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REGULAR ARTICLE

Impact of high temperature on sucrose translocation, sugar content and inulin yield in *Cichorium intybus* L. var. *sativum*

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Abstract
Background and aim *Cichorium intybus* is a biennal species storing inulin in taproot during the first year and flowering after vernalization. Heat impact on sugar distribution and inulin yield remains poorly documented.
Methods Plants were cultivated under ambient or high (ambient+5 °C) temperature for 27 weeks. Plants were monthly harvested and morphological parameters, bolting rate, sugar translocation, soluble sugars and inulin content were determined.
Results Heat reduced shoot and root growth and unexpectedly induced precocious bolting. It increased fructose contents in roots and leaves, increased root *myo*-inositol and reduced leaf sucrose content. At harvest, inulin content was higher in heat-treated than in control roots but total amount of inulin produced per plant was lower. Heat inhibited sugar translocation from leaves to secondary roots. Total soluble sugar content was lower in leaves but higher in roots of bolted plants compared to non-bolted ones. Bolting induced an increase in the mean degree of polymerization of inulin and root lignification.
Conclusion High temperatures impaired inulin production as a result of root growth inhibition and reduced sugar translocation from the leaves to the roots. Heat induced precocious bolting on non-vernalized plants. Bolting reinforced root growth inhibition and thus inulin yield decrease.

Keywords *Cichorium intybus* · Climate change · Flowering · Global warming · Heat stress · Inulin · Root chicory

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Introduction

Since 1980s, root chicory is mainly cultivated for inulin production (Desprez et al. 1999). Inulin is a polysaccharide type fructan that the plant stores in its taproot. It constitutes a polymer of fructose linked by β (2 → 1) bonds with a terminal glucose (Hendry 1993; Vergauwen et al. 2003). Depending on the degree of polymerization (DP), industry is interested by inulin for food and non-food applications (Stevens et al. 2001). Fructans have some health-promoting effects (Van Arkel et al. 2013) and inulin is widely used as prebiotic agent with numerous antioxidant properties (Stoyanova et al. 2011; Roberfroid and Delzenne 1998).

Root chicory is a biennal plant that grows as a leafy rosette and stores inulin during its first year

of growing season. Inulin is then used to sustain bolting and flowering occurring after vernalization during the second year of the cycle (Van Laere and Van den Ende 2002). Druart et al. (2001) showed that the biosynthesis of inulin is induced when the radial root growth starts approximatively 7 weeks after sowing. Van Arkel et al. (2012) distinguish 3 phases for inulin metabolism. The first phase starts with the inulin biosynthesis and is characterized by a steady increase of DP and yield. The second phase is characterised by a decrease of DP while inulin yield still increases. Then during the last phase, the DP continues to decrease and the yield becomes constant. In root chicory, the mean inulin polymer length is around 9–10 at harvest and the average yield is about 11,000 kg per hectare (Wilson et al. 2004; Van Arkel et al. 2012).

Root chicory has now been introduced in numerous countries from the Southern hemisphere where extremely high temperatures may occur during the growing seasons (Patel et al. 2000; Wang and Cui 2011; Khagami et al. 2012; Seghatoleslami et al. 2014). Even in temperate countries, rising temperatures and changing precipitation patterns are expected in the future to have negative impacts on crop yield. Global warming is indeed an unequivocal phenomenon compromising food security (Luo et al. 2017). Climate changes will not only increase the mean temperatures but it will also increase the frequencies of drought events or, alternatively, increase local precipitations leading to more frequent flooding stress in some areas (Pathak et al. 2018).

Several data are available regarding water stress impact on root chicory. Schittenhelm (1999) indeed provided clear evidences that water stress hampered agronomic performances of root chicory in the field. Monti et al. (2005) reported that moderate water stress intensity affected stomatal conductance leading to a transient decrease in photosynthetic activity but did not affect yield-related parameters at the final harvest. In contrast, Vandoorne et al. (2012a) demonstrated that high water stress intensity inhibited enzyme activities involved in inulin synthesis (sucrose:sucrose 1-fructosyltransferase (EC 2.4.1.99) and fructan:fructan 1-fructosyltransferase (EC 2.4.1.100)) and slightly increased inulin-degrading fructan 1-exohydrolase activity (EC 3.2.1.153). Moreover, Vandoorne et al. (2012b) showed that the root system of *C. intybus* displays a high plasticity under water shortage and that a passive compensation mechanism at the root level may start before the plants have to reduce their transpiration rate. Root plasticity was also observed in flooded conditions since taproot appeared shorter and larger but final quantity of inulin produced per plant remained unaffected even if the mean degree of polymerization (DP) of the inulin chain was reduced by flooding (Vandoorne et al. 2014). Lignification was also sometimes observed in the upper part of the root and compromises industrial process of inulin extraction after harvest (Wang and Cui 2011; Van Hercke, personal communication).

Surprisingly, fewer information are available regarding impact of high temperatures on root chicory although elevated ambient temperatures such as those expected under climate change may trigger numerous molecular responses in higher plants (Fei et al. 2018). As a biennal plant species, *C. intybus* encounters a vernalization process by low temperature to allow flowering after winter during the second year of its cycle. Several studies thus focused on vernalization and impact of cold events occurring at the young seedling stage and which could induce premature unwanted bolting and flowering during the first year of the cycle (Neefs et al. 2000; Dielen et al. 2005; Périlleux et al. 2013). Such a premature flowering is expected to reduce root yield and affect carbohydratye composition (Wilson et al. 2004) but quantitative data regarding this impact are lacking. Vernalization is not the only process leading to flowering in *C. intybus*. Indeed, Mathieu et al. (2014) demonstrated that heat stress may also induce flowering in non-vernalized plants of *C. intybus*. The molecular basis of such a heat-induced flowering process still needs to be identified. Its impact on the root soluble sugar and inulin content also requires further investigation in relation to sugar distribution in the root profile.

Inulin synthesis in the root is highly dependent on sugar translocation from the leaves. In *C. intybus*, Mathieu et al. (2014) showed that photosynthesis remained unaffected or even increased at high temperature. The sink-to-source relationships however remained poorly documented. Wei et al. (2016) recently demonstrated that

genes coding for sucrose transporters and cell wall invertases putatively involved in phloem loading and unloading are differentially regulated during plant development and may, to some extent, be controlled by environmental parameters such as N starvation and cold but no data are available regarding the influence of high temperatures.

This present study was therefore undertaken in order to i) determine the influence of high temperature on carbohydrate profile in roots and leaves of *C. intybus* during the growing period at monthly intervals; ii) quantify the impact of heat stress on quantitative and qualitative yield-related parameters at the end of the growing period; iii) precise the impact of heat stress-induced bolting and flowering on these properties and iv) analyze the impact of high temperature on sugar translocation. Considering the physiological plasticity of the chicory taproot, sugar content was analyzed in three parts (upper, intermediate and lower parts) of the root.

Material and methods

Plant material and growing conditions

Seeds of *C. intybus* L. var. *sativum* (cultivar Melci provided by Warcoing S.A.; Belgium) were sown at the end of February on a sand and loam substrate (3:1, *v*/v) in a growth chamber (Economic Delux model ECD01E; Snijders Scientific, Tilburg, Netherlands) at 17 °C. Three weeks later, young seedlings were transplanted in pots containing 2 L of the same substrate and placed in a temperate greenhouse during one week. After that, half of the plants were kept in this temperate greenhouse for control condition and half of the plants were transferred in a heated-greenhouse for heat treatment. Both greenhouses were equipped with lamps (Philips HPLR 400 W) to obtain a 16 h-photoperiod and a minimum PAR of 220 $\mu mol.m^{-2}.s^{-1}$. Temperatures and relative humidity were recorded in both greenhouses every 15 min (Temperature Probe SDTS and hydrometer HIH7000 supervised by the ARIA software). The maximum, minimum and mean temperatures and relative humidity per week are presented in Fig. 1. The mean, maximum and minimum temperatures were on average 5 °C higher in the heated greenhouse than in the control one during all the experiment (Fig. 1). The mean and minimum relative humidity remained similar in control and heated greenhouses while the maximum relative humidity was slightly higher in the heated greenhouse at the beginning of the experiment only.

Ten days after sowing, three plants per treatment were harvested (between 10.0 am and 11:30 am) each month until the end of the experiment (27 weeks after sowing). The leaves and the roots of each plant were separated. The main roots were divided in 3 sections of equal size: the upper, the intermediate and the lower parts.

Plant growth and water content

The number of rosette leaves and the area of the youngest fully expanded leaf were recorded on 6 plants per treatment every two weeks. The number of rosette leaves was used to determine the plastochron index by dividing the number of days between two measurements by the number of leaves appearing between the measurement times. At each harvest, total root mass was measured on each plant. The youngest fully expanded leaf was also weighed to determine fresh mass (FW) and further incubated during 48 h in an oven at 72 °C to determine dry mass (DW). The rate of bolting (plants with a visible developing floral stem) was also recorded each two weeks.

To assess precocious lignification of the root system, transversal hand cross-sections were performed using a razor blade just below the crown and stained with phloroglucin 1% in HCl 6 N during 10 min according to Rogers et al. (2005). The intensity of the colouring of the stained zone was evaluated to the naked eye or under the binocular and bright-field illuminated cross-sections were categorized in two groups exhibiting low (light orange) or high (dark brown) coloration.

Sugar content

Leaves and roots samples (c.a. 0.4 g FW) were ground to a fine powder in liquid nitrogen and extracted three times with 4 mL of 70% ethanol (*w*/*v*) and centrifuged fort 15 min at 6600 *g* at 4 °C. For total soluble sugar quantification, 200 μL of the supernatant were added to 1 mL of anthrone solution (0.5 g anthrone, 250 mL H_2SO_4 95% and 12.5 mL distilled water) during 10 min at 100 °C

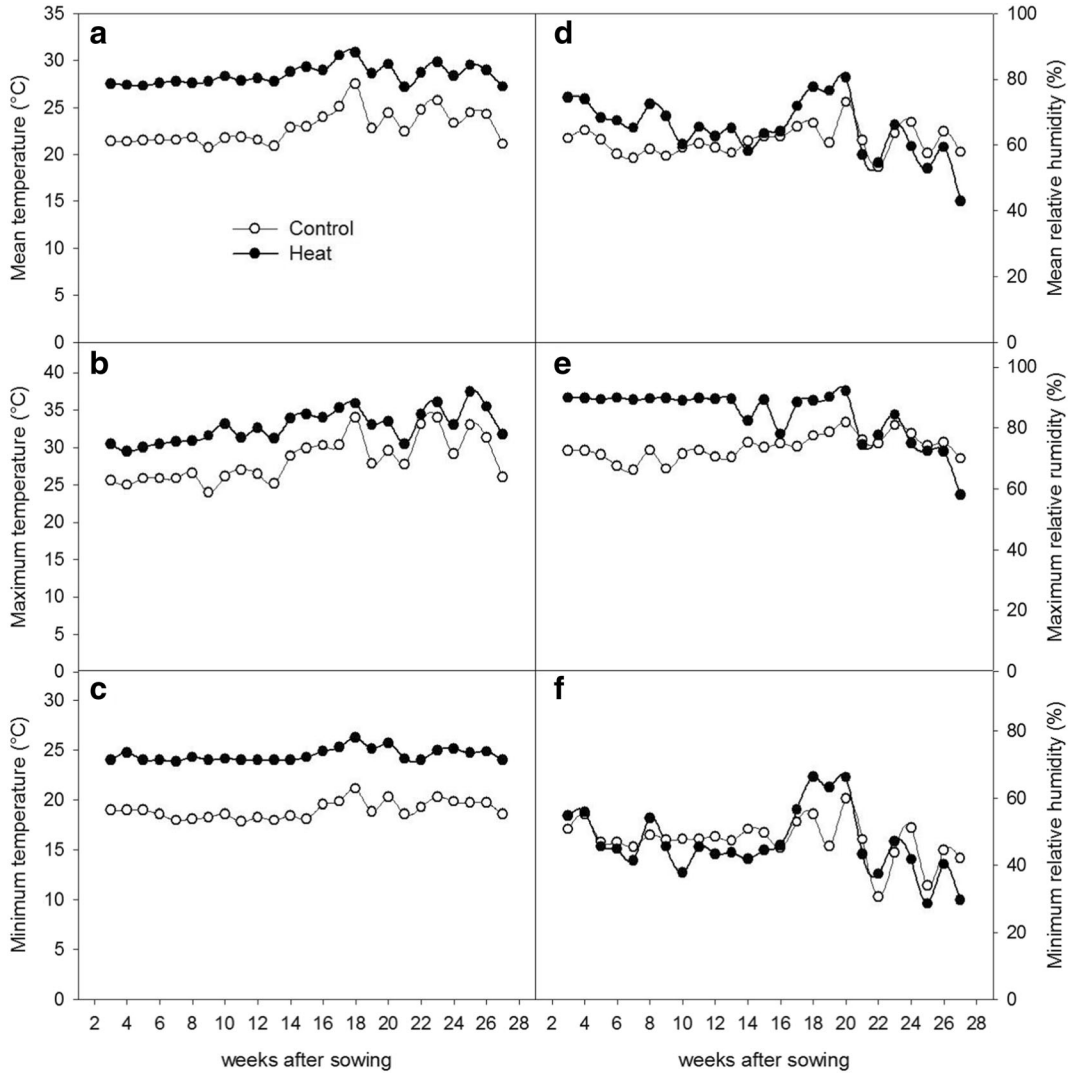

Fig. 1 Changes in the temperatures and the relative humidity in the control and heated greenhouses. All values were estimated on a weekly basis **a**: mean temperature; **b**: maximum temperature **c**: minimum temperature **d**: mean relative humidity; E: maximum relative humidity and F: minimum relative humidity

according to Yemm and Willis (1954). The absorbance was read at 625 nm (Spectophotometer UV, 1800, Shimadzu, Kyoto, Japan) and a standard curve was established using glucose. Sugar concentration was expressed on a DW basis considering the water content of the analysed samples.

The remaining supernatants were dried for identification and quantification of individual free soluble sugars (glucose, fructose, sucrose, kestose, raffinose, galactose and *myo*-inositol) and for inulin quantification. For inulin quantification, the dried supernatant was diluted in water and digested with inulase (Fructozyme, Sigma) during 1 h at 37 °C to completely depolymerize all the inulin in single components (glucose and fructose). Mono-, di- and trisaccharide concentrations were determined by gas chromatography (Perkin Elmer, Autosystem XL, Colonne CP-Sil5 – 25 m × 0.32 mm ID. × 0.25 μm, Whatham, USA) using helium as a carrier gas at a flow rate of 1 mL. min^{-1}. Two microliters were injected in a FID detector with a split ratio of 1:20. The injector and flame ionization detector temperatures were 270 and 350 °C, respectively. The oven temperature was held at 120 °C for 3 min and then incremented to 230 °C at 3 °C.min^{-1}. The oven was maintained at

this temperature for 12 min, then heated to 300 °C at 20 °C.min^{-1} and held for 15 min.

The mean degree of polymerization (DP) was calculated according to Baert (1997):

$$DP\text{mean} = \frac{F_{\text{hydrol}} - F_{\text{free}} - S_{\text{free}}}{G_{\text{hydrol}} - G_{\text{free}} - S_{\text{free}}} + 1$$

where F_{hydrol} and G_{hydrol} are the concentrations in fructose (F) and glucose (G) after the hydrolysis with inulase and F_{free}, G_{free} and S_{free} are the concentrations of free fructose (F), glucose (G) and Sucrose (S) estimated before hydrolysis. The percentage of inulin in the root was estimated using a formula adapted from Baert (1997):

$$Percentage_{\text{inul}} = \frac{\left(F_{\text{hydrol}} - F_{\text{free}} - S_{\text{free}} + G_{\text{hydrol}} - G_{\text{free}} - S_{\text{free}}\right) * \left(180 - \frac{18^{*}(DP_{\text{mean}} - 1)}{DP_{\text{mean}}}\right)}{10000^{*}(1 - WC)}$$

considering that each addition of one fructose unit to the inulin chain induces the loss of one molecule of water (18 g.mole^{-1}).

[^{14}C]sucrose transport

Sucrose transport was quantified on young seedlings issued from seeds germinated in glass dishes on distilled water at 17 °C during 2 weeks. Seedlings were then transferred on polystyrene plates floating on aerated half-strength Hoagland nutrient solution containing: 5 mM KNO_3, 5.5 mM $Ca(NO_3)_2$, 1 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4$, 25 μM KCl, 10 μM H_3BO_4, 1 μM $MnSO_4$, 0.25 μM $CuSO_4$, 1 μM $ZnSO_4$, 10 μM $(NH_4)_6Mo_7O$ and 1.87 g l^{-1} Fe-EDTA. Seedlings were kept at 17 °C in a plant growth chamber (Economic Delux model ECD01E; Snijders Scientific, Tilburg, Netherlands) during 2 weeks before half of them were transferred to a similar growth chamber but exposed to 38 °C for heat treatment during 10 days. Forty day-old plants with 5 leaves were used for sucrose transport quantification.

A small portion of the upper epidermis (*c.a.* 2 cm^2) of the youngest fully expanded leaf was abraded with Carborundum (0.06 mm) as described by Grusak et al. (1990). Ten microliter of a solution containing 37 kBq of [^{14}C]Sucrose in 20 mM-KOH buffer (pH 5) was applied on the abraded part of the leaf. Six hours after application, leaves (treated leaf, young leaves and old leaves harvested separately), roots (main root and secondary roots separately) and crown were harvested. Samples were incubated during 16 h at 60 °C with a buffer containing 30% H_2O_2, 10% Triton-X100 and $HClO_4$ in the ratio 2:2:1 by vol. After cooling and addition of 4 ml of scintillation liquid (Ultima Gold, PerkinElmer), level of ^{14}C was counted by liquid scintillation spectroscopy using an external standard for correction of quenching (Tri-carB 1600TR Liquid Scintillation Analyzer; Packard Instruments). Radioactivity of each parts of the plant was then expressed as a percentage of total ^{14}C measured in the plant.

Statistical treatment

Normality of the data was verified using Shapiro-Wilk tests and data were transformed when required. ANOVA 1 and ANOVA 2 were performed at a significance level of $p < 0.05$; $P < 0.01$ or $P < 0.001$ using SAS Enterprise Guide 6.1 (SAS 9.4 system for windows). For the ANOVA1, the treatment (presence or absence of stress) or the physiological state (bolting or non-bolting) were considered as factor. For the ANOVA2, the treatment (presence or absence of stress) and the part of the root (superior part, intermediate part and inferior part) were considered as factor. Post-hoc analyses were performed using Student-Newman-Keuls test at a 5% level. Results are presented as means ± standard errors.

Results

Plant growth and development

Heat hastened leaf production (Fig. 2a) but reduced the mean leaf area (Fig. 2b). Whatever the treatment leaf production transiently decreased 14–16 weeks after sowing and then re-increased. It has to be mentioned that the decrease of plastochron occurred earlier in heat-treated than in control plants (Fig. 2a). The leaf area of the youngest unfolded leaf of control plants increased

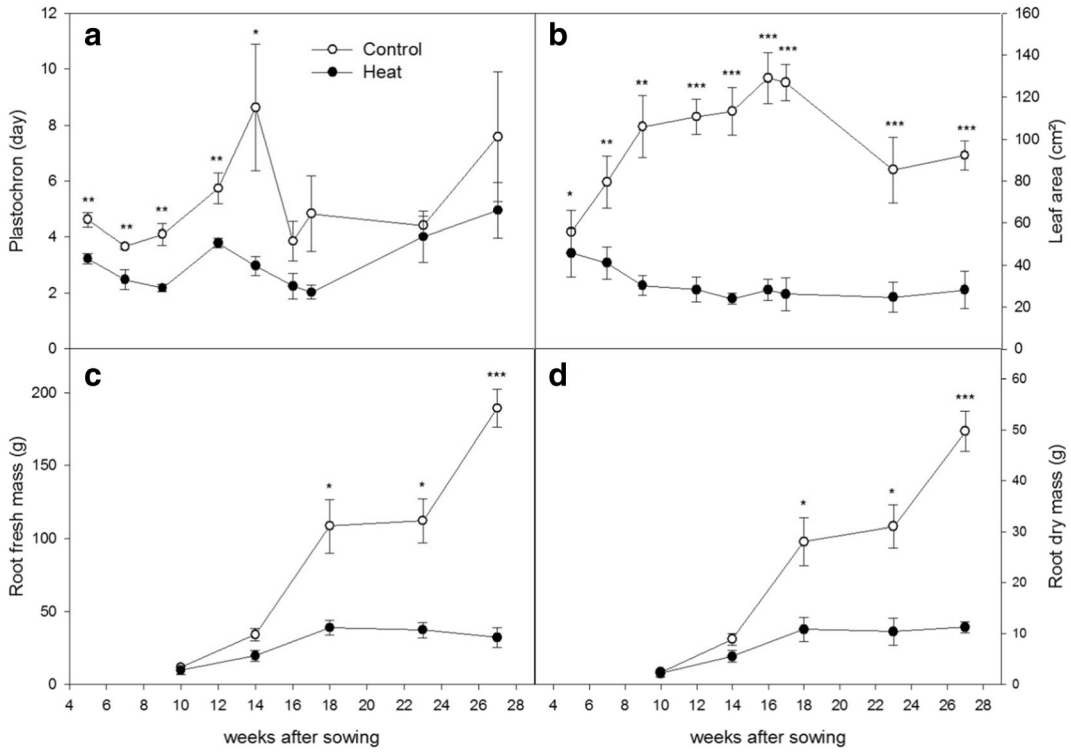

Fig. 2 Changes in the plastochron (**a**), area of the youngest fully expanded leaf (**b**) and root fresh (**c**) and dry masses (**d**) in control and heat-treated plants of *C. intybus*. Heat treatment starts 4 weeks after sowing. Each value is the mean of 3 replicates and vertical bars are standard errors. The stars indicate the presence of a statistical difference between the treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

during the first 18 weeks of culture and then decreased. In contrast, the leaf area of heat-treated plants remained more or less constant (Fig. 2b). The leaves of heat-treated plants exhibited a purple coloration and were more rigid and curly compared to control plants (Fig. S1). The total leaf fresh mass markedly increased up to 18 weeks and was higher in control plants (87.0 ± 12 g) than in heat-treated plants (63.1 g ± 9 g). It then only slightly increased until the end of the experiment and remained higher in control than in stressed plants.

The mean root fresh and dry masses were also clearly lower in heat-treated plants than in control ones, the difference starting to be significant after 18 weeks (Fig. 2c, d). In control plants, root fresh mass increased from 10 weeks after sowing until the end of the experiment while it was not the case in treated plants. Heat did not affect the water content of leaves and roots ($F = 0.07$, $p = 0.78$ and $F = 0.51$, $P = 0.4844$ for leaves and roots respectively) which fluctuated between 80 and 85% for leaves and between 70 and 75% for roots (detailed data not shown).

No bolting was observed in control plants. In contrast, bolting was observed in heat-treated plants from 20 weeks after sowing until the end of experiment. The bolting rate was 33% 23 weeks after sowing and 42% at the end of the experimental period (Fig. S1). Some bolted plants bore flowers on the floral stem while other did not, suggesting that heat-induced bolting does not always lead to complete flowering process. In addition to bolting, heat also induced development of axillary branches. Total fresh mass of aerial part was slightly higher in bolted (68.4 ± 1.7 g) than in non-bolted plants (60.9 ± 2.8 g) and bolting also reduced the root mass (Table 1).

Two contrasting intensities of lignin coloration were observed in root sections below the crown: low intensity (Fig. 3a) that corresponds to a low accumulation of lignin and high intensity (Fig. 3b). In the control plants, we only observed low intensity of lignin coloration (Fig. 3d). In heat-treated plants, bolted plants always presented a high intensity of lignin. In non-bolted heat-treated plants, low and high intensity of coloration were detected depending on the considered plants.

Table 1 Mean degree of polymerization, inulin content, root fresh mass, root dry mass and quantity of inulin per plant in the roots of bolted and non-bolted plants of *C. intybus* in heat treatment at 27 weeks after sowing (end of the experiment)

	Mean degree of Polymerization (DP)	Inulin content (g inulin/100 g DW)	Root fresh mass (g)	Root dry mass (g)	Quantity of inulin/plant (g)
Non-bolted	9.79 ± 0.36	75.76 ± 0.36	45.89 ± 2.81	11.48 ± 0.05	6.99 ± 0.79
Bolted	11.63 ± 0.31**	63.38 ± 9.75	33.61 ± 5.25*	7.87 ± 1.08*	3.96 ± 0.44**

Each value is the mean of 3 replicates and vertical bars are standard errors. The stars indicate the presence of a statistical difference between the treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

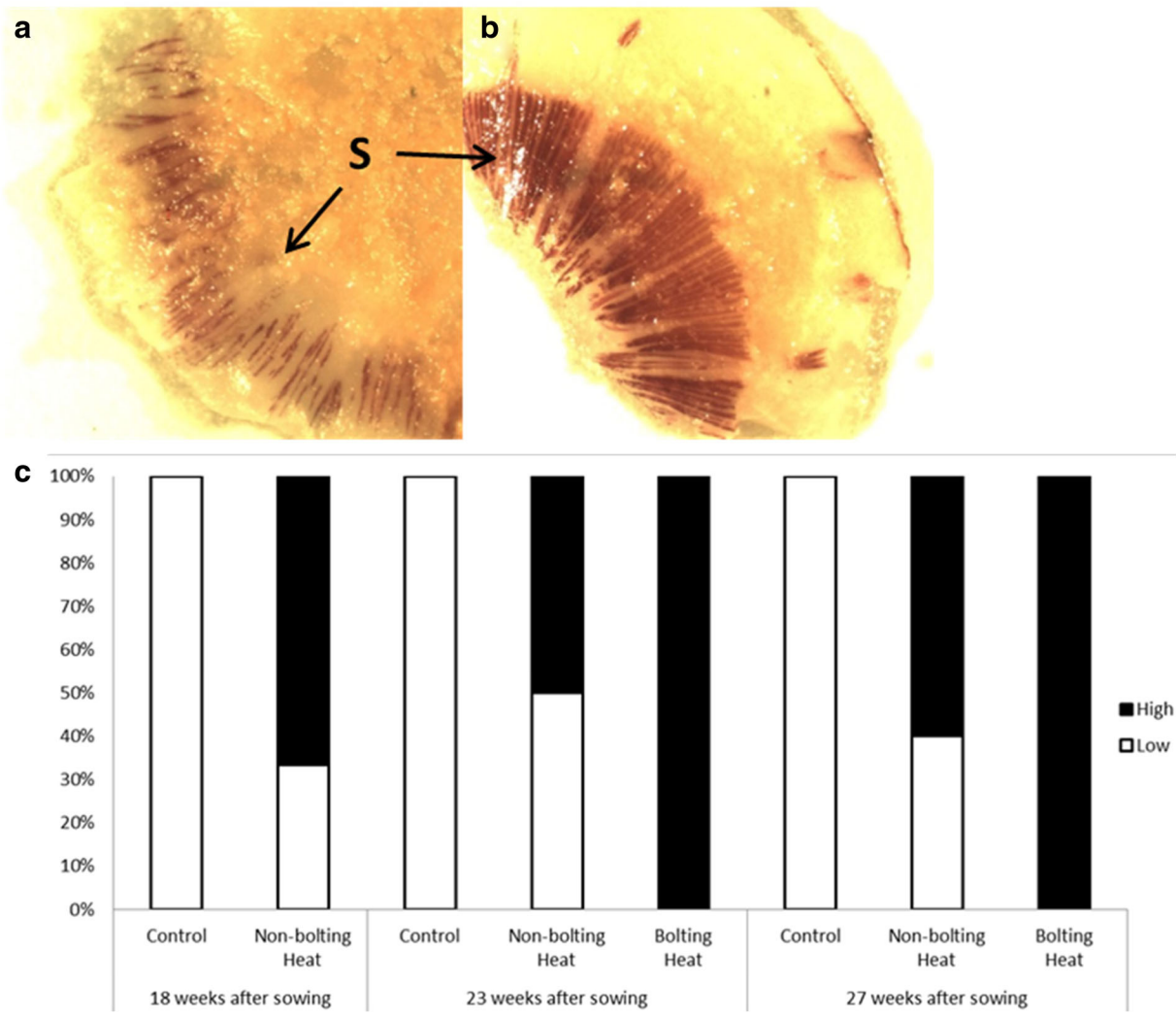

Fig. 3 Lignin accumulation in roots of control and heat-treated plants of *C. intybus*. (**a**-**b**) Root sections below the crown stained with phloroglucin to detect lignin (S). A corresponds to a low intensity of lignin coloration and B corresponds to a high intensity of lignin coloration. (**c**) Changes in lignin coloration intensity in control and non-bolted and bolted heat-treated plants. Heat treatment starts 4 weeks after sowing and bolting start from 20 weeks after sowing

Sugar content and inulin yield

Sugar content was analyzed in leaves and in roots at each harvest date. As bolting could affect the sugar concentration and distribution in the plant, we first analyzed the impact of heat on non-bolted plants and we then investigated the specific impact of bolting in heat-treated ones.

Impact of heat treatment

As shown in Fig. 4, the total soluble sugars in leaves were lower in heat-treated plants than in control 14 and 23 weeks after sowing while an opposite trend was observed at 10 and 27 weeks. In roots, the total soluble sugar concentrations were lower at 10 weeks after sowing and higher at 14, 23 and 27 weeks after sowing for heat-treated plants as compared with controls. When comparing the organ behaviour, an interesting opposite trend was recorded for leaves and roots at 10, 14 and 18 weeks after sowing ($F = 8.45$, $P < 0.004$; $F = 17.18$, $P < 0.0001$; $F = 4.53$, $P < 0.0345$; for 10, 14 and 18 weeks after sowing respectively). In roots, a higher concentration of total soluble sugars was observed in the intermediate comparatively to the upper part in both control and heat-treated plants at 10 weeks after sowing (Fig. S2; $F = 66.79$, $P < 0.0001$). In all root parts total soluble sugar concentrations were higher in the heat-treated plants than in the control ones at the end of the experiment.

Leaf fructose content was higher in heat-treated plants than in controls throughout the experiment duration

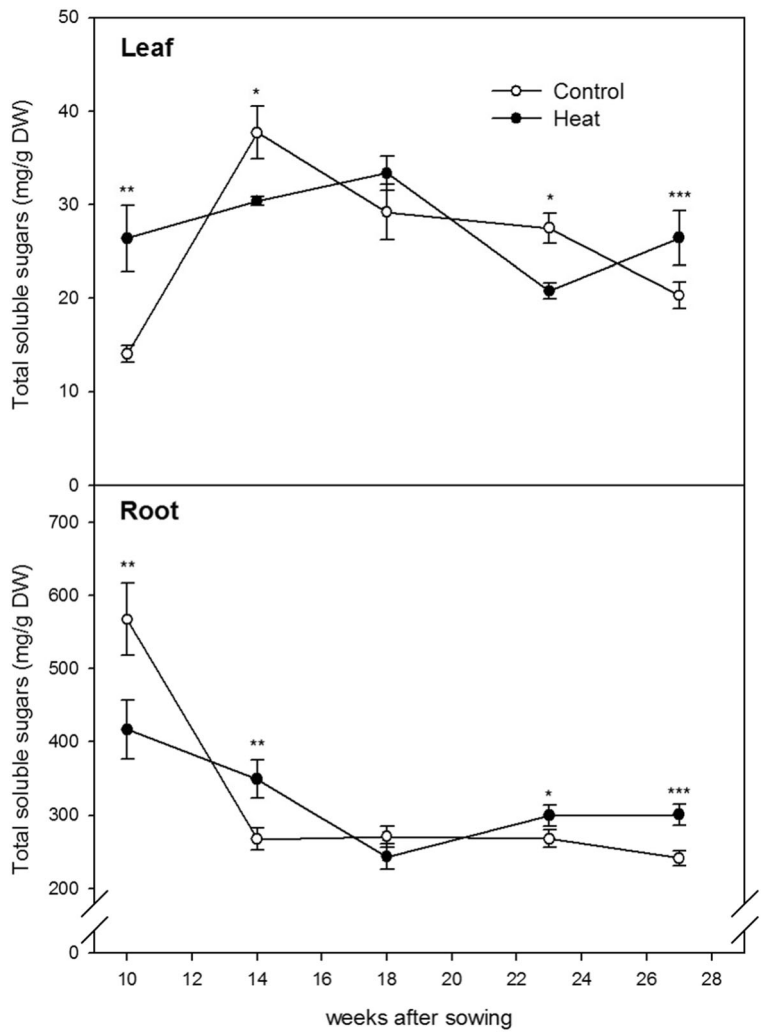

Fig. 4 Changes in the total soluble sugars in the leaves and the roots of control and heat-treated plants of *C. intybus*. Heat treatment was applied 4 weeks after sowing. Each value is the mean of 3 replicates and vertical bars are standard errors. The stars indicate the presence of a statistical difference between the treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

(Fig. 5a). On the opposite, the sucrose content was lower in heat-treated than in control plants, except 10 and 27 weeks after sowing (Fig. 5b). Glucose content increased until 18 weeks after sowing and was at this time higher in control plants than in stressed ones. The leaf glucose content then dropped in control plants and started to be higher in heat-treated ones than in controls (Fig. 5c). *Myo*-inositol content followed the same trend than sucrose (Fig. 5d): the content was higher at 10 and 27 weeks and lower at 14 weeks in heat-treated plants compare to controls (Fig. 5d). Kestose was not detected in leaves. Raffinose and galactose were also detected in leaves with mean contents around 0.98 ± 0.18 μmol g^{-1} DW for raffinose and 1.05 ± 0.16 μmol g^{-1} DW for galactose. The contents of these two sugars remained unaffected in response to heat treatment (data not shown).

Root fructose and *myo*-inositol contents were higher in heat-treated plants than in controls (Fig. 6a and d). For both sucrose and glucose, the recorded contents in roots were lower in heat-treated plants than in controls until week 18. An opposite trend was however recorded at the end of the experiment (Fig. 6b, c). Kestose was also higher in heat-treated plants than in controls at the end of the experiment (Fig. 7a). We also observed that for these 5 sugars, the root content in both control and heat-treated plants decreased from 10 until 18–23 weeks after sowing and then re-increased 27 weeks after sowing (Figs. 6 and 7a). Ten weeks after sowing comparison between different root parts showed that contents for these five sugars were higher in the intermediate and lower parts of the root than in the upper part for both control and heat-treated plants (Fig. S3 and S4; $F = 35.85$, $P < 0.0001$; $F = 17.05$, $P < 0.0001$; $F = 19.66$, $P < 0.0001$; $F = 27.05$, $P < 0.0001$ and $F = 51.34$, $P < 0.0001$ for fructose, glucose, sucrose, *myo*-inositol and kestose respectively). Root raffinose and galactose contents were not affected by heat treatment (data not shown; mean content around 0.26 ± 0.03 μmol g^{-1} DW and 0.15 ± 0.01 μmol g^{-1} DW for raffinose and galactose respectively).

The mean inulin content was lower in heat-treated plants than in controls, except at the end of the experiment where it was higher in heat-treated plants than in control plants (Fig. 7b). The mean degree of

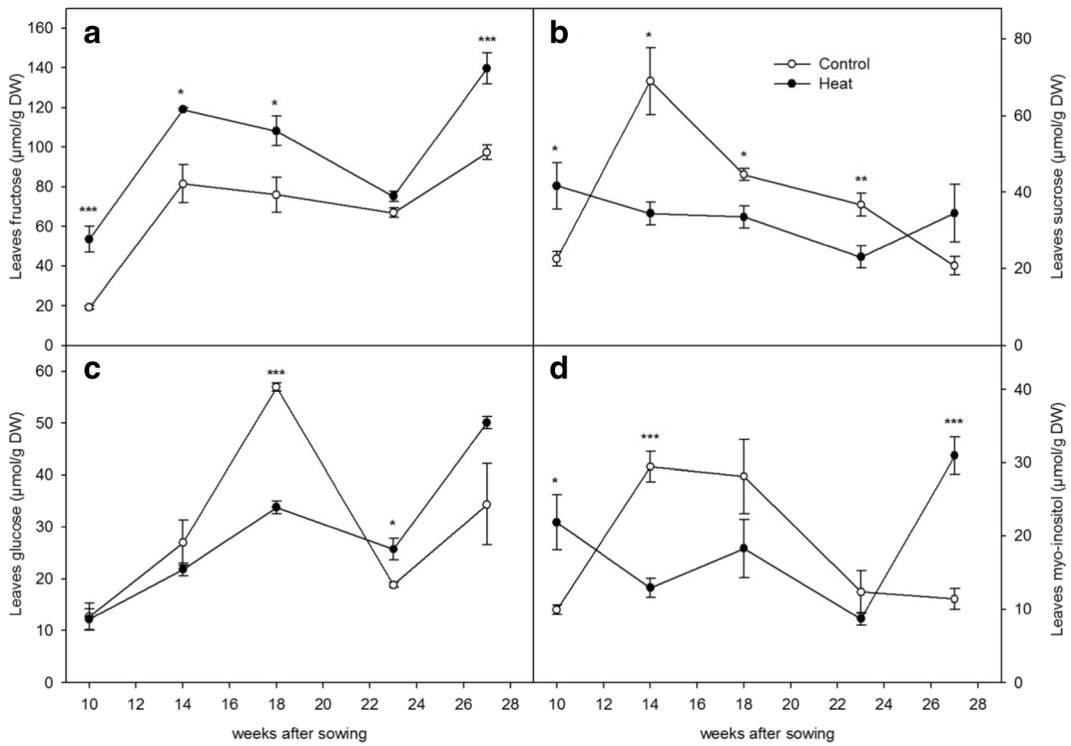

Fig. 5 Changes in soluble sugars content in leaves of control and heat-treated plants of *C. intybus*: (**a**) fructose, (**b**) sucrose, (**c**) glucose and (**d**) *myo*-inositol. Heat treatment started 4 weeks after sowing. Each value is the mean of 3 replicates and vertical bars are standard errors. The stars indicate the presence of a statistical difference between the treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

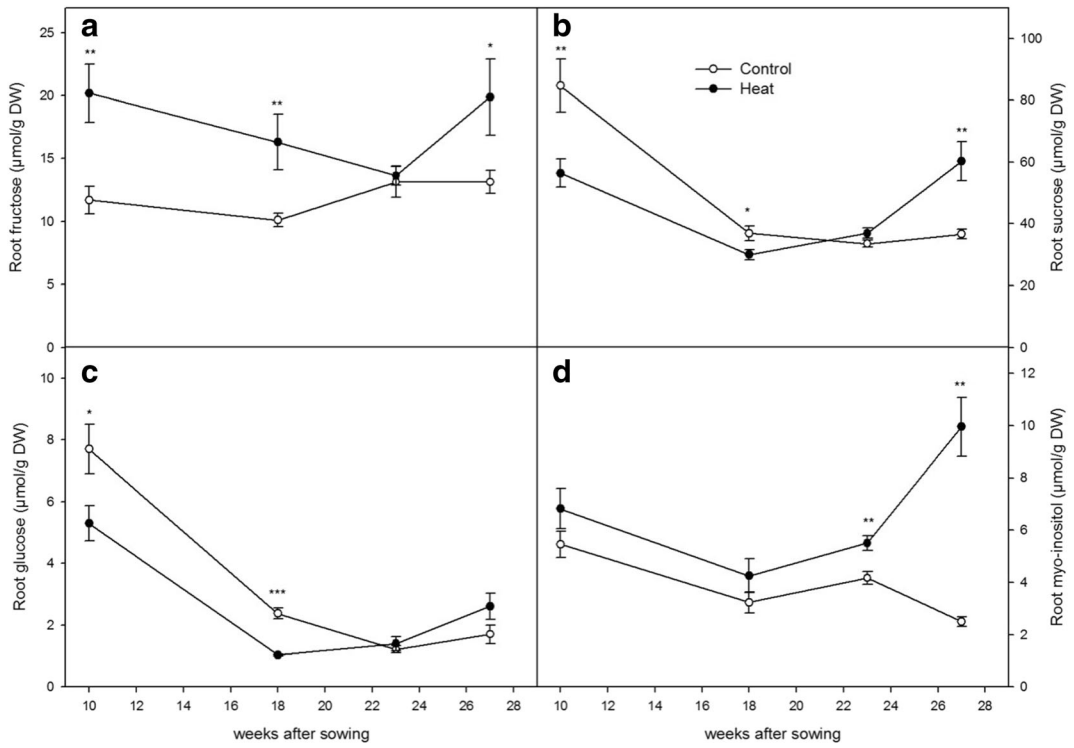

Fig. 6 Changes in soluble sugars content in roots of control and heat-treated plants of *C. intybus*: (**a**) fructose, (**b**) sucrose, (**c**) glucose and (**d**) *myo*-inositol. Heat treatment started 4 weeks after sowing. Each value is the mean of 3 replicates and vertical bars are standard errors. The stars indicate the presence of a statistical difference between the treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

polymerization (DP) remained similar in the two treatments, except after 23 weeks where it was higher in control than in stressed plants (Fig. 7c). These observations were valid for the upper and intermediate parts of the root but not for the lower part (Fig. S4). Indeed in the lower part, inulin content was clearly lower in heat-treated plants than in controls except at the end of the experiment and heat treatment increased DP (Fig. S4). Overall, total amount of inulin per plant was by far lower in heat-treated plants than in controls and the difference increased with time (Fig. 7d). At the end of the experiment, the total amount of inulin per plant was 32.32 ± 2.86 and 6.79 ± 0.79 g/plant for control and heat-treated plants respectively.

Impact of bolting

Total soluble sugar content was lower in leaves but higher in roots of bolted plants compared to non-bolted ones (Fig. 8). The lower content observed in the leaves of bolted plants could be related to a decrease in fructose, glucose and *myo*-inositol concentrations (Fig. 9) while bolting did not affect leaf sucrose content. Similarly, the recorded increase of soluble sugar in roots of bolted plants might be related to a conspicuous increase in fructose content (Fig. 9), whatever the considered root part (Fig. S5). The root contents of glucose, sucrose and *myo*-inositol remained similar in bolted and non-bolted plants and kestose concentration was lower in roots of bolted plants than in roots of non-bolted plants (Fig. 9). Similar results were obtained in the different root parts except for glucose (Fig. S6). In the intermediate part of the root, glucose content was indeed higher in bolted plants compared to non-bolted plants (Fig. S6).

As shown in Table 1, the mean DP was significantly higher in bolted comparatively with non-bolted plants while the recorded decrease in inulin content was not significant. The root fresh mass was significantly lower in bolted plants comparatively to non-bolted ones and the total amount of inulin produced per plant was consequently lower in the former than in the latter. Similar observations were recorded in the upper part of the taproot (Fig. S6) but not in the intermediate or lower parts.

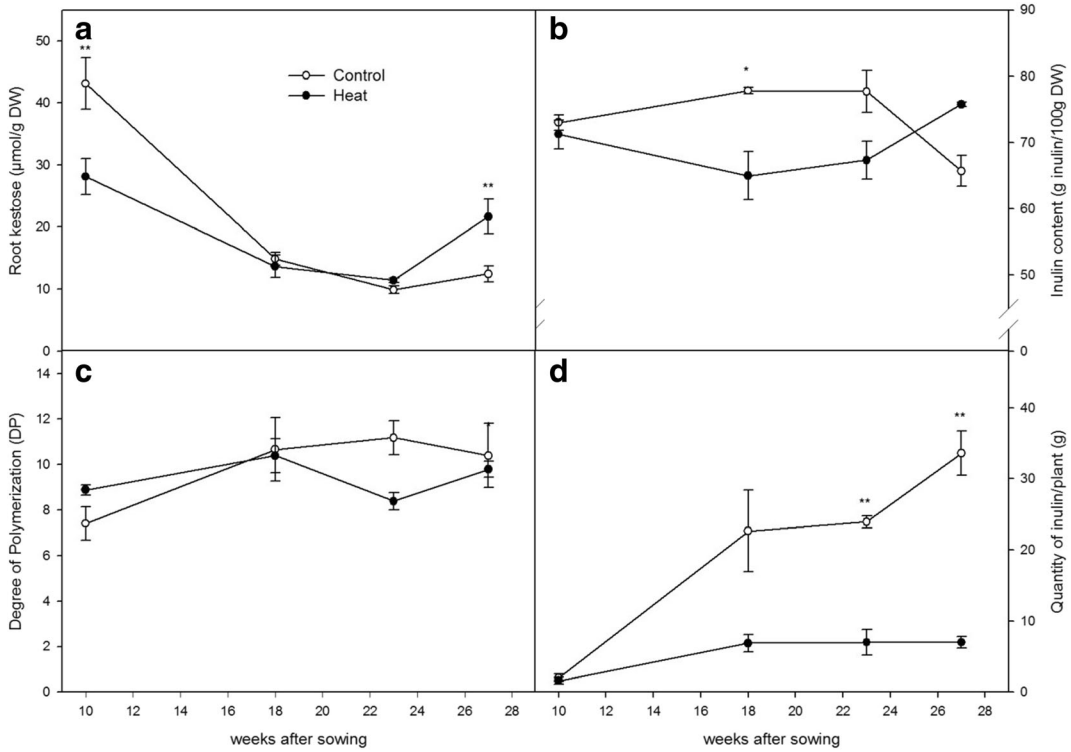

Fig. 7 Changes in kestose content (**a**), inulin content (**b**), mean degree of polymerization of the inulin chains (DP) (**c**) and quantity of inulin per plant (**d**) in roots of control and heat-treated plants in *C. intybus*. Each value is the mean of 3 replicates and vertical bars are standard errors. The stars indicate the presence of a statistical difference between the treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

[^{14}C]sucrose transport

Six hours after application of radiolabelled sucrose, the percentage of radioactivity was higher in old leaves of heat-treated plants than in controls. The percentage was similar for control and heat-treated plants in the treated leaf itself, as well as in the young leaves, the crown and the main root (Fig. 10). However, percentage of radioactivity was 8 times higher in the secondary roots of control plants than in heat-treated ones.

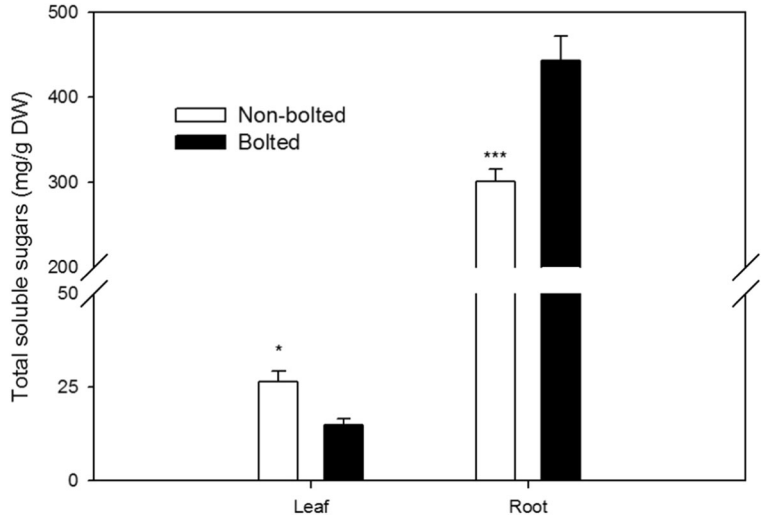

Fig. 8 Total soluble sugars in leaves and in roots of bolted and non-bolted heat-treated plants at 27 weeks after sowing (end of the experiment). Each value is the mean of 6 replicates and vertical bars are standard errors. The stars indicate the presence of a statistical difference between the treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

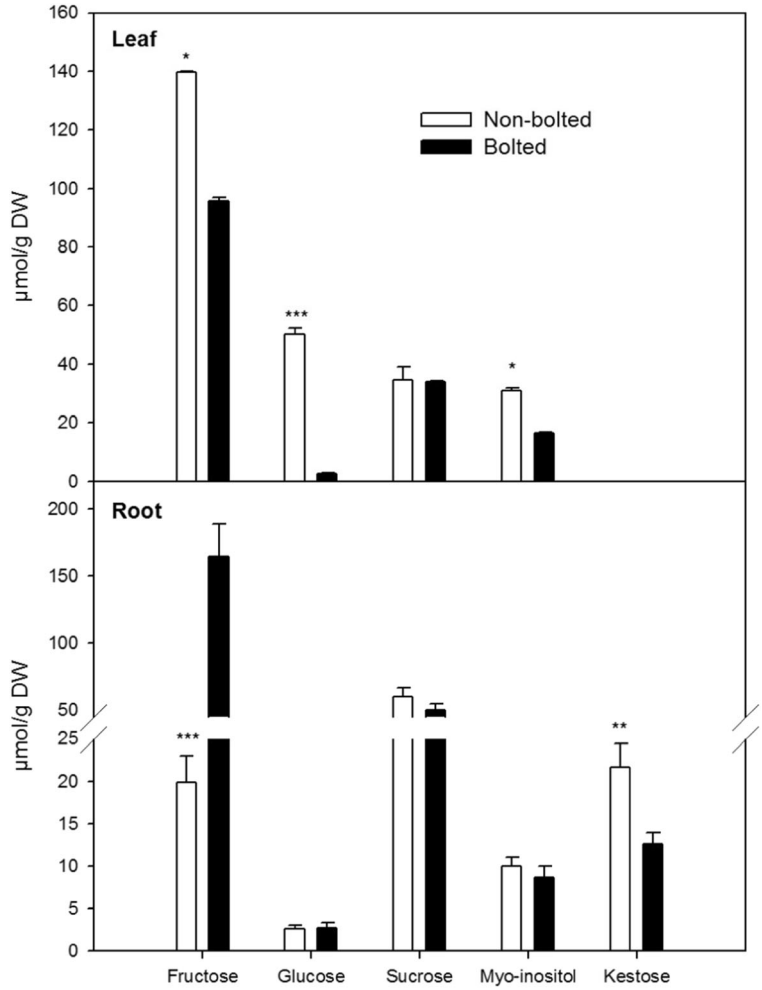

Fig. 9 Soluble sugars content (fructose, glucose, sucrose, *myo*-inositol and kestose) in leaves and roots of bolted and non-bolted heat-treated plants at 27 weeks after sowing (end of the experiment). Each value is the mean of 3 replicates and vertical bars are standard errors. The stars indicate the presence of a statistical difference between the treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

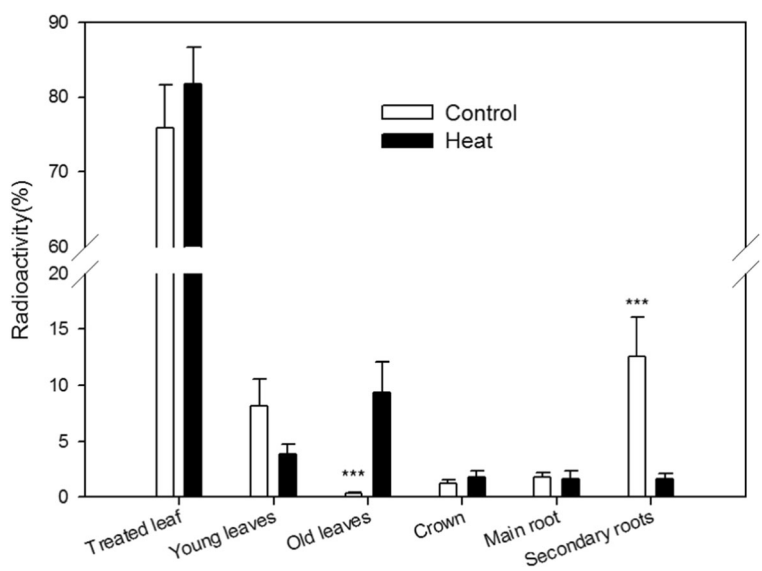

Fig. 10 Translocation of [^{14}C]sucrose between leaves and roots of chicory plants. Radioactivity is expressed as a percentage (%) of total ^{14}C measured in the plant. Level of [^{14}C] was measured in the treated leaf, the young leaves, the old leaves, the crown, the main root and the secondary roots after 6 h of exposure. Each value is the mean of 10 replicates and vertical bars are standard errors. The stars indicate the presence of a statistical difference between the treatments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

Discussion

The present work provides evidences that a difference in the mean temperature of 5 °C during the whole growing season is sufficient to drastically impact plant growth and inulin yield in *C. intybus*. The overall consequences of high temperature on the sugar profile in roots and leaves however fluctuated during plant development suggesting an influence of plant phenology on the biochemical behavior of heat-treated plants.

Mathieu et al. (2014) demonstrated that heat treatment under similar environmental conditions had no negative impact on photosynthesis or even increased photosynthesis efficiency in root chicory. In the present study, heat leads to a higher leaf total soluble sugar content at the beginning and at the end of the experiment but this was not the case anymore during the treatment where leaf total soluble sugars were higher in control than in stressed plants. According to Das et al. (2017), sugar contents in heat-treated plants are frequently reduced as a consequence of an increase in respiration providing energy required by metabolic processes involved in stress resistance. A lower sucrose content recorded after 14, 18 and 23 weeks in the leaves of heat-treated plants supports this hypothesis. Similarly, a lower leaf glucose content recorded in stressed plants until week 23 might result from the stimulation of glycolysis converting glucose into pyruvate while fructose also resulting from sucrose hydrolysis accumulated.

Data regarding heat stress impact on sucrose metabolizing enzymes remain scarce, especially for vegetative organs. Guo et al. (2007) reported that high temperature in tomato decreased leaf invertase (EC 3.2.1.26) and sucrose synthase (SuSy; EC 2.4.1.13). In root chicory, Vandoorne et al. (2012a) reported that even high water stress intensities only had a marginal impact on leaf SuSy, suggesting that this enzyme remains stable under harsh environmental conditions and this is confirmed by Wei et al. (2016) for N starvation. Leaf *myo*-inositol also displayed a typical response, being higher in stressed plants than in control at week 10 and 27, but presenting an inverse trend for the intermediate durations of stress. Many eukaryotes use inositol-based cytosolic solutes as protective compounds under stress conditions (Valluru and Van den Ende 2011). Some *myo*-inositol derivaties such as galactinol, raffinose-family oligosaccharides or pinitol may act as compatible osmoprotectants while other play a key role in signal transduction, membrane trafficking and mRNA-exports (Gillaspy 2011; Valluru and Van den Ende 2011). It might be hypothesized that the high *myo*-inositol content recorded at week 10 corresponds to an attempt to limit the deleterious impact of environmental constraint. After this initial burst, the *myo*-inotol content decreased but then increased again at the end of the growing season when plants were not able anymore to cope with the consequences of a long-term heat stress.

Sugar translocation from source to sink organs is an important factor for plant growth and development. In *C. intybus*, taproot is the main sink organ at the vegetative stage. In other plant species, especially in cereals, impairment of sucrose loading by high temperature was frequently considered as the major cause of photosyntates partitioning disruption leading to sink organ abortion (Suwa et al. 2010). However, Mathieu et al. (2014) hypothesized that heat also affects assimilate partitioning in *C. intybus*. The present work demonstrates that heat impact on sugar translocation did not lead to a decrease in sugar provision of the main root but rather disturbed the sugar supply to secondary (lateral) roots. For practical reason, sugar translocation was here quantified at the seedling stage while root growth was quantified throughout the experiment until week 27. Nevertheless, alteration in sugar supply might explain a strong heat-induced decrease in root growth. Moreover, the fact that ^{14}C-labelled sucrose was translocated from young to old leaves in heat-treated plants only also suggests that source-sink regulation was clearly affected in response to high temperature.

Root sugar content is mainly regulated by root growth, sugar translocation from leaves and sugar polymerization. A strong decrease in the total root soluble sugars was recorded between weeks 10 and 14 suggesting that root growth may be responsible for a diluting effect. At week 14, root soluble sugar content was higher in stressed plants which may be regarded as the consequence of root growth inhibition occurring in stressed plants. Week 18 appeared as a crucial stage for root development: indeed, after 18 weeks, root growth was completely abolished in stressed plants and a slight, although significant increase in root total soluble sugars concentration might be regarded as the consequence of the maintenance of a low sugar supply through phloem.

Although fructose accumulated in the roots of heat-treated plants, such an accumulation did not appear as the consequence of inhibition of inulin synthesis. Indeed, at week 10, fructose content was two times higher in

stressed plants than in controls while inulin concentration was similar in the two types of plants. Conversely, root fructose content was similar in the two types of plants at week 24 while inulin concentration was clearly higher in controls than in stressed plants. Finally, both fructose and inulin content increased in stressed plants at week 27. Hence, fructose and inulin content did not correlate but heat modified their relative proportion and this is an important qualitative aspect since fructose and fructans clearly have different biochemical and physiological properties in the human body (Di Bartolomeo and Van den Ende 2015). At this time, *myo*-inositol strongly increased in the root of stressed plants, as previously discussed for leaves, while it conversely presented minimal values in the root of control plants.

Sugars were not evenly distributed in the roots: an important percentage of soluble sugars was recorded in the intermediate portion while heat significantly reduced inulin content of the lower part only. Root lignification in contrast was only observed in the upper root part of heat-treated plants. In maize, abiotic stress such as water deficit was reported to increase the expression of genes involved in lignin biosynthesis and to reduce the mechanical extensibility of the root cell wall and this process occurs in the elongation zone just behind the tip (Fan et al. 2006). In root chicory, which is producing a taproot system, lignification of the upper part of the root might be related to an attempt to limit root volume increase in a context where shoot growth is itself altered.

In plants of *C. intybus* encountering a normal phenological development, bolting and flowering occur during the second year of the plant cycle after vernalization by low winter temperatures. This implies that the long-term overwintering reserve of inulin in the taproot is mobilized to provide material and energy for flowering processes. The presence of inulin in reproductive organs has been reported in some plant species such as *Campanula rapunculoides* where inulin plays a key role in flower opening (Vergauwen et al. 2000). In Asteraceae species like *Taraxacum officinale*, fructans concentrations are extremely low in stalk but higher in receptacle (Van den Ende et al. 2000). Nevertheless, root inulin cleavage and translocation of the resulting soluble sugars are required for bolting and flowering in *C. intybus*. The present work confirms that heat may induce precocious flowering during the first year of the plant cycle, at a time when stored inulin is not fully available and could not be efficiently mobilized to allow bolting. It is noteworthy that root soluble sugar concentration was higher in bolted plants than in non-bolted ones while an inverse trend was recorded for leaves. This supports the hypothesis that some sugars produced in the leaves are diverted to developing reproductive structures and that heat-induced bolting did not rely only on root inulin mobilization. Inulin decrease during bolting mainly occurred in the upper part of the root (Fig. S6). The content of kestose, one of inulin releasing fructose, was obviously lower in upper and lower parts of the roots in bolted plants which suggests that smaller DP inulin was hydrolyzed for bolting and resulted in decrease in mean DP. The ultimate effect of heat-induced bolting may consists in a significant decrease in root growth occurring as a consequence of root lignification, and as the consequence of a competition between taproot and reproductive sink organs for photosynthate supply. The fact that some bolted plants remained unable to produce mature flowers suggests that photosyntate supply might be insufficient for a full flowering process.

It is noteworthy that control plants never bolted and never exhibited root lignification. On the other hand, it has to be mentioned that root lignification was observed in heat-treated plants that did not bolt. It could not be excluded that root lignification limiting the root volume is a pre-requisite for heat-induced bolting since it allows more sugar transfer to reproductive structures but that other cues such as hormonal signals are involved to allow flowering.

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