity: Different Foes, Similar Battleground. *Cell Host Microbe* 12, 484–495. doi:10.1016/j.chom.2012.09.003.
- Down, G. J., Grenville, L. J., and Clarkson, J. M. (2002). Phylogenetic analysis of *Spongospora* and implications for the taxonomic status of the plasmodiophorids. *Mycol. Res.* 106, 1060–1065. doi:10.1017/S0953756202006391.
- Draycott, A. P. (1993). "Nutrition.," in *The Sugar Beet Crop: Science into practice* (London: Cooke DA & Scott RK), 265–270.
- Draycott, A. P. (2008). Sugar beet.
- Driskel, B. A., Doss, P., Littlefield, L. J., Walker, N. R., and Verchot-Lubicz, J. (2004). Soilborne Wheat Mosaic Virus Movement Protein and RNA and Wheat Spindle Streak Mosaic Virus Coat Protein Accumulate Inside Resting Spores of Their Vector,

- Polymyxa graminis*. *Mol. Plant. Microbe Interact.* 17, 739–748. doi:10.1094/MPMI.2004.17.7.739.
- Dubois, F., Sangwan, R. S., and Sangwan-Norreel, B. S. (1994). Immunogold Labeling and Electron Microscopic Screening of Beet Necrotic Yellow Vein Virus in the Fungus *Polymyxa betae* Infecting Beta vulgaris Root Cortical Parenchyma Cells. *Int. J. Plant Sci.* 155, 545–552.
- Dünser, K., Gupta, S., Herger, A., Feraru, M. I., Ringli, C., and Kleine-Vehn, J. (2019). Extracellular matrix sensing by FERONIA and Leucine-Rich Repeat Extensins controls vacuolar expansion during cellular elongation in Arabidopsis thaliana. *EMBO J.* 38, e100353.
- Eisenhaber, B., Bork, P., and Eisenhaber, F. (1999). Prediction of potential GPI-modification sites in proprotein sequences. *J. Mol. Biol.* 292, 741–758. doi:10.1006/jmbi.1999.3069.
- Elmore, J. M., Perovic, D., Ordon, F., Schweizer, P., and Wise, R. P. (2018). “A Genomic View of Biotic Stress Resistance,” in *The Barley Genome Compendium of Plant Genomes.*, eds. N. Stein and G. J. Muehlbauer (Cham: Springer International Publishing), 233–257. doi:10.1007/978-3-319-92528-8_14.
- Emanuelsson, O., Nielsen, H., Brunak, S., and von Heijne, G. (2000). Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *J. Mol. Biol.* 300, 1005–1016. doi:10.1006/jmbi.2000.3903.
- Erhardt, M., Dunoyer, P., Guilley, H., Richards, K., Jonard, G., and Bouzoubaa, S. (2001). Beet necrotic yellow vein virus particles localize to mitochondria during infection. *Virology* 286, 256–262. doi:10.1006/viro.2001.0931.
- Erhardt, M., Morant, M., Ritzenthaler, C., Stussi-Garaud, C., Guilley, H., Richards, K., et al. (2000). P42 Movement Protein of Beet necrotic yellow vein virus Is Targeted by the Movement Proteins P13 and P15 to Punctate Bodies Associated with Plasmodesmata. *Mol. Plant-Microbe Interactions®* 13, 520–528. doi:10.1094/MPMI.2000.13.5.520.
- Erhardt, M., Vetter, G., Gilmer, D., Bouzoubaa, S., Richards, K., Jonard, G., et al. (2005). Subcellular localization of the Triple Gene Block movement proteins of Beet necrotic yellow vein virus by electron microscopy. *Virology* 340, 155–166. doi:10.1016/j.virol.2005.06.012.
- European Commission (2021). Sugar. *Eur. Comm. - Eur. Comm.* Available at: https://ec.europa.eu/info/food-farming-fisheries/plants-and-plant-products/plant-products/sugar_en [Accessed September 17, 2021].
- Evans, E., and Messerschmidt, U. (2017). Review: Sugar beets as a substitute for grain for lactating dairy cattle. *J. Anim. Sci. Biotechnol.* 8, 25. doi:10.1186/s40104-017-0154-8.
- Falloon, R. E., Curtin, D., Lister, R. A., and Butler, R. C. (2004). The obligate soilborne pathogen *Spongospora subterranea* affects host (*Solanum tuberosum*) root function. in *Proc. 3rd Australasian Soilborne Diseases Symp. South Australian Research and Development Inst. Adelaide, Australia*, 30–31.
- Falloon, R. E., Genet, R. A., Wallace, A. R., and Butler, R. C. (2003). Susceptibility of potato (*Solanum tuberosum*) cultivars to powdery scab (caused by *Spongospora subterranea* f. sp. *subterranea*), and relationships between tuber and root infection. *Australas. Plant Pathol.* 32, 377–385. doi:10.1071/AP03040.

REFERENCES

- Falloon, R. E., Merz, U., Butler, R. C., Curtin, D., Lister, R. A., and Thomas, S. M. (2015). Root infection of potato by *Spongospora subterranea*: knowledge review and evidence for decreased plant productivity. *Plant Pathol.* 65, 422–434. doi:10.1111/ppa.12419.
- Falloon, R. E., Merz, U., Butler, R. C., Curtin, D., Lister, R. A., and Thomas, S. M. (2016). Root infection of potato by *Spongospora subterranea*: knowledge review and evidence for decreased plant productivity. *Plant Pathol.* 65, 422–434. doi:10.1111/ppa.12419.
- Fan, H., Sun, H., Wang, Y., Zhang, Y., Wang, X., Li, D., et al. (2014). Deep Sequencing–Based Transcriptome Profiling Reveals Comprehensive Insights into the Responses of *Nicotiana benthamiana* to Beet necrotic yellow vein virus Infections Containing or Lacking RNA4. *PLOS ONE* 9, e85284. doi:10.1371/journal.pone.0085284.
- Fan, H., Zhang, Y., Sun, H., Liu, J., Wang, Y., Wang, X., et al. (2015a). Transcriptome Analysis of *Beta macrocarpa* and Identification of Differentially Expressed Transcripts in Response to Beet Necrotic Yellow Vein Virus Infection. *PLOS ONE* 10, e0132277. doi:10.1371/journal.pone.0132277.
- Fan, J., Ning, K., Zeng, X., Luo, Y., Wang, D., Hu, J., et al. (2015b). Genomic Foundation of Starch-to-Lipid Switch in Oleaginous *Chlorella* spp. *Plant Physiol.* 169, 2444–2461. doi:10.1104/pp.15.01174.
- FAOSTAT (2021). Cultures et produits animaux. Available at: <http://www.fao.org/faostat/> [Accessed September 17, 2021].
- Fei, W., Feng, J., Rong, S., Strelkov, S. E., Gao, Z., and Hwang, S.-F. (2015). Infection and Gene Expression of the Clubroot Pathogen *Plasmodiophora brassicae* in Resistant and Susceptible Canola Cultivars. *Plant Dis.* 100, 824–828. doi:10.1094/PDIS-11-15-1255-RE.
- Feng, J., Hwang, R., Hwang, S.-F., Strelkov, S. E., Gossen, B. D., Zhou, Q.-X., et al. (2010). Molecular characterization of a serine protease Pro1 from *Plasmodiophora brassicae* that stimulates resting spore germination. *Mol. Plant Pathol.* 11, 503–512. doi:10.1111/j.1364-3703.2010.00623.x.
- Fernando Gil, J., Liebe, S., Thiel, H., Lennefors, B.-L., Kraft, T., Gilmer, D., et al. (2018). Massive up-regulation of LBD transcription factors and EXPANSINS highlights the regulatory programs of rhizomania disease. *Mol. Plant Pathol.* 19, 2333–2348. doi:10.1111/mpp.12702.
- Fernando Gil, J., Wibberg, D., Eini, O., Savenkov, E. I., Varrelmann, M., and Liebe, S. (2020). Comparative Transcriptome Analysis Provides Molecular Insights into the Interaction of Beet necrotic yellow vein virus and Beet soil-borne mosaic virus with Their Host Sugar Beet. *Viruses* 12, 76. doi:10.3390/v12010076.
- Finn, R. D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R. Y., Eddy, S. R., et al. (2014). Pfam: the protein families database. *Nucleic Acids Res.* 42, D222–D230. doi:10.1093/nar/gkt1223.
- Flegontova, O., Flegontov, P., Malviya, S., Audic, S., Wincker, P., de Vargas, C., et al. (2016). Extreme Diversity of Diplonemid Eukaryotes in the Ocean. *Curr. Biol.* 26, 3060–3065. doi:10.1016/j.cub.2016.09.031.
- Flobinus, A., Chevigny, N., Charley, P. A., Seissler, T., Klein, E., Bleykasten-Grosshans, C., et al. (2018). Beet necrotic yellow vein virus noncoding rna production depends on a 5'→3' xrn exoribonuclease activity. *Viruses* 10. doi:10.3390/v10030137.

- Flobinus, A., Hleibieh, K., Klein, E., Ratti, C., Bouzoubaa, S., and Gilmer, D. (2016). A viral noncoding RNA complements a weakened viral RNA silencing suppressor and promotes efficient systemic host infection. *Viruses* 8. doi:10.3390/v8100272.
- Foley, R. C., and Singh, K. B. (2004). TGA5 acts as a positive and TGA4 acts as a negative regulator of ocs element activity in Arabidopsis roots in response to defence signals. *FEBS Lett.* 563, 141–145. doi:https://doi.org/10.1016/S0014-5793(04)00288-1.
- Forouhar, F., Yang, Y., Kumar, D., Chen, Y., Fridman, E., Park, S. W., et al. (2005). Structural and biochemical studies identify tobacco SABP2 as a methyl salicylate esterase and implicate it in plant innate immunity. *Proc. Natl. Acad. Sci.* 102, 1773–1778. doi:10.1073/pnas.0409227102.
- Francis, S. A. (2006). “Development of sugar beet,” in, ed. A. P. Draycott (Blackwell Publishing Oxford), 9–29. Available at: <https://repository.rothamsted.ac.uk/item/898x5/development-of-sugar-beet> [Accessed September 17, 2021].
- Fuchs, W. H., and Madelin, M. F. (1966). Liberation and behaviour of spores of *Polymyxa betae* Keskin. *Fungus Spore*, 111–112.
- Fujisawa, I., and Sugimoto, T. (1977). Transmission of beet necrotic yellow vein virus by *Polymyxa betae*. *Ann. Phytopathol. Soc. Jpn.* 43, 583–586.
- Galein, Y., Legrève, A., and Bragard, C. (2018). Long Term Management of Rhizomania Disease—Insight Into the Changes of the Beet necrotic yellow vein virus RNA-3 Observed Under Resistant and Non-resistant Sugar Beet Fields. *Front. Plant Sci.* 9. doi:10.3389/fpls.2018.00795.
- Gao, F., Zhang, B.-S., Zhao, J.-H., Huang, J.-F., Jia, P.-S., Wang, S., et al. (2019). Deacetylation of chitin oligomers increases virulence in soil-borne fungal pathogens. *Nat. Plants* 5, 1167–1176. doi:10.1038/s41477-019-0527-4.
- García, M. L., Bó, E. D., da Graça, J. V., Gago-Zachert, S., Hammond, J., Moreno, P., et al. (2018). Corrigendum: ICTV Virus Taxonomy Profile: Ophioviridae. *J. Gen. Virol.* 99, 949. doi:10.1099/jgv.0.001093.
- Gattiker, A., Gasteiger, E., and Bairoch, A. (2002). ScanProsite: a reference implementation of a PROSITE scanning tool. *Appl. Bioinformatics* 1, 107–108.
- Gau, R. D., Merz, U., Falloon, R. E., and Brunner, P. C. (2013). Global Genetics and Invasion History of the Potato Powdery Scab Pathogen, *Spongospora subterranea* f.sp. *subterranea*. *PLOS ONE* 8, e67944. doi:10.1371/journal.pone.0067944.
- Gawryluk, R. M. R., del Campo, J., Okamoto, N., Strasser, J. F. H., Lukeš, J., Richards, T. A., et al. (2016). Morphological Identification and Single-Cell Genomics of Marine Diplonemids. *Curr. Biol.* 26, 3053–3059. doi:10.1016/j.cub.2016.09.013.
- George Thompson, A. M., Iancu, C. V., Neet, K. E., Dean, J. V., and Choe, J. (2017). Differences in salicylic acid glucose conjugations by UGT74F1 and UGT74F2 from *Arabidopsis thaliana*. *Sci. Rep.* 7, 46629. doi:10.1038/srep46629.
- Gerik, J. S., and Duffus, J. E. (1988). Differences in vectoring ability and aggressiveness of isolates of *Polymyxa betae*. *Phytopathology* 78, 1340–1343.
- Gilchrist, E., Soler, J., Merz, U., and Reynaldi, S. (2011). Powdery scab effect on the potato *Solanum tuberosum* ssp. *andigena* growth and yield. *Trop. Plant Pathol.* 36, 350–355. doi:10.1590/S1982-56762011000600002.

REFERENCES

- Gilmer, D. (2016). "Molecular biology and replication of beet necrotic yellow vein virus," in *Rhizomania*, 85–107. doi:10.1007/978-3-319-30678-0_4.
- Gilmer, D., Bouzoubaa, S., Hehn, A., Guilley, H., Richards, K., and Jonard, G. (1992). Efficient cell-to-cell movement of beet necrotic yellow vein virus requires 3' proximal genes located on RNA 2. *Virology* 189, 40–47. doi:10.1016/0042-6822(92)90679-J.
- Gilmer, D., and Ratti, C. (2017). ICTV Virus Taxonomy Profile: Benyviridae. *J. Gen. Virol.* 98, 1571–1572. doi:10.1099/jgv.0.000864.
- Glazebrook, J. (2005). Contrasting Mechanisms of Defense Against Biotrophic and Necrotrophic Pathogens. *Annu. Rev. Phytopathol.* 43, 205–227. doi:10.1146/annurev.phyto.43.040204.135923.
- Glöckner, G., Hülsmann, N., Schleicher, M., Noegel, A. A., Eichinger, L., Gallinger, C., et al. (2014). The Genome of the Foraminiferan *Reticulomyxa filosa*. *Curr. Biol.* 24, 11–18. doi:10.1016/j.cub.2013.11.027.
- Gomez-Valero, L., Rusniok, C., Cazalet, C., and Buchrieser, C. (2011). Comparative and functional genomics of legionella identified eukaryotic like proteins as key players in host-pathogen interactions. *Front. Microbiol.* 2, 208. doi:10.3389/fmicb.2011.00208.
- Graaf, P. V. D., Wale, S. J., and Lees, A. K. (2007). Factors affecting the incidence and severity of *Spongopora subterranea* infection and galling in potato roots. *Plant Pathol.* 56, 1005–1013. doi:10.1111/j.1365-3059.2007.01686.x.
- Grau, C. R., and Leonard, K. J. (1978). Zea Mays, A New Host for *Ligniera Junci* (Plasmodiophorales). *Mycologia* 70, 41–46. doi:10.1080/00275514.1978.12020200.
- Grsic-Rausch, S., Kobelt, P., Siemens, J. M., Bischoff, M., and Ludwig-Müller, J. (2000). Expression and Localization of Nitrilase during Symptom Development of the Clubroot Disease in *Arabidopsis*. *Plant Physiol.* 122, 369–378.
- Gullner, G., Komives, T., Király, L., and Schröder, P. (2018). Glutathione S-Transferase Enzymes in Plant-Pathogen Interactions. *Front. Plant Sci.* 9. doi:10.3389/fpls.2018.01836.
- Gust, A. A., Pruitt, R., and Nürnberger, T. (2017). Sensing Danger: Key to Activating Plant Immunity. *Trends Plant Sci.* 22, 779–791. doi:10.1016/j.tplants.2017.07.005.
- Gutiérrez, P., Bulman, S., Alzate, J., Ortiz, M. C., and Marín, M. (2016). Mitochondrial genome sequence of the potato powdery scab pathogen *Spongopora subterranea*. *Mitochondrial DNA Part A* 27, 58–59. doi:10.3109/19401736.2013.873898.
- Haas, B., and Papanicolaou, A. (2016). TransDecoder (find coding regions within transcripts). Available at: <https://github.com/TransDecoder/>.
- Habyarimana, F., Al-khodori, S., Kalia, A., Graham, J. E., Price, C. T., Garcia, M. T., et al. (2008). Role for the Ankyrin eukaryotic-like genes of *Legionella pneumophila* in parasitism of protozoan hosts and human macrophages. *Environ. Microbiol.* 10, 1460–1474. doi:10.1111/j.1462-2920.2007.01560.x.
- Haeberle, A. M., and Stussi-Garaud, C. (1995). In situ localization of the non-structural protein P25 encoded by beet necrotic yellow vein virus RNA 3. *J. Gen. Virol.* 76, 643–650. doi:10.1099/0022-1317-76-3-643.
- Haeberlé, A. M., Stussi-Garaud, C., Schmitt, C., Garaud, J. C., Richards, K. E., Guilley, H., et al. (1994). Detection by immunogold labelling of P75 readthrough protein near an extremity of beet necrotic yellow vein virus particles. *Arch. Virol.* 134, 195–203. doi:10.1007/BF01379118.

- Hahn, C., Bachmann, L., and Chevreux, B. (2013). Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. *Nucleic Acids Res.* 41, e129. doi:10.1093/nar/gkt371.
- Han, J.-H., Shin, J.-H., Lee, Y.-H., and Kim, K. S. (2018). Distinct roles of the YPEL gene family in development and pathogenicity in the ascomycete fungus *Magnaporthe oryzae*. *Sci. Rep.* 8, 14461. doi:10.1038/s41598-018-32633-6.
- Hanse, B., Schneider, J. H. M., Termorshuizen, A. J., and Varrelmann, M. (2011). Pests and diseases contribute to sugar beet yield difference between top and averagely managed farms. *Crop Prot.* 30, 671–678. doi:10.1016/j.cropro.2011.02.018.
- Harju, V. A., Mumford, R. A., Blockley, A., Boonham, N., Clovert, G. R. G., Weekes, R., et al. (2002). Occurrence in the United Kingdom of Beet necrotic yellow vein virus isolates which contain RNA 5. *Plant Pathol.* 51, 811. doi:10.1046/j.1365-3059.2002.00781.x.
- Haudecoeur, R., Ahmed-Belkacem, A., Yi, W., Fortuné, A., Brillet, R., Belle, C., et al. (2011). Discovery of Naturally Occurring Aurones That Are Potent Allosteric Inhibitors of Hepatitis C Virus RNA-Dependent RNA Polymerase. *J. Med. Chem.* 54, 5395–5402. doi:10.1021/jm200242p.
- Hehn, A., Bouzoubaa, S., Twell, D., Marbach, J., Richards, K., Guilley, H., et al. (1995). The small cysteine-rich protein P14 of beet necrotic yellow vein virus regulates accumulation of RNA 2 in cis and coat protein in trans. *Virology* 210, 73–81. doi:10.1006/viro.1995.1318.
- Hehn, A., Fritsch, C., Richards, K. E., Guilley, H., and Jonard, G. (1997). Evidence for in vitro and in vivo autocatalytic processing of the primary translation product of beet necrotic yellow vein virus RNA 1 by a papain-like proteinase. *Arch. Virol.* 142, 1051–1058. doi:10.1007/s007050050141.
- Heidel, G. B., Rush, C. M., Kendall, T. L., Lommel, S. A., and French, R. C. (1997). Characteristics of Beet Soilborne Mosaic Virus, a Furo-like Virus Infecting Sugar Beet. *Plant Dis.* 81, 1070–1076. doi:10.1094/PDIS.1997.81.9.1070.
- Heijbroek, W., Musters, P. M. S., and Schoone, A. H. L. (1999). Variation in pathogenicity and multiplication of beet necrotic yellow vein virus (BNYVV) in relation to the resistance of sugar-beet cultivars. *Eur. J. Plant Pathol.* 105, 397–405. doi:10.1023/A:1008705111232.
- Henry, C. M., Jones, R. a. C., and Coutts, R. H. A. (1986). Occurrence of a soil-borne virus of sugar beet in England. *Plant Pathol.* 35, 585–591. doi:10.1111/j.1365-3059.1986.tb02059.x.
- Hittorf, M., Letsch-Praxmarer, S., Windegger, A., Bass, D., Kirchmair, M., and Neuhauser, S. (2020). Revised Taxonomy and Expanded Biodiversity of the Phytomyxea (Rhizaria, Endomyxa). *J. Eukaryot. Microbiol.* 67, 648–659. doi:https://doi.org/10.1111/jeu.12817.
- Hoagland, D. R., and Arnon, D. I. (1950). The water-culture method for growing plants without soil. *Circ. Calif. Agric. Exp. Stn.* 347. Available at: <https://www.cabdirect.org/cabdirect/abstract/19500302257> [Accessed October 5, 2017].
- Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., and Vinh, L. S. (2018). UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Mol. Biol. Evol.* 35, 518–522. doi:10.1093/molbev/msx281.

REFERENCES

- Hoff, K. J., Lomsadze, A., Borodovsky, M., and Stanke, M. (2019). Whole-Genome Annotation with BRAKER. *Methods Mol. Biol. Clifton NJ* 1962, 65–95. doi:10.1007/978-1-4939-9173-0_5.
- Horak, I., and Schlösser, E. (1980). Rizomania. II. Effect of temperature on development of beet necrotic yellow vein virus and tobacco necrosis virus on sugar beet seedlings. *Proc. 5th Congr. Mediterr. Phytopathol. Union*, 31–32.
- Horton, P., Park, K.-J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. J., et al. (2007). WoLF PSORT: protein localization predictor. *Nucleic Acids Res.* 35, W585–W587. doi:10.1093/nar/gkm259.
- Hossain, M. M., Pérez-López, E., Todd, C. D., Wei, Y., and Bonham-Smith, P. C. (2021). Endomembrane-Targeting Plasmodiophora brassicae Effectors Modulate PAMP Triggered Immune Responses in Plants. *Front. Microbiol.* 12, 651279. doi:10.3389/fmicb.2021.651279.
- Hou, S., Liu, Z., Shen, H., and Wu, D. (2019). Damage-Associated Molecular Pattern-Triggered Immunity in Plants. *Front. Plant Sci.* 10, 646. doi:10.3389/fpls.2019.00646.
- Huerta-Cepas, J., Forslund, K., Coelho, L. P., Szklarczyk, D., Jensen, L. J., von Mering, C., et al. (2017). Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. *Mol. Biol. Evol.* 34, 2115–2122. doi:10.1093/molbev/msx148.
- Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., et al. (2019). eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res.* 47, D309–D314. doi:10.1093/nar/gky1085.
- Hughes, I. K. (1980). Powdery scab (*Spongospora subterranea*) of potatoes in Queensland: occurrence, cultivar susceptibility, time of infection, effect of soil pH, chemical control and temperature relations. *Aust. J. Exp. Agric.* 20, 625–632. doi:10.1071/ea9800625.
- Hull, R. (2013). *Plant Virology*. Academic Press.
- Hwang, I. S., Choi, D. S., Kim, N. H., Kim, D. S., and Hwang, B. K. (2014). Pathogenesis-related protein 4b interacts with leucine-rich repeat protein 1 to suppress PR4b-triggered cell death and defense response in pepper. *Plant J.* 77, 521–533. doi:https://doi.org/10.1111/tpj.12400.
- Hwang, S.-F., Strelkov, S. E., Feng, J., Gossen, B. D., and Howard, R. J. (2012). Plasmodiophora brassicae: a review of an emerging pathogen of the Canadian canola (*Brassica napus*) crop. *Mol. Plant Pathol.* 13, 105–113. doi:10.1111/j.1364-3703.2011.00729.x.
- Irani, S., Todd, C. D., Wei, Y., and Bonham-Smith, P. C. (2019). Changes in phenylpropanoid pathway gene expression in roots and leaves of susceptible and resistant *Brassica napus* lines in response to *Plasmodiophora brassicae* inoculation. *Physiol. Mol. Plant Pathol.* 106, 196–203. doi:10.1016/j.pmpp.2019.02.007.
- Irani, S., Trost, B., Waldner, M., Nayidu, N., Tu, J., Kusalik, A. J., et al. (2018). Transcriptome analysis of response to *Plasmodiophora brassicae* infection in the *Arabidopsis* shoot and root. *BMC Genomics* 19, 23. doi:10.1186/s12864-017-4426-7.
- Islam, Z., Nagampalli, R. S. K., Fatima, M. T., and Ashraf, G. M. (2018). New paradigm in ankyrin repeats: Beyond protein-protein interaction module. *Int. J. Biol. Macromol.* 109, 1164–1173. doi:10.1016/j.ijbiomac.2017.11.101.

- Ivanovic, M., MacFarlane, I., and Wood, R. D. (1983). Viruses of sugarbeet associated with *Polymyxa betae*. *Annu. Rep. Rothamsted Exp. Stn.* 1982, 189–190.
- Jahn, L., Mucha, S., Bergmann, S., Horn, C., Staswick, P., Steffens, B., et al. (2013). The Clubroot Pathogen (*Plasmodiophora brassicae*) Influences Auxin Signaling to Regulate Auxin Homeostasis in *Arabidopsis*. *Plants* 2, 726–749. doi:10.3390/plants2040726.
- Jashni, M. K., Mehrabi, R., Collemare, J., Mesarich, C. H., and de Wit, P. J. G. M. (2015). The battle in the apoplast: further insights into the roles of proteases and their inhibitors in plant–pathogen interactions. *Front. Plant Sci.* 6. doi:10.3389/fpls.2015.00584.
- Jernigan, K. K., and Bordenstein, S. R. (2014). Ankyrin domains across the Tree of Life. *PeerJ* 2, e264. doi:10.7717/peerj.264.
- Jernigan, K. K., and Bordenstein, S. R. (2015). Tandem-repeat protein domains across the tree of life. *PeerJ* 3, e732. doi:10.7717/peerj.732.
- Ji, R., Gao, S., Bi, Q., Wang, Y., Lv, M., Ge, W., et al. (2021). The Salicylic Acid Signaling Pathway Plays an Important Role in the Resistant Process of *Brassica rapa* L. ssp. *pekinensis* to *Plasmodiophora brassicae* Woronin. *J. Plant Growth Regul.* 40, 405–422. doi:10.1007/s00344-020-10105-4.
- Jia, H., Wei, X., Yang, Y., Yuan, Y., Wei, F., Zhao, Y., et al. (2017). Root RNA-seq analysis reveals a distinct transcriptome landscape between clubroot-susceptible and clubroot-resistant Chinese cabbage lines after *Plasmodiophora brassicae* infection. *Plant Soil* 421, 93–105. doi:10.1007/s11104-017-3432-5.
- Jiang, C., Kan, J., Ordon, F., Perovic, D., and Yang, P. (2020a). Bymovirus-induced yellow mosaic diseases in barley and wheat: viruses, genetic resistances and functional aspects. *Theor. Appl. Genet.* 133, 1623–1640. doi:10.1007/s00122-020-03555-7.
- Jiang, Z., Zhang, K., Li, Z., Li, Z., Yang, M., Jin, X., et al. (2020b). The Barley stripe mosaic virus *yb* protein promotes viral cell-to-cell movement by enhancing ATPase-mediated assembly of ribonucleoprotein movement complexes. *PLoS Pathog.* 16. doi:10.1371/journal.ppat.1008709.
- Johnson, D. A., and Cummings, T. F. (2015). Effect of Powdery Scab Root Galls on Yield of Potato. *Plant Dis.* 99, 1396–1403. doi:10.1094/PDIS-11-14-1170-RE.
- Jones, J. D. G., and Dangl, J. L. (2006). The plant immune system. *Nature* 444, 323–329. doi:10.1038/nature05286.
- Jones, J. D. G., Vance, R. E., and Dangl, J. L. (2016). Intracellular innate immune surveillance devices in plants and animals. *Science* 354, aaf6395. doi:10.1126/science.aaf6395.
- Jones, P., Binns, D., Chang, H.-Y., Fraser, M., Li, W., McAnulla, C., et al. (2014). InterProScan 5: genome-scale protein function classification. *Bioinformatics* 30, 1236–1240. doi:10.1093/bioinformatics/btu031.
- Jones, R. a. C., and Harrison, B. D. (1969). The behaviour of potato mop-top virus in soil, and evidence for its transmission by *Spongospora subterranea* (Wallr.) Lagerh. *Ann. Appl. Biol.* 63, 1–17. doi:https://doi.org/10.1111/j.1744-7348.1969.tb05461.x.
- Jubault, M., Lariagon, C., Taconnat, L., Renou, J.-P., Gravot, A., Delourme, R., et al. (2013). Partial resistance to clubroot in *Arabidopsis* is based on changes in the host

REFERENCES

- primary metabolism and targeted cell division and expansion capacity. *Funct. Integr. Genomics* 13, 191–205. doi:10.1007/s10142-013-0312-9.
- Jung, J., Kim, J. I., Jeong, Y.-S., and Yi, G. (2018). AGORA: organellar genome annotation from the amino acid and nucleotide references. *Bioinforma. Oxf. Engl.* 34, 2661–2663. doi:10.1093/bioinformatics/bty196.
- Jupin, I., Guilley, H., Richards, K. E., and Jonard, G. (1992). Two proteins encoded by beet necrotic yellow vein virus RNA 3 influence symptom phenotype on leaves. *EMBO J.* 11, 479–488. doi:10.1002/j.1460-2075.1992.tb05078.x.
- Jurkiewicz, P., Melser, S., Maucourt, M., Ayeb, H., Veljanovski, V., Maneta-Peyret, L., et al. (2018). The multistress-induced Translocator protein (TSPO) differentially modulates storage lipids metabolism in seeds and seedlings. *Plant J.* 96, 274–286. doi:https://doi.org/10.1111/tpj.14028.
- Kadereit, G., Hohmann, S., and Kadereit, J. W. (2006). A Synopsis of Chenopodiaceae Subfam. Betoideae and Notes on the Taxonomy of Beta. *Willdenowia* 36, 9–19.
- Kakani, K., Robbins, M., and Rochon, D. (2003). Evidence that Binding of Cucumber Necrosis Virus to Vector Zoospores Involves Recognition of Oligosaccharides. *J. Virol.* 77, 3922–3928. doi:10.1128/JVI.77.7.3922-3928.2003.
- Käll, L., Krogh, A., and Sonnhammer, E. L. L. (2007). Advantages of combined transmembrane topology and signal peptide prediction--the Phobius web server. *Nucleic Acids Res.* 35, W429–432. doi:10.1093/nar/gkm256.
- Kalunke, R. M., Tundo, S., Benedetti, M., Cervone, F., De Lorenzo, G., and D'Ovidio, R. (2015). An update on polygalacturonase-inhibiting protein (PGIP), a leucine-rich repeat protein that protects crop plants against pathogens. *Front. Plant Sci.* 6. doi:10.3389/fpls.2015.00146.
- Kalvari, I., Nawrocki, E. P., Argasinska, J., Quinones-Olvera, N., Finn, R. D., Bateman, A., et al. (2018). Non-coding RNA analysis using the Rfam database. *Curr. Protoc. Bioinforma.* 62, e51. doi:10.1002/cpbi.51.
- Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., and Jermini, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589. doi:10.1038/nmeth.4285.
- Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., and Morishima, K. (2017). KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* 45, D353–D361. doi:10.1093/nar/gkw1092.
- Kang, D. D., Froula, J., Egan, R., and Wang, Z. (2015). MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. *PeerJ* 3, e1165. doi:10.7717/peerj.1165.
- Kanyuka, K., Ward, E., and Adams, M. J. (2003). Polymyxa graminis and the cereal viruses it transmits: a research challenge. *Mol. Plant Pathol.* 4, 393–406. doi:10.1046/j.1364-3703.2003.00177.x.
- Karling, J. S. (1981). Woronina leptolegniae n. sp., a plasmodiophorid parasite of Leptolegnia. *Nova Hedwig. Z. Kryptogamenkd.* Available at: <https://agris.fao.org/agris-search/search.do?recordID=US201302618008> [Accessed August 16, 2021].
- Kassanis, B., and Macfarlane, I. (1964). Transmission of Tobacco Necrosis Virus by Zoospores of Olpidium brassicae. *Microbiology* 36, 79–93. doi:10.1099/00221287-36-1-79.

- Katoh, K., Rozewicki, J., and Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* 20, 1160–1166. doi:10.1093/bib/bbx108.
- Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384. doi:10.1038/ni.1863.
- Keeling, P. J., Burki, F., Wilcox, H. M., Allam, B., Allen, E. E., Amaral-Zettler, L. A., et al. (2014). The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): Illuminating the Functional Diversity of Eukaryotic Life in the Oceans through Transcriptome Sequencing. *PLOS Biol.* 12, e1001889. doi:10.1371/journal.pbio.1001889.
- Kemen, E., and Jones, J. D. G. (2012). Obligate biotroph parasitism: can we link genomes to lifestyles? *Trends Plant Sci.* 17, 448–457. doi:10.1016/j.tplants.2012.04.005.
- Keskin, B. (1964). Polymyxa betae n.sp., ein Parasit in den Wurzeln von Beta vulgaris Tournefort, besonders während der Jugendentwicklung der Zuckerrübe. *Arch. Für Mikrobiol.* 49, 348–374. doi:10.1007/BF00406857.
- Keskin, B., and Fuchs, W. H. (1969). Der Infektionsvorgang bei Polymyxa betae. *Arch. Für Mikrobiol.* 68, 218–226. doi:10.1007/BF00409914.
- Kiguchi, T., Saito, M., and Tamada, T. (1996). Nucleotide sequence analysis of RNA-5 of five isolates of beet necrotic yellow vein virus and the identity of a deletion mutant. *J. Gen. Virol.* 77, 575–580. doi:10.1099/0022-1317-77-4-575.
- Kim, D., Langmead, B., and Salzberg, S. L. (2015). HISAT: a fast spliced aligner with low memory requirements. *Nat. Methods* 12, 357–360. doi:10.1038/nmeth.3317.
- Kim, D. S., and Hwang, B. K. (2014). An important role of the pepper phenylalanine ammonia-lyase gene (PAL1) in salicylic acid-dependent signalling of the defence response to microbial pathogens. *J. Exp. Bot.* 65, 2295–2306. doi:10.1093/jxb/eru109.
- Kingsnorth, C. S., Asher, M. J. C., Keane, G. J. P., Chwarszczynska, D. M., Luterbacher, M. C., and Mutasa-Göttgens, E. S. (2003a). Development of a recombinant antibody ELISA test for the detection of Polymyxa betae and its use in resistance screening. *Plant Pathol.* 52, 673–680. doi:10.1111/j.1365-3059.2003.00928.x.
- Kingsnorth, C. S., Kingsnorth, A. J., Lyons, P. A., Chwarszczynska, D. M., and Asher, M. J. C. (2003b). Real-time analysis of Polymyxa betae GST expression in infected sugar beet. *Mol. Plant Pathol.* 4, 171–176. doi:10.1046/j.1364-3703.2003.00160.x.
- Klee, E. W., and Ellis, L. B. (2005). Evaluating eukaryotic secreted protein prediction. *BMC Bioinformatics* 6, 256. doi:10.1186/1471-2105-6-256.
- Klein, E., Link, D., Schirmer, A., Erhardt, M., and Gilmer, D. (2007). Sequence variation within Beet necrotic yellow vein virus p25 protein influences its oligomerization and isolate pathogenicity on Tetragonia expansa. *Virus Res.* 126, 53–61. doi:10.1016/j.virusres.2006.12.019.
- Koenig, R. (2000). Deletions in the KTER-encoding domain, which is needed for Polymyxa transmission, in manually transmitted isolates of Beet necrotic yellow vein benyvirus. *Arch. Virol.* 145, 165–170. doi:10.1007/s007050050013.

REFERENCES

- Koenig, R., and Burgermeister, W. (1989). Mechanical Inoculation of Sugarbeet Roots With Isolates of Beet Necrotic Yellow Vein Virus Having Different RNA Compositions. *J. Phytopathol.* 124, 249–255. doi:10.1111/j.1439-0434.1989.tb04920.x.
- Koenig, R., Haeberlé, A.-M., and Commandeur, U. (1997). Detection and characterization of a distinct type of beet necrotic yellow vein virus RNA 5 in a sugarbeet growing area in Europe. *Arch. Virol.* 142, 1499–1504. doi:10.1007/s007050050176.
- Koenig, R., Jarausch, W., Li, Y., Commandeur, U., Burgermeister, W., and Gehrke Luddecke, M. P. (1991). Effect of recombinant beet necrotic yellow vein virus with different RNA compositions on mechanically inoculated sugarbeets. *J. Gen. Virol.* 72, 2243–2246. doi:10.1099/0022-1317-72-9-2243.
- Koenig, R., and Lennefors, B.-L. (2000). Molecular analyses of European A, B and P type sources of Beet necrotic yellow vein virus and detection of the rare P type in Kazakhstan. *Arch. Virol.* 145, 1561–1570. doi:10.1007/s007050070076.
- Koenig, R., Loss, S., Specht, J., Varrelmann, M., Lüddecke, P., and Demi, G. (2009). A single U/C nucleotide substitution changing alanine to valine in the Beet necrotic yellow vein virus P25 protein promotes increased virus accumulation in roots of mechanically inoculated, partially resistant sugar beet seedlings. *J. Gen. Virol.* 90, 759–763. doi:10.1099/vir.0.007112-0.
- Koenig, R., Luddecke, P., and Haeberle, A. M. (1995). Detection of beet necrotic yellow vein virus strains, variants and mixed infections by examining single-strand conformation polymorphisms of immunocapture RT-PCR products. *J. Gen. Virol.* 76, 2051–2055. doi:10.1099/0022-1317-76-8-2051.
- Koenig, R., and Stein, B. (1990). Distribution of beet necrotic yellow vein virus in mechanically inoculated sugarbeet plantlets of cultivars with different degrees of rizomania resistance. *Proc. First Symp. Int. Work. Group Plant Viruses Fungal Vectors* 1, 87–90.
- Kogel, K.-H., Franken, P., and Hückelhoven, R. (2006). Endophyte or parasite – what decides? *Curr. Opin. Plant Biol.* 9, 358–363. doi:10.1016/j.pbi.2006.05.001.
- Kohorn, B., and Kohorn, S. (2012). The cell wall-associated kinases, WAKs, as pectin receptors. *Front. Plant Sci.* 3, 88. doi:10.3389/fpls.2012.00088.
- Kondo, H., Hirano, S., Chiba, S., Andika, I. B., Hirai, M., Maeda, T., et al. (2013). Characterization of burdock mottle virus, a novel member of the genus Benyvirus, and the identification of benyvirus-related sequences in the plant and insect genomes. *Virus Res.* 177, 75–86. doi:10.1016/j.virusres.2013.07.015.
- Kong, G., Wan, L., Deng, Y. Z., Yang, W., Li, W., Jiang, L., et al. (2019). Pectin acetyltransferase PAE5 is associated with the virulence of plant pathogenic oomycete *Peronosphythora litchii*. *Physiol. Mol. Plant Pathol.* 106, 16–22. doi:10.1016/j.pmp.2018.11.006.
- Kourelis, J., and Hoorn, R. A. L. van der (2018). Defended to the Nines: 25 Years of Resistance Gene Cloning Identifies Nine Mechanisms for R Protein Function. *Plant Cell* 30, 285–299. doi:10.1105/tpc.17.00579.
- Kriventseva, E. V., Kuznetsov, D., Tegenfeldt, F., Manni, M., Dias, R., Simão, F. A., et al. (2019). OrthoDB v10: sampling the diversity of animal, plant, fungal, protist, bacterial and viral genomes for evolutionary and functional annotations of orthologs. *Nucleic Acids Res.* 47, D807–D811. doi:10.1093/nar/gky1053.

- Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E. L. (2001). Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J. Mol. Biol.* 305, 567–580. doi:10.1006/jmbi.2000.4315.
- Kruse, M., Koenig, R., Hoffmann, A., Kaufmann, A., Commandeur, U., Solovyev, A. G., et al. (1994). Restriction fragment length polymorphism analysis of reverse transcription-PCR products reveals the existence of two major strain groups of beet necrotic yellow vein virus. *J. Gen. Virol.* 75, 1835–1842. doi:10.1099/0022-1317-75-8-1835.
- Kumar, D., and Klessig, D. F. (2003). High-affinity salicylic acid-binding protein 2 is required for plant innate immunity and has salicylic acid-stimulated lipase activity. *Proc. Natl. Acad. Sci.* 100, 16101–16106. doi:10.1073/pnas.0307162100.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* 35, 1547–1549. doi:10.1093/molbev/msy096.
- Kuraku, S., Zmasek, C. M., Nishimura, O., and Katoh, K. (2013). aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Res.* 41, W22–W28. doi:10.1093/nar/gkt389.
- Kutluk Yilmaz, N. D., Arli-Sokmen, M., and Kaya, R. (2018a). p25 pathogenicity factor deletion mutants of beet necrotic yellow vein virus occurring in sugar beet fields in Turkey. *J. Plant Dis. Prot.* 125, 89–98. doi:10.1007/s41348-017-0142-4.
- Kutluk Yilmaz, N. D., Kaya Altop, E., Phillippo, C. J., and Mennan, H. (2016). New natural weed host *Raphanus raphanistrum* L. (Brassicaceae) for Beet necrotic yellow vein virus and its vector *Polomyxa betae* Keskin. *Turk. J. Agric. For.* 40, 120–126. doi:10.3906/TAR-1410-113.
- Kutluk Yilmaz, N. D., Uzunbacak, H., Arli-Sokmen, M., and Kaya, R. (2018b). Distribution of resistance-breaking isolates of beet necrotic yellow vein virus differing in virulence in sugar beet fields in Turkey. *Acta Agric. Scand. Sect. B Soil Plant Sci.* 68, 546–554. doi:10.1080/09064710.2018.1441432.
- Lai, Y.-T., Chang, Y.-Y., Hu, L., Yang, Y., Chao, A., Du, Z.-Y., et al. (2015). Rapid labeling of intracellular His-tagged proteins in living cells. *Proc. Natl. Acad. Sci. U. S. A.* 112, 2948–2953. doi:10.1073/pnas.1419598112.
- Langenberg, W. G., and Giunchedi, L. (1982). Ultrastructure of fungal plant virus vectors *Polomyxa graminis* in Soilborne wheat mosaic virus-infected wheat and *P. betae* in Beet necrotic yellow vein virus-infected sugar beet. *Phytopathology* 72, 1152–1158.
- Langmead, B., and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359. doi:10.1038/nmeth.1923.
- Larson, R. L., Wintermantel, W. M., Hill, A., Fortis, L., and Nunez, A. (2008). Proteome changes in sugar beet in response to Beet necrotic yellow vein virus. *Physiol. Mol. Plant Pathol.* 72, 62–72. doi:10.1016/j.pmpp.2008.04.003.
- Lauber, E., Bleykasten-Grosshans, C., Erhardt, M., Bouzoubaa, S., Jonard, G., Richards, K. E., et al. (1998a). Cell-to-cell movement of beet necrotic yellow vein virus: I. Heterologous complementation experiments provide evidence for specific interactions among the triple gene block proteins. *Mol. Plant. Microbe Interact.* 11, 618–625. doi:10.1094/MPMI.1998.11.7.618.
- Lauber, E., Guilley, H., Tamada, T., Richards, K. E., and Jonard, G. (1998b). Vascular movement of beet necrotic yellow vein virus in *Beta macrocarpa* is probably

REFERENCES

- dependent on an RNA 3 sequence domain rather than a gene product. *J. Gen. Virol.* 79, 385–393. doi:10.1099/0022-1317-79-2-385.
- Laufer, M., Mohammad, H., Christ, D. S., Riedel, D., Maiss, E., Varrelmann, M., et al. (2018a). Fluorescent labelling of beet necrotic yellow vein virus and beet soil-borne mosaic virus for co-and superinfection experiments in *nicotiana benthamiana*. *J. Gen. Virol.* 99, 1321–1330. doi:10.1099/jgv.0.001122.
- Laufer, M., Mohammad, H., Maiss, E., Richert-Pöggeler, K., Dall'Ara, M., Ratti, C., et al. (2018b). Biological properties of Beet soil-borne mosaic virus and Beet necrotic yellow vein virus cDNA clones produced by isothermal in vitro recombination: Insights for reassortant appearance. *Virology* 518, 25–33. doi:10.1016/j.virol.2018.01.029.
- Lavenus, J., Goh, T., Roberts, I., Guyomarc'h, S., Lucas, M., De Smet, I., et al. (2013). Lateral root development in *Arabidopsis*: fifty shades of auxin. *Trends Plant Sci.* 18, 450–458. doi:10.1016/j.tplants.2013.04.006.
- Law, C. W., Alhamdoosh, M., Su, S., Dong, X., Tian, L., Smyth, G. K., et al. (2018). RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR. *F1000Research* 5, 1408. doi:10.12688/f1000research.9005.3.
- Law, C. W., Chen, Y., Shi, W., and Smyth, G. K. (2014). voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol.* 15, R29. doi:10.1186/gb-2014-15-2-r29.
- Ledingham, G. A. (1939). Studies on *Polymyxa Graminis*, N. Gen. N. Sp., a Plasmodiophoraceous Root Parasite of Wheat. *Can. J. Res.* 17c, 40–51. doi:10.1139/cjr39c-005.
- Lee, L., Telford, E. B., Batten, J. S., Scholthof, K.-B. G., and Rush, C. M. (2001). Complete nucleotide sequence and genome organization of Beet soilborne mosaic virus, a proposed member of the genus Benyvirus. *Arch. Virol.* 146, 2443–2453. doi:10.1007/s007050170014.
- Legrève, A., Delfosse, P., and Maraite, H. (2002). Phylogenetic analysis of *Polymyxa* species based on nuclear 5.8S and internal transcribed spacers ribosomal DNA sequences. *Mycol. Res.* 106, 138–147. doi:10.1017/S0953756201005391.
- Legrève, A., Delfosse, P., Ribonnet, L., Paridaens, A. M., Lurkin, R., and Maraite, H. (2005a). Diversity of *Polymyxa graminis* associated with cereals in West Africa. *Parasitica* 61, 5–10.
- Legrève, A., Delfosse, P., Vanpee, B., Goffin, A., and Maraite, H. (1998). Differences in temperature requirements between *Polymyxa* sp. of Indian origin and *Polymyxa graminis* and *Polymyxa betae* from temperate areas. *Eur. J. Plant Pathol.* 104, 195–205. doi:10.1023/A:1008612903927.
- Legrève, A., Desoignies, N., Dieryck, B., and Bragard, C. (2011). Future challenges in virus transmission by *Polymyxa*. in (Louvain-La-Neuve, Belgium: International Working Group on Plant Viruses with Fungal Vectors), 47–51.
- Legrève, A., and Maraite, H. (1991). Influence du calcium et du pH sur le potentiel infectieux du sol en *Polymyxa betae* Keskin et son expression en champ.
- Legrève, A., Vanpee, B., Delfosse, P., and Maraite, H. (2000). Host Range of Tropical and Sub-tropical Isolates of *Polymyxa graminis*. *Eur. J. Plant Pathol.* 106, 379–389. doi:10.1023/A:1008784823899.
- Legrève, Schmit, J., Bragard, C., and Maraite, H. (2005b). The role of climate and alternative hosts in the epidemiology of rhizomanina. in *Symposium of the*

- International Working Group on Plant Viruses with Fungal Vectors*, 129–132. Available at: https://www.researchgate.net/profile/Salah_Bouzoubaa/publication/207541056_Analysis_of_the_vascular_movement_of_Beet_necrotic_yellow_vein_virus_in_Nicotiana_benthamiana_and_Beta_vulgaris_spp._maritima_M8_using_a_GFP-expressing_virus/links/0fcfd50c9b61352e15000000.pdf#page=138 [Accessed November 3, 2016].
- Lekota, M., Muzhinji, N., and Waals, J. E. van der (2019). Identification of differentially expressed genes in tolerant and susceptible potato cultivars in response to *Spongospora subterranea* f. sp. *subterranea* tuber infection. *Plant Pathol.* 68, 1196–1206. doi:<https://doi.org/10.1111/ppa.13029>.
- Letunic, I., and Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res.* 47, W256–W259. doi:10.1093/nar/gkz239.
- Li, D. W., Yu, J. L., Han, C. G., Lui, T., Quin, S. C., Lui, Y., et al. (1999). Detection and nucleotide sequence analysis of Beet necrotic yellow vein virus RNA 5 isolated from China. *Chin J Biotechnol* 15, 461–465.
- Li, H., Li, X., Xuan, Y., Jiang, J., Wei, Y., and Piao, Z. (2018). Genome Wide Identification and Expression Profiling of SWEET Genes Family Reveals Its Role During *Plasmodiophora brassicae*-Induced Formation of Clubroot in *Brassica rapa*. *Front. Plant Sci.* 9. doi:10.3389/fpls.2018.00207.
- Li, J., Mahajan, A., and Tsai, M.-D. (2006). Ankyrin repeat: a unique motif mediating protein-protein interactions. *Biochemistry* 45, 15168–15178. doi:10.1021/bi062188q.
- Li, M., Liu, T., Wang, B., Han, C., Li, D., and Yu, J. (2008). Phylogenetic analysis of Beet necrotic yellow vein virus isolates from China. *Virus Genes* 36, 429–432. doi:10.1007/s11262-008-0202-8.
- Liebe, S., Wibberg, D., Maiss, E., and Varrelmann, M. (2020). Application of a Reverse Genetic System for Beet Necrotic Yellow Vein Virus to Study Rz1 Resistance Response in Sugar Beet. *Front. Plant Sci.* 10. doi:10.3389/fpls.2019.01703.
- Link, D., Schmidlin, L., Schirmer, A., Klein, E., Erhardt, M., Geldreich, A., et al. (2005). Functional characterization of the Beet necrotic yellow vein virus RNA-5-encoded p26 protein: Evidence for structural pathogenicity determinants. *J. Gen. Virol.* 86, 2115–2125. doi:10.1099/vir.0.80937-0.
- Littlefield, L. J., Delfosse, P., Whallon, J. H., Hassan, Z. M., Sherwood, J. L., and Reddy, D. V. R. (1997). Anatomy of sporosori of *Polymyxa graminis*, the vector of Indian peanut clump virus, in roots of *Sorghum bicolor*. *Can. J. Plant Pathol.* 19, 281–288. doi:10.1080/07060669709500524.
- Littlefield, L. J., Whallon, J. H., Doss, P. J., and Hassan, Z. M. (1998). Postinfection development of *Polymyxa graminis* in roots of *Triticum aestivum*. *Mycologia* 90, 869–882. doi:10.2307/3761328.
- Liu, H., and Lewellen, R. T. (2007). Distribution and differentiation of resistance-breaking isolates of Beet necrotic yellow vein virus in the United States. *Gen. Meet. Am. Soc. Sugar Beet Technol.*
- Liu, H.-Y., Sears, J. L., and Lewellen, R. T. (2005). Occurrence of resistance-breaking Beet necrotic yellow vein virus of sugar beet. *Plant Dis.* 89, 464–468. doi:10.1094/PD-89-0464.
- Liu, J., Fan, H., Wang, Y., Han, C., Wang, X., Yu, J., et al. (2020a). Genome-Wide microRNA Profiling Using Oligonucleotide Microarray Reveals Regulatory Networks

REFERENCES

- of microRNAs in *Nicotiana benthamiana* during Beet Necrotic Yellow Vein Virus Infection. *Viruses* 12. doi:10.3390/v12030310.
- Liu, J.-Y., Fan, H.-Y., Wang, Y., Zhang, Y.-L., Li, D.-W., Yu, J.-L., et al. (2017). Characterization of microRNAs of Beta macrocarpa and their responses to Beet necrotic yellow vein virus infection. *PLoS ONE* 12. doi:10.1371/journal.pone.0186500.
- Liu, R., Chen, K., Jansz, N., Blewitt, M. E., and Ritchie, M. E. (2015a). Transcriptional profiling of the epigenetic regulator Smchd1. *Genomics Data* 7, 144–147. doi:10.1016/j.gdata.2015.12.027.
- Liu, R., Holik, A. Z., Su, S., Jansz, N., Chen, K., Leong, H. S., et al. (2015b). Why weight? Modelling sample and observational level variability improves power in RNA-seq analyses. *Nucleic Acids Res.* 43, e97. doi:10.1093/nar/gkv412.
- Liu, T., Han, C., Li, D., Cai, Z., Yu, J., and Liu, Y. (2003). Effect of the RNA5 component on pathogenicity of beet necrotic yellow vein virus. *Chin. Sci. Bull.* 48, 796–799. doi:10.1360/02wc0502.
- Liu, T., Song, T., Zhang, X., Yuan, H., Su, L., Li, W., et al. (2014a). Unconventionally secreted effectors of two filamentous pathogens target plant salicylate biosynthesis. *Nat. Commun.* 5, 4686. doi:10.1038/ncomms5686.
- Liu, X., Grabherr, H. M., Willmann, R., Kolb, D., Brunner, F., Bertsche, U., et al. (2014b). Host-induced bacterial cell wall decomposition mediates pattern-triggered immunity in Arabidopsis. *eLife* 3, e01990. doi:10.7554/eLife.01990.
- Liu, X., Zhang, C., Zhang, L., Huang, J., Dang, C., Xie, C., et al. (2020b). TaRPP13-3, a CC-NBS-LRR-like gene located on chr 7D, promotes disease resistance to wheat powdery mildew in Brock. *J. Phytopathol.* 168, 688–699. doi:10.1111/jph.12949.
- Livak, K. J., and Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2- $\Delta\Delta$ CT Method. *Methods* 25, 402–408. doi:10.1006/meth.2001.1262.
- Lomsadze, A., Burns, P. D., and Borodovsky, M. (2014). Integration of mapped RNA-Seq reads into automatic training of eukaryotic gene finding algorithm. *Nucleic Acids Res.* 42, e119–e119. doi:10.1093/nar/gku557.
- Lorenz, M. C., and Fink, G. R. (2002). Life and Death in a Macrophage: Role of the Glyoxylate Cycle in Virulence. *Eukaryot. Cell* 1, 657–662. doi:10.1128/EC.1.5.657-662.2002.
- Lorenzo, O., Piqueras, R., Sánchez-Serrano, J. J., and Solano, R. (2003). ETHYLENE RESPONSE FACTOR1 Integrates Signals from Ethylene and Jasmonate Pathways in Plant Defense[W]. *Plant Cell* 15, 165–178. doi:10.1105/tpc.007468.
- Lovelock, D. A., Donald, C. E., Conlan, X. A., and Cahill, D. M. (2013). Salicylic acid suppression of clubroot in broccoli (*Brassica oleracea* var. *italica*) caused by the obligate biotroph *Plasmodiophora brassicae*. *Australas. Plant Pathol.* 42, 141–153. doi:10.1007/s13313-012-0167-x.
- Lowe, T. M., and Eddy, S. R. (1997). tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25, 955–964.
- Lozano, I., and Morales, F. (2009). Molecular characterisation of Rice stripe necrosis virus as a new species of the genus Benyvirus. *Eur. J. Plant Pathol.* 124, 673–680. doi:10.1007/s10658-009-9453-z.

- Lu, S., and Edwards, M. C. (2016). Genome-Wide Analysis of Small Secreted Cysteine-Rich Proteins Identifies Candidate Effector Proteins Potentially Involved in *Fusarium graminearum*–Wheat Interactions. *Phytopathology*® 106, 166–176. doi:10.1094/PHYTO-09-15-0215-R.
- Lubicz, J. V., Rush, C. M., Payton, M., and Colberg, T. (2007). Beet necrotic yellow vein virus accumulates inside resting spores and zoosporangia of its vector *Polymyxa betae* BNYVV infects *P. betae*. *Viol. J.* 4, 37. doi:10.1186/1743-422X-4-37.
- Ludwig-Müller, J., Auer, S., Jülke, S., and Marschollek, S. (2017). “Manipulation of Auxin and Cytokinin Balance During the *Plasmodiophora brassicae*–*Arabidopsis thaliana* Interaction,” in *Auxins and Cytokinins in Plant Biology: Methods and Protocols* Methods in Molecular Biology., eds. T. Dandekar and M. Naseem (New York, NY: Springer), 41–60. doi:10.1007/978-1-4939-6831-2_3.
- Ludwig-Müller, J., Jülke, S., Geiß, K., Richter, F., Mithöfer, A., Šola, I., et al. (2015). A novel methyltransferase from the intracellular pathogen *Plasmodiophora brassicae* methylates salicylic acid: Salicylic acid methyltransferase from *P. brassicae*. *Mol. Plant Pathol.* 16, 349–364. doi:10.1111/mpp.12185.
- Ludwig-Müller, J., Prinsen, E., Rolfe, S. A., and Scholes, J. D. (2009). Metabolism and Plant Hormone Action During Clubroot Disease. *J. Plant Growth Regul.* 28, 229–244. doi:10.1007/s00344-009-9089-4.
- Ma, L.-J., van der Does, H. C., Borkovich, K. A., Coleman, J. J., Daboussi, M.-J., Di Pietro, A., et al. (2010). Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* 464, 367–373. doi:10.1038/nature08850.
- Mahillon, M. (2020). Reverse genetic tools for exploring the plant-vector-virus pathosystems of beet black scorch virus and beet soil-borne virus. Available at: <https://dial-uclouvain-be.proxy.bib.ucl.ac.be:2443/pr/boreal/object/boreal:237669> [Accessed September 4, 2021].
- Mahillon, M., Decroës, A., Peduzzi, C., Romay, G., Legrève, A., and Bragard, C. (2021). RNA silencing machinery contributes to inability of BSBV to establish infection in *Nicotiana benthamiana*: evidence from characterization of agroinfectious clones of Beet soil-borne virus. *J. Gen. Virol.* 102, 001530. doi:10.1099/jgv.0.001530.
- Malinowski, R., Novák, O., Borhan, M. H., Spíchal, L., Strnad, M., and Rolfe, S. A. (2016). The role of cytokinins in clubroot disease. *Eur. J. Plant Pathol.* 145, 543–557. doi:10.1007/s10658-015-0845-y.
- Malinowski, R., Smith, J. A., Fleming, A. J., Scholes, J. D., and Rolfe, S. A. (2012). Gall formation in clubroot-infected *Arabidopsis* results from an increase in existing meristematic activities of the host but is not essential for the completion of the pathogen life cycle. *Plant J.* 71, 226–238. doi:10.1111/j.1365-313X.2012.04983.x.
- Malinowski, R., Truman, W., and Blicharz, S. (2019). Genius Architect or Clever Thief—How *Plasmodiophora brassicae* Reprograms Host Development to Establish a Pathogen-Oriented Physiological Sink. *Mol. Plant-Microbe Interactions*® 32, 1259–1266. doi:10.1094/MPMI-03-19-0069-CR.
- Marçais, G., and Kingsford, C. (2011). A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. *Bioinformatics* 27, 764–770. doi:10.1093/bioinformatics/btr011.
- Mathesius, U. (2018). Flavonoid Functions in Plants and Their Interactions with Other Organisms. *Plants* 7. doi:10.3390/plants7020030.

REFERENCES

- McCarthy, D. J., and Smyth, G. K. (2009). Testing significance relative to a fold-change threshold is a TREAT. *Bioinformatics* 25, 765–771. doi:10.1093/bioinformatics/btp053.
- McGrann, G. R. D., and Adams, M. J. (2003). Characterisation of resistance to Barley mild mosaic virus. *Characterisation Resist. Barley Mild Mosaic Virus*, 72–75.
- McGrann, G. R. D., Grimmer, M. K., Mutasa-Göttgens, E. S., and Stevens, M. (2009). Progress towards the understanding and control of sugar beet rhizomania disease. *Mol. Plant Pathol.* 10, 129–141. doi:https://doi.org/10.1111/j.1364-3703.2008.00514.x.
- McGrann, G. R. D., Martin, L. D., Kingsnorth, C. S., Asher, M. J. C., Adams, M. J., and Mutasa-Göttgens, E. S. (2007). Screening for genetic elements involved in the nonhost response of sugar beet to the plasmodiophorid cereal root parasite *Polymyxa graminis* by representational difference analysis. *J. Gen. Plant Pathol.* 73, 260–265. doi:10.1007/s10327-007-0024-x.
- McGrann, G. R. D., and Mutasa-Göttgens, E. S. (2009). Barley root morphology affects the zoospore production rate by the obligate root parasite and soil-borne virus vector *Polymyxa graminis*. *J. Phytopathol.* 157, 262–264. doi:10.1111/j.1439-0434.2008.01478.x.
- McGrann, G. R. D., Townsend, B. J., Antoniwi, J. F., Asher, M. J. C., and Mutasa-Göttgens, E. S. (2009). Barley elicits a similar early basal defence response during host and non-host interactions with *Polymyxa* root parasites. *Eur. J. Plant Pathol.* 123, 5–15. doi:10.1007/s10658-008-9332-z.
- Medina, E. M., and Buchler, N. E. (2020). Chytrid fungi. *Curr. Biol.* 30, R516–R520.
- Merz, U., and Falloon, R. E. (2009). Review: Powdery Scab of Potato—Increased Knowledge of Pathogen Biology and Disease Epidemiology for Effective Disease Management. *Potato Res.* 52, 17–37. doi:10.1007/s11540-008-9105-2.
- Meunier, A., Schmit, J.-F., and Bragard, C. (2002). Sequences analysis of Belgian BNYYV and development of a simultaneous detection of soil-borne sugar-beet viruses by RT-PCR. *Proc. Fifth Symp. Int. Work. Group Plant Viruses Fungal Vectors*, 9–12.
- Meunier, A., Schmit, J.-F., Stas, A., Kutluk, N., and Bragard, C. (2003). Multiplex Reverse Transcription-PCR for Simultaneous Detection of Beet Necrotic Yellow Vein Virus, Beet Soilborne Virus, and Beet Virus Q and Their Vector *Polymyxa betae* KESKIN on Sugar Beet. *Appl. Environ. Microbiol.* 69, 2356–2360. doi:10.1128/AEM.69.4.2356-2360.2003.
- Miedes, E., Vanholme, R., Boerjan, W., and Molina, A. (2014). The role of the secondary cell wall in plant resistance to pathogens. *Front. Plant Sci.* 0. doi:10.3389/fpls.2014.00358.
- Miller, C. E., Martin, R. W., and Dylewski, D. P. (1985). The ultrastructure of plasmodia, sporangia, and cystosori of *Ligniera verrucosa* (Plasmodiophoromycetes). *Can. J. Bot.* 63, 263–273. doi:10.1139/b85-030.
- Mitchell, A. L., Attwood, T. K., Babbitt, P. C., Blum, M., Bork, P., Bridge, A., et al. (2019). InterPro in 2019: improving coverage, classification and access to protein sequence annotations. *Nucleic Acids Res.* 47, D351–D360. doi:10.1093/nar/gky1100.
- Mithen, R., and Magrath, R. (1992). A contribution to the life history of *Plasmodiophora brassicae*: secondary plasmodia development in root galls of *Arabidopsis thaliana*. *Mycol. Res.* 96, 877–885. doi:10.1016/S0953-7562(09)81035-6.

- Miyanishi, M., Kusume, T., Saito, M., and Tamada, T. (1999). Evidence for three groups of sequence variants of beet necrotic yellow vein virus RNA 5. *Arch. Virol.* 144, 879–892. doi:10.1007/s007050050553.
- Morales, F. J., Ward, E., Castaño, M., Arroyave, J. A., Lozano, I., and Adams, M. J. (1999). Emergence and partial characterization of rice stripe necrosis virus and its fungus vector in South America. *Eur. J. Plant Pathol.* 105, 643–650. doi:10.1023/A:1008786832634.
- Mou, B., Richardson, K., Benzen, S., and Liu, H.-Y. (2012). Effects of beet necrotic yellow vein virus in spinach cultivars. *Plant Dis.* 96, 618–622. doi:10.1094/PDIS-09-11-0748.
- Mou, Z., Fan, W., and Dong, X. (2003). Inducers of Plant Systemic Acquired Resistance Regulate NPR1 Function through Redox Changes. *Cell* 113, 935–944. doi:10.1016/S0092-8674(03)00429-X.
- Mouhanna, A. M., Langen, G., and Schlösser, E. (2008). Weeds as alternative hosts for BSBV, BNYVV, and the vector Polymyxa betae (German isolate). *J. Plant Dis. Prot.* 115, 193–198. doi:10.1007/BF03356263.
- Moxham, S. E., and Buczacki, S. T. (1983). Chemical composition of the resting spore wall of Plasmodiophora brassicae. *Trans. Br. Mycol. Soc.* 80, 297–304. doi:10.1016/S0007-1536(83)80013-8.
- Mukherjee, S., Stamatis, D., Bertsch, J., Ovchinnikova, G., Sundaramurthi, J. C., Lee, J., et al. (2021). Genomes OnLine Database (GOLD) v.8: overview and updates. *Nucleic Acids Res.* 49, D723–D733. doi:10.1093/nar/gkaa983.
- Murakami, H., Tsushima, S., Kuroyanagi, Y., and Shishido, Y. (2002). Reduction of resting spore density of Plasmodiophora brassicae and clubroot disease severity by liming. *Soil Sci. Plant Nutr.* 48, 685–691. doi:10.1080/00380768.2002.10409258.
- Muszevska, A., Steczkiewicz, K., Stepniewska-Dziubinska, M., and Ginalski, K. (2019). Transposable elements contribute to fungal genes and impact fungal lifestyle. *Sci. Rep.* 9, 1–10. doi:10.1038/s41598-019-40965-0.
- Mutasa, E. S., Ward, E., Adams, M. J., Collier, C. R., Chwarszczynska, D. M., and Asher, M. J. C. (1993). A sensitive DNA probe for the detection of Polymyxa betae in sugar beet roots. *Physiol. Mol. Plant Pathol.* 43, 379–390. doi:10.1006/pmpp.1993.1066.
- Mutasa-Gottgens, E. S., Chwarszczynska, D. M., Halsey, K., and Asher, M. J. C. (2000). Specific polyclonal antibodies for the obligate plant parasite Polymyxa— a targeted recombinant DNA approach. *Plant Pathol.* 49, 276–287. doi:10.1046/j.1365-3059.2000.00446.x.
- Neuhauser, S., Bulman, S., and Kirchmair, M. (2010). “Plasmodiophorids: The Challenge to Understand Soil-Borne, Obligate Biotrophs with a Multiphasic Life Cycle,” in *Molecular Identification of Fungi*, eds. Y. Gherbawy and K. Voigt (Berlin, Heidelberg: Springer Berlin Heidelberg), 51–78. doi:10.1007/978-3-642-05042-8_3.
- Neuhauser, S., Huber, L., and Kirchmair, M. (2009). Sorosphaera viticola, a plasmodiophorid parasite of grapevine. *Phytopathol. Mediterr.* 48, 136–139.
- Neuhauser, S., Kirchmair, M., Bulman, S., and Bass, D. (2014). Cross-kingdom host shifts of phytomyxid parasites. *BMC Evol. Biol.* 14, 33. doi:10.1186/1471-2148-14-33.

REFERENCES

- Neuhauser, S., Kirchmair, M., and Gleason, F. H. (2011). The ecological potentials of Phytomyxea ("plasmodiophorids") in aquatic food webs. *Hydrobiologia* 659, 23–35. doi:10.1007/s10750-010-0508-0.
- Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., and Minh, B. Q. (2015). IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* 32, 268–274. doi:10.1093/molbev/msu300.
- Nieberl, P., Ehrl, C., Pommerrenig, B., Graus, D., Marten, I., Jung, B., et al. (2017). Functional characterisation and cell specificity of BvSUT1, the transporter that loads sucrose into the phloem of sugar beet (*Beta vulgaris* L.) source leaves. *Plant Biol. Stuttg. Ger.* 19, 315–326. doi:10.1111/plb.12546.
- Niesbach-Klösgen, U., Guilley, H., Jonard, G., and Richards, K. (1990). Immunodetection in Vivo of beet necrotic yellow vein virus-encoded proteins. *Virology* 178, 52–61. doi:10.1016/0042-6822(90)90378-5.
- Nurk, S., Meleshko, D., Korobeynikov, A., and Pevzner, P. A. (2017). metaSPAdes: a new versatile metagenomic assembler. *Genome Res.* 27, 824–834. doi:10.1101/gr.213959.116.
- Ohki, T., Sasaya, T., Sayama, M., and Maoka, T. (2013). Phylogenetic analysis and host compatibility of Polymyxa graminis in Japan (abstract in Japanese). *Jpn J Phytopathol* 79, 224.
- Ohki, T., Sasaya, T., Sayama, M., and Maoka, T. (2017). Diversity of rDNA-ITS sequences of Polymyxa graminis from wheat and barley in Japan. *J. Gen. Plant Pathol.* 83, 226–230. doi:10.1007/s10327-017-0713-z.
- Ökmen, B., and Doehlemann, G. (2014). Inside plant: biotrophic strategies to modulate host immunity and metabolism. *Curr. Opin. Plant Biol.* 20, 19–25. doi:10.1016/j.pbi.2014.03.011.
- Pakdel, A., Mounier, C., Klein, E., Hleibieh, K., Monsion, B., Mutterer, J., et al. (2015). On the interaction and localization of the beet necrotic yellow vein virus replicase. *Virus Res.* 196, 94–104. doi:pakd.
- Panella, L. (2010). Sugar Beet as an Energy Crop. *Sugar Tech* 12, 288–293. doi:10.1007/s12355-010-0041-5.
- Panella, L. W., and Biancardi, E. (2016). "Genetic Resistances," in *Rhizomania*, eds. E. Biancardi and T. Tamada (Cham: Springer International Publishing), 195–220. doi:10.1007/978-3-319-30678-0_9.
- Park, S.-W., Kaimoyo, E., Kumar, D., Mosher, S., and Klessig, D. F. (2007). Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science* 318, 113–116. doi:10.1126/science.1147113.
- Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., and Tyson, G. W. (2015). CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* 25, 1043–1055. doi:10.1101/gr.186072.114.
- Parra, G., Bradnam, K., and Korf, I. (2007). CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. *Bioinformatics* 23, 1061–1067. doi:10.1093/bioinformatics/btm071.
- Päsold, S., Siegel, I., Seidel, C., and Ludwig-Müller, J. (2010). Flavonoid accumulation in *Arabidopsis thaliana* root galls caused by the obligate biotrophic pathogen *Plasmodiophora brassicae*. *Mol. Plant Pathol.* 11, 545–562. doi:https://doi.org/10.1111/j.1364-3703.2010.00628.x.

- Paul, H., Henken, B., Bock, Th. S. M. de, and Lange, W. (1992). Resistance to *Polymyxa betae* in Beta Species of the Section Procumbentes, in Hybrids with *B. vulgaris* and in Monosomic Chromosome Additions of *B. procumbens* in *B. vulgaris*. *Plant Breed.* 109, 265–273. doi:10.1111/j.1439-0523.1992.tb00184.x.
- Paul, H., Henken, B., Scholten, O. E., Bock, T. S. M. de, and Lange, W. (1993). Variation in the level of infection with *Polymyxa betae* and its effect on infection with beet necrotic yellow vein virus in beet accessions of the sections Beta and Corollinae. *Proc. Second Symp. Int. Work. Group Plant Viruses Fungal Vectors McGill Univ. Montr. Can. 25-27 July 1993*, 133–136.
- Pauwels, L., Inzé, D., and Goossens, A. (2009). Jasmonate-inducible gene: what does it mean? *Trends Plant Sci.* 14, 87–91. doi:10.1016/j.tplants.2008.11.005.
- Pavli, O. I., Kelaidi, G. I., Tampakaki, A. P., and Skaracis, G. N. (2011a). The HrpZ gene of *Pseudomonas syringae* pv. phaseolicola enhances resistance to rhizomania disease in transgenic *Nicotiana benthamiana* and sugar beet. *PLoS ONE* 6. doi:10.1371/journal.pone.0017306.
- Pavli, O. I., Tampakaki, A. P., and Skaracis, G. N. (2012). High Level Resistance against Rhizomania Disease by Simultaneously Integrating Two Distinct Defense Mechanisms. *PLoS ONE* 7. doi:10.1371/journal.pone.0051414.
- Pavli, O., Prins, M., Goldbach, R., and Skaracis, G. N. (2011b). Efficiency of Rz1-based rhizomania resistance and molecular studies on BNYVV isolates from sugar beet cultivation in Greece. *Eur. J. Plant Pathol.* 130, 133–142. doi:10.1007/s10658-010-9639-4.
- Peltier, C., Hleibieh, K., Thiel, H., Elodie, K., Bragard, C., and Gilmer, D. (2008). Molecular Biology of the Beet necrotic yellow vein virus. *Plant Viruses* 2, 14–24.
- Peltier, C., Klein, E., Hleibieh, K., D'Alonzo, M., Hammann, P., Bouzoubaa, S., et al. (2012). Beet necrotic yellow vein virus subgenomic RNA3 is a cleavage product leading to stable non-coding RNA required for long-distance movement. *J. Gen. Virol.* 93, 1093–1102. doi:10.1099/vir.0.039685-0.
- Peltier, C., Schmidlin, L., Klein, E., Taconnat, L., Prinsen, E., Erhardt, M., et al. (2011). Expression of the Beet necrotic yellow vein virus p25 protein induces hormonal changes and a root branching phenotype in *Arabidopsis thaliana*. *Transgenic Res.* 20, 443–466. doi:10.1007/s11248-010-9424-3.
- Pérez-López, E., Hossain, M. M., Tu, J., Waldner, M., Todd, C. D., Kusalik, A. J., et al. (2020). Transcriptome Analysis Identifies *Plasmodiophora brassicae* Secondary Infection Effector Candidates. *J. Eukaryot. Microbiol.* 67, 337–351. doi:10.1111/jeu.12784.
- Pérez-López, E., Waldner, M., Hossain, M., Kusalik, A. J., Wei, Y., Bonham-Smith, P. C., et al. (2018). Identification of *Plasmodiophora brassicae* effectors - A challenging goal. *Virulence* 9, 1344–1353. doi:10.1080/21505594.2018.1504560.
- Pertea, G., and Pertea, M. (2020). GFF Utilities: GffRead and GffCompare. *F1000Research* 9. doi:10.12688/f1000research.23297.2.
- Pertea, M., Pertea, G. M., Antonescu, C. M., Chang, T.-C., Mendell, J. T., and Salzberg, S. L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat. Biotechnol.* 33, 290–295. doi:10.1038/nbt.3122.
- Pferdmenges, F., Korf, H., and Varrelmann, M. (2009). Identification of rhizomania-infected soil in Europe able to overcome Rz1 resistance in sugar beet and

REFERENCES

- comparison with other resistance-breaking soils from different geographic origins. *Eur. J. Plant Pathol.* 124, 31–43. doi:10.1007/s10658-008-9388-9.
- Pferdmenges, F., and Varrelmann, M. (2009). Breaking of Beet necrotic yellow vein virus resistance in sugar beet is independent of virus and vector inoculum densities. *Eur. J. Plant Pathol.* 124, 231–245. doi:10.1007/s10658-008-9408-9.
- Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annu. Rev. Cell Dev. Biol.* 28, 489–521. doi:10.1146/annurev-cellbio-092910-154055.
- Pieterse, C. M. J., van Wees, S. C. M., van Pelt, J. A., Knoester, M., Laan, R., Gerrits, H., et al. (1998). A Novel Signaling Pathway Controlling Induced Systemic Resistance in Arabidopsis. *Plant Cell* 10, 1571–1580. doi:10.1105/tpc.10.9.1571.
- Pollini, C. P., and Giunchedi, L. (1989). Comparative histopathology of Sugar beets that are susceptible and partially resistant to Rhizomania. *Phytopathol. Mediterr.* 28, 16–21.
- Pollini, C. P., Masia, A., and Giunchedi, L. (1990). Free indole-3-acetic acid in sugar-beet root of rhizomania-susceptible and moderately resistant cultivars. *Phytopathol. Mediterr.* 29, 191–195.
- Pozo, M. J., and Azcón-Aguilar, C. (2007). Unraveling mycorrhiza-induced resistance. *Curr. Opin. Plant Biol.* 10, 393–398. doi:10.1016/j.pbi.2007.05.004.
- Prillwitz, H., and Schlösser, E. (1992). Beet soil-borne virus: occurrence, symptoms and effect on plant development. *Meded. Van Fac. Landbouwwet. Univ. Gent* 57, 295–302.
- Prillwitz, H., and Schlösser, E. (1993). Interactions between beet soil-borne virus (BSBV-2) and beet necrotic yellow vein virus (BNYVV). 71–74.
- Pritchard, L., and Birch, P. R. J. (2014). The zigzag model of plant–microbe interactions: is it time to move on? *Mol. Plant Pathol.* 15, 865–870. doi:10.1111/mpp.12210.
- Putz, C. (1977). Composition and structure of beet necrotic yellow vein virus. *J. Gen. Virol.* 35, 397–401. doi:10.1099/0022-1317-35-2-397.
- Putz, C., Pinck, M., Fritsch, C., and Pinck, L. (1983). Identification of the 3' - and 5' - ends of beet necrotic yellow vein virus RNAs. *FEBS Lett.* 156, 41–46. doi:10.1016/0014-5793(83)80244-0.
- Qu, X., and Christ, B. J. (2006a). Single Cystosorus Isolate Production and Restriction Fragment Length Polymorphism Characterization of the Obligate Biotroph *Spongospora subterranea* f. sp. *subterranea*. *Phytopathology* 96, 1157–1163. doi:10.1094/PHYTO-96-1157.
- Qu, X., and Christ, B. J. (2006b). The host range of *Spongospora subterranea* f. sp. *subterranea* in the United States. *Am. J. Potato Res.* 83, 343–347. doi:10.1007/BF02871595.
- Qu, X., and Christ, B. J. (2007). In vitro culture of the obligate parasite *Spongospora subterranea* (cercozoa; plasmodiophorida) associated with root-inducing transferred-DNA transformed potato hairy roots. *J. Eukaryot. Microbiol.* 54, 465–467. doi:10.1111/j.1550-7408.2007.00289.x.
- Quillet, L., Guilley, H., Jonard, G., and Richards, K. (1989). In vitro synthesis of biologically active beet necrotic yellow vein virus RNA. *Virology* 172, 293–301. doi:10.1016/0042-6822(89)90131-1.

- Rahim, M. D., Andika, I. B., Han, C., Kondo, H., and Tamada, T. (2007). RNA4-encoded p31 of beet necrotic yellow vein virus is involved in efficient vector transmission, symptom severity and silencing suppression in roots. *J. Gen. Virol.* 88, 1611–1619. doi:10.1099/vir.0.82720-0.
- Rao, A. S., and Brakke, M. K. (1969). Relation of soil-borne Wheat mosaic virus and its fungal vector, *Polymyxa graminis*. *Phytopathology* 59, 581–587.
- Ratna, A. S., Rao, A. S., Reddy, A. S., Molt, B. L., Reddy, D. V. R., Vijayalakshmi, M., et al. (1991). Studies on transmission of Indian peanut clump virus disease by *Polymyxa graminis*. *Ann. Appl. Biol.* 118, 71–78. doi:10.1111/j.1744-7348.1991.tb06086.x.
- Ratti, C., and Biancardi, E. (2016). "Control of the disease," in *Rhizomania*, 175–193. doi:10.1007/978-3-319-30678-0_8.
- Ratti, C., Hleibieh, K., Bianchi, L., Schirmer, A., Autonell, C. R., and Gilmer, D. (2009). Beet soil-borne mosaic virus RNA-3 is replicated and encapsidated in the presence of BNYVV RNA-1 and -2 and allows long distance movement in Beta macrocarpa. *Virology* 385, 392–399. doi:10.1016/j.virol.2008.12.013.
- Reavy, B., Arif, M., Cowan, G. H., and Torrance, L. (1998). Association of sequences in the coat protein/readthrough domain of potato mop-top virus with transmission by *Spongospora subterranea*. *J. Gen. Virol.* 79, 2343–2347. doi:10.1099/0022-1317-79-10-2343.
- Rejeb, I. B., Pastor, V., and Mauch-Mani, B. (2014). Plant Responses to Simultaneous Biotic and Abiotic Stress: Molecular Mechanisms. *Plants* 3, 458–475. doi:10.3390/plants3040458.
- Ren, D., Navarro, B., Perez, G., Jackson, A. C., Hsu, S., Shi, Q., et al. (2001). A sperm ion channel required for sperm motility and male fertility. *Nature* 413, 603–609. doi:10.1038/35098027.
- Richard-Molard, M. (Institut T. F. de la B. I. (1985). Rhizomania - The situation in 1985 [Beet necrotic yellow vein virus; *Polymyxa betae*]. in (IIRB. Secretariat General). Available at: <http://agris.fao.org/agris-search/search.do?recordID=BE8511218> [Accessed July 14, 2016].
- Richards, K. E., and Tamada, T. (1992). Mapping functions on the multipartite genome of beet necrotic yellow vein virus. *Annu Rev Phytopathol* 30, 291–313.
- Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., et al. (2011). Integrative Genomics Viewer. *Nat. Biotechnol.* 29, 24–26. doi:10.1038/nbt.1754.
- Rochon, D., Kakani, K., Robbins, M., and Reade, R. (2004). Molecular Aspects of Plant Virus Transmission by Olpidium and Plasmodiophorid Vectors. *Annu. Rev. Phytopathol.* 42, 211–241. doi:10.1146/annurev.phyto.42.040803.140317.
- Rodriguez-R, L. M., and Konstantinidis, K. T. (2014). Bypassing Cultivation To Identify Bacterial Species: Culture-independent genomic approaches identify credibly distinct clusters, avoid cultivation bias, and provide true insights into microbial species. *Microbe Mag.* 9, 111–118. doi:10.1128/microbe.9.111.1.
- Rodriguez-R, L. M., and Konstantinidis, K. T. (2016). The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ Inc.* doi:10.7287/peerj.preprints.1900v1.

REFERENCES

- Rolfe, S. A., Strelkov, S. E., Links, M. G., Clarke, W. E., Robinson, S. J., Djavaheri, M., et al. (2016). The compact genome of the plant pathogen *Plasmodiophora brassicae* is adapted to intracellular interactions with host *Brassica* spp. *BMC Genomics* 17, 272. doi:10.1186/s12864-016-2597-2.
- Rush, C. M. (2003). *Ecology and Epidemiology of Benyviruses and Plasmodiophorid Vectors*. doi:10.1146/annurev.phyto.41.052002.095705.
- Rush, C. M., Liu, H.-Y., Lewellen, R. T., and Acosta-Leal, R. (2006). The Continuing Saga of Rhizomania of Sugar Beets in the United States. *Plant Dis.* 90, 4–15. doi:10.1094/PD-90-0004.
- Rysanek, P., Stocky, G., Haeberlé, A. M., and Putz, C. (1992). Immunogold labelling of beet necrotic yellow vein virus particles inside its fungal vector, *Polymyxa betae* K. *Agronomie* 12, 651–659. doi:10.1051/agro:19920808.
- Saary, P., Mitchell, A. L., and Finn, R. D. (2019). Estimating the quality of eukaryotic genomes recovered from metagenomic analysis. *bioRxiv*, 2019.12.19.882753. doi:10.1101/2019.12.19.882753.
- Saito, M., Kiguchi, T., Kusume, T., and Tamada, T. (1996). Complete nucleotide sequence of the Japanese isolate S of beet necrotic yellow vein virus RNA and comparison with European isolates. *Arch. Virol.* 141, 2163–2175. doi:10.1007/BF01718223.
- Sayama, M., Momota, Y., and Takenaka, S. (2006). Fluorescent staining of resting spores of *Polymyxa betae*. *J. Gen. Plant Pathol.* 72, 190–192. doi:10.1007/s10327-005-0269-1.
- Schirmer, A., Link, D., Cognat, V., Moury, B., Beuve, M., Meunier, A., et al. (2005). Phylogenetic analysis of isolates of Beet necrotic yellow vein virus collected worldwide. *J. Gen. Virol.* 86, 2897–2911. doi:10.1099/vir.0.81167-0.
- Schlosser, E. (1988). Epidemiology and management of *Polymyxa betae* and beet necrotic yellow vein virus. *Dev. Appl. Biol.* Available at: <http://agris.fao.org/agris-search/search.do?recordID=US201301733518> [Accessed November 15, 2016].
- Schlösser, E. (1990). Rizomania. XII. Effect of BNYVV and BSBV on the development of *Polymyxa betae* Keskin. *Rizomania XII Eff. BNYVV BSBV Dev. Polymyxa Betae Keskin* 55, 1069–1071.
- Schmidlin, L., Bruyne, E. D., Weyens, G., Lefebvre, M., and Gilmer, D. (2008). Identification of differentially expressed root genes upon rhizomania disease. *Mol. Plant Pathol.* 9, 741–751. doi:https://doi.org/10.1111/j.1364-3703.2008.00498.x.
- Schmitt, C., Balmori, E., Jonard, G., Richards, K. E., and Guilley, H. (1992). In vitro mutagenesis of biologically active transcripts of beet necrotic yellow vein virus RNA 2: evidence that a domain of the 75-kDa readthrough protein is important for efficient virus assembly. *Proc. Natl. Acad. Sci.* 89, 5715–5719. doi:10.1073/pnas.89.13.5715.
- Scholten, O. E., and Lange, W. (2000). Breeding for resistance to rhizomania in sugar beet: A review. *Euphytica* 112, 219–231. doi:10.1023/A:1003988003165.
- Schuller, A., Kehr, J., and Ludwig-Müller, J. (2014). Laser Microdissection Coupled to Transcriptional Profiling of Arabidopsis Roots Inoculated by *Plasmodiophora brassicae* Indicates a Role for Brassinosteroids in Clubroot Formation. *Plant Cell Physiol.* 55, 392–411. doi:10.1093/pcp/pct174.
- Schwacke, R., Ponce-Soto, G. Y., Krause, K., Bolger, A. M., Arsova, B., Hallab, A., et al. (2019). MapMan4: A Refined Protein Classification and Annotation Framework

- Applicable to Multi-Omics Data Analysis. *Mol. Plant* 12, 879–892. doi:10.1016/j.molp.2019.01.003.
- Schwelm, A., Badstöber, J., Bulman, S., Desoignies, N., Etemadi, M., Falloon, R. E., et al. (2018). Not in your usual Top 10: protists that infect plants and algae. *Mol. Plant Pathol.* 19, 1029–1044. doi:10.1111/mpp.12580.
- Schwelm, A., Dixelius, C., and Ludwig-Müller, J. (2015a). New kid on the block – the clubroot pathogen genome moves the plasmodiophorids into the genomic era. *Eur. J. Plant Pathol.* 145, 531–542. doi:10.1007/s10658-015-0839-9.
- Schwelm, A., Fogelqvist, J., Knaust, A., Jülke, S., Lilja, T., Bonilla-Rosso, G., et al. (2015b). The Plasmodiophora brassicae genome reveals insights in its life cycle and ancestry of chitin synthases. *Sci. Rep.* 5, 11153. doi:10.1038/srep11153.
- Sedaghatkish, A., Gossen, B. D., Yu, F., Torkamaneh, D., and McDonald, M. R. (2019). Whole-genome DNA similarity and population structure of Plasmodiophora brassicae strains from Canada. *BMC Genomics* 20, 744. doi:10.1186/s12864-019-6118-y.
- Sels, J., Mathys, J., De Coninck, B. M. A., Cammue, B. P. A., and De Bolle, M. F. C. (2008). Plant pathogenesis-related (PR) proteins: A focus on PR peptides. *Plant Physiol. Biochem.* 46, 941–950. doi:10.1016/j.plaphy.2008.06.011.
- Sergeeva, L. I., Keurentjes, J. J. B., Bentsink, L., Vonk, J., van der Plas, L. H. W., Koornneef, M., et al. (2006). Vacuolar invertase regulates elongation of Arabidopsis thaliana roots as revealed by QTL and mutant analysis. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2994–2999. doi:10.1073/pnas.0511015103.
- Shah, F. A., Falloon, R. E., Butler, R. C., and Lister, R. A. (2012). Low amounts of Spongospora subterranea sporosorus inoculum cause severe powdery scab, root galling and reduced water use in potato (Solanum tuberosum). *Australas. Plant Pathol.* 41, 219–228. doi:10.1007/s13313-011-0110-6.
- Shah, N., Li, Q., Xu, Q., Liu, J., Huang, F., Zhan, Z., et al. (2020). CRb and PbBa8.1 Synergically Increases Resistant Genes Expression upon Infection of Plasmodiophora brassicae in Brassica napus. *Genes* 11, 202. doi:10.3390/genes11020202.
- Shindo, T., and Hoorn, R. a. L. V. D. (2008). Papain-like cysteine proteases: key players at molecular battlefields employed by both plants and their invaders. *Mol. Plant Pathol.* 9, 119–125. doi:10.1111/j.1364-3703.2007.00439.x.
- Shrode, L. B., Lewis, Z. A., White, L. D., Bell-Pedersen, D., and Ebbole, D. J. (2001). vvd Is Required for Light Adaptation of Conidiation-Specific Genes of Neurospora crassa, but Not Circadian Conidiation. *Fungal Genet. Biol.* 32, 169–181. doi:10.1006/fgbi.2001.1264.
- Sibbald, S. J., and Archibald, J. M. (2017). More protist genomes needed. *Nat. Ecol. Evol.* 1, 1–3. doi:10.1038/s41559-017-0145.
- Siemens, J., González, M.-C., Wolf, S., Hofmann, C., Greiner, S., Du, Y., et al. (2011). Extracellular invertase is involved in the regulation of clubroot disease in Arabidopsis thaliana. *Mol. Plant Pathol.* 12, 247–262. doi:https://doi.org/10.1111/j.1364-3703.2010.00667.x.
- Siemens, J., Graf, H., Bulman, S., In, O., and Ludwig-Müller, J. (2009). Monitoring expression of selected Plasmodiophora brassicae genes during clubroot development in Arabidopsis thaliana. *Plant Pathol.* 58, 130–136. doi:https://doi.org/10.1111/j.1365-3059.2008.01943.x.

REFERENCES

- Siemens, J., Keller, I., Sarx, J., Kunz, S., Schuller, A., Nagel, W., et al. (2006). Transcriptome Analysis of Arabidopsis Clubroots Indicate a Key Role for Cytokinins in Disease Development. *Mol. Plant-Microbe Interactions®* 19, 480–494. doi:10.1094/MPMI-19-0480.
- Sierra, R., Cañas-Duarte, S. J., Burki, F., Schwelm, A., Fogelqvist, J., Dixelius, C., et al. (2016). Evolutionary Origins of Rhizarian Parasites. *Mol. Biol. Evol.* 33, 980–983. doi:10.1093/molbev/msv340.
- Sigrist, C. J. A., de Castro, E., Cerutti, L., Cuche, B. A., Hulo, N., Bridge, A., et al. (2013). New and continuing developments at PROSITE. *Nucleic Acids Res.* 41, D344–347. doi:10.1093/nar/gks1067.
- Simpson, A. G. B., and Roger, A. J. (2004). The real 'kingdoms' of eukaryotes. *Curr. Biol.* 17, R693–R696. doi:10.1016/j.cub.2004.08.038.
- Singh, K., Tzelepis, G., Zouhar, M., Ryšánek, P., and Dixelius, C. (2018). The immunophilin repertoire of Plasmodiophora brassicae and functional analysis of PbCYP3 cyclophilin. *Mol. Genet. Genomics* 293, 381–390. doi:10.1007/s00438-017-1395-0.
- Singhal, K., and Mohanty, S. (2019). Genome organisation and comparative genomics of four novel Wolbachia genome assemblies from Indian Drosophila host. *Funct. Integr. Genomics* 19, 617–632. doi:10.1007/s10142-019-00664-5.
- Slykhuis, J. T., and Barr, D. J. S. (1978). Confirmation of Polymyxa graminis as a vector of wheat spindle streak mosaic virus. *Phytopathology* 68, 639–643.
- Smith, M. J., Adams, M. J., and Ward, E. (2011). Evidence that Polymyxa species may infect Arabidopsis thaliana. *FEMS Microbiol. Lett.* 318, 35–40. doi:10.1111/j.1574-6968.2011.02236.x.
- Smith, M. J., Adams, M. J., and Ward, E. (2013). Ribosomal DNA analyses reveal greater sequence variation in Polymyxa species than previously thought and indicate the possibility of new ribotype-host-virus associations. *Environ. Microbiol. Rep.* 5, 143–150. doi:https://doi.org/10.1111/1758-2229.12026.
- Smith, M., and Rush, C. (2016). "CHAPTER 25: Polymyxa Species and the Viruses They Vector?With Emphasis on Benyviruses and Furoviruses," in *Vector-Mediated Transmission of Plant Pathogens* General Plant Pathology., eds. Madeleine Smith and Charles Rush (The American Phytopathological Society), 353–364. doi:10.1094/9780890545355.025.
- Smyth, G. K. (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* 3, Article3. doi:10.2202/1544-6115.1027.
- Spandl, J., White, D. J., Peychl, J., and Thiele, C. (2009). Live Cell Multicolor Imaging of Lipid Droplets with a New Dye, LD540. *Traffic* 10, 1579–1584. doi:https://doi.org/10.1111/j.1600-0854.2009.00980.x.
- Spanu, P. D. (2012). The Genomics of Obligate (and Nonobligate) Biotrophs. *Annu. Rev. Phytopathol.* 50, 91–109. doi:10.1146/annurev-phyto-081211-173024.
- Sperschneider, J., Dodds, P. N., Gardiner, D. M., Singh, K. B., and Taylor, J. M. (2018). Improved prediction of fungal effector proteins from secretomes with EffectorP 2.0. *Mol. Plant Pathol.* 19, 2094–2110. doi:10.1111/mpp.12682.

- Stanke, M., Diekhans, M., Baertsch, R., and Haussler, D. (2008). Using native and syntenically mapped cDNA alignments to improve de novo gene finding. *Bioinformatics* 24, 637–644. doi:10.1093/bioinformatics/btn013.
- Staswick, P. E., Tiryaki, I., and Rowe, M. L. (2002). Jasmonate Response Locus JAR1 and Several Related Arabidopsis Genes Encode Enzymes of the Firefly Luciferase Superfamily That Show Activity on Jasmonic, Salicylic, and Indole-3-Acetic Acids in an Assay for Adenylation. *Plant Cell* 14, 1405–1415. doi:10.1105/tpc.000885.
- Stegmann, M., Monaghan, J., Smakowska-Luzan, E., Rovenich, H., Lehner, A., Holton, N., et al. (2017). The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. *Science* 355, 287–289. doi:10.1126/science.aal2541.
- Steven, A. C., Trus, B. L., Putz, C., and Wurtz, M. (1981). The molecular organization of beet necrotic yellow vein virus. *Virology* 113, 428–438. doi:10.1016/0042-6822(81)90172-0.
- Stjelja, S., Fogelqvist, J., Tellgren-Roth, C., and Dixelius, C. (2019). The architecture of the Plasmodiophora brassicae nuclear and mitochondrial genomes. *Sci. Rep.* 9, 15753. doi:10.1038/s41598-019-52274-7.
- Strausbaugh, C. A., Neher, O., Rearick, E., and Eujayl, I. A. (2015). Influence of harvest timing, fungicides, and beet necrotic yellow vein virus on sugar beet storage. *Plant Dis.* 99, 1296–1309. doi:10.1094/PDIS-10-14-0998-RE.
- Šubr, Z. W., Kastirr, U., and Kühne, T. (2002). Subtractive Cloning of DNA from Polymyxa graminis— an Obligate Parasitic Plasmodiophorid. *J. Phytopathol.* 150, 564–568. doi:10.1046/j.1439-0434.2002.00795.x.
- Sun, X., Zhu, Y., Wang, L., Liu, H., Ling, Y., Li, Z., et al. (2017). The Catsper channel and its roles in male fertility: a systematic review. *Reprod. Biol. Endocrinol. RBE* 15. doi:10.1186/s12958-017-0281-2.
- Tamada, T. (2007). Susceptibility and resistance of Beta vulgaris subsp. maritima to foliar rub-inoculation with Beet necrotic yellow vein virus. *J. Gen. Plant Pathol.* 73, 76–80. doi:10.1007/s10327-006-0318-4.
- Tamada, T. (2016). “General Features of Beet Necrotic Yellow Vein Virus,” in *Rhizomania*, eds. E. Biancardi and T. Tamada (Cham: Springer International Publishing), 55–83. doi:10.1007/978-3-319-30678-0_3.
- Tamada, T., Abe, H., and Baba, T. (1975). Beet necrotic yellow vein virus and its relation to the fungus Polymyxa betae. *Proc Int Congr Int Assoc Microbiol Soc 1st* 3, 313–320.
- Tamada, T., and Abe, H. (Hokkaido C. A. E. S. (1989). Evidence that beet necrotic yellow vein virus RNA-4 is essential for efficient transmission by the fungus Polymyxa betae. *J. Gen. Virol. U. K.* 70, 3391–3398. doi:10.1099/0022-1317-70-12-3391.
- Tamada, T., and Asher, M. J. C. (2016a). “Ecology and Epidemiology,” in *Rhizomania*, eds. E. Biancardi and T. Tamada (Springer International Publishing), 155–171. doi:10.1007/978-3-319-30678-0_7.
- Tamada, T., and Asher, M. J. C. (2016b). “The Plasmodiophorid Protist Polymyxa betae,” in *Rhizomania*, eds. E. Biancardi and T. Tamada (Cham: Springer International Publishing), 135–153. doi:10.1007/978-3-319-30678-0_6.
- Tamada, T., and Baba, T. (1973). Beet necrotic yellow vein virus from rizomania-affected sugar beet in Japan. *Ann. Phytopathol. Soc. Jpn.* 39, 325–332. doi:10.3186/jjphytopath.39.325.

REFERENCES

- Tamada, T., Baba, T., and Abe, H. (1971). A virus isolated from sugar beet showing 'Rhizomania'-like symptoms and its transmission in soil. *Proc Sugar Beet Res Assoc Jpn* 13, 179–186.
- Tamada, T., and Kondo, H. (2013). Biological and genetic diversity of plasmodiophorid-transmitted viruses and their vectors. *J. Gen. Plant Pathol.* 79, 307–320. doi:10.1007/s10327-013-0457-3.
- Tamada, T., Kondo, H., and Chiba, S. (2016). "Genetic diversity of beet necrotic yellow vein virus," in *Rhizomania*, 109–131. doi:10.1007/978-3-319-30678-0_5.
- Tamada, T., and Kusume, T. (1991). Evidence that the 75K readthrough protein of beet necrotic yellow vein virus RNA-2 is essential for transmission by the fungus *Polymyxa betae*. *J. Gen. Virol.* 72, 1497–1504. doi:10.1099/0022-1317-72-7-1497.
- Tamada, T., Kusume, T., Uchino, H., Kiguchi, T., and Saito, M. (1996a). Evidence that Beet necrotic yellow vein virus RNA 5 is involved in symptom development of sugar beet roots. *Proc. Third Symp. Int. Work. Group Plant Viruses Fungal Vectors*, 49–52.
- Tamada, T., Schmitt, C., Saito, M., Guillely, H., Richards, K., and Jonard, G. (1996b). High resolution analysis of the readthrough domain of beet necrotic yellow vein virus readthrough protein: a KTER motif is important for efficient transmission of the virus by *Polymyxa betae*. *J. Gen. Virol.* 77, 1359–1367. doi:10.1099/0022-1317-77-7-1359.
- Tamada, T., Shirako, Y., Abe, H., Saito, M., Kiguchi, T., and Harada, T. (1989). Production and pathogenicity of isolates of beet necrotic yellow vein virus with different numbers of RNA components. *J. Gen. Virol.* 70, 3399–3409. doi:10.1099/0022-1317-70-12-3399.
- Tamada, T., Uchino, H., Kusume, T., Iketani-Saito, M., Chiba, S., Andika, I. B., et al. (2021). Pathogenetic roles of beet necrotic yellow vein virus RNA5 in the exacerbation of symptoms and yield reduction, development of scab-like symptoms, and Rz1-resistance breaking in sugar beet. *Plant Pathol.* 70, 219–232. doi:10.1111/ppa.13266.
- Tamada, T., Uchino, H., Kusume, T., and Saito, M. (1999). RNA 3 deletion mutants of beet necrotic yellow vein virus do not cause rhizomania disease in sugar beets. *Phytopathology* 89, 1000–1006. doi:10.1094/PHYTO.1999.89.11.1000.
- Tang, D., Wang, G., and Zhou, J.-M. (2017). Receptor Kinases in Plant-Pathogen Interactions: More Than Pattern Recognition. *Plant Cell* 29, 618–637. doi:10.1105/tpc.16.00891.
- Tauzin, A. S., and Giardina, T. (2014). Sucrose and invertases, a part of the plant defense response to the biotic stresses. *Front. Plant Sci.* 5, 293. doi:10.3389/fpls.2014.00293.
- Teixeira, P. J. P., Colaianni, N. R., Fitzpatrick, C. R., and Dangl, J. L. (2019). Beyond pathogens: microbiota interactions with the plant immune system. *Curr. Opin. Microbiol.* 49, 7–17. doi:10.1016/j.mib.2019.08.003.
- Temmink, J. H. M., Campbell, R. N., and Smith, P. R. (1970). Specificity and Site of in vitro Acquisition of Tobacco Necrosis Virus by Zoospores of *Olpidium brassicae*. *J. Gen. Virol.* 9, 201–213. doi:10.1099/0022-1317-9-3-201.
- Terefe, T. G., Visser, B., Botha, W., Kozana, A., Roberts, R., Thompson, G. D., et al. (2021). Detection and molecular characterization of Wheat stripe mosaic virus on wheat in South Africa. *Crop Prot.* 143. doi:10.1016/j.cropro.2020.105464.

- Ter-Hovhannisyan, V., Lomsadze, A., Chernoff, Y. O., and Borodovsky, M. (2008). Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training. *Genome Res.* 18, 1979–1990. doi:10.1101/gr.081612.108.
- Thiel, H., Hleibieh, K., Gilmer, D., and Varrelmann, M. (2012). The P25 pathogenicity factor of Beet necrotic yellow vein virus targets the sugar beet 26S proteasome involved in the induction of a hypersensitive resistance response via interaction with an F-box protein. *Mol. Plant. Microbe Interact.* 25, 1058–1072. doi:10.1094/MPMI-03-12-0057-R.
- Thiel, H., and Varrelmann, M. (2009). Identification of Beet necrotic yellow vein virus P25 pathogenicity factor-interacting sugar beet proteins that represent putative virus targets or components of plant resistance. *Mol. Plant. Microbe Interact.* 22, 999–1010. doi:10.1094/MPMI-22-8-0999.
- Thouvenel, J. C., and Fauquet, C. (1981). Further properties of peanut clump virus and studies on its natural transmission. *Ann. Appl. Biol.* 97, 99–107. doi:10.1111/j.1744-7348.1981.tb02998.x.
- Tian, T., Liu, Y., Yan, H., You, Q., Yi, X., Du, Z., et al. (2017). agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. *Nucleic Acids Res.* 45, W122–W129. doi:10.1093/nar/gkx382.
- Tillich, M., Lehwark, P., Pellizzer, T., Ulbricht-Jones, E. S., Fischer, A., Bock, R., et al. (2017). GeSeq – versatile and accurate annotation of organelle genomes. *Nucleic Acids Res.* 45, W6–W11. doi:10.1093/nar/gkx391.
- Torrance, L., Wright, K., Crutzen, F., Cowan, G., Lukhovitskaya, N., Bragard, C., et al. (2011). Unusual Features of Pomoviral RNA Movement. *Front. Microbiol.* 2. doi:10.3389/fmicb.2011.00259.
- Toruño, T. Y., Stergiopoulos, I., and Coaker, G. (2016). Plant-Pathogen Effectors: Cellular Probes Interfering with Plant Defenses in Spatial and Temporal Manners. *Annu. Rev. Phytopathol.* 54, 419–441. doi:10.1146/annurev-phyto-080615-100204.
- Tuitert, G., Musters-van Oorschot, P. M. S., and Heijbroek, W. (1994). Effect of sugar beet cultivars with different levels of resistance to beet necrotic yellow vein virus on transmission of virus by *Polymyxa betae*. *Eur. J. Plant Pathol.* 100, 201–220. doi:10.1007/BF01876236.
- Uritskiy, G. V., DiRuggiero, J., and Taylor, J. (2018). MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. *Microbiome* 6, 158. doi:10.1186/s40168-018-0541-1.
- Vaïanopoulos, C. (2008). Widespread occurrence of soil-borne mosaic viruses in Belgium: preferential associations of *Polymyxa graminis* and mosaic viruses on cereals. Available at: <https://dial.uclouvain.be/pr/boreal/object/boreal:206520> [Accessed July 10, 2021].
- Vaïanopoulos, C., Bragard, C., Moreau, V., Maraite, H., and Legrève, A. (2007). Identification and Quantification of *Polymyxa graminis* f. sp. *temperata* and *P. graminis* f. sp. *tepida* on Barley and Wheat. *Plant Dis.* 91, 857–864. doi:10.1094/PDIS-91-7-0857.
- Vaïanopoulos, C., Legrève, A., Lorca, C., Moreau, V., Steyer, S., Maraite, H., et al. (2006). Widespread Occurrence of Wheat spindle streak mosaic virus in Belgium. *Plant Dis.* 90, 723–728. doi:10.1094/PD-90-0723.

REFERENCES

- Vaïanopoulos, C., Legreve, A., Moreau, V., Steyer, S., Maraite, H., and Bragard, C. (2005). Occurrence of bymo- and furoviruses on wheat in Belgium. *Parasitica* 61, 47–53.
- Valentin, C., Dunoyer, P., Vetter, G., Schalk, C., Dietrich, A., and Bouzoubaa, S. (2005). Molecular basis for mitochondrial localization of viral particles during Beet necrotic yellow vein virus infection. *J. Virol.* 79, 9991–10002. doi:10.1128/JVI.79.15.9991-10002.2005.
- van Aubel, G., Cambier, P., Dieu, M., and Van Cutsem, P. (2016). Plant immunity induced by COS-OGA elicitor is a cumulative process that involves salicylic acid. *Plant Sci.* 247, 60–70. doi:10.1016/j.plantsci.2016.03.005.
- Van de Weyer, A.-L., Monteiro, F., Furzer, O. J., Nishimura, M. T., Cevik, V., Witek, K., et al. (2019). A Species-Wide Inventory of NLR Genes and Alleles in *Arabidopsis thaliana*. *Cell* 178, 1260-1272.e14. doi:10.1016/j.cell.2019.07.038.
- van Loon, L. c., Rep, M., and Pieterse, C. m. j. (2006). Significance of Inducible Defense-related Proteins in Infected Plants. *Annu. Rev. Phytopathol.* 44, 135–162. doi:10.1146/annurev.phyto.44.070505.143425.
- van Wees, S. C. M., Luijendijk, M., Smoorenburg, I., van Loon, L. C., and Pieterse, C. M. J. (1999). Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. *Plant Mol. Biol.* 41, 537–549. doi:10.1023/A:1006319216982.
- Veltri, D., Wight, M. M., and Crouch, J. A. (2016). SimpleSynteny: a web-based tool for visualization of microsynteny across multiple species. *Nucleic Acids Res.* 44, W41–45. doi:10.1093/nar/gkw330.
- Verchot-Lubicz, J., Torrance, L., Solovyev, A. G., Morozov, S. Y., Jackson, A. O., and Gilmer, D. (2010). Varied movement strategies employed by triple gene block-encoding viruses. *Mol. Plant-Microbe Interact. MPMI* 23, 1231–1247. doi:10.1094/MPMI-04-10-0086.
- Verkhatsky, A., and Parpura, V. (2014). Calcium signalling and calcium channels: Evolution and general principles. *Eur. J. Pharmacol.* 739, 1–3. doi:10.1016/j.ejphar.2013.11.013.
- Vetter, G., Hily, J.-M., Klein, E., Schmidlin, L., Haas, M., Merkle, T., et al. (2004). Nucleo-cytoplasmic shuttling of the beet necrotic yellow vein virus RNA-3-enclosed p25 protein. *J. Gen. Virol.* 85, 2459–2469. doi:10.1099/vir.0.80142-0.
- Vivek-Ananth, R. P., Mohanraj, K., Vandanashree, M., Jhingran, A., Craig, J. P., and Samal, A. (2018). Comparative systems analysis of the secretome of the opportunistic pathogen *Aspergillus fumigatus* and other *Aspergillus* species. *Sci. Rep.* 8. doi:10.1038/s41598-018-25016-4.
- Vogt, T. (2010). Phenylpropanoid Biosynthesis. *Mol. Plant* 3, 2–20. doi:10.1093/mp/ssp106.
- Voronin, D. A., and Kiseleva, E. V. (2008). Functional role of proteins containing ankyrin repeats. *Cell Tissue Biol.* 2, 1–12. doi:10.1134/S1990519X0801001X.
- Vuittenez, A., Arnold, J., Spindler, C., and De Larambergue, H. (1977). Test de transmission du virus de la rhizomanie de la betterave (beet necrotic yellow vein virus) par le champignon *Polymyxa betae* Kesik myxomyc-*te* associé aux racines. Application à la prospection de la maladie (Abst.). *Ann. Phytopathol.* 9, 537.

- Wagh, S. G., Alam, M. M., Kobayashi, K., Yaeno, T., Yamaoka, N., Toriba, T., et al. (2016). Analysis of rice RNA-dependent RNA polymerase 6 (OsRDR6) gene in response to viral, bacterial and fungal pathogens. *J. Gen. Plant Pathol.* 82, 12–17. doi:10.1007/s10327-015-0630-y.
- Wakeham, A. J., and White, J. G. (1996). Serological detection in soil of *Plasmodiophora brassicae* resting spores. *Physiol. Mol. Plant Pathol.* 48, 289–303. doi:10.1006/pmpp.1996.0024.
- Walker, P. J., Blasdel, K. R., Calisher, C. H., Dietzgen, R. G., Kondo, H., Kurath, G., et al. (2018). ICTV Virus Taxonomy Profile: Rhabdoviridae. *J. Gen. Virol.* 99, 447–448. doi:10.1099/jgv.0.001020.
- Walsh, J. A., Merz, U., and Harrison, G. (1996). Serological detection of spore balls of *Spongopora subterranea* and quantification in soil. *Plant Pathol.* 45, 884–895. doi:10.1111/j.1365-3059.1996.tb02899.x.
- Wang, D., Tian, L., Zhang, D.-D., Song, J., Song, S.-S., Yin, C.-M., et al. (2020). Functional analyses of small secreted cysteine-rich proteins identified candidate effectors in *Verticillium dahliae*. *Mol. Plant Pathol.* 21, 667–685. doi:10.1111/mpp.12921.
- Wang, Y., Fan, H., Wang, X.-B., Li, M., Han, C., Li, D., et al. (2011). Detection and characterization of spontaneous internal deletion mutants of Beet Necrotic yellow vein virus RNA3 from systemic host *Nicotiana benthamiana*. *Virology* 8, 422X-8-335. doi:10.1186/1743-422X-8-335.
- Ward, E., and Adams, M. J. (1998). Analysis of ribosomal DNA sequences of *Polymyxa* species and related fungi and the development of genus- and species-specific PCR primers. *Mycol. Res.* 102, 965–974. doi:10.1017/S0953756297005881.
- Ward, E., Adams, M. J., Mutasa, E. S., Collier, C. R., and Asher, M. J. C. (1994). Characterization of *Polymyxa* species by restriction analysis of PCR-amplified ribosomal DNA. *Plant Pathol.* 43, 872–877. doi:10.1111/j.1365-3059.1994.tb01631.x.
- Waterhouse, G. (1973). *Plasmodiophoromycetes*. *Fungi B* 4, 75–82.
- Webb, K. M., Broccardo, C. J., Prenni, J. E., and Wintermantel, W. M. (2014). Proteomic profiling of sugar beet (*Beta vulgaris*) leaves during rhizomania compatible interactions. *Proteomes* 2, 208–223. doi:10.3390/proteomes2020208.
- Webb, K. M., Wintermantel, W. M., Kaur, N., Prenni, J. E., Broccardo, C. J., Wolfe, L. M., et al. (2015). Differential abundance of proteins in response to Beet necrotic yellow vein virus during compatible and incompatible interactions in sugar beet containing Rz1 or Rz2. *Physiol. Mol. Plant Pathol.* 91, 96–105. doi:10.1016/j.pmpp.2015.06.006.
- Webb, K. M., Wintermantel, W. M., Wolfe, L., Yao, L., Hladky, L. J., Broeckling, C. D., et al. (2020). Metabolic disturbances in sugar beet (*Beta vulgaris*) during infection with Beet necrotic yellow vein virus. *Physiol. Mol. Plant Pathol.* 112, 101520. doi:10.1016/j.pmpp.2020.101520.
- Weiland, J. J., Bornemann, K., Neubauer, J. D., Khan, M. F. R., and Bolton, M. D. (2019). Prevalence and distribution of beet necrotic yellow vein virus strains in North Dakota and Minnesota. *Plant Dis.* 103, 2083–2089. doi:10.1094/PDIS-02-19-0360-RE.
- Wetzel, V., Willems, G., Darracq, A., Galein, Y., Liebe, S., and Varrelmann, M. (2021). The *Beta vulgaris*-derived resistance gene Rz2 confers broad-spectrum resistance

REFERENCES

- against soilborne sugar beet-infecting viruses from different families by recognizing triple gene block protein 1. *Mol. Plant Pathol.* 22, 829–842. doi:10.1111/mpp.13066.
- Williams, B. A. P., Slamovits, C. H., Patron, N. J., Fast, N. M., and Keeling, P. J. (2005). A high frequency of overlapping gene expression in compacted eukaryotic genomes. *Proc. Natl. Acad. Sci.* 102, 10936–10941. doi:10.1073/pnas.0501321102.
- Wisler, G. C., and Duffus, J. E. (2000). A century of plant virus management in the Salinas Valley of California, “East of Eden.” *Virus Res.* 71, 161–169. doi:10.1016/S0168-1702(00)00196-9.
- Wisler, G. C., Liu, H. Y., and Duffus, J. E. (1994). Beet necrotic yellow vein virus and its relationship to eight sugar beet furo-like viruses from the United States. *Plant Dis. USA* 78, 995–1001. doi:10.1094/PD-78-0995.
- Wormit, A., and Usadel, B. (2018). The Multifaceted Role of Pectin Methylesterase Inhibitors (PMEIs). *Int. J. Mol. Sci.* 19. doi:10.3390/ijms19102878.
- Woronin (1877). “*Plasmodiophora brassicae*, Urheber der Kohlpflanzen-Hernie.” in *Jahrbücher für Wissenschaftliche Botanik*, 548–574.
- Wu, H.-C., Bulgakov, V. P., and Jinn, T.-L. (2018). Pectin Methylesterases: Cell Wall Remodeling Proteins Are Required for Plant Response to Heat Stress. *Front. Plant Sci.* 9. doi:10.3389/fpls.2018.01612.
- Wu, W.-Q., Fan, H.-Y., Jiang, N., Wang, Y., Zhang, Z.-Y., Zhang, Y.-L., et al. (2014). Infection of Beet necrotic yellow vein virus with RNA4-encoded P31 specifically up-regulates pathogenesis-related protein 10 in *Nicotiana benthamiana*. *Viol. J.* 11. doi:10.1186/1743-422X-11-118.
- Xu, L., Dong, Z., Fang, L., Luo, Y., Wei, Z., Guo, H., et al. (2019). OrthoVenn2: a web server for whole-genome comparison and annotation of orthologous clusters across multiple species. *Nucleic Acids Res.* 47, W52–W58. doi:10.1093/nar/gkz333.
- Xu, Q., Wang, J., Zhao, J., Xu, J., Sun, S., Zhang, H., et al. (2020). A polysaccharide deacetylase from *Puccinia striiformis* f. sp. *tritici* is an important pathogenicity gene that suppresses plant immunity. *Plant Biotechnol. J.* 18, 1830–1842. doi:10.1111/pbi.13345.
- Xu, Y., Hu, L., Li, L., Zhang, Y., Sun, B., Meng, X., et al. (2018). Ribotypes of *Polymyxa graminis* in Wheat Samples Infected with Soilborne Wheat Viruses in China. *Plant Dis.* 102, 948–954. doi:10.1094/PDIS-09-17-1394-RE.
- Xue, S., Cao, T., Howard, R. J., Hwang, S. F., and Strelkov, S. E. (2008). Isolation and Variation in Virulence of Single-Spore Isolates of *Plasmodiophora brassicae* from Canada. *Plant Dis.* 92, 456–462. doi:10.1094/PDIS-92-3-0456.
- Ye, J., Fang, L., Zheng, H., Zhang, Y., Chen, J., Zhang, Z., et al. (2006). WEGO: a web tool for plotting GO annotations. *Nucleic Acids Res.* 34, W293-297. doi:10.1093/nar/gkl031.
- Ye, J., Zhang, Y., Cui, H., Liu, J., Wu, Y., Cheng, Y., et al. (2018). WEGO 2.0: a web tool for analyzing and plotting GO annotations, 2018 update. *Nucleic Acids Res.* 46, W71–W75. doi:10.1093/nar/gky400.
- Yin, C., Ramachandran, S. R., Zhai, Y., Bu, C., Pappu, H. R., and Hulbert, S. H. (2019). A novel fungal effector from *Puccinia graminis* suppressing RNA silencing and plant defense responses. *New Phytol.* 222, 1561–1572. doi:10.1111/nph.15676.
- Yoshida, K., Saunders, D. G. O., Mitsuoka, C., Natsume, S., Kosugi, S., Saitoh, H., et al. (2016). Host specialization of the blast fungus *Magnaporthe oryzae* is associated

- with dynamic gain and loss of genes linked to transposable elements. *BMC Genomics* 17, 370. doi:10.1186/s12864-016-2690-6.
- Yu, F., Wang, S., Zhang, W., Tang, J., Wang, H., Yu, L., et al. (2019). Genome-wide identification of genes encoding putative secreted E3 ubiquitin ligases and functional characterization of PbRING1 in the biotrophic protist *Plasmodiophora brassicae*. *Curr. Genet.* 65, 1355–1365. doi:10.1007/s00294-019-00989-5.
- Zhang, H., Yohe, T., Huang, L., Entwistle, S., Wu, P., Yang, Z., et al. (2018). dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Res.* 46, W95–W101. doi:10.1093/nar/gky418.
- Zhang, L., Wang, Z., Wang, X., Li, D., Han, C., Zhai, Y., et al. (2005). Two virus-encoded RNA silencing suppressors, P14 of Beet necrotic yellow vein virus and S6 of Rice black streak dwarf virus. *Chin. Sci. Bull.* 50, 305–310. doi:10.1360/03wb0058.
- Zhang, X., Liu, Y., Fang, Z., Li, Z., Yang, L., Zhuang, M., et al. (2016). Comparative Transcriptome Analysis between Broccoli (*Brassica oleracea* var. *italica*) and Wild Cabbage (*Brassica macrocarpa* Guss.) in Response to *Plasmodiophora brassicae* during Different Infection Stages. *Front. Plant Sci.* 7. doi:10.3389/fpls.2016.01929.
- Zhao, Y., Bi, K., Gao, Z., Chen, T., Liu, H., Xie, J., et al. (2017). Transcriptome Analysis of *Arabidopsis thaliana* in Response to *Plasmodiophora brassicae* during Early Infection. *Front. Microbiol.* 8. doi:10.3389/fmicb.2017.00673.
- Zhou, F., Emonet, A., Dénervaud Tendon, V., Marhavy, P., Wu, D., Lahaye, T., et al. (2020). Co-incidence of Damage and Microbial Patterns Controls Localized Immune Responses in Roots. *Cell* 180, 440–453.e18. doi:10.1016/j.cell.2020.01.013.
- Zhou, J.-M., and Zhang, Y. (2020). Plant Immunity: Danger Perception and Signaling. *Cell* 181, 978–989. doi:10.1016/j.cell.2020.04.028.
- Zhou, M., Zhang, K., Sun, Z., Yan, M., Chen, C., Zhang, X., et al. (2017). LNK1 and LNK2 Corepressors Interact with the MYB3 Transcription Factor in Phenylpropanoid Biosynthesis. *Plant Physiol.* 174, 1348–1358. doi:10.1104/pp.17.00160.
- Zhu, X., Soliman, A., Islam, M. R., Adam, L. R., and Daayf, F. (2017). *Verticillium dahliae*'s Isochorismatase Hydrolase Is a Virulence Factor That Contributes to Interference with Potato's Salicylate and Jasmonate Defense Signaling. *Front. Plant Sci.* 8. doi:10.3389/fpls.2017.00399.
- Zhuo, N., Jiang, N., Zhang, C., Zhang, Z.-Y., Zhang, G.-Z., Han, C.-G., et al. (2015). Genetic diversity and population structure of beet necrotic yellow vein virus in China. *Virus Res.* 205, 54–62. doi:10.1016/j.virusres.2015.05.009.
- Ziegler, A., Fomitcheva, V., Zakri, A. M., and Kastirr, U. (2016). Occurrence of *Polymyxa graminis* Ribotypes in Germany and Their Association with Different Host Plants and Viruses. *Cereal Res. Commun.* 44, 251–262. doi:10.1556/0806.43.2015.051.
- Ziegler-Graff, V., Bouzoubaa, S., Jupin, I., Guilley, H., Jonard, G., and Richards, K. (1988). Biologically Active Transcripts of Beet Necrotic Yellow Vein Virus RNA-3 and RNA-4. *J. Gen. Virol.* 69, 2347–2357. doi:10.1099/0022-1317-69-9-2347.
- Ziegler-Graff, V., Richards, K., Guilley, H., Jonard, G., and Putz, C. (1985). Cell-free Translation of Beet Necrotic Yellow Vein Virus: Readthrough of the Coat Protein Cistron. *J. Gen. Virol.* 66, 2079–2087. doi:10.1099/0022-1317-66-10-2079.

APPENDIX

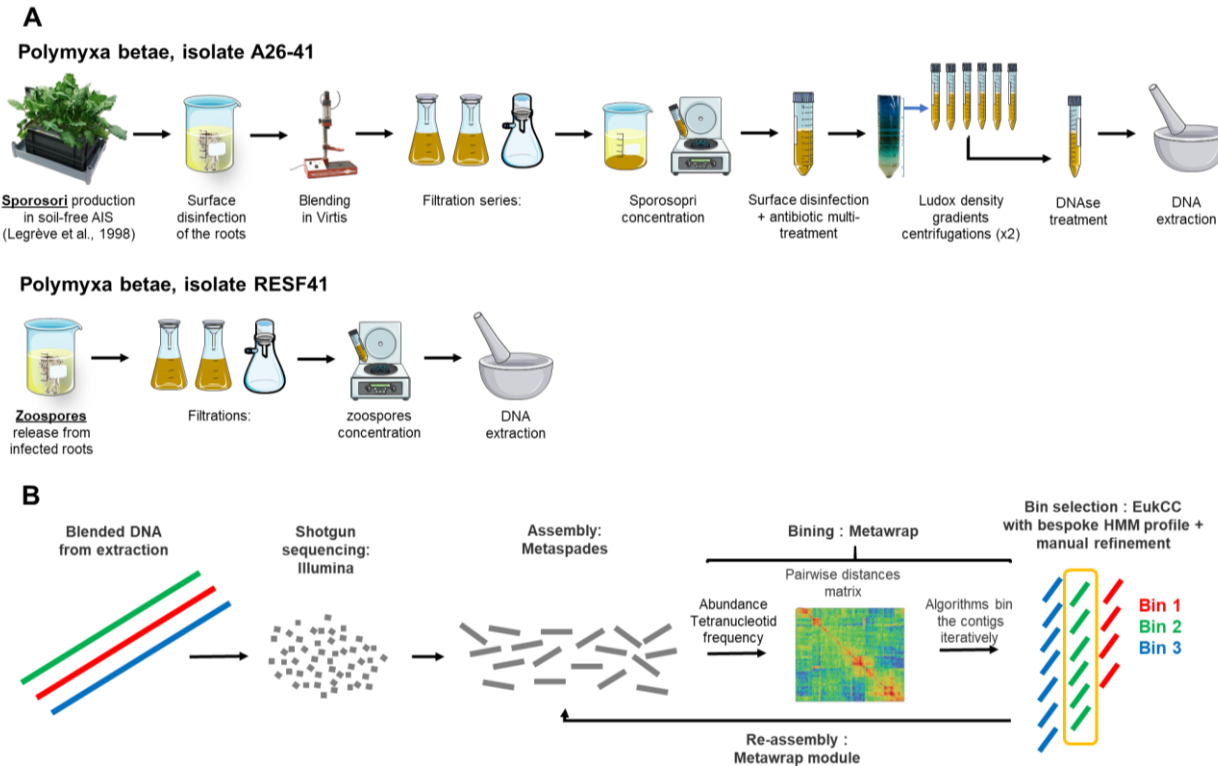
1. Methodology adopted for document search in Chapter II

The Scopus search engine was used (29th April 2021) to find documents with the following key search : (TITLE-ABS-KEY ("*Polymyxa betae*") OR TITLE-ABS-KEY ("*Polymyxa graminis*") OR TITLE-ABS-KEY ("*Beet necrotic yellow vein virus*") OR TITLE-ABS-KEY (bnyvv) AND NOT TITLE-ABS-KEY ("*acillus polymyxa")). All primary documents (617) and secondary documents (415) were retrieved, excluding patents. Double entries were sorted by the Zotero software, resulting in 957 documents. From these, we kept only main studies which bring valuable information to current knowledge on the interactions between the *Polymyxa spp.* and transmitted viruses, *Polymyxa spp.* and host plants as well as BNYVV and host plants. Studies focusing on the characterization of a single organism without considering interactions with at least one of the other rhizomania protagonists were selected on a case-by-case basis for general considerations on rhizomania and BNYVV. Field surveys regarding the prevalence of BNYVV or *Polymyxa betae*. alone without added value were not included. Studies aiming to find molecular markers of resistance, to screen plant accessions for resistance or describing the obtention of resistant plant accessions without discussing the mechanisms underlying these resistances were mostly not considered. Pure methodology descriptions (e.g. for the detection of BNYVV and *Polymyxa sp.*) were also discarded. The initial search was completed with further references obtained from citations within references and major reviews published in the field.

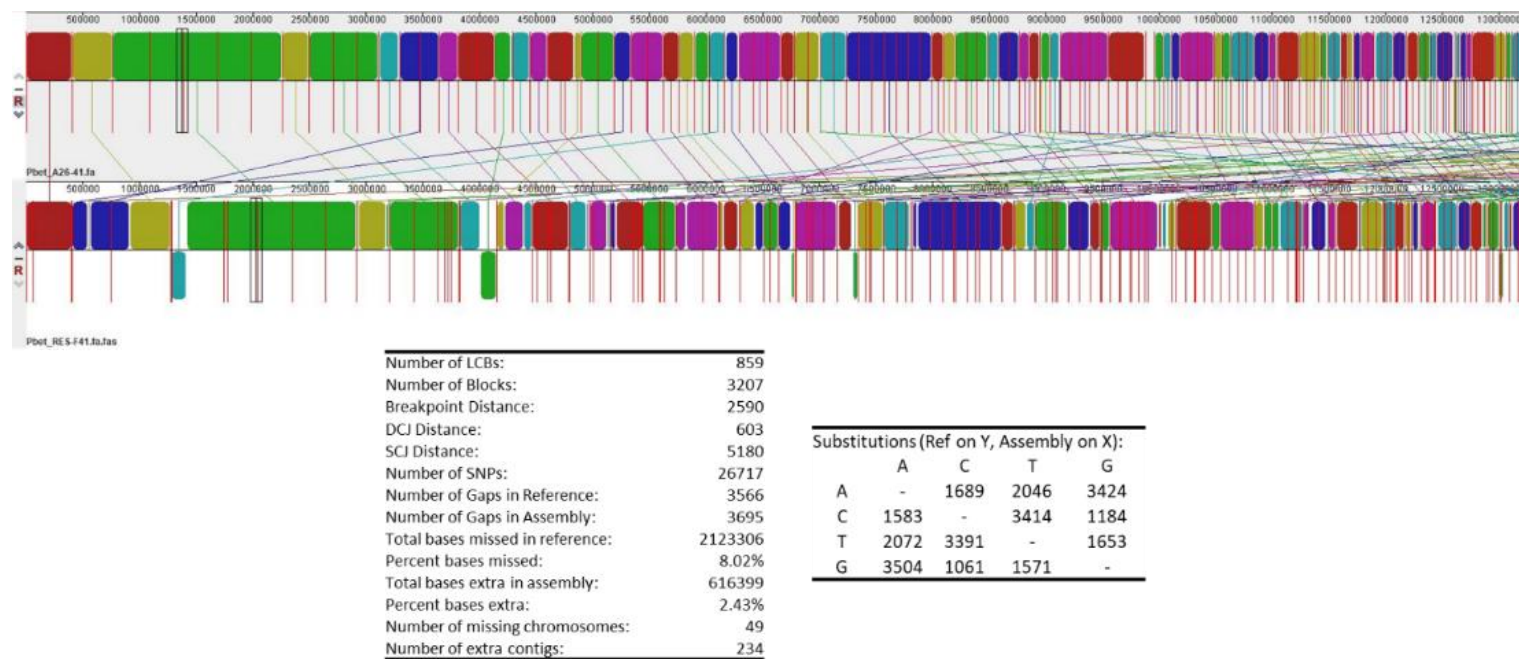
2. Supplementary information – Chapter IV

a. Supplementary figures

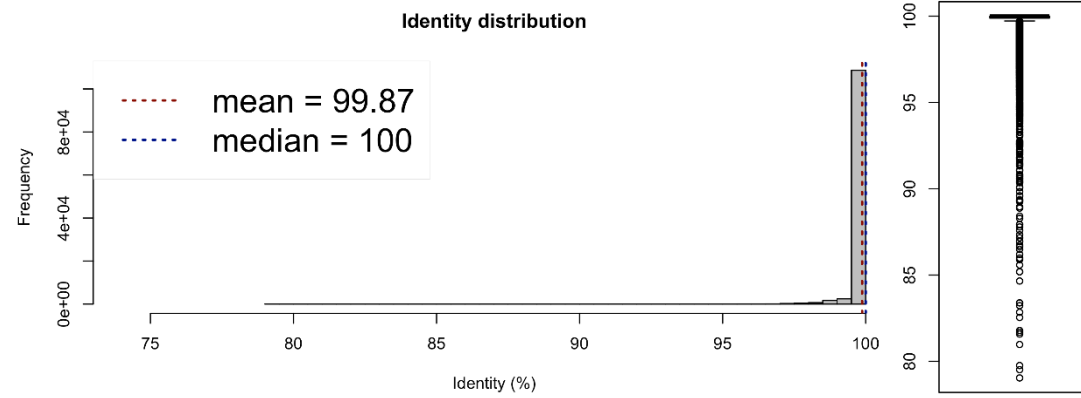
The first figure is presented on next page.



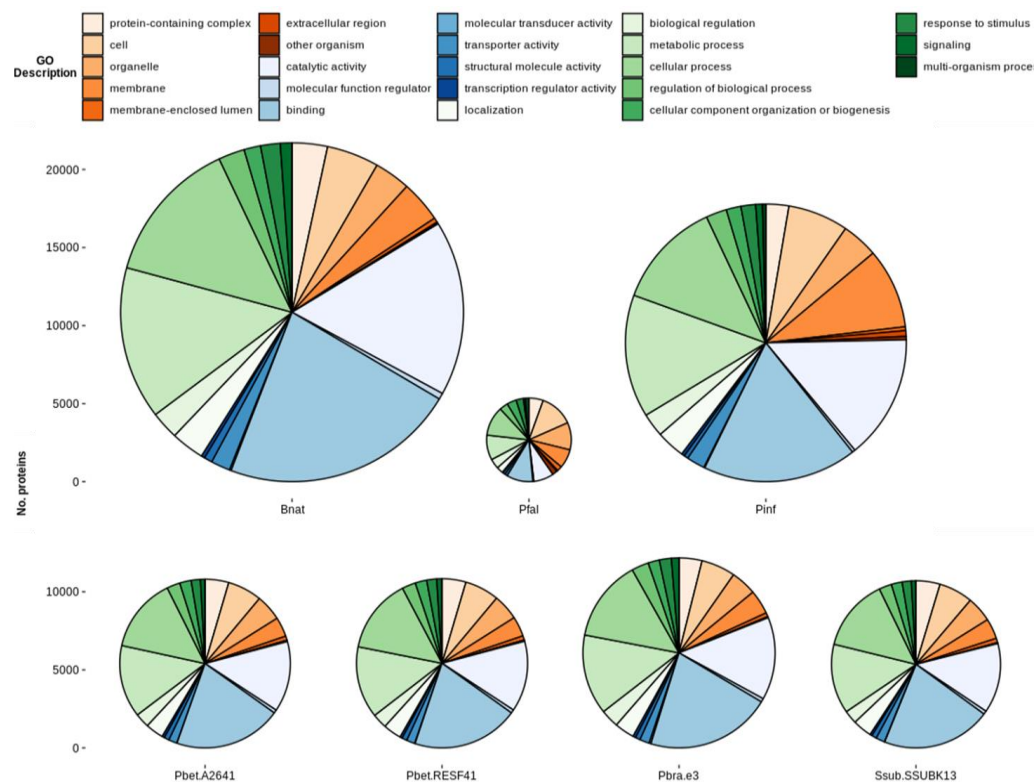
Supplementary Figure 1. Schematic representation of the method applied to sequence and assemble two *P. betae* genomes from different starting materials. A : Purification steps performed to collect sporosori from isolate A26-41 (up) and zoospores from isolate RESF41 (down). B : Representation of the method used to select *P. betae* contigs from blended DNA extract (contaminated with bacteria).



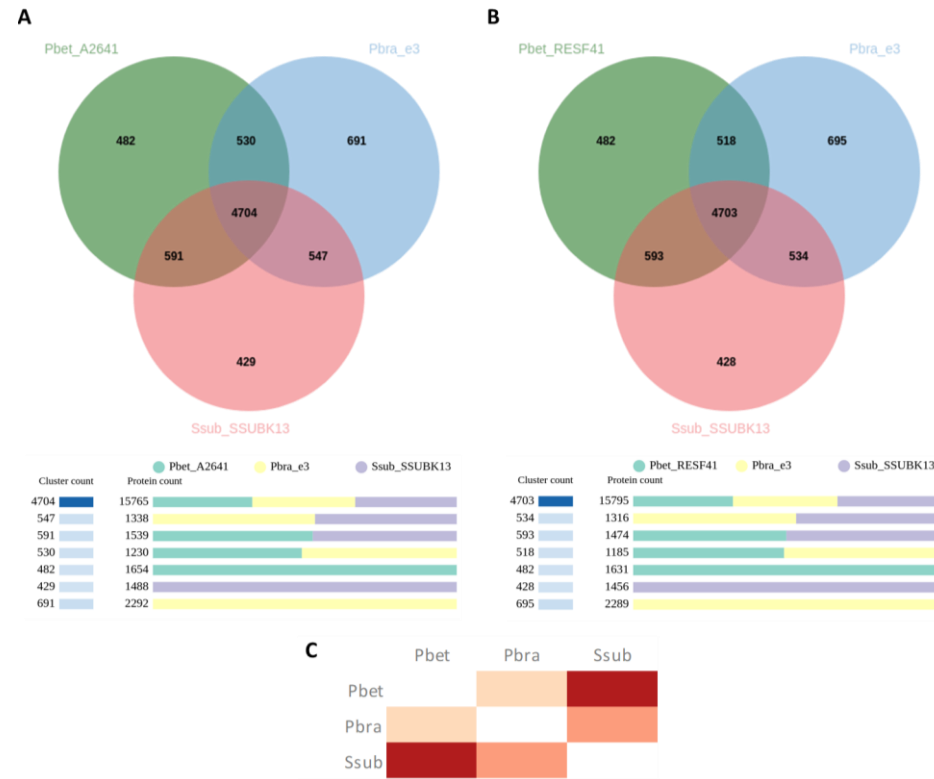
Supplementary Figure 2. ProgressiveMauve alignments of *P. betae* genomes with *P. betae* A26-41 set as reference (at the top) and RES F41 (at the bottom) reordered according to the reference contigs order. Locally Collinear Blocks (LCBs) with a minimum weight of 3064.corresponding to the first 50% of A26-41 genome are shown.



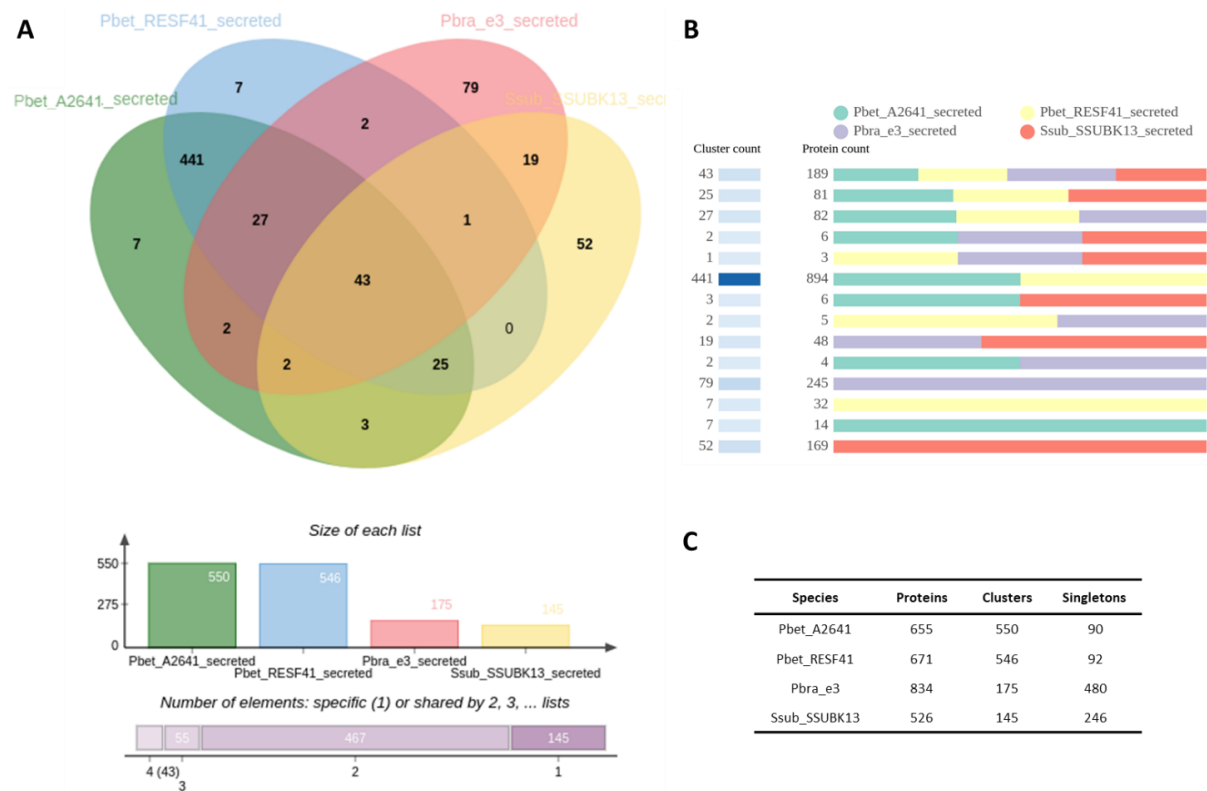
Supplementary Figure 3. Identity distribution of aligned fragments from *P. betae* A26-41 and RES F41 draft genomes. The figure was generated with ani.rb (Rodriguez-R and Konstantinidis, 2016).



Supplementary Figure 4. Comparison of selected general GO functional categories (level 2) predicted for *P. betae* (Pbet) drafts genomes A26-41 and RES F41, *P. brassicae* (Pbra) e3 and *S. subterranea* (Ssub) SSUBK13 with InterProScan. These are compared to GO from *Bigelowiella natans* (Bnat), *Plasmodium falciparum* (Pfal) and *Phytophthora infestans* (Pinf) retrieved with Biomart.

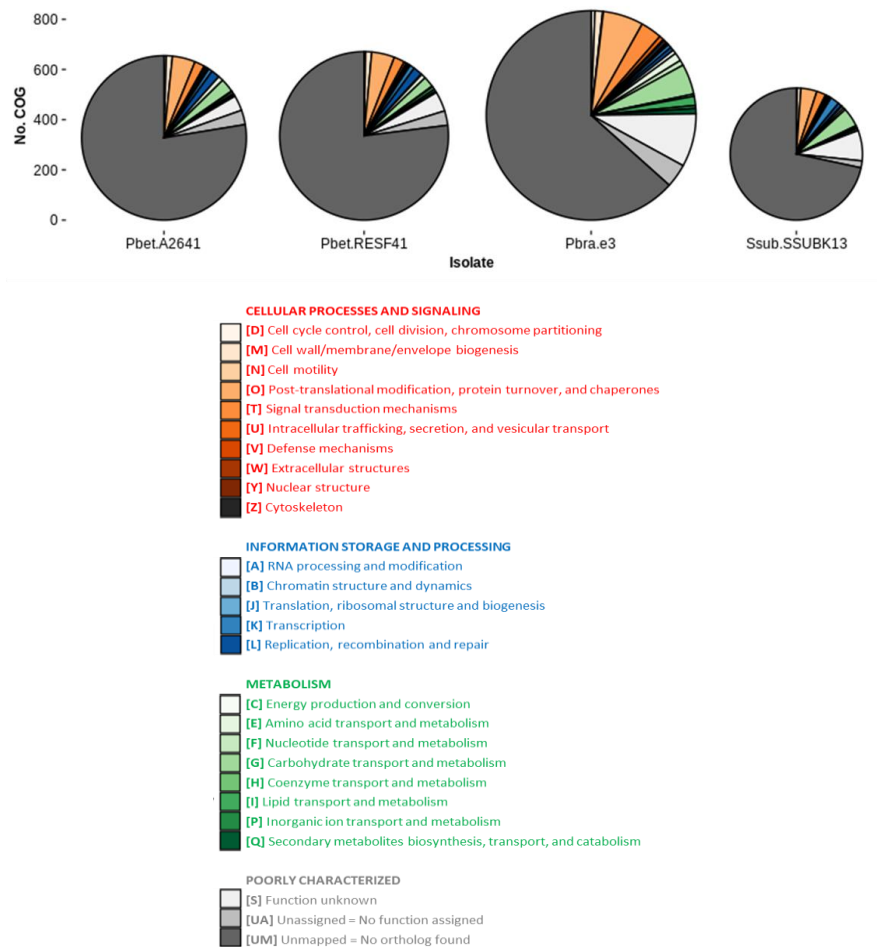


Supplementary Figure 5. Protein clustering from *P. betae* (Pbet) drafts genomes A26-41 and RES F41 *P. brassicae* (Pbra) e3 and *S. subterranea* (Ssub) SSUBK13 with OrthoVenn2. A: Venn diagram of ortholog clusters and cluster counts with the A26-41 isolate; B: Venn diagram of ortholog clusters and cluster counts with the RES F41 isolate; C: similarity heatmap computed with A26-41 data.

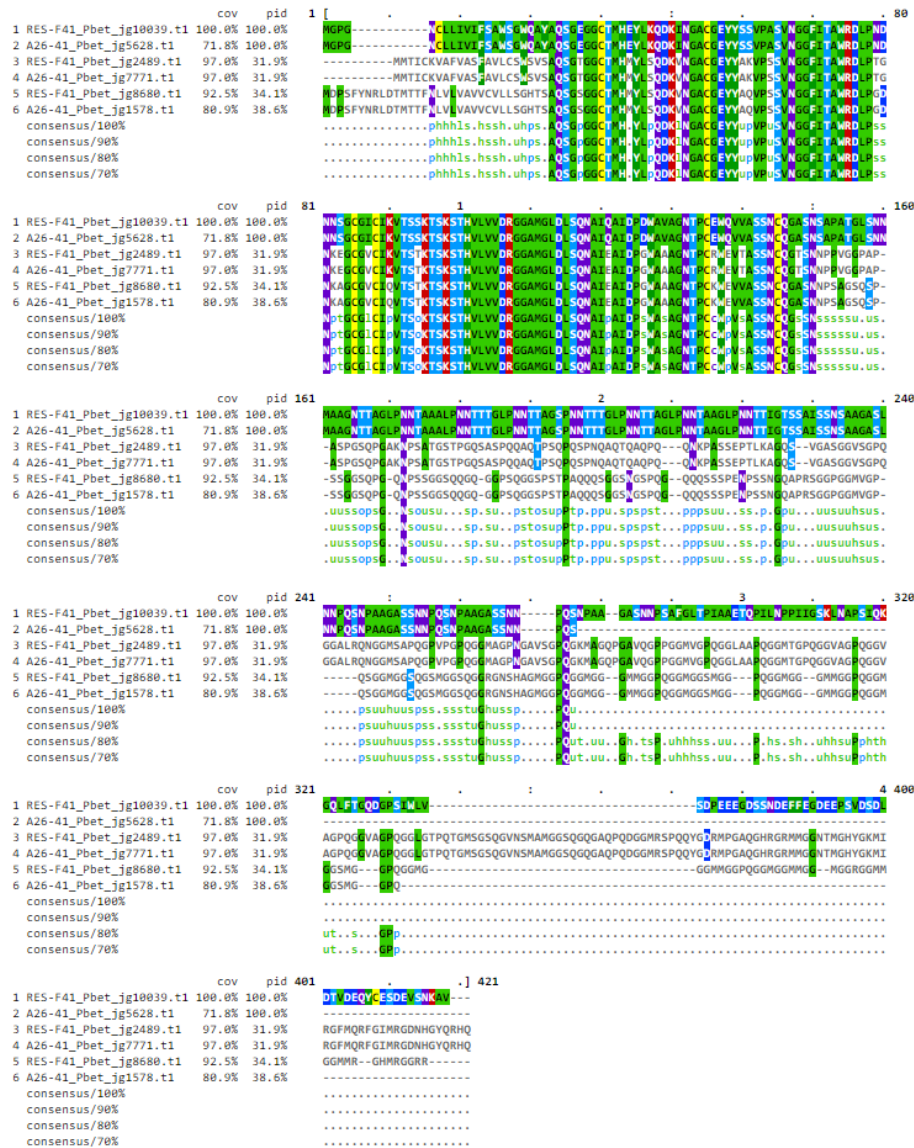


Supplementary Figure 6. Secreted protein clustering from *P. betae* (Pbet) drafts genomes A26-41 and RES F41 *P. brassicae* (Pbra) e3 and *S. subterranea* (Ssub) SSUBK13 with OrthoVenn2. A: Venn diagram of ortholog clusters. B: cluster and protein counts; C: summary table, comprising singletons.

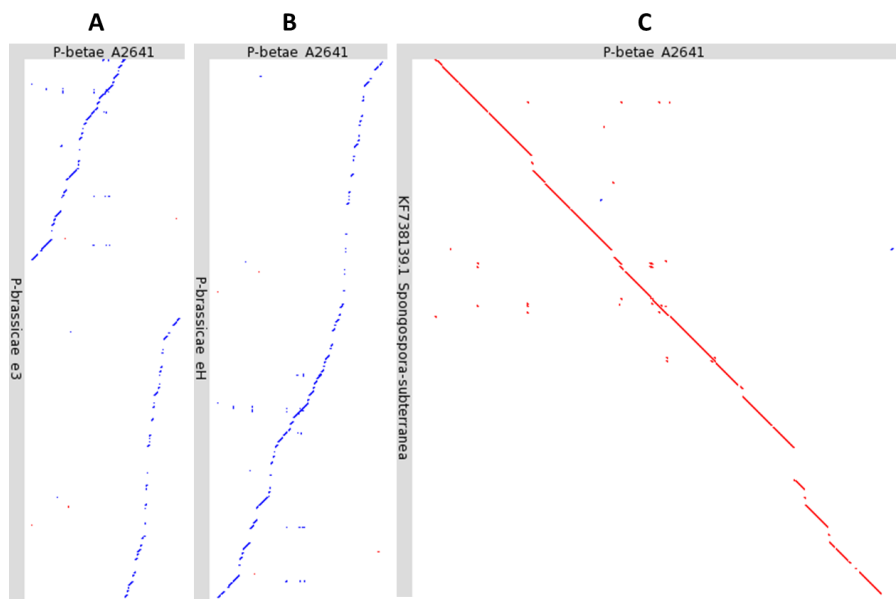
Supplementary information – CHAPTER IV



Supplementary Figure 7. Comparison of COG categories from secreted proteins predicted for *P. betae* (Pbet) drafts genomes A26-41 and RES F41, *P. brassicae* (Pbra) e3 and *S. subterranea* (Ssub) SSUBK13. The diameter of pie charts is proportional to the number of proteins represented



Supplementary Figure 8. Multiple alignment of proteins annotated as "defense response" related ontologies. Alignments were performed with MUSCLE and viewed with Mview.



Supplementary Figure 9. Mitochondrial genomes dot-plot comparisons performed with LAST (Kielbasa et al., 2011). A. *P. betae* A26-41 vs *P. brassicae* e3 (Stjelja et al., 2019). B. *P. betae* A26-41 vs *P. brassicae* eH (Daval et al., 2019). C. *P. betae* A26-41 vs *S. subterranea* (Gutiérrez et al., 2014).

b. Supplementary Tables

Supplementary Table 1. Genome completeness and contamination assessments of putative *Polymyxa* Metawrap bins from the A26-41 and RES F41 *P. betae* (Pbet) isolates. Evaluation of completeness and contamination was performed with EukCC and a bespoke Rhizaria reference profile HMMs set.

Source data	Bin No.	Completeness (%)	Contamination (%)
Pbet A26-41	C-bin.22	92.92	2.99
	M-bin.4	92.30	2.99
Pbet RES F41	C-bin.12	92.30	2.99
	M-bin.13	52.04	1.42

Supplementary Table 2. Taxonomic assignment of bmtaWRAP bins considered to be medium/high quality in Metaspades assembly from both P. betae datasets. These bins were assigned a quality score (QS) calculated as “completeness” - (5* “contamination”). Bins with a QS > 50 are considered to be medium/high quality.

Isolate Bin	Completeness (%)	Contamination (%)	Quality score	Family classification	Genus classification	Species classification
A26-41.bin.5	99.71	0.968	94.87	Rhizobiaceae	FKL33	-
A26-41.bin.7	91.59	0.420	89.49	Oligoflexaceae	Oligoflexus	-
A26-41.bin.1	90.56	1.161	84.755	Devosiaceae	Devosia	-
A26-41.bin.4	80.26	1.010	75.21	Microbacteriaceae	Schumannella	-
A26-41.bin.6	76.71	1.801	67.705	Dermatophilaceae	Phycococcus	-
A26-41.bin.3	64.29	1.481	56.885	Microbacteriaceae	Microbacterium	-
A26-41.bin.2	73.71	4.273	52.345	Microtrichaceae	IMCC26207	-
RES F41.bin.6	100.0	0.0	100	Crocinitomicaceae	Fluviicola	-
RES F41.bin.13	99.50	0.492	97.04	Chitinophagaceae	Sediminibacterium	-
RES F41.bin.34	99.86	0.602	96.85	Burkholderiaceae	Duganella	-
RES F41.bin.42	99.35	0.750	95.6	Sphingomonadaceae	Sphingomonas	-
RES F41.bin.36	95.28	0.08	94.88	Burkholderiaceae	Herminiimonas	-
RES F41.bin.17	97.42	0.643	94.205	Rhodobacteraceae	Tabrizicola	-
RES F41.bin.37	96.53	0.546	93.8	Pseudomonadaceae	Pseudomonas_A	Pseudomonas_A sp000765155
RES F41.bin.16	98.21	0.967	93.375	Bdellovibrionaceae	Ga0074137	-
RES F41.bin.19	97.05	0.750	93.3	Pseudomonadaceae	Pseudomonas_E	-
RES F41.bin.29	98.80	1.177	92.915	Flavobacteriaceae	Flavobacterium	-
RES F41.bin.2	99.52	1.428	92.38	Sphingobacteriaceae	-	-
RES F41.bin.25	99.90	1.543	92.185	Burkholderiaceae	Undibacterium	-
RES F41.bin.31	93.76	0.345	92.035	Rhodocyclaceae	Zoogloea	-
RES F41.bin.8	93.88	1.011	88.825	Rhizobiaceae	-	-
RES F41.bin.43	97.13	1.815	88.055	Hyphomonadaceae	UBA7672	-
RES F41.bin.28	97.08	1.811	88.025	Burkholderiaceae	Janthinobacterium	Janthinobacterium lividum_C
RES F41.bin.12	96.00	1.642	87.79	Chitinophagaceae	Flavipsychrobacter	-
RES F41.bin.27	94.94	1.488	87.5	Spirosomaceae	Emticicia	-
RES F41.bin.1	94.25	1.351	87.495	SM23-39	2-02-FULL-45-22_A	-
RES F41.bin.38	96.22	1.844	87	Devosiaceae	Devosia	-
RES F41.bin.22	96.87	2.023	86.755	Flavobacteriaceae	Flavobacterium	-
RES F41.bin.24	99.47	2.550	86.72	Sphingomonadaceae	Sphingopyxis	Sphingopyxis sp001427085
RES F41.bin.30	93.73	1.435	86.555	Sphingobacteriaceae	Pedobacter	Pedobacter ginsengisoli
RES F41.bin.35	95.46	1.978	85.57	Burkholderiaceae	Methylibium	-

Supplementary information – CHAPTER IV

RES F41.bin.15	88.65	0.630	85.5	Oligoflexaceae	Oligoflexus	-
RES F41.bin.32	84.94	0.0	84.94	Chitinophagaceae	Sediminibacterium	-
RES F41.bin.20	89.99	1.459	82.695	Rhizobiaceae	Rhizobium_A	Rhizobium_A sp001427345
RES F41.bin.10	89.39	1.515	81.815	Burkholderiaceae	Acidovorax_D	Acidovorax_D sp001633105
RES F41.bin.7	88.95	1.620	80.85	Methylophilaceae	Methylophilus	-
RES F41.bin.21	86.39	1.571	78.535	Sphingomonadaceae	Novosphingobium	-
RES F41.bin.39	83.45	1.172	77.59	Burkholderiaceae	Pseudorhododerax	Pseudorhododerax soli
RES F41.bin.14	78.68	1.092	73.22	UBA7675	UBA7675	-
RES F41.bin.4	93.46	4.709	69.915	Burkholderiaceae	Herminiimonas	Herminiimonas sp003852535
RES F41.bin.9	91.48	4.818	67.39	Burkholderiaceae	Ramlibacter	-
RES F41.bin.11	73.90	1.439	66.705	Flavobacteriaceae	Flavobacterium	-
RES F41.bin.44	77.12	2.188	66.18	Micavibrionaceae	UBA6139	-
RES F41.bin.18	60.67	0.611	57.615	Polyangiaceae	NIC37A-2	-
RES F41.bin.23	59.93	0.470	57.58	Burkholderiaceae	Acidovorax_D	Acidovorax_D sp001411535
RES F41.bin.3	56.89	0.0	56.89	Rhizobiaceae	Agrobacterium	Agrobacterium sp900013535
RES F41.bin.41	59.78	1.801	50.775	Bdellovibrionaceae	-	-

Supplementary Table 3. *Polymyxa betae* drafts genomes statistics for the isolates A26-41 and RES F41

	<i>P. betae</i> A2641	<i>P. betae</i> RES F41
number of contigs	1040	1476
total contigs length	26458542	25372353
contigs > 10K nt	597	496
contigs > 100K nt	49	43
N50	48520	56926
L50	124	99
N80	16377	13961
L80	402	373
Genes No.	10224	10222
Genes %	63.3	63.9
Mean gene length nt	1637	1585
mRNAs No.	10799	10808
CDS %	52.4	54.3
Mean CDS length	1284	1274
mean intron number	~5	~5

Supplementary Table 4. Enriched GO terms in *P. betae* A26-41, *P. brassicae* e3 and *S. subterranea* SSUBK proteomes. GO terms were derived from InterProScan annotation of full proteomes. GO terms were compared with a background encompassing the proteomes of the three species. GO mapped on a minimum of five genes of one species and with a P-value < 0,05 are represented. The column “Ont.” refer to the three main aspects of gene ontology classification : P = biological process, F = molecular function, C = cellular component. “P-val” are the Hochberg adjusted P-values. The yellow-red scale colors represent the enrichment significance of each term in each of the secretomes 1 (Pbra e3); 2 (Ssub SSUBK13); 3 (Pbet A26-41) and 4 (Pbet RES F41).

GO Information							Pbra e3		Ssub SSUBK13		Pbet A26-41		Pbet RES F41	
GO Term	Ont.	Description	1	2	3	4	P-val	No.	P-val	No.	P-val	No.	P-val	No.
GO:0007186	P	G-protein coupled receptor signaling pathway	Yellow				7.8e-11	82	---	---	---	---	---	---
GO:0007165	P	signal transduction	Yellow				0.011	208	---	---	---	---	---	---
GO:0044700	P	single organism signaling	Yellow				0.011	208	---	---	---	---	---	---
GO:0007154	P	cell communication	Yellow				0.011	208	---	---	---	---	---	---
GO:0023052	P	signaling	Yellow				0.011	208	---	---	---	---	---	---
GO:0099600	F	transmembrane receptor activity	Red				1.1e-14	55	---	---	---	---	---	---
GO:0004888	F	transmembrane signaling receptor activity	Red				1.3e-14	54	---	---	---	---	---	---
GO:0004930	F	G-protein coupled receptor activity	Red				2e-14	53	---	---	---	---	---	---
GO:0060089	F	molecular transducer activity	Red				2.1e-13	62	---	---	---	---	---	---
GO:0004872	F	receptor activity	Red				2.1e-13	62	---	---	---	---	---	---
GO:0038023	F	signaling receptor activity	Red				3.9e-13	61	---	---	---	---	---	---
GO:0004871	F	signal transducer activity	Red				7e-11	68	---	---	---	---	---	---
GO:0004965	F	G-protein coupled GABA receptor activity	Red				5.3e-09	29	---	---	---	---	---	---
GO:0016917	F	GABA receptor activity	Red				5.3e-09	29	---	---	---	---	---	---
GO:0003676	F	nucleic acid binding			Yellow	Yellow	---	---	---	---	2.7e-05	824	0.005	783
GO:1901363	F	heterocyclic compound binding			Yellow	Yellow	---	---	---	---	0.0029	1529	0.043	1489
GO:0097159	F	organic cyclic compound binding			Yellow	Yellow	---	---	---	---	0.0029	1529	0.043	1489

Supplementary Table 5. Enriched GO terms in overlapping ortholog clusters of *P. betae* A26-41, *P. brassicae* e3 and *S. subterranea* SSUBK. GO terms were derived from InterProScan annotation of proteins in clusters containing proteins of at least two species as determined with Orthovenn2. GO terms were compared with a background encompassing the proteomes of the three species. GO mapped on a minimum of five genes of one species and with a P-value < 0,05 are represented.

GO_acc	Term type	Term	Queryitem	Querytotal	Refitem	Reftotal	P-value	FDR
Pbet $\cap$ Pbra								
GO:0044743	P	intracellular protein transmembrane import	9	647	23	15395	2.1e-07	4.3e-05
GO:0065002	P	intracellular protein transmembrane transport	9	647	26	15395	7.0e-07	4.9e-05
GO:0071806	P	protein transmembrane transport	9	647	26	15395	7.0e-07	4.9e-05
GO:0015074	P	DNA integration	16	647	97	15395	3.3e-06	0.00014
GO:1990542	P	mitochondrial transmembrane transport	7	647	17	15395	3.2e-06	0.00014
GO:0046146	P	tetrahydrobiopterin metabolic process	5	647	9	15395	1.5e-05	0.00043
GO:0006729	P	tetrahydrobiopterin biosynthetic process	5	647	9	15395	1.5e-05	0.00043
GO:0072655	P	establishment of protein localization to mitochondrion	7	647	24	15395	4.4e-05	0.0009
GO:0070585	P	protein localization to mitochondrion	7	647	24	15395	4.4e-05	0.0009
GO:0006626	P	protein targeting to mitochondrion	7	647	24	15395	4.4e-05	0.0009
GO:1902582	P	single-organism intracellular transport	15	647	107	15395	4.8e-05	0.0009
GO:0017038	P	protein import	9	647	43	15395	6.6e-05	0.0011
GO:1902580	P	single-organism cellular localization	15	647	114	15395	0.0001	0.0016
GO:0006839	P	mitochondrial transport	7	647	33	15395	0.00039	0.0057
GO:0032446	P	protein modification by small protein conjugation	11	647	87	15395	0.0011	0.015
GO:0046856	P	phosphatidylinositol dephosphorylation	5	647	21	15395	0.0015	0.019
GO:0046839	P	phospholipid dephosphorylation	5	647	21	15395	0.0015	0.019
GO:0007186	P	G-protein coupled receptor signaling pathway	12	647	113	15395	0.0031	0.035
GO:0008124	F	4-alpha-hydroxytetrahydrobiopterin dehydratase activity	5	647	9	15395	1.5e-05	0.0021
GO:0036442	F	hydrogen-exporting ATPase activity	7	647	29	15395	0.00016	0.0048
GO:0003700	F	transcription factor activity, sequence-specific DNA binding	16	647	131	15395	0.00015	0.0048
GO:0001071	F	nucleic acid binding transcription factor activity	16	647	131	15395	0.00015	0.0048
GO:0015662	F	ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism	7	647	29	15395	0.00016	0.0048
GO:0008553	F	hydrogen-exporting ATPase activity, phosphorylative mechanism	5	647	17	15395	0.00054	0.01
GO:0042626	F	ATPase activity, coupled to transmembrane movement of substances	16	647	149	15395	0.00063	0.01

GO:0043492	F	ATPase activity, coupled to movement of substances	16	647	149	15395	0.00063	0.01
GO:0016820	F	hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	16	647	149	15395	0.00063	0.01
GO:0022890	F	inorganic cation transmembrane transporter activity	23	647	261	15395	0.00086	0.011
GO:0004553	F	hydrolase activity, hydrolyzing O-glycosyl compounds	13	647	111	15395	0.00087	0.011
GO:0004842	F	ubiquitin-protein transferase activity	16	647	159	15395	0.0013	0.012
GO:0022853	F	active ion transmembrane transporter activity	9	647	63	15395	0.0013	0.012
GO:0019829	F	cation-transporting ATPase activity	9	647	63	15395	0.0013	0.012
GO:0042625	F	ATPase coupled ion transmembrane transporter activity	9	647	63	15395	0.0013	0.012
GO:0015399	F	primary active transmembrane transporter activity	16	647	163	15395	0.0016	0.013
GO:0015405	F	P-P-bond-hydrolysis-driven transmembrane transporter activity	16	647	163	15395	0.0016	0.013
GO:0042623	F	ATPase activity, coupled	18	647	192	15395	0.0015	0.013
GO:0019787	F	ubiquitin-like protein transferase activity	16	647	165	15395	0.0018	0.014
GO:0016798	F	hydrolase activity, acting on glycosyl bonds	13	647	126	15395	0.0027	0.02
GO:0051082	F	unfolded protein binding	9	647	80	15395	0.0066	0.046
GO:0015095	F	magnesium ion transmembrane transporter activity	6	647	41	15395	0.0071	0.047
Pbet ∩ Ssub								
GO:0015074	P	DNA integration	26	606	97	15395	8.3e-15	1.7e-12
GO:0046394	P	carboxylic acid biosynthetic process	20	606	98	15395	1.8e-09	1.8e-07
GO:0030317	P	sperm motility	6	606	6	15395	3.8e-09	2.6e-07
GO:0006952	P	defense response	6	606	7	15395	2.6e-08	7.7e-07
GO:0009085	P	lysine biosynthetic process	6	606	7	15395	2.6e-08	7.7e-07
GO:0008652	P	cellular amino acid biosynthetic process	16	606	72	15395	2.0e-08	7.7e-07
GO:0006553	P	lysine metabolic process	6	606	7	15395	2.6e-08	7.7e-07
GO:0016053	P	organic acid biosynthetic process	20	606	116	15395	3.6e-08	9.3e-07
GO:0009067	P	aspartate family amino acid biosynthetic process	8	606	21	15395	7.8e-07	1.8e-05
GO:0044283	P	small molecule biosynthetic process	21	606	157	15395	1.4e-06	2.8e-05
GO:1901607	P	alpha-amino acid biosynthetic process	13	606	71	15395	4.1e-06	7.6e-05
GO:0009066	P	aspartate family amino acid metabolic process	8	606	26	15395	5.0e-06	8.6e-05
GO:0048870	P	cell motility	6	606	20	15395	9.2e-05	0.0013
GO:0051674	P	localization of cell	6	606	20	15395	9.2e-05	0.0013
GO:0040011	P	locomotion	6	606	20	15395	9.2e-05	0.0013
GO:1901605	P	alpha-amino acid metabolic process	13	606	119	15395	0.00092	0.012
GO:0005975	P	carbohydrate metabolic process	29	606	408	15395	0.0022	0.026
GO:0009084	P	glutamine family amino acid biosynthetic process	5	606	25	15395	0.0026	0.03
GO:0072657	P	protein localization to membrane	6	606	40	15395	0.0046	0.047
GO:0090150	P	establishment of protein localization to membrane	6	606	40	15395	0.0046	0.047
GO:0008061	F	chitin binding	18	606	108	15395	2.8e-07	9.6e-06
GO:0005227	F	calcium activated cation channel activity	6	606	9	15395	2.9e-07	9.6e-06

Supplementary information – CHAPTER IV

GO:0022839	F	ion gated channel activity	6	606	9	15395	2.9e-07	9.6e-06
GO:0005261	F	cation channel activity	11	606	66	15395	5.4e-05	0.0011
GO:0003676	F	nucleic acid binding	131	606	2262	15395	4.9e-05	0.0011
GO:0042910	F	xenobiotic transporter activity	5	606	13	15395	9.5e-05	0.0016
GO:0022803	F	passive transmembrane transporter activity	16	606	153	15395	0.00042	0.0046
GO:0015267	F	channel activity	16	606	153	15395	0.00042	0.0046
GO:0022836	F	gated channel activity	10	606	68	15395	0.00034	0.0046
GO:0008270	F	zinc ion binding	40	606	575	15395	0.00061	0.006
GO:0046914	F	transition metal ion binding	46	606	719	15395	0.0014	0.013
GO:0005216	F	ion channel activity	14	606	145	15395	0.002	0.015
GO:0022838	F	substrate-specific channel activity	14	606	145	15395	0.002	0.015
GO:0051287	F	NAD binding	7	606	49	15395	0.003	0.021
GO:0046983	F	protein dimerization activity	16	606	187	15395	0.0034	0.023
GO:0046872	F	metal ion binding	76	606	1410	15395	0.0064	0.04
GO:0016620	F	oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor	5	606	32	15395	0.008	0.044
GO:0015297	F	antiporter activity	5	606	32	15395	0.008	0.044
GO:0043169	F	cation binding	76	606	1432	15395	0.0089	0.047
GO:0036128	C	CatSper complex	14	606	14	15395	2.5e-20	1.7e-18
GO:0034704	C	calcium channel complex	14	606	18	15395	6.6e-17	1.5e-15
GO:0005891	C	voltage-gated calcium channel complex	14	606	18	15395	6.6e-17	1.5e-15
GO:0034702	C	ion channel complex	14	606	28	15395	5.9e-13	8.1e-12
GO:0034703	C	cation channel complex	14	606	28	15395	5.9e-13	8.1e-12
GO:1902495	C	transmembrane transporter complex	14	606	38	15395	9.8e-11	1.1e-09
GO:1990351	C	transporter complex	14	606	43	15395	6.5e-10	6.4e-09
GO:0098797	C	plasma membrane protein complex	14	606	46	15395	1.8e-09	1.5e-08
GO:0044459	C	plasma membrane part	14	606	51	15395	8.0e-09	6.00E-08
GO:0005886	C	plasma membrane	14	606	55	15395	2.3e-08	1.6e-07
GO:0071944	C	cell periphery	14	606	89	15395	1.1e-05	6.9e-05
GO:0098796	C	membrane protein complex	26	606	287	15395	0.0001	0.00058
GO:0005681	C	spliceosomal complex	6	606	37	15395	0.0031	0.016
Pbra ∩ Ssub								
GO:0006427	P	histidyl-tRNA aminoacylation	5	700	5	15395	6.2e-20	1.3e-17
GO:0030154	P	cell differentiation	5	700	10	15395	9.4e-10	9.8e-08
GO:0043648	P	dicarboxylic acid metabolic process	6	700	23	15395	9.0e-06	0.00063
GO:0016573	P	histone acetylation	6	700	28	15395	0.00013	0.007
GO:0018205	P	peptidyl-lysine modification	8	700	47	15395	0.00019	0.0079
GO:0006475	P	internal protein amino acid acetylation	6	700	32	15395	0.00063	0.016
GO:0018394	P	peptidyl-lysine acetylation	6	700	32	15395	0.00063	0.016

GO:0018393	P	internal peptidyl-lysine acetylation	6	700	32	15395	0.00063	0.016
GO:0032259	P	methylation	12	700	99	15395	0.00082	0.019
GO:0006473	P	protein acetylation	6	700	35	15395	0.0016	0.033
GO:0004821	F	histidine-tRNA ligase activity	5	700	5	15395	6.2e-20	7.5e-18
GO:0004181	F	metallocarboxypeptidase activity	6	700	10	15395	2.4e-14	1.5e-12
GO:0004402	F	histone acetyltransferase activity	6	700	15	15395	2.7e-09	1.1e-07
GO:0019207	F	kinase regulator activity	7	700	23	15395	5.6e-08	1.7e-06
GO:0061733	F	peptide-lysine-N-acetyltransferase activity	6	700	19	15395	3.7e-07	7.5e-06
GO:0034212	F	peptide N-acetyltransferase activity	6	700	19	15395	3.7e-07	7.5e-06
GO:0010181	F	FMN binding	7	700	29	15395	4.3e-06	7.5e-05
GO:0008238	F	exopeptidase activity	11	700	66	15395	1.1e-05	0.00017
GO:0008235	F	metalloexopeptidase activity	6	700	25	15395	3.0e-05	0.00041
GO:0004180	F	carboxypeptidase activity	6	700	26	15395	5.2e-05	0.00063
GO:0016810	F	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	13	700	114	15395	0.0011	0.012
GO:0008080	F	N-acetyltransferase activity	10	700	80	15395	0.0018	0.018
GO:0016747	F	transferase activity, transferring acyl groups other than amino-acyl groups	19	700	204	15395	0.0022	0.021
GO:0070011	F	peptidase activity, acting on L-amino acid peptides	40	700	542	15395	0.0029	0.025
GO:0016410	F	N-acyltransferase activity	10	700	85	15395	0.0036	0.029
GO:0030234	F	enzyme regulator activity	16	700	171	15395	0.0051	0.038
GO:0016407	F	acetyltransferase activity	10	700	90	15395	0.0066	0.045
GO:0022843	F	voltage-gated cation channel activity	6	700	41	15395	0.0067	0.045
GO:0034066	C	RIC1-RGP1 guanyl-nucleotide exchange factor complex	8	700	8	15395	2.1e-33	7.5e-32
GO:0032045	C	guanyl-nucleotide exchange factor complex	8	700	8	15395	2.1e-33	7.5e-32
GO:0005770	C	late endosome	6	700	14	15395	5.2e-10	1.3e-08
GO:0005768	C	endosome	8	700	36	15395	3.1e-06	4.5e-05
GO:0044440	C	endosomal part	8	700	36	15395	3.1e-06	4.5e-05
GO:0010008	C	endosome membrane	6	700	26	15395	5.2e-05	0.00063
GO:0034702	C	ion channel complex	6	700	28	15395	0.00013	0.0012
GO:0034703	C	cation channel complex	6	700	28	15395	0.00013	0.0012
GO:0044437	C	vacuolar part	8	700	53	15395	0.00085	0.0062
GO:0005773	C	vacuole	8	700	53	15395	0.00085	0.0062
GO:1902495	C	transmembrane transporter complex	6	700	38	15395	0.0035	0.021
GO:0012505	C	endomembrane system	26	700	316	15395	0.0032	0.021

Supplementary Table 6. Enriched Pfam domains in *P. betae* A26-41, *P. brassicae* e3, *S. subterranea* SSUBK proteomes. Pfam domains were derived from hmmscan results of predicted proteins from each data set. Pfam domains for each species/species pairs were compared with the full proteome background from the three species. For species pair results, only domains widely shared by both species were kept (Pfam ratio > 1:3) in order to avoid to consider enrichments originated only from one species of the pair (already considered in single-species-analyses).

PFAM Domain	Enrichment	Adjusted P-val	No. in subset	No. in total set
<i>Pbet-vs-all</i>				
Domain of unknown function (DUF4939) (DUF4939)	2.6	4,00E-02	13	16
gag-polypeptide of LTR copia-type (Retrotran_gag_2)	2.3	9,00E-06	35	49
BTB/POZ domain (BTB)	2.2	9,00E-04	27	39
Reverse transcriptase (RNA-dependent DNA polymerase) (RVT_2)	2.2	4,00E-07	51	76
Integrase core domain (rve)	1.8	7,00E-02	29	51
IPT/TIG domain (TIG)	1.5	1,00E-06	139	288
<i>Ssub-vs-all</i>				
Ankyrin repeats (many copies) (Ank_5)	2.2	2,00E-16	100	150
Ankyrin repeats (many copies) (Ank_4)	2.1	4,00E-22	144	223
Ankyrin repeats (3 copies) (Ank_2)	1.3	1,00E-06	315	775
<i>Plasmob-vs-all</i>				
DYW family of nucleic acid deaminases (DYW_deaminase)	2.6	3,00E-14	40	40
Bacterial signalling protein N terminal repeat (MHYT)	2.6	2,00E-02	10	10
Receptor family ligand binding region (ANF_receptor)	2.6	5,00E-04	14	14
GPCR proteolysis site, GPS, motif (GPS)	2.6	5,00E-04	14	14
Endonuclease-reverse transcriptase (Exo_endo_phos_2)	2.6	8,00E-03	11	11
Bacterial extracellular solute-binding protein (SBP_bac_1)	2.6	1,00E-01	8	8
Domain of unknown function (DUF4218) (DUF4218)	2.4	9,00E-03	13	14
Leucine rich repeat (LRR_8)	2.4	4,00E-81	280	309
7 transmembrane sweet-taste receptor of 3 GCPR (7tm_3)	2.3	1,00E-12	51	57
Cid1 family poly A polymerase (PAP_assoc)	2.3	4,00E-05	24	27
PPR repeat (PPR)	2.2	1,00E-08	41	48
NHL repeat (NHL)	2.1	4,00E-05	32	40
Protein of unknown function (DUF563) (DUF563)	1.7	8,00E-02	31	49
PPR repeat family (PPR_2)	1.6	1,00E-01	38	64
Regulator of G protein signaling domain (RGS)	1.4	3,00E-03	96	177
Ankyrin repeats (3 copies) (Ank_2)	1.3	4,00E-10	392	775
<i>Pbet&Ssub-vs-all</i>				
gag-polypeptide of LTR copia-type (Retrotran_gag_2)	1.6	5,00E-05	47	49
IPT/TIG domain (TIG)	1.3	1,00E-05	224	288
WD domain, G-beta repeat (WD40)	1.1	8,00E-02	442	642

Pbet&Plasmob-vs-all					
Fungal Zn(2)-Cys(6) binuclear cluster domain	(Zn_clus)	1.3	3,00E-02	43	46
Reverse transcriptase (RNA-dependent DNA polymerase)	(RVT_2)	1.3	1,00E-03	70	76
Regulator of G protein signaling domain	(RGS)	1.2	3,00E-03	149	177
Ssub&Plasmob-vs-all					
Ankyrin repeats (many copies)	(Ank_5)	1.4	6E-13	143	150
Ankyrin repeats (3 copies)	(Ank_2)	1.3	4E-49	707	775
Ankyrin repeats (many copies)	(Ank_4)	1.3	2E-11	201	223

Supplementary Table 7. Enriched Pfam domains in *P. betae* A26-41, *P. brassicae* e3, *S. subterranea* SSUBK secretomes. Pfam domains were derived from hmmscan results of predicted proteins included the computational secretome for each data set. Pfam domains for each secretome were compared with the full proteome background from the three species. Shared enrichments are highlighted: red stands for hits shared by *P. betae* and *S. subterranea*; green stands for hits shared by *S. subterranea* and *P. brassicae*; yellow stands for hits shared by the three species. Each species is compared to a background encompassing the three compared species.

PFAM Domain	Enrichment	Adjusted P-val	No. in subset	No. in total set
Pbet-vs-all				
PT repeat (PT)	127.6	0,000004	4	4
Barwin family (Barwin)	102	0,00002	4	5
Chitinase class I (Glyco hydro 19)	54.7	0,006	3	7
Kazal-type serine protease inhibitor domain (Kazal 2)	47.8	0,007	3	8
MAC/Perforin domain (MACPF)	47.8	0,007	3	8
PA14 domain (PA14)	47.8	0,007	3	8
Filamin/ABP280 repeat (Filamin)	42.5	0,000004	6	18
Fasciclin domain (Fasciclin)	42.5	0,01	3	9
Papain family cysteine protease (Peptidase C1)	31.9	0,0003	5	20
Cathepsin propeptide inhibitor domain (I29) (Inhibitor I29)	31.9	0,003	4	16
PHD-zinc-finger like domain (zf-HC5HC2H 2)	26.9	0,005	4	19
Chitin recognition protein (Chitin bind 1)	25.9	5E-14	15	74
PHD-finger (PHD 2)	23.9	0,05	3	16
Cysteine-rich secretory protein family (CAP)	17.2	0,005	5	37
Polysaccharide deacetylase (Polysacc deac 1)	12	0,01	5	53
Galactose binding lectin domain (Gal Lectin)	7.9	0,09	5	81
Ring finger domain (zf-RING 2)	5.9	0,04	7	151
Ssub-vs-all				
Filamin/ABP280 repeat (Filamin)	42.2	0,000003	6	18

Supplementary information – CHAPTER IV

Papain family cysteine protease (Peptidase C1)	38	0,000005	6	20
Cathepsin propeptide inhibitor domain (I29) (Inhibitor I29)	31.7	0,002	4	16
Serine carboxypeptidase (Peptidase S10)	31.7	0,002	4	16
Cysteine-rich secretory protein family (CAP)	17.1	0,003	5	37
Polysaccharide deacetylase (Polysacc deac 1)	16.7	0,0001	7	53
Ankyrin repeats (many copies) (Ank 5)	12.7	0,000000003	15	150
Chitin recognition protein (Chitin bind 1)	12	0,0009	7	74
Ankyrin repeats (many copies) (Ank 4)	8.5	0,0000003	15	223
Ankyrin repeats (3 copies) (Ank 2)	4.7	0,000000003	29	775
Plasmob-vs-all				
EGF domain (EGF 3)	49.3	0,00008	4	4
Partial alpha/beta-hydrolase lipase region (Abhydro lipase)	49.3	0,06	2	2
Carbohydrate/starch-binding module (family 21) (CBM 21)	49.3	0,06	2	2
SnoAL-like polyketide cyclase (SnoAL)	49.3	0,06	2	2
Spherulation-specific family 4 (Spherulin4)	49.3	0,06	2	2
Phage Tail Collar Domain (Collar)	49.3	0,003	3	3
D-mannose binding lectin (B lectin)	39.4	0,0003	4	5
Variant-surface-glycoprotein phospholipase C (Varsurf PPLC)	24.6	0,04	3	6
Serine carboxypeptidase S28 (Peptidase S28)	18.5	0,06	3	8
Fasciclin domain (Fasciclin)	16.4	0,09	3	9
Papain family cysteine protease (Peptidase C1)	14.8	0,0008	6	20
Common central domain of tyrosinase (Tyrosinase)	13.7	0,006	5	18
Galactose binding lectin domain (Gal Lectin)	13.4	4E-16	22	81
Leucine rich repeat (LRR 8)	13.1	1E-65	82	309
Glycosyl hydrolases family 16 (Glyco hydro 16)	12.3	0,05	4	16
Cysteine-rich secretory protein family (CAP)	9.3	0,003	7	37
Eukaryotic aspartyl protease (Asp)	8.8	0,05	5	28
Chitin recognition protein (Chitin bind 1)	8	0,00002	12	74
Ankyrin repeats (3 copies) (Ank 2)	7.6	5E-70	120	775
Glycosyl hydrolases family 18 (Glyco hydro 18)	6.6	0,05	6	45
Polysaccharide deacetylase (Polysacc deac 1)	6.5	0,02	7	53
Protein of unknown function (DUF563) (DUF563)	6	0,06	6	49
Ankyrin repeats (many copies) (Ank 5)	5.3	0,00004	16	150
Ankyrin repeats (many copies) (Ank 4)	4.6	0,000005	21	223
Protein nase domain (Pkinase)	2.3	0,03	25	538

Supplementary Table 8. Enriched GO terms in *P. betae* A26-41, *P. betae* RES F41, *P. brassicae* e3 and *S. subterranea* SSUBK secretomes. GO terms were derived from InterProScan annotation of predicted proteins included the computational secretome for each data set. For each organism, Generic GO-Slim terms in secretomes were compared with the full proteome background from the same data set. “No.” refers to the number of proteins linked to specific GO terms, in each secretome. The column “Ont.” refer to the three main aspects of gene ontology classification : P = biological process, F = molecular function, C = cellular component. “P-val” are the Hochberg adjusted P-values. The yellow-red scale colors represent the enrichment significance of each term in each of the secretomes 1 (Pbra e3); 2 (Ssub SSUBK13); 3 (Pbet A26-41) and 4 (Pbet RES F41).

GO-Slim Information							Pbra e3		Ssub SSUBK13		Pbet A26-41		Pbet RES F41	
GO Term	Ont.	Description	1	2	3	4	P-val	No.	P-val	No.	P-val	No.	P-val	No.
GO:0005975	P	carbohydrate metabolic process					3.8e-09	34	2.7e-10	22	3.9e-07	17	0.00038	13
GO:0030246	F	carbohydrate binding					1.4e-07	14	2.8e-05	7	0.012	5	---	---
GO:0008233	F	peptidase activity					0.0005	27	0.0024	14	3.3e-06	20	5.1e-06	20
GO:0005515	F	protein binding					0.0005	129	---	---	---	---	---	---
GO:0016787	F	hydrolase activity					0.0027	77	0.00068	42	0.00057	42	0.0052	39
GO:0009607	P	response to biotic stimulus					---	---	---	---	0.00027	3	0.036	2
GO:0009605	P	response to external stimulus					---	---	---	---	0.0007	3	0.047	2
GO:0051704	P	multi-organism process					---	---	---	---	0.0013	3	---	---

Supplementary Table 9. Enriched GO terms in *P. betae* A26-41, *P. betae* RES F41, *P. brassicae* e3, *S. subterranea* SSUBK membranomes. GO terms were derived from InterProScan annotation of predicted proteins included the computational membranome for each data set. For each organism, Generic GO-Slim terms in membranomes were compared with the full proteome background from the same data set. “No.” refers to the number of proteins linked to specific GO terms, in each secretome. The column “Ont.” refer to the three main aspects of gene ontology classification : P = biological process, F = molecular function, C = cellular component. “P-val” are the Hochberg adjusted P-values. The yellow-red scale colors represent the enrichment significance of each term in each of the secretomes 1 (Pbra e3); 2 (Ssub SSUBK13); 3 (Pbet A26-41) and 4 (Pbet RES F41).

GO-Slim Information							Pbra e3		Ssub SSUBK13		Pbet A26-41		Pbet RES F41	
GO Term	Ont.	Description	1	2	3	4	P-val	No.	P-val	No.	P-val	No.	P-val	No.
GO:0006810	P	transport					8.4e-85	290	9.1e-77	266	6.6e-68	243	2.3e-64	239
GO:0051234	P	establishment of localization					8.4e-85	291	9.1e-77	267	6.6e-68	244	2.3e-64	240
GO:0051179	P	localization					3.8e-84	293	7.5e-76	269	6.9e-67	247	1.9e-63	243
GO:0006811	P	ion transport					2.4e-67	123	4.9e-63	113	3.4e-59	108	9.9e-59	108
GO:0006629	P	lipid metabolic process					1.6e-12	65	1.4e-11	52	1.1e-07	46	5.5e-06	41
GO:0007165	P	signal transduction					2.4e-11	73	---	---	---	---	0.021	31
GO:0007154	P	cell communication					2.4e-11	73	---	---	---	---	0.021	31
GO:0050896	P	response to stimulus					0.00094	77	---	---	---	---	---	---
GO:0005215	F	transporter activity (act.)					2.2e-116	219	1.8e-98	192	2.6e-91	179	7.2e-90	177
GO:0022857	F	transmembrane transporter act.					2.2e-116	215	2.6e-97	186	2.1e-91	176	3.0e-89	174
GO:0015075	F	ion transmembrane transporter act.					4.6e-69	121	2.9e-62	111	1.7e-58	108	5.2e-58	107
GO:0022891	F	substrate-specific transmembrane transporter act.					2.1e-64	131	1.5e-59	122	4.6e-57	118	1.7e-56	117
GO:0022892	F	substrate-specific transporter act.					2.1e-64	134	1.2e-60	127	1.7e-56	120	6.2e-56	119
GO:0022803	F	passive transmembrane transporter act.					1.4e-36	49	8.0e-37	51	3.6e-34	46	3.9e-32	44
GO:0015267	F	channel act.					1.4e-36	49	8.0e-37	51	3.6e-34	46	3.9e-32	44
GO:0005216	F	ion channel act.					4.1e-35	47	1.3e-34	48	6.0e-32	43	7.0e-30	41
GO:0022838	F	substrate-specific channel act.					4.1e-35	47	1.3e-34	48	6.0e-32	43	7.0e-30	41
GO:0060089	F	molecular transducer act.					8.8e-35	54	0.045	3	9.4e-06	8	7.7e-05	8
GO:0004872	F	receptor act.					8.8e-35	54	0.045	3	9.4e-06	8	7.7e-05	8
GO:0004871	F	signal transducer act.					1.1e-30	54	---	---	0.0014	8	0.0032	8
GO:0016462	F	pyrophosphatase act.					0.0056	71	---	---	---	---	---	---
GO:0016818	F	hydrolase act. in phosphorus-containing anhydrides					0.0091	71	---	---	---	---	---	---
GO:0016817	F	hydrolase act., acting on acid anhydrides					0.0091	71	---	---	---	---	---	---
GO:0019825	F	oxygen binding					0.019	4	---	---	---	---	---	---

GO:0017111	F	nucleoside-triphosphatase act.		0.02	65	---	---	---	---	---	---
GO:0016020	C	membrane		2e-197	483	6e-160	386	5e-141	360	4e-133	350
GO:0005886	C	plasma membrane		3.5e-08	14	6.7e-10	18	1.1e-05	11	1.9e-08	15
GO:0005783	C	endoplasmic reticulum		2.3e-05	20	6.9e-07	21	8.7e-08	23	9.9e-06	19
GO:0012505	C	endomembrane system		2.3e-05	36	0.014	29	0.00012	33	0.0039	29
GO:0000323	C	lytic vacuole		0.026	3	---	---	---	---	---	---
GO:0005764	C	lysosome		0.026	3	---	---	---	---	---	---
GO:0033036	P	macromolecule localization		---	---	0.022	35	---	---	---	---
GO:0008233	F	peptidase act.		---	---	0.02	40	---	---	---	---
GO:0030246	F	carbohydrate binding		---	---	---	---	0.011	12	9.8e-05	16
GO:0016740	F	transferase act.		---	---	---	---	0.033	122	0.03	122

Supplementary Table 10. Enriched Pfam domains in overlapping ortholog clusters of *P. betae* A26-41, *P. brassicae* e3, *S. subterranea* SSUBK. Pfam domains were derived from hmmscan results of proteins comprised in clusters containing proteins of at least two species as determined with Orthovenn2. Pfam domains were compared with a background encompassing the proteomes of the three species.

PFAM Domain	Enrichment	Adjusted val	P-	No. in Query	No. in Ref
Pbet ∩ Ssub					
Anticodon-binding domain (AD)	26.6	1,00E-02		3	3
Domain of unknown function (DUF4504)	26.6	1,00E-02		3	3
Tetraspanin family (Tetraspanin)	26.6	1,00E-02		3	3
Barwin family (Barwin)	26.6	5,00E-05		5	5
Zinc knuckle (zf-CCHC_3)	20	3,00E-02		3	4
Eukaryotic translation initiation factor 3 subunit 8 N-terminus (eIF-3c_N)	17.7	7,00E-03		4	6
Semialdehyde dehydrogenase, NAD binding domain (Semialdehyde_dh)	16.6	1,00E-03		5	8
4'-phosphopantetheinyl transferase superfamily (ACPS)	13.3	2,00E-02		4	8
Alanine dehydrogenase/PNT, N-terminal domain (AlaDh_PNT_N)	13.3	2,00E-02		4	8
MatE (MatE)	12.1	4,00E-06		10	22
Tc5 transposase DNA-binding domain (HTH_Tnp_Tc5)	11.8	7,00E-05		8	18
DDE superfamily endonuclease (DDE_1)	11.5	3,00E-10		16	37
Retrotransposon gag protein (Retrotrans_gag)	10.4	5,00E-05		9	23
MULE transposase domain (MULE)	10	4,00E-03		6	16
GAG-pre-integrase domain (gag_pre-integr)	8.9	2,00E-04		9	27
Myb/SANT-like DNA-binding domain (Myb_DNA-bind_3)	8	1,00E-02		6	20

Supplementary information – CHAPTER IV

Integrase core domain (rve)	6.8	4,00E-05	13	51
gag-polypeptide of LTR copia-type (Retrotran_gag_2)	5.4	4,00E-03	10	49
Glycosyl hydrolases family 18 (Glyco_hydro_18)	5.3	9,00E-03	9	45
Chromo (CHRromatin Organisation MOdifier) domain (Chromo)	5	1,00E-02	9	48
Chitin recognition protein (Chitin_bind_1)	4.7	1,00E-03	13	74
Zinc finger, C2H2 type (zf-C2H2)	2.9	4,00E-04	26	235
Pbet ∩ Pbra				
Anaphase-promoting complex subunit 11 RING-H2 finger (zf-ANAPC11)	23.5	2,00E-02	3	3
Protein of unknown function (DUF3645)	23.5	5,00E-03	4	4
RNA-binding, Nab2-type zinc finger (zf-CCCH_2)	20.2	1,00E-04	6	7
Maf-like protein (Maf)	18.8	7,00E-03	4	5
FGGY family of carbohydrate kinases, C-terminal domain (FGGY_C)	15.7	1,00E-02	4	6
FGGY family of carbohydrate kinases, N-terminal domain (FGGY_N)	15.7	1,00E-02	4	6
Isochorismatase family (Isochorismatase)	14.7	5,00E-03	5	8
Protein-L-isoaspartate(D-aspartate) O-methyltransferase (PCMT)	13.5	3,00E-02	4	7
Protein of unknown function (DUF3638)	11.8	4,00E-02	4	8
Dynein light chain type 1 (Dynein_light)	10.9	5,00E-03	6	13
Zn-finger in Ran binding protein and others (zf-RanBP)	10.9	5,00E-03	6	13
Polycystin cation channel (PKD_channel)	10.1	6,00E-03	6	14
Retroviral aspartyl protease (RVP_2)	8.8	1,00E-02	6	16
ENTH domain (ENTH)	7.1	3,00E-02	6	20
HSF-type DNA-binding (HSF_DNA-bind)	6.7	4,00E-02	6	21
CBS domain (CBS)	4.5	6,00E-03	12	63
Ssub ∩ Pbra				
Aromatic amino acid lyase (Lyase_aromatic)	23.2	2,00E-03	4	4
N-terminal glutamine amidase (Nt_Gln_amidase)	23.2	2,00E-03	4	4
Prephenate dehydratase (PDT)	23.2	2,00E-03	4	4
NAD-dependent DNA ligase adenylation domain (DNA_ligase_aden)	23.2	2,00E-02	3	3
NAD-dependent DNA ligase OB-fold domain (DNA_ligase_OB)	23.2	2,00E-02	3	3
Caenorhabditis protein of unknown function (DUF268)	23.2	2,00E-02	3	3
MOZ/SAS family (MOZ_SAS)	18.6	6,00E-03	4	5
Endonuclease-reverse transcriptase (Exo_endo_phos_2)	16.9	3,00E-06	8	11
Zinc carboxypeptidase (Peptidase_M14)	13.9	1,00E-03	6	10
NHL repeat (NHL)	13.3	4,00E-18	23	40
twin BRCT domain (PTCB-BRCT)	11.6	4,00E-02	4	8
RNA binding activity-knot of a chromodomain (Tudor-knot)	11.6	4,00E-02	4	8
Stress-induced bacterial acidophilic repeat motif (KGG)	10.8	1,00E-03	7	15

Histidyl-tRNA synthetase (tRNA-synt_His)	9.9	6,00E-03	6	14
BRCA1 C Terminus (BRCT) domain (BRCT)	8.9	3,00E-02	5	13
Prolyl oligopeptidase family (Peptidase_S9)	7.3	3,00E-02	6	19
Pbet $\cap$ Ssub $\cap$ Pbra				
Armadillo/beta-catenin-like repeat (Arm)	1.3	2,00E-02	37	37
Proteasome subunit (Proteasome)	1.3	6,00E-03	43	43
Prenyltransferase and squalene oxidase repeat (Prenyltrans)	1.3	3,00E-02	35	35
ATPase family associated with various cellular activities (AAA)	1.2	3,00E-08	149	155
Mitochondrial carrier protein (Mito_carr)	1.2	1,00E-12	264	279
Sel1 repeat (Sel1)	1.2	3,00E-04	112	119
Regulator of chromosome condensation (RCC1) repeat (RCC1)	1.2	3,00E-12	290	310
Ras family (Ras)	1.2	2,00E-02	93	101
WD domain, G-beta repeat (WD40)	1.2	1,00E-19	588	642
Regulator of chromosome condensation (RCC1) repeat (RCC1_2)	1.2	1,00E-03	157	173
Helicase conserved C-terminal domain (Helicase_C)	1.1	2,00E-02	216	248
RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain) (RRM_1)	1.1	9,00E-03	253	292

Supplementary Table 11. General statistics of Pfam domain signatures scanning from *P. betae* A26-41, *P. betae* (Pbet) RES F41, *P. brassicae* (Pbra) e3 and *S. subterranea* (Ssub) SSUBK13. Results from *de novo* predicted proteins for *P. brassicae* and *S. subterranea* were compared with results obtained with published (pub) protein sequence set. All matches mean all domains found with hmmscan. Best non-overlapping matches were parsed from those results in order to keep only one domain by sequence region.

Proteome	Pfam (E-val < 10 ⁻⁴)		Proteins with >= 1 hit	
	All matches	Best non-overlapping matches	All matches	Best non-overlapping matches
Pbet A26-41	12606	9068	5794	5603
Pbet RES F41	12563	9019	5787	5595
Ssub (pub.)	13603	9181	5776	5546
Pbra (pub.)	15804	10224	6542	6362
Ssub (<i>de novo</i>)	13203	9044	5733	5521
Pbra (<i>de novo</i>)	17396	11180	7094	6862

Supplementary Table 12 contains ncRNA predictions. These results were kept in a separate excel file since they are too large to be included in this manuscript. We will be happy to send a copy of this additional file on request.

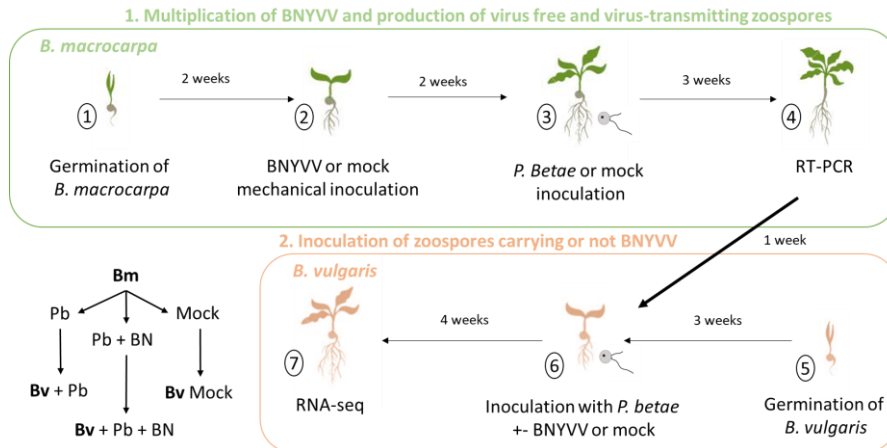
c. Supplementary notes

Clustering analysis obtained with OrthoVenn2 for both *P. betae* isolates are highly similar. The *P. betae* A26-41 and RES F41 isolates share 4704 and 4703 clusters, respectively, with *P. brassicae* e3 and *S. subterranea* SSUBK13 (Supplementary Figure S4 A and B). For the A26-41 clustering (A), those clusters include 5159 *P. betae* proteins, 5330 *P. brassicae* proteins and 5266 *S. subterranea* proteins. Beside this, 591 clusters are shared only by *P. betae* and *S. subterranea*, 547 are shared by *S. subterranea* and *P. brassicae* and 530 are shared by *P. betae* and *P. brassicae*. A total of 2573, 3190 and 2593 proteins have been classified as singletons in *P. betae* A26-41, *P. brassicae* and *S. subterranea*, respectively. For the RES F41 clustering (A), those clusters include 5131 *P. betae* proteins, 5371 *P. brassicae* proteins and 5293 *S. subterranea* proteins. Beside this, 593 clusters are shared only by *P. betae* and *S. subterranea*, 534 are shared by *S. subterranea* and *P. brassicae* and 518 are shared by *P. betae* and *P. brassicae*. A total of 2729, 3202 and 2594 proteins have been classified as singletons in *P. betae* RES F41, *P. brassicae* and *S. subterranea*, respectively.

The same analysis was performed with the *P. betae* A26-41 dataset and published *P. brassicae* and *S. subterranea* proteomes instead of *de novo* prediction. *S. subterranea* data were kindly provided by the *S. subterranea* genome paper authors (Ciaghi et al., 2018) while the *P. brassicae* proteome UP000039324 was retrieved from the UniProtKB/TrEMBL database. A total of 4516 protein clusters are shared by the three organisms, accounting for 5001 *P. betae* proteins, 4748 *P. brassicae* proteins and 5307 *S. subterranea* proteins. A higher number of *P. betae* proteins are found in clusters shared with *P. brassicae* and *S. subterranea* using *de novo* predicted proteins than using “published” proteins. It tends to confirm the usefulness of performing *de novo* gene prediction on *S. subterranea* and *P. brassicae* before making three-way comparisons. The numbers of two-species shared clusters are also consistent between organisms.

3. Supplementary information – Chapter V

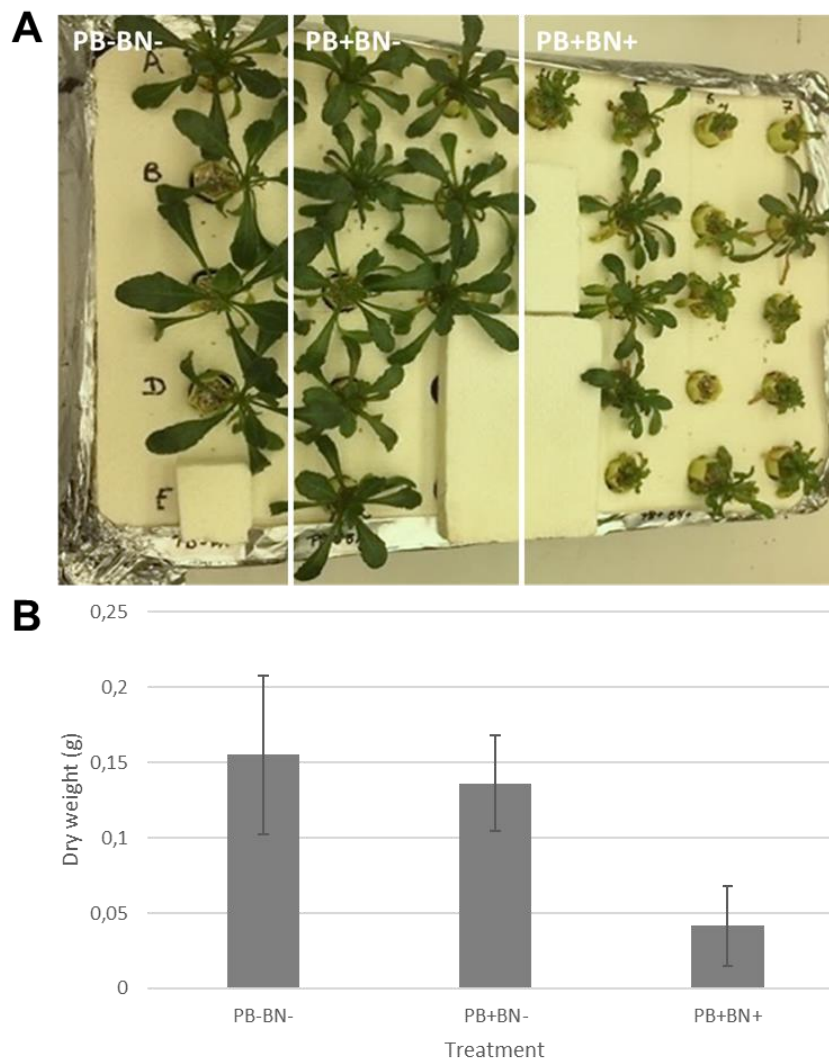
a. Supplementary figures



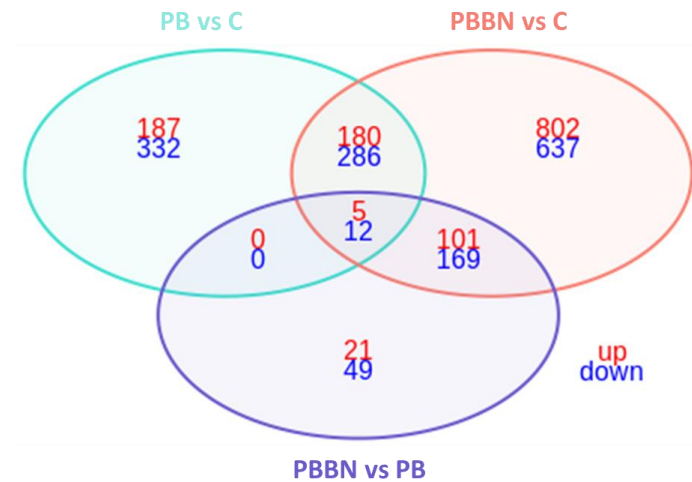
Supplementary Figure 1. Schematic representation of the seven successive steps of the bioassay performed to produce sugar beets infected with *P. betae* and Beet necrotic yellow vein virus (BNYVV) from aviruliferous zoospores of the vector and BNYVV inoculum (infected sap of *Beta macrocarpa* symptomatic leaves), including virus-transmitting zoospores production on *B. macrocarpa* (1 to 4, upper part) and their inoculation on *Beta vulgaris* (5 to 7, bottom part). Timing are shown on arrows. A summary diagram is shown at the bottom right. Bm = *B. macrocarpa*, Bv = *B. vulgaris*, Pb = *P. betae*, BN = BNYVV, Mock = inoculum produced and inoculated with the same method but in absence of Pb and/or BN.



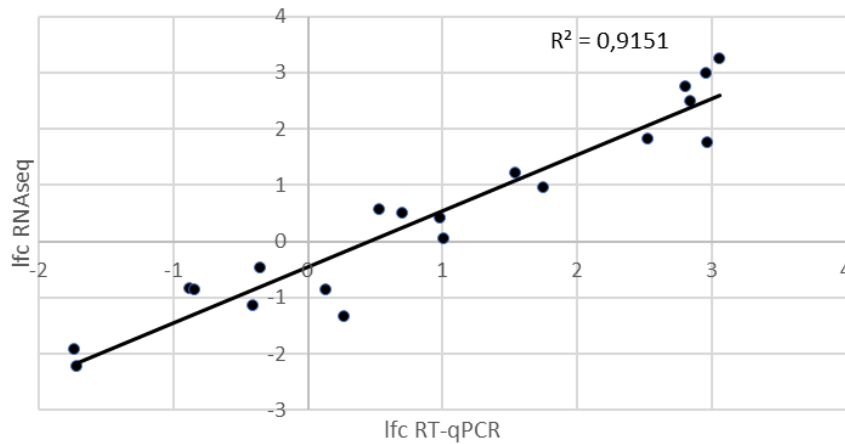
Supplementary Figure 2. Systemic symptoms observed on *B. macrocarpa* 8, 15, 22, 41 days after mechanical inoculation, at roots level, with the BNYVV. First yellow spots appeared on leaves eight day after inoculation. One week later, new leaves showed vein yellowing, mosaic symptoms and distortions. Symptomatic leaves eventually started to become necrotic, whereas no significant effect was noticed on plant inoculated with *P. betae* alone



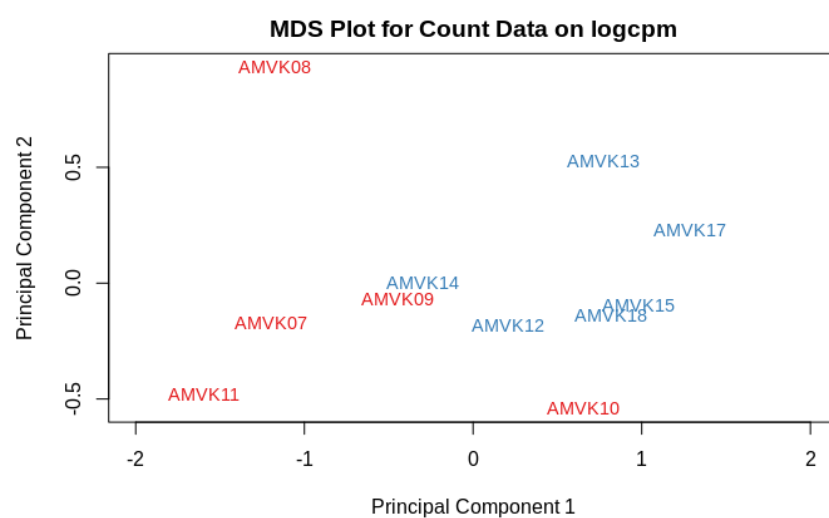
Supplementary Figure 3. Stunting (A) and reduction of root dry weight (B) of *B. macrocarpa* infected with the BNYVV. PB+ (PB-) = inoculated (or not) with *P. betae*, BN+ (BN-) = inoculated (or not) with the BNYVV



Supplementary Figure 4. Venn diagram showing the total of up- and down-regulated *B. vulgaris* genes between the three inoculation modalities. C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV. Each circle represents one side of the three-way comparison. Figures in red and blue are the numbers of up- and down-regulated genes, respectively.

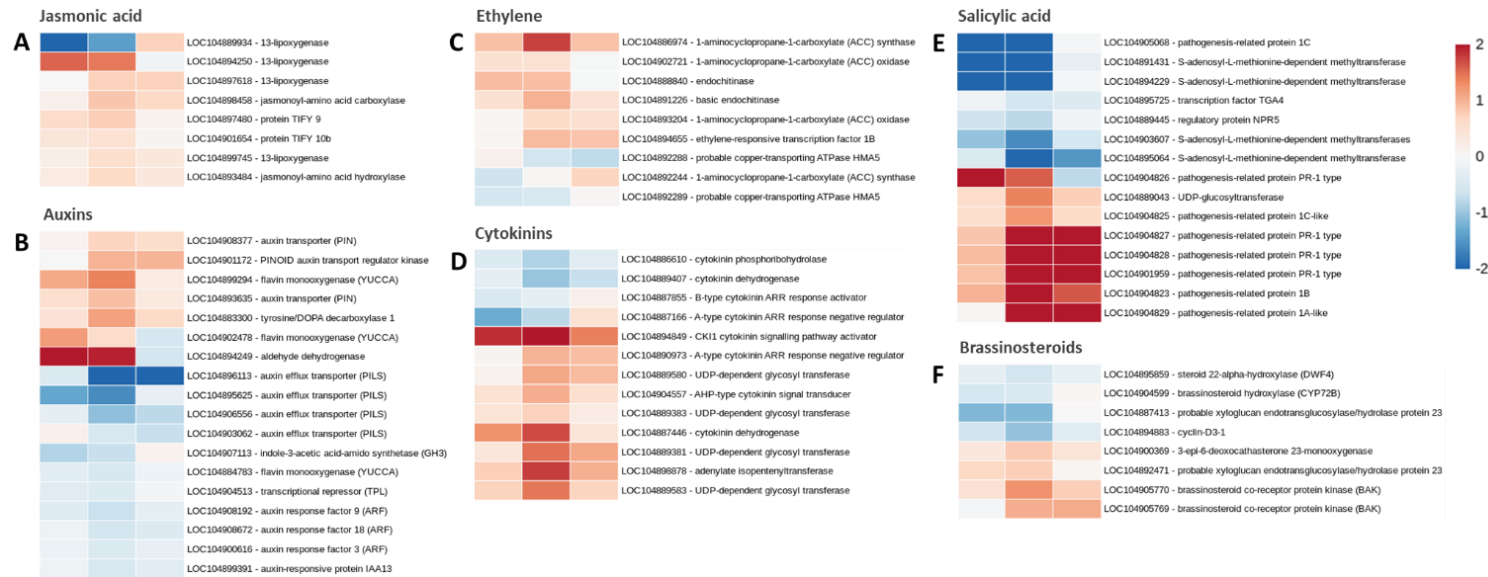


Supplementary Figure 5. Comparison of RNA-seq and RT-qPCR results. Mean log fold changes (lfc) of ten genes were determined in both inoculation status in comparison to non-inoculated controls using the RNA-seq data and by RT-qPCR and were compared to validate the expression changes found in the transcriptomic study.



Supplementary Figure 6. Multidimensional scaling (MDS) analysis of *P. betae* gene expression data. In red, libraries from *P. betae* alone, in plant roots. In blue, libraries from *P. betae* co-inoculated with BNYVV.

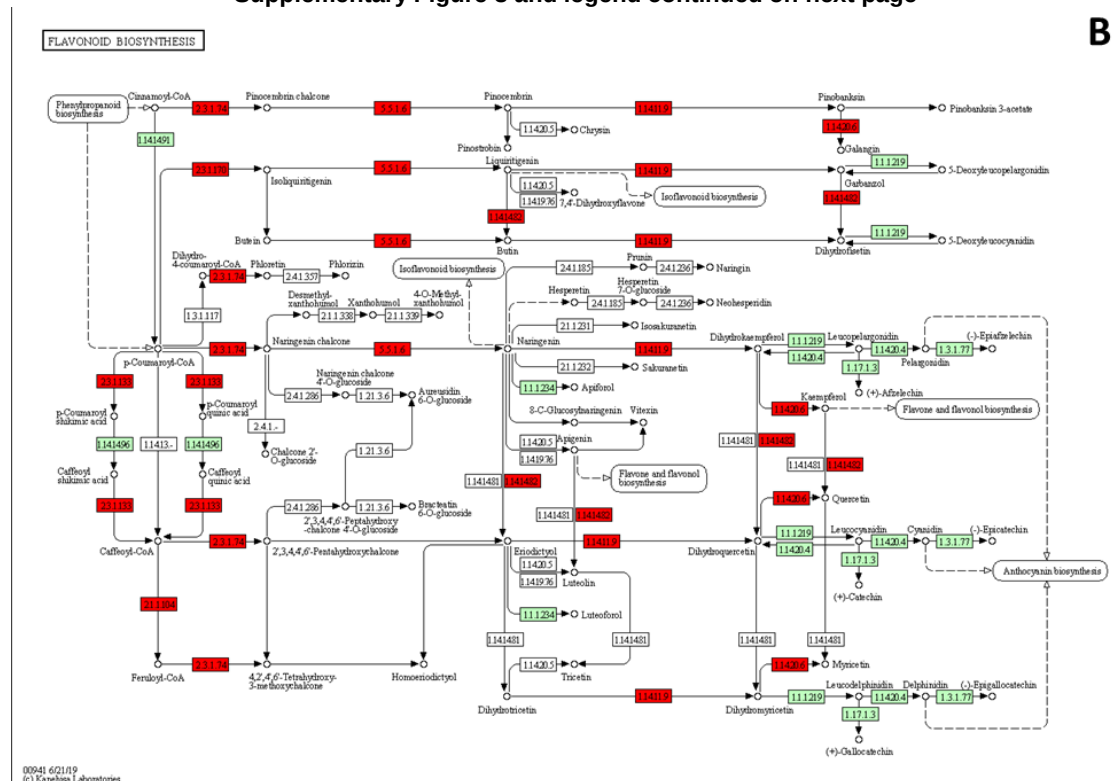
Supplementary information – CHAPTER V



Supplementary Figure 7. Phytohormone action in response to *P. betae* +/- BNYVV inoculation. The heatmaps of DEGs related to jasmonic acid (A), auxins (B), ethylene (C), cytokinins (D), salicylic acid (E) and brassinosteroids (F) are shown. Up- and down-regulation are represented in red and blue, respectively. The scale is based on log₂ fold changes. C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV. Genes were classified according to Mercator/MapMan4 and KEGG Annotations comprised the NCBI Gene symbol and the MapMan4 or KEGG annotation (if not classified by MapMan).



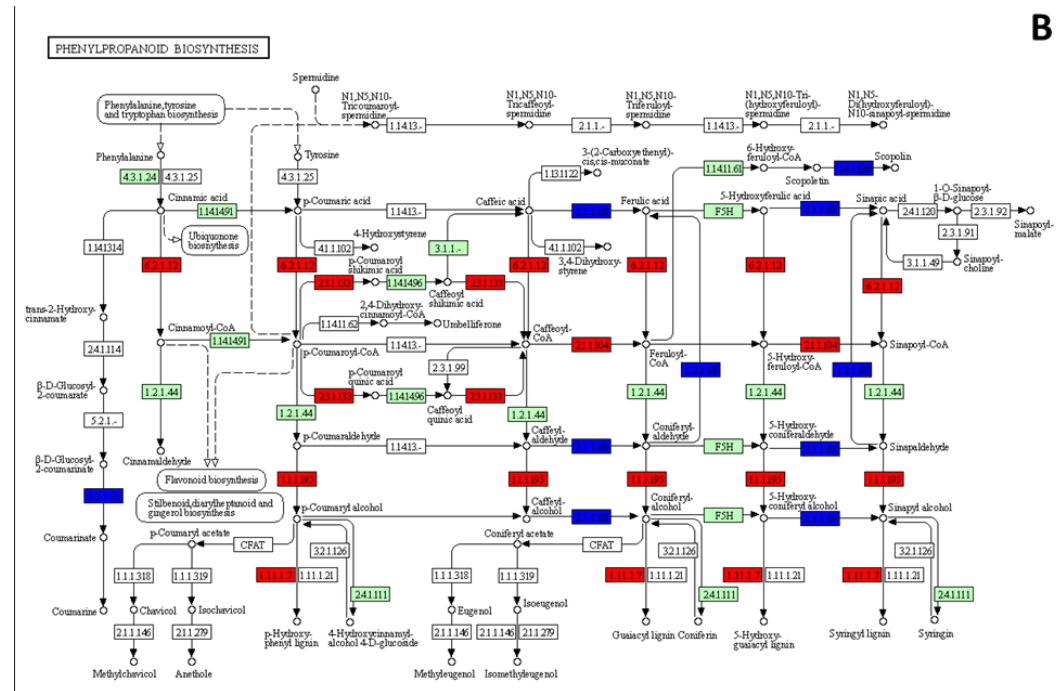
Supplementary Figure 8 and legend continued on next page



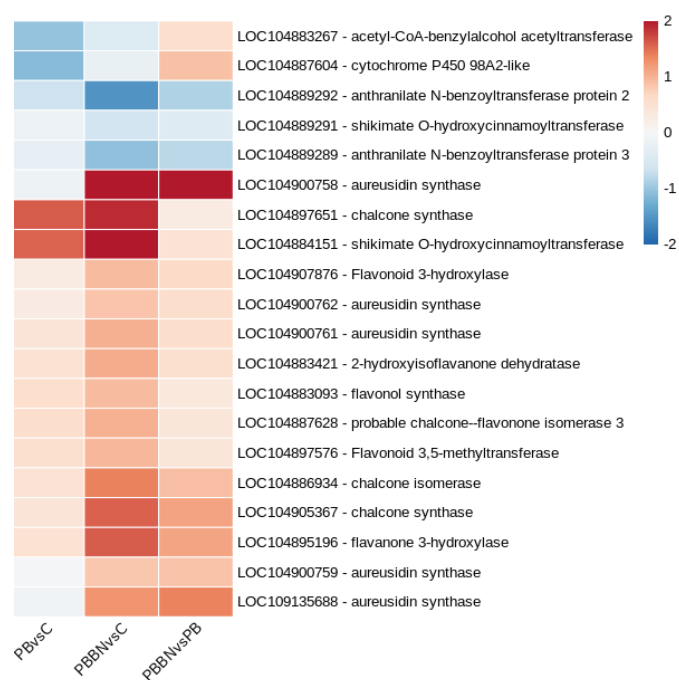
Supplementary Figure 8. KEGG representation of DEGs related to flavonoids metabolism in response to *P. betae* alone (A) or *P. betae* + BNYPV (B) inoculation. Up- and down-regulation compared to mock-inoculated controls are represented in red and blue, respectively

A



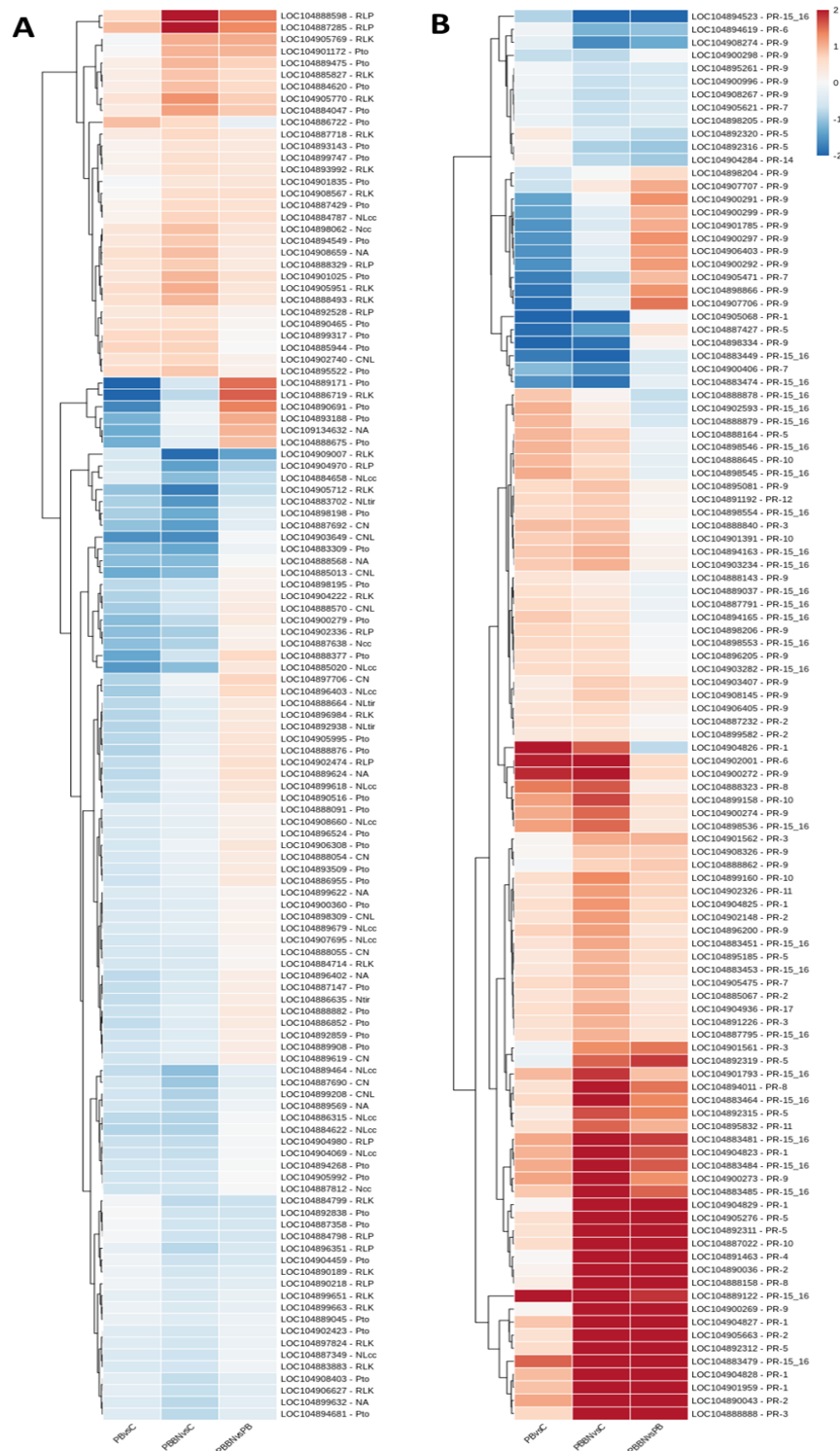


Supplementary Figure 9. KEGG representation of DEGs related to phenylpropanoids metabolism in response to *P. betae* alone (A) or *P. betae* + BNYVV (B) inoculation. Up- and down-regulation compared to mock-inoculated controls are represented in red and blue, respectively.



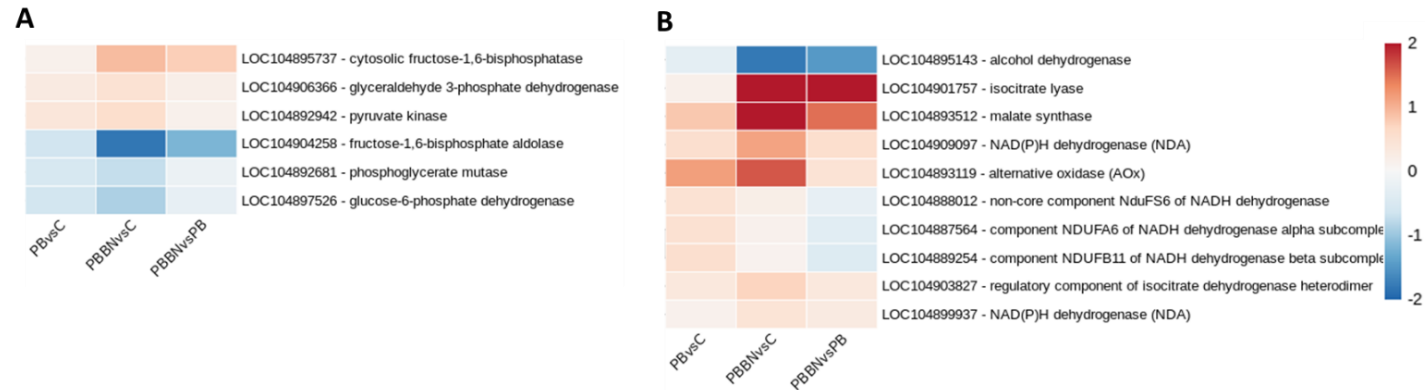
Supplementary Figure 10. Heatmap of the flavonoids metabolism in response to *P. betae* +/- BNYVV inoculation. Up- and down-regulation are represented in red and blue, respectively. The scale is based on $\log_2$ fold changes. C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBN = plants infected with *P. betae* and the BNYVV. Genes were classified according to Mercator/MapMan4 and KEGG. Annotations comprised the NCBI Gene symbol and the MapMan4 or KEGG annotation (if not classified by MapMan).

Supplementary information – CHAPTER V

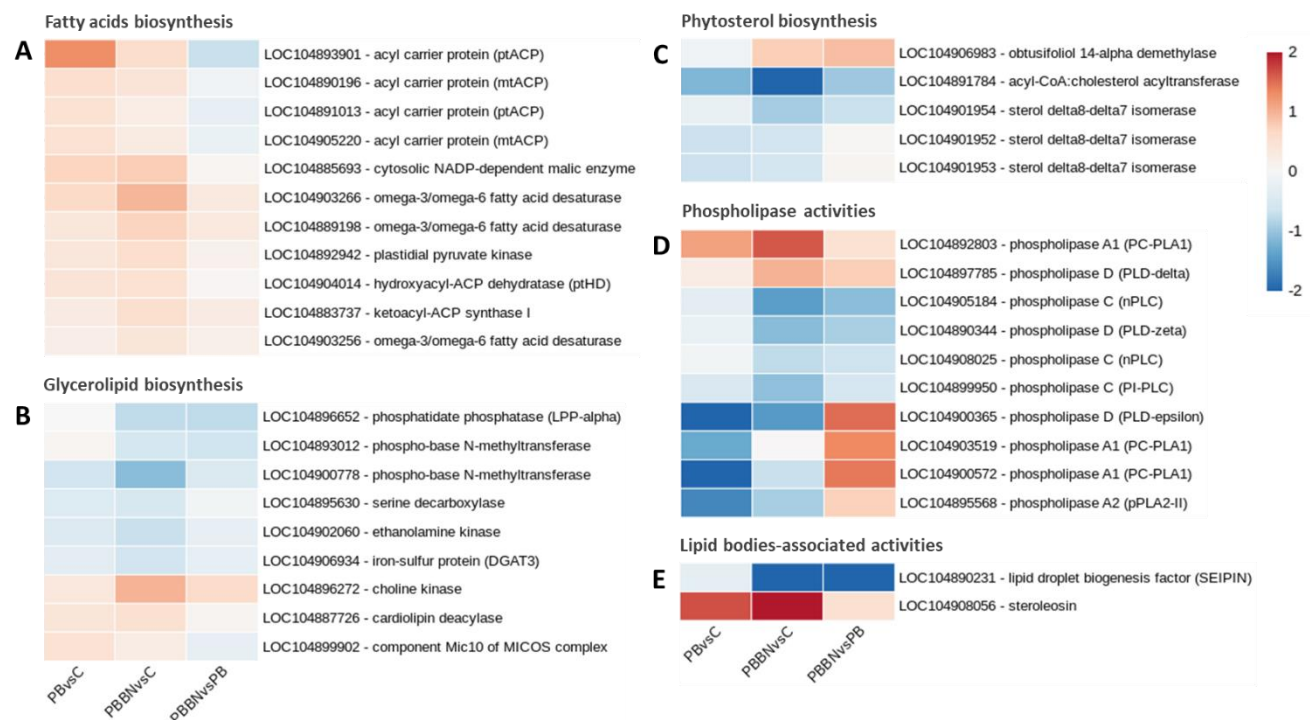


Supplementary Figure 11: legend on next page

Supplementary Figure 11. Resistance genes analogues (RGAs) (A) and pathogenesis-related proteins encoding genes (*PR* + *PR-like*) (B) DEGs heatmaps in response to *P. betae* +/- BNYVV inoculation. Up- and down-regulation are represented in red and blue, respectively. The scale is based on log₂ fold changes. C = mock-inoculated plants. PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV. RGAs were classified according to Dohm et al. (2014) according to the presence of domains known to be involved in resistance to pathogens: coiled-coil (CC), leucine zipper (LZ), nucleotide-binding site (NBS), leucine rich repeat (LRR), serine-threonine kinase (STK), Toll/Interleukin-1 receptor (TIR) and transmembrane (TM) domains Groups were constructed as follow: CNL if NBS_CC and LRR and CC OR LZ, TNL if NBS_TIR and TIR and LRR, RLK if STK and TM and LRR, RLP if NOT STK and TM and LRR, Pto if STK and NOT TM, Tx if NOT NBS_TIR and TIR, TN if NBS_TIR and TIR and NOT LRR, Ntir if NBS_TIR and NOT TIR and NOT LRR, Nltir if NBS_TIR and NOT TIR and LRR, NLcc if NBS_CC and LRR and NOT CC and NOT LZ, Ncc if NBS_CC and NOT LRR and NOT CC and NOT LZ, CN if NBS_CC and NOT LRR and CC OR LZ. PR and PR-like were searched using tBLASTn on *B. vulgaris* refseq_rna (E-value < e⁻⁶, Bit-score >50). Annotations comprised the NCBI Gene symbol protein family. RGAs families are detailed in Dohm et al. (2014).



Supplementary Figure 12. Part of the carbohydrate metabolism in response to *P. betae* +/- BNYVV inoculation. The heatmaps of DEGs related to plastidial glycolysis, gluconeogenesis, oxidative pentose phosphate pathway (A) as well as fermentation, Krebs cycle, Glyoxylate shunt and oxidative phosphorylation (B) are shown. Up- and down-regulation are represented in red and blue, respectively. The scale is based on log₂ fold changes. All DEGs are computed in comparison to mock-inoculated plants. C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV. Genes were classified according to Mercator/MapMan4. Annotations comprised the NCBI Gene symbol and the MapMan4 annotation.



Supplementary Figure 13. Lipid metabolism in response to *P. betae* +/- BNYVV inoculation. The heatmaps of DEGs related to fatty acids biosynthesis (A), glycerolipid biosynthesis (B), phytosterol biosynthesis (C), phospholipase activities (D) and lipid bodies-associated activities (E) are shown. Up- and down-regulation are represented in red and blue, respectively. The scale is based on log₂ fold changes. C = mock-inoculated plants, PB = plants infected with *P. betae* alone, PBBN = plants infected with *P. betae* and the BNYVV. Genes were classified according to Mercator/MapMan4. Annotations comprised the NCBI Gene symbol and the MapMan4 annotation.

b. Supplementary Tables

Supplementary Table 1. List of primers to amplify the four RNAs of BNYVV by RT-PCR in BNYVV and *P. betae* infected *B. vulgaris*.

Target	Primers name	Sequences	Amplicon size
RNA1	BNYVV1(dup)for	5'-CTGGTTCAAAGATGGGTACTGCTG-3'	161bp
	BNYVV1(dup)rev	5'-GTCCCGTTAAATCCACTTCAAC-3'	
RNA2	BNYVV2(2)for	5'-CGAGTAATAAGTAGCCGCCGTC-3'	834bp
	BNYVV2(2)rev	5'-CAAAAGGAAGACCATGTGAGGA-3'	
RNA3	BNYVV3(1)for	5'-CAGTTTATGATTAGGGCACA-3'	635bp
	BNYVV3(1)rev	5'-ATCATCATCAACACCGTCAG-3'	
RNA4	p31-Nt	5'-GCTGATGGAGAGATATGTCGG-3'	843bp
	p31-Ct	5'-ATCGTGATAAAAGACAAACC-3'	

Supplementary Table 2. List of primers used for RT-qPCR quantification of *P. betae* relatively to *B. vulgaris* 18S rRNA gene expression.

Target	Primers name	Sequences	Amplicon size
RNA1	Bv18for	5'-GAAAGACGAACAAGTGCAGAA-3'	112bp
	Bv18rev	5'-CATCGTTTATGTTGAGACTAGGA-3'	
RNA2	PB1	5'-GGAATTTGAACAAGTGACTTGG-3'	204bp
	PSP2rev	5'-AGGGCTCTCGAAAGCGCAA-3'	

Supplementary Table 3. List of *B. vulgaris* genes targeted for expression analysis by RT-qPCR at 1, 7, 14 and 21 dpi.

Pathway	Gene	Refseq annotation	Primer name	Primer sequence	length
Jasmonate	LOC104901654	protein TIFY 10b	BvJAZ1F	TGCTGTGCCTAGTGTGTCTG	91
			BvJAZ1R	TTTGCCCAAGCCGGTC	
	LOC104890828	Probable linoleate 9S-lipoxygenase 5	BvLOX5F	TCCTGAGGACCATTTCAAGCA	95
			BvLOX5R	CCCAAGGTAGACCTCGTCAG	
Salicylate	LOC104894469	UDP-glycosyltransferase 74G1	BvUGT74G1F	GTTCTGTGGGACAATACGGGT	110
			BvUGT74G1R	AGCCATCCAATCAATCACCTCT	
redox homeostasis	LOC104900197	putative respiratory burst oxidase homolog protein H	BvRbohF	TGGTGTCTGAAAGCCGGATAAG	150
			BvRbohR	GGCAAAGCTTCCTCAATGGT	
	LOC104907706	peroxidase 44	BvPER44F	CCTTCCATGACAGTTGCACA	92
			BvPER44R	ACAGCGTGATAACCTAGAAGG	
	LOC104895166	L-ascorbate peroxidase 3	BvAPX3F	ATGTTCAACTATATGCTAAGGACGA	100
			BvAPX3R	GGCAACTTGGGTGGGTTGA	
	LOC104899212	glutathione S-transferase U8	BvGSTU8F	GCCAAATTCATAGATGAAAAGTGTA	177
			BvGSTU8R	TGCAACTATGTCCACAAAACCA	
	LOC104883638	glutaredoxin-C5	BvGRXS9F	CTGCCAGCCGTGTTCTATTG	91
			BvGRXS9R	TGACCATTTGGAACGAGAGCG	
R genes	LOC104885013	putative disease resistance protein RGA3	BvRGA3F	AGACACTGGAGATCTGGGGA	144
			BvRGA3R	TTAGGCAAGCAATGCCTGGA	
PR genes	LOC104904829	pathogenesis-related protein 1A-like	BvPR1AF	TGCAACTATAGTCCACCAGGA	115
			BvPR1AR	CGGAGTTATATTAATTGTCCCTGC	
	LOC104905276	thaumatin-like protein 1b	BvTLP1F	CACTGGCAACTTCAGGTGTATG	96
			BvTLP1R	AATTCTGCTAGGGTTGCTGGT	
	LOC104888158	Acidic endochitinase SE2	BvSE2F	GCCGGCAGTGGTTTTATTCC	124
			BvSE2R	CACTGCTGTACCCACTGTCA	
Reference genes	LOC104890174	V-type proton ATPase subunit H	BvVHA-HF	TCTGCTCCCCAAAGGAACAT	108
			BvVHA-HR	GCCTCCACAAGGTCCTCATC	
	LOC104896095	polypyrimidine tract-binding protein homolog 3	BvPTB3F	GCTGTTCACTTTCTGAAGGGGATT	86
			BvPTB3R	GCACCTGTTGTGATTTGAGGA	

Supplementary information – CHAPTER V

Supplementary Table 4. RT-PCR detection rates of BNYVV and *P. betae* in *B. macrocarpa* roots in the three bioassays.

	No. samples	BNYVV positive rate (RT-PCR)	<i>P. betae</i> positive rate (RT-PCR)
BIOASSAY 1	19	52.63-57.89%	100 %
BIOASSAY 2	15	66.67-100%	100 %
BIOASSAY 3	33	48.48-69%	100 %

Supplementary Table 5. RT-PCR detection rates of BNYVV and *P. betae* in *B. vulgaris* roots in the three bioassays.

	No. samples	BNYVV positive rate (RT-PCR)	<i>P. betae</i> positive rate (RT-PCR)
BIOASSAY 1	30	53.33-80%	100 %
BIOASSAY 2	30	76.67-90%	100 %
BIOASSAY 3	60	58.33-76.67%	100 %

Supplementary Table 6. Overview of the RNA-seq results statistics and mapping percentage on *P. betae*, *B. vulgaris* and BNYVV genomes. PB = plants infected with *P. betae* ; PBBN = plants infected with *P. betae* and BNYVV. Control = mock inoculated plants.

Library	Yield (Mb)	%PF	No. Clusters	% Q30	Mean quality	% <i>P. betae</i>	% <i>B. vulgaris</i>	% BNYVV
Control 1	11.111	100	55.559.869	93.31	35.79	0	97.44	0
Control 2	9.99	100	49.952.854	92.76	35.66	0	97.32	0
Control 3	7.873	100	39.365.122	93.09	35.72	0	96.95	0
Control 4	9.886	100	49.430.500	93.51	35.82	0	98.14	0
Control 5	10.743	100	53.715.258	92.6	35.63	0	97.37	0
PB 1	7.406	100	37.033.407	91.85	35.43	32.26	63.82	0
PB 2	8.598	100	42.992.093	92.32	35.54	32.81	63.43	0
PB 3	8.238	100	41.194.342	92.57	35.6	33.73	61.37	0
PB 4	9.249	100	46.246.587	92.53	35.59	29.15	66.33	0
PB 5	11.247	100	56.238.774	93.43	35.8	34.31	61.88	0
PBBN 1	10.032	100	50.160.846	92.33	35.57	26.92	67.97	0.83
PBBN 2	10.887	100	54.439.838	93.3	35.77	14.43	75.15	6.9
PBBN 3	11.049	100	55.245.943	92.57	35.6	18.85	73.71	3.89
PBBN 4	10.671	100	53.359.886	93.52	35.81	16.69	72.15	6.93
PBBN 5	11.921	100	59.607.046	91.62	35.46	17.06	77.76	2.04
PBBN 6	10.018	100	50.091.793	92.31	35.55	15.44	76.04	4.18

Supplementary Table 7. Differentially expressed genes of *P. betae* in sugar beet roots in the presence of BNYVV compared to without BNYVV. The top blast hit on Swiss-Prot database obtained from ORF encoded protein (predicted with TransDecoder) is reported as well as the percentage of identity (id.) and corresponding E-value. If no blast hit was found, the best PFAM hit is mentioned (see PFAM accessions). Blank cell means that no BLASTP or PFAM correspondence was found.

Gene expression change			Top BLASTP or PFAM hit		
gene.id	logFC	adj.P.Val	Id. (%)	E-val	Name
MSTRG.6647	-1,30	0,032	28.8	2.68e-27	Retrotransposon-derived protein PEG10
MSTRG.5216	-0,98	0,034			
MSTRG.4037	-0,80	0,031			
MSTRG.10146	-0,77	0,046			
MSTRG.3100	-0,72	0,047			
MSTRG.2958	-0,71	0,040	31.492	3.35e-44	Fe(2+) transport protein 1
jg2261	-0,70	0,033			
MSTRG.8471	-0,68	0,032			
MSTRG.5013	-0,66	0,039			
MSTRG.7961	-0,65	0,045			
MSTRG.7233	-0,65	0,027			
MSTRG.5300	-0,65	0,038			
MSTRG.8740	-0,63	0,032			
MSTRG.4292	-0,63	0,050			
MSTRG.8435	-0,62	0,031			
MSTRG.7268	-0,61	0,040			
MSTRG.7685	-0,61	0,047	32.381	7.4e-05	Putative bacterial sensory transduction regulator (PF10722.11)
MSTRG.1168	-0,61	0,033			
MSTRG.1984	-0,60	0,047			
MSTRG.3954	-0,60	0,044			
MSTRG.2322	-0,59	0,045			
MSTRG.3781	-0,58	0,045			
MSTRG.668	-0,58	0,034			
MSTRG.7470	-0,58	0,031			
MSTRG.3381	-0,56	0,035			
MSTRG.1877	-0,56	0,041			
MSTRG.6563	-0,55	0,046	50.0	2.31e-68	Ras-related protein Rab-28
MSTRG.2462	-0,54	0,033			
MSTRG.3205	-0,54	0,048	50.0	2.31e-68	Ras-related protein Rab-28
MSTRG.10107	-0,54	0,039			
MSTRG.6809	-0,54	0,038			

Supplementary information – CHAPTER V

MSTRG.5936	-0,54	0,036	42.857	6.3e-06	Protein FAM177A1
MSTRG.6499	-0,53	0,036	46.602	1.78e-25	Zinc finger protein 596
MSTRG.6758	-0,53	0,032	60.345	2.14e-94	28 kDa inner dynein arm light chain, axonemal
MSTRG.6488	-0,51	0,038	50.893	4.25e-40	Dynein light chain Tctex-type protein 2B
MSTRG.1803	-0,50	0,038			
MSTRG.8712	-0,49	0,043			
MSTRG.10281	-0,49	0,038	34.94	2.18e-07	Regulator of G-protein signaling rgs-2
MSTRG.3116	-0,49	0,041	48.556	6.9e-122	Anaphase-promoting complex subunit cdc20
MSTRG.4545	-0,48	0,048			
MSTRG.1042	-0,48	0,038			
MSTRG.7390	-0,48	0,040	28.0	2.22e-23	Thioredoxin domain-containing protein 5
MSTRG.9047	-0,48	0,036	40.816	1.2e-49	Protein DPCD
MSTRG.545	-0,47	0,032	38.532	9.85e-16	TRAF3-interacting protein 1
MSTRG.9660	-0,47	0,045			
jg6675	-0,47	0,034	33.01	3.27e-09	FK506-binding protein 5
MSTRG.10343	-0,46	0,032	34.409	4.45e-34	Probable aquaporin PIP-type 7a
MSTRG.6292	-0,45	0,042	32.407	2.37e-10	Phosphatidylglycerol/phosphatidylinositol transfer protein
MSTRG.8434	-0,44	0,033	53.279	7.37e-35	Zinc finger protein 271
MSTRG.4417	-0,44	0,032		3.1e-10	GYF domain (PF14237.8)
MSTRG.1691	-0,43	0,050			
MSTRG.9192	-0,43	0,038	50.0	1,00E-60	Guanylate kinase
MSTRG.373	-0,43	0,031	40.059	2.86e-77	DnaJ homolog subfamily B member 4
MSTRG.2192	-0,42	0,032			
MSTRG.7283	-0,41	0,041	31.783	1.26e-10	DNA-binding protein LIV4
MSTRG.3909	-0,41	0,039	40.484	5.99e-47	Protein phosphatase 2C and cyclic nucleotide-binding/kinase domain-containing protein
MSTRG.5198	-0,41	0,042		0.0064	PAN domain (PF14295.8)
MSTRG.8062	-0,41	0,043			
MSTRG.432	-0,40	0,046			
MSTRG.7261	-0,40	0,039			
MSTRG.1164	-0,39	0,043	25.065	1.31e-18	RIB43A-like with coiled-coils protein 1
MSTRG.6256	-0,38	0,047	51.887	2.77e-30	BoIA-like protein 1
MSTRG.3162	-0,38	0,039			
MSTRG.2827	-0,37	0,038		2.1e-45	Protoglobin (PF11563.10)
MSTRG.7027	-0,36	0,046			
MSTRG.9341	-0,36	0,032	51.163	4.82e-23	Calmodulin-related protein
MSTRG.9174	-0,35	0,032			
MSTRG.8622	-0,35	0,048	41.64	5.86e-74	CYFIP-related Rac1 interactor homolog
MSTRG.4387	-0,35	0,039	36.517	8.63e-37	Cysteine dioxygenase 1
MSTRG.9140	-0,35	0,038			
MSTRG.1428	-0,34	0,038	38.462	1,00E-20	Zinc finger protein 787

MSTRG.503	-0,33	0,043			
MSTRG.2725	0,29	0,049	48.153	0	DNA replication licensing factor mcm6
MSTRG.7136	0,31	0,047	49.335	0	Lon protease homolog, mitochondrial
MSTRG.9181	0,32	0,045	68.158	0	Histone deacetylase 1
MSTRG.7509	0,33	0,047	31.21	1.58e-34	Protein GLUTELIN PRECURSOR ACCUMULATION 3
MSTRG.6129	0,34	0,039	27.592	5.45e-113	Structural maintenance of chromosomes protein 6
MSTRG.4705	0,34	0,038	55.03	0	Crooked neck-like protein 1
MSTRG.5270	0,35	0,042	44.732	5.39e-113	SNW/SKI-interacting protein A
MSTRG.1930	0,36	0,049	31.167	1.12e-82	Fanconi-associated nuclease 1 homolog
MSTRG.2586	0,40	0,041	43.137	1.55e-87	Histone deacetylase 5
MSTRG.1001	0,41	0,044	37.963	0	Structural maintenance of chromosomes protein 4
MSTRG.2832	0,41	0,032	31.865	5.82e-155	Regulator of nonsense transcripts UPF2
MSTRG.147	0,44	0,036	53.451	0	Eukaryotic translation initiation factor 5B
MSTRG.2861	0,45	0,042	29.545	1.18e-07	Suppressor of RPS4-RLD 1
MSTRG.3695	0,45	0,032	25.951	2.28e-49	Serine/threonine-protein phosphatase 4 regulatory subunit 3
MSTRG.8259	0,50	0,036	24.352	4.67e-47	Cullin-associated NEDD8-dissociated protein 1
MSTRG.7206	0,51	0,048	37.22	3.77e-33	DNA repair protein RAD5B
MSTRG.6735	0,52	0,041	22.692	1.37e-10	Mechanosensitive ion channel protein 9
MSTRG.2455	0,54	0,032	33.891	4.44e-144	Transcription elongation factor SPT5
MSTRG.9897	0,58	0,032	26.6	1.99e-31	Importin subunit alpha-4
MSTRG.7910	0,63	0,032	24.485	1.36e-28	Tetratricopeptide repeat protein 6
MSTRG.4974	0,65	0,049			
MSTRG.9042	0,66	0,045			
MSTRG.9211	0,67	0,043			
MSTRG.8978	0,68	0,031			
MSTRG.6299	0,87	0,038			
MSTRG.10021	0,92	0,035			
MSTRG.24	0,93	0,038	35.606	8.13e-42	tRNA (guanine(9)-N1)-methyltransferase
MSTRG.779	0,97	0,044			
MSTRG.5386	1,00	0,038			
MSTRG.5238	1,00	0,035		2e-27	Protein of unknown function (PF04343.15)
MSTRG.2550	1,02	0,031			
jg2047	1,07	0,035			
MSTRG.243	1,08	0,042	33.131	9.48e-45	Pumilio homolog 12
MSTRG.4486	1,10	0,032	26.256	1.68e-61	Protein pianissimo A
MSTRG.3586	1,12	0,038			
MSTRG.6089	1,16	0,032			
MSTRG.3690	1,17	0,032			
MSTRG.6642	1,17	0,032			
jg6782	1,20	0,049	30.035	2.35e-07	Late embryogenesis abundant protein At3g53040

Supplementary information – CHAPTER V

ig3300	1,24	0,047			
MSTRG.310	1,24	0,032			
MSTRG.6475	1,27	0,046			
MSTRG.132	1,28	0,042	39.928	0	DNA polymerase alpha catalytic subunit
MSTRG.311	1,32	0,031			
MSTRG.8310	1,34	0,039			
MSTRG.837	1,40	0,032			
MSTRG.2635	1,41	0,043			
MSTRG.8004	1,44	0,032	31.673	2.34e-24	Calcium-dependent protein kinase 4
ig5863	1,45	0,031	47.403	2.71e-91	Cathepsin L-like
MSTRG.5965	1,48	0,035			
MSTRG.9398	1,49	0,045			
MSTRG.6731	1,50	0,047			
MSTRG.4668	1,52	0,043			
MSTRG.6833	1,53	0,032	45.021	7.63e-131	Catalase
MSTRG.464	1,55	0,043	39.831	1.74e-15	22.0 kDa heat shock protein
MSTRG.3957	1,55	0,034			
MSTRG.9059	1,56	0,046			
MSTRG.332	1,57	0,032			
MSTRG.7412	1,57	0,039			
MSTRG.754	1,58	0,031	38.4	1.7e-33	Ankyrin homolog
MSTRG.9968	1,61	0,042		1.2e-06	PB1 domain (PF00564.26)
MSTRG.6146	1,67	0,047	26.908	5.3e-11	Lipase
MSTRG.652	1,70	0,032			
MSTRG.1023	1,73	0,036	26.087	9.63e-06	Probable metallo-hydrolase YybB
MSTRG.9014	1,76	0,045	55.294	0	RNA-splicing ligase RtcB homolog 1
MSTRG.440	1,83	0,038			
MSTRG.866	1,87	0,045			
MSTRG.8913	1,91	0,031	39.015	2.44e-64	Calcium/calmodulin-dependent protein kinase type 1D
ig7392	1,92	0,032	40.984	5.58e-14	BTB/POZ domain-containing protein KCTD9
MSTRG.8693	1,99	0,034	58.824	4.66e-10	Conidiation-specific protein 10
MSTRG.5306	2,00	0,049		0.00061	CBS domain (PF00571.30)
MSTRG.9018	2,01	0,032	43.056	9.96e-27	Protein archease
MSTRG.9295	2,10	0,039	43.023	5.87e-19	Protein yippe-like 3
MSTRG.4612	2,10	0,031			
MSTRG.8692	2,14	0,044			
MSTRG.8630	2,15	0,032	26.415	1.03e-17	Serine/threonine kinase SAD-1
MSTRG.706	2,17	0,032			
MSTRG.131	2,24	0,043			
ig9471	2,26	0,032	29.762	2.26e-11	Lipase

MSTRG.409	2,28	0,032	41.499	1.22e-85	Alcohol dehydrogenase
MSTRG.6695	2,31	0,032			
MSTRG.5924	2,38	0,049			
MSTRG.6144	2,41	0,032	24.364	3.56e-11	Pre-pro-metalloprotease PrtV
MSTRG.3369	2,44	0,034			
MSTRG.9254	2,50	0,036			
MSTRG.1077	2,64	0,032		6.2e-07	BTB/POZ domain (PF00651.33)
MSTRG.9843	2,67	0,031			
MSTRG.10478	2,76	0,040			
MSTRG.985	2,99	0,043	25.765	1.26e-16	Lysosomal acid phosphatase
MSTRG.9239	3,00	0,033		1.9e-07	BTB/POZ domain (PF00651.33)
MSTRG.3290	3,03	0,031	27.937	2.42e-21	Pre-pro-metalloprotease PrtV {ECO:0000303 PubMed:23905008}
MSTRG.6669	3,37	0,031			
MSTRG.6670	3,68	0,042			

Supplementary Table 8. TOP 20 of the most down- and up-regulated *B. vulgaris* genes in plants infected with *P. betae* alone in comparison to mock-inoculated plants.

TOP20 down-regulated genes <i>P. betae</i> alone vs Control			
IDENTIFIER	logFC	DESCRIPTION – Mapman	DESCRIPTION – ncbi
LOC104900933	-5.176544	pectin methylesterase	probable pectinesterase
LOC104882806	-4.602125	no hits	uncharacterized protein
LOC104891524	-4.017623	no hits	DNA-directed RNA polymerase II subunit RPB1-like
LOC104903772	-3.989863	no hits	uncharacterized protein
LOC104892667	-3.952520	no hits	extensin-1-like
LOC104884038	-3.518837	copper-containing amine oxidase (CuAO)	primary amine oxidase
LOC104891150	-3.511638	no hits	uncharacterized protein
LOC104892665	-3.454923	Extensin-1*	extensin-1-like
LOC109135932	-3.348110	no hits	uncharacterized protein
LOC104885262	-3.297319	glycosylase	polygalacturonase
LOC104905068	-3.045940	Pathogenesis-related protein 1C*	pathogenesis-related protein 1C
LOC109134609	-2.985720	no hits	U-box domain-containing protein 35-like
LOC104900365	-2.976613	phospholipase D (PLD-epsilon)	phospholipase D alpha 4
LOC104900624	-2.963081	Protein DMP4*	uncharacterized protein
LOC104902219	-2.894875	MLP-like protein*	MLP-like protein 43
LOC109134982	-2.732998	Extensin-1*	repetitive proline-rich cell wall protein 1-like
LOC104902171	-2.680168	transcription factor (bHLH)	transcription factor PIF3
LOC109135155	-2.661970	protein kinase (WAK/WAKL)	uncharacterized protein
LOC104884961	-2.659433	no hits	uncharacterized protein
LOC104902279	-2.605948	GASA precursor polypeptide	peamaclein
TOP20 up-regulated genes <i>P. betae</i> alone vs Control			
IDENTIFIER	logFC	DESCRIPTION – Mapman	DESCRIPTION – ncbi
LOC104905546	5.229937	anion transporter (NRT1/PTR)	protein NRT1/ PTR FAMILY 7.3-like
LOC104885894	4.807008	no hits	uncharacterized protein
LOC104898578	4.447281	glycosyltransferase	UDP-glycosyltransferase 73C2
LOC104889122	3.942373	Putative germin-like protein 2-1*	putative germin-like protein 2-1
LOC104897041	3.288200	GDSL esterase/lipase*	GDSL esterase/lipase
LOC104892975	2.978898	no hits	uncharacterized protein
LOC104904417	2.867068	no hits	uncharacterized protein
LOC104899212	2.758942	class tau glutathione S-transferase	glutathione S-transferase U8
LOC104883635	2.623483	glutaredoxin	glutaredoxin-C11
LOC104894249	2.563844	oxidoreductase acting on aldehyde or oxo group of donor	aldehyde dehydrogenase family 2 member B4, mitochondrial
LOC104901601	2.532880	no hits	uncharacterized protein

LOC104904826	2.368139	Pathogenesis-related protein PR-1 type*	pathogenesis-related protein PR-1 type
LOC104908694	2.292147	cytosolic polyamine oxidase (PAO1)	polyamine oxidase-like
LOC104890889	2.196618	Dynamin-related protein 4C	ynamin-related protein 4C
LOC104887958	2.132711	no hits	uncharacterized protein
LOC104904340	2.066261	transcription factor (MYB-related)	protein RADIALIS-like
LOC104905250	1.993011	no hits	fasciclin-like arabinogalactan protein 21
LOC104892034	1.974705	pEARL1-like lipid transfer protein 2*	14 kDa proline-rich protein DC2.15
LOC104902001	1.960860	Glu S.griseus protease inhibitor*	glu S.griseus protease inhibitor
LOC104887960	1.947601	transcription factor (ERF)	ethylene-responsive transcription factor ERF098

Supplementary Table 9. TOP 20 of the most down- and up-regulated *B. vulgaris* genes in plants infected with both *P. betae* and BNYVV in comparison to mock-inoculated plants.

TOP20 down-regulated genes <i>P. betae</i> + BNYVV vs Control			
IDENTIFIER	logFC	DESCRIPTION – Mapman	DESCRIPTION – ncbi
LOC104882862	-5.530511	no hits	uncharacterized protein
LOC104904208	-5.382440	beta-galactosidase (BGAL)	uncharacterized protein
LOC104889603	-3.852079	transcription factor (bHLH)	transcription factor bHLH101
LOC104892481	-3.801442	oxidoreductase acting on paired donor with incorporation or reduction of molecular oxygen	CYP72A219-like
LOC104887305	-3.745222	plasma membrane intrinsic protein (PIP)	aquaporin PIP1-2
LOC104905072	-3.598602	class-C-I small heat-shock-responsive protein	18.3 kDa class I heat shock protein
LOC104891851	-3.593080	BURP domain protein USPL1*	BURP domain protein USPL1
LOC104895160	-3.546685	no hits	mannose/glucose-specific lectin
LOC104883456	-3.422643	no hits	uncharacterized protein
LOC104887949	-3.360988	phosphate transporter (PHT1)	probable inorganic phosphate transporter 1-3
LOC104896999	-3.232256	class-ER small heat-shock-responsive protein	17.6 kDa class I heat shock protein
LOC104887571	-3.128492	no hits	protein LNK3 ; protein LNK4
LOC104905068	-3.108410	Pathogenesis-related protein 1C*	pathogenesis-related protein 1C
LOC104891760	-3.082512	no hits	protein GOS9
LOC104907728	-3.081873	no hits	uncharacterized protein
LOC104891824	-3.076825	no hits	uncharacterized protein
LOC104884995	-3.058449	no hits	uncharacterized protein
LOC104887760	-3.048779	Antiviral protein alpha*	ribosome-inactivating protein lychnin-like
LOC104901408	-3.023264	transcription factor (MYB-related)	protein LHY
LOC104901308	-2.981664	no hits	uncharacterized protein

Supplementary information – CHAPTER V

TOP20 up-regulated genes <i>P. betae</i> + BNYVV vs Control			
IDENTIFIER	logFC	DESCRIPTION – Mapman	DESCRIPTION – ncbi
LOC104900551	5.970368	hydrolase acting on peptide bond (peptidase)	zingipain-2
LOC104889122	5.788924	Putative germin-like protein 2-1*	putative germin-like protein 2-1
LOC104904467	5.363815	no hits	uncharacterized protein
LOC104904827	5.277591	Pathogenesis-related protein PR-1 type*	pathogenesis-related protein PR-1 type
LOC104889751	5.166398	solute transporter (UmamiT)	WAT1-related protein At1g68170-like
LOC104905663	4.808010	glycosylase	glucan endo-1,3-beta-glucosidase
LOC104900269	4.754115	Cationic peroxidase 1*	cationic peroxidase 1
LOC104908474	4.577608	Matrixin-type metalloprotease	metalloendoproteinase 1-like
LOC104892312	4.526055	Thaumatococcus-like protein*	thaumatococcus-like protein 1
LOC104904828	4.460779	Pathogenesis-related protein PR-1 type*	pathogenesis-related protein PR-1 type
LOC104900344	4.459196	no hits	uncharacterized protein
LOC104901959	4.289308	Pathogenesis-related protein PR-1 type*	pathogenesis-related protein PR-1 type
LOC104890043	4.178907	glycosylase	glucan endo-1,3-beta-glucosidase
LOC104883635	4.109535	glutaredoxin	glutaredoxin-C11
LOC104887732	3.990455	NA	uncharacterized protein
LOC104888888	3.977193	Acidic endochitinase SP2*	acidic endochitinase SP2
LOC104883479	3.973833	Auxin-binding protein ABP19b*	auxin-binding protein ABP19b-like
LOC104894255	3.971373	sugar efflux transporter (SWEET)	bidirectional sugar transporter SWEET5
LOC104904469	3.934609	no hits	uncharacterized protein
LOC104898578	3.917387	glycosyltransferase	UDP-glycosyltransferase 73C2

Supplementary Table 10. Regulation of genes encoding proteins belonging to pathogenesis-related (PR and PR-like) families in response to inoculation with *P. betae* alone (PBvsC) or *P. betae* + BNYVV (PBBNvsC) inoculation. Significant up-regulation (up) and down-regulation (down) in comparison to mock-inoculated plants are summarized. PR and PR-like were identified through tBLASTn searches on *B. vulgaris* refseq_rna (E-value < e^{-6}).

These results were kept in a separate excel file since they are too large to be included in this manuscript. We will be happy to send a copy of this additional file on request.

Supplementary Table 11. Regulation of genes belonging to resistance genes analogues (RGA) families in response to inoculation with *P. betae* alone (PBvsC) or *P. betae* + BNYVV (PBBNvsC) inoculation. Significant expression up-regulation (up) and down regulation (down) in comparison to mock-inoculated plants are summarized. RGAs were identified and classified by Dohm et al. (2014) according to the presence of domains known to be involved in resistance to pathogens: coiled-coil (CC), leucine zipper (LZ), nucleotide-binding site (NBS), leucine rich repeat (LRR), serine-threonine kinase (STK), Toll/Interleukin-1 receptor (TIR) and transmembrane (TM) domains.

These results were kept in a separate excel file since they are too large to be included in this manuscript. We will be happy to send a copy of this additional file on request.

Supplementary Table 12. DEGs belonging to all principal TF families in response to *P. betae* alone (PBvsC) or *P. betae* + BNYVV (PBBNvsC) inoculation. Significant expression up-regulation (up) and down regulation (down) in comparison to mock-inoculated plants are summarized. TF were classified according to MapMan4. *P. betae* and BNYVV (*P. betae* alone) inoculation.

These results were kept in a separate excel file since they are too large to be included in this manuscript. We will be happy to send a copy of this additional file on request.