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Sleep tight and wake-up early: nocturnal transpiration traits to increase wheat drought tolerance in a Mediterranean environment

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Abstract. In wheat, night-time transpiration rate (TR_N) could amount to 14–55% of daytime transpiration rate (TR), depending on the cultivar and environment. Recent evidence suggests that TR_N is much less responsive to soil drying than daytime TR, and that such 'wasteful' water losses would increase the impact of drought on yields. In contrast, other evidence indicates that pre-dawn, circadian increases in TR_N may enable enhanced radiation use efficiency, resulting in increased productivity under water deficit. Until now, there have been no attempts to evaluate these seemingly conflicting hypotheses in terms of their impact on yields in any crop. Here, using the Mediterranean environment of Tunisia as a case study, we undertook a simulation modelling approach using SSM-Wheat to evaluate yield outcomes resulting from these TR_N trait modifications. TR_N represented 15% of daytime TR-generated yield penalties of up to 20%, and these worsened when TR_N was not sensitive to soil drying TR. For the same TR_N level (15%), simulating a predawn increase in TR_N alleviated yield penalties, leading to yield gains of up to 25%. Overall, this work suggests that decreasing TR_N but increasing pre-dawn circadian control would be a viable breeding target to increase drought tolerance in a Mediterranean environment.

Additional keywords: circadian clock, food security, physiological trade-offs, process-based crop model, water conservation.

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Introduction

Water use in non-Crassulacean acid metabolism crops is often assumed to take place only during the day, due to total or near total stomata closure during the night. As a result, crop models commonly assume null transpiration rates (TR) during the night-time. There is, however, evidence showing non-negligible levels of nocturnal TR (TR_N) for many plant species (see reviews by Caird *et al*. 2007*a*; Marks and Lechowicz 2007; Resco de Dios *et al*. 2019; Duursma *et al*. 2019). In crops, studies using gas-exchange or lysimetric approaches found TR_N values ranging from 8–55% of daytime TR for alfalfa (*Medicago sativa* L., Abdel-Aziz *et al*. 1964), kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F. Liang & A.R.Ferguson), apple (*Malus domestica* Borkh., 1803; (Green *et al*. 1989), cotton (*Gossypium hirsutum* L., Tolk *et al*. 2006), tomato (*Solanum lycopersicum* L., Caird *et al*. 2007*b*), almond (*Prunus dulcis* (Mill.) D. A. Webb, Fuentes *et al*. 2013), bean (*Phaseolus vulgaris* L., Resco de Dios *et al*. 2015), and, more recently, barley (*Hordeum vulgare* L., Even *et al*. 2018; Sadok and Tamang 2019). In grapevine (*Vitis vinifera* L.), large genetic variability in TR_N has been found, and the magnitude of TR_N is driving breeding and irrigation efforts to optimise crop water use (e.g. Rogiers *et al*. 2009, 2012; Fuentes *et al*. 2014; Coupel-Ledru *et al*. 2016).

In wheat, substantial TR_N values have been reported depending on the genotype and the experimental conditions, particularly vapour pressure deficit (VPD). For instance, using a gas-exchange approach, Richards *et al*. (1986) and Rawson and Clarke (1988) identified wheat genotypes with TR_N that was 23 and 26% of daytime TR respectively. More recently, Schoppach *et al*. (2014), using a gravimetric method for estimating whole-plant transpiration, reported substantial increases in TR_N when nocturnal atmospheric VPD was increased from 0.4 to 2.2 kPa, leading to TR_N values that could potentially represent 13.8–55% of daytime TR, the latter being measured at a daytime VPD of 3.2 kPa.

Such non-negligible values could be highly problematic from a water budget perspective, particularly for rain-fed wheat grown under semiarid to arid environments where average nightly VPD often exceeds 1 kPa during the summer and could be as high as 2 kPa (Rawson and Clarke 1988; Tolk *et al*. 2006; Alvarado-Barrientos *et al*. 2015; Bazié *et al*. 2018). In wheat, Rawson and Clarke (1988) estimated that 'profligate' genotypes grown in South Australia could lose as much as 0.5 mm per night under relatively well-watered conditions, adding that 'such measurements of night water loss in wheat crops suggest totals of 40 mm for the season are possible'.

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Another lesser known body of evidence suggests that even under water deficit conditions, some wheat genotypes may continue transpiring at high rates during the night, and their daytime TR is being reduced by progressive soil drying, resulting in an increasing contribution of TR_N to total water use under drought. Richards *et al*. (1986) observed that the ratio of TR_N to daytime TR increased from 12 to 27% in plants subjected to well-watered and water-deficit treatments, respectively, and more recently, Claverie *et al*. (2018) used a gravimetric approach and reported a strikingly similar finding on two Australian wheat genotypes. Combined, the above observations indicate that reducing TR_N under a variety of water availability regimes is potentially a worthy breeding target as decreasing TR_N would result in reducing seemingly 'wasteful' night-time water loss, that is, water that is not traded for CO_2 fixation (Sadok and Jagadish 2020).

Besides the magnitude of nightly water use, the temporal dynamics of TR_N during a given night may play a role in controlling plant fitness and productivity under water-deficit conditions. In Eucalyptus, Resco de Dios *et al*. (2016) found that pre-dawn, circadian increases in stomatal conductance (g_s) were associated with faster stomatal opening in response to early-morning increases in photosynthetically active radiation (PAR), thereby leading to higher carbon assimilation rates and early-morning radiation use efficiency (RUE). This, in turn, was associated with increasing productivity and fitness under drought (i.e. the 'anticipative hypothesis'). In wheat, evidence indicates that this phenomenon may exist. Tamang *et al*. (2019) identified late-night circadian signatures in TR_N time courses among wheat genotypes, showing that such pre-dawn increases positively correlated with maximal daytime canopy conductance (G_s). More unexpectedly, such circadian control was found to be positively associated with the year of release of 23 drought-adapted south-Australian wheat cultivars released between 1890 and 2008, in a manner that indicated gains of productivity arising from a stronger increase in pre-dawn TR_N over time (Tamang *et al*. 2019).

Taken together, the outlined evidence points to the general hypothesis that variation in nocturnal water use may contribute to wheat yield gains under water-limited environments via two mechanisms: (i) water conservation by reducing water loss overnight; and (ii) a steep, late-night increase in night-time G_s (G_{sN}) leading to an increased RUE. Yet to date, there has been no effort to examine the relevance of these hypotheses in terms of their quantitative impacts on yield performance. In the past, such efforts have been successfully conducted to estimate yield gains or penalties arising from physiological alterations that impact daytime TR, using process-based crop models (e.g. Sinclair *et al*. 2005, 2010, 2014; Messina *et al*. 2015; Guiguitant *et al*. 2017; Sadok *et al*. 2019). Although such approaches have proven highly effective in informing breeding programs, crop models have not been applied to test physiological hypotheses examining nocturnal water use.

The main aim of the present investigation was therefore to use a mechanistic crop model (SSM-Wheat, Soltani and Sinclair 2012; Soltani *et al*. 2013) to examine the extent of yield gains or penalties, arising from the above physiological traits. Because the default option of crop models is typically a G_{sN} set at zero, a first goal was to characterise the impacts on yield of two levels of constant G_{sN}, reflecting TR_N values representing 15 and 44% of daytime TR, which cover most of the range reported in the literature on wheat. A second goal was to examine the consequence of these strategies while assuming an insensitivity of TR_N to progressive soil drying as suggested by Richards *et al*. (1986) and Claverie *et al*. (2018). A third goal of the simulation was to characterise the effects of simulating a pre-dawn increase in G_{sN} that led to increasing daytime G_s and dry matter production for a given solar energy unit captured expressed as an increase of 25% of RUE. This approach was used to simulate the circadian mechanism suggested by Resco de Dios *et al*. (2016) and Tamang *et al*. (2019).

The simulation analysis was applied to the highly divergent environmental conditions of Tunisia, a Mediterranean country where wheat food supply derived from wheat is threatened by recurrent drought events that regularly reduce yields by up to 50% (WFP 2011). The SSM-Wheat model was recently used successfully to simulate yield gains and penalties resulting from various daytime water use traits and accurately predicted yield potential gradients encountered within that country (Sadok *et al*. 2019). We hypothesised that (i) increases in nocturnal water use will lead to decreasing yields; (ii) its insensitivity to progressive soil drying would severely aggravate that penalty; but (iii) low nocturnal water use coupled with a pre-dawn increase in G_{sN} would lead to increasing yields.

Materials and methods

Description of the studied Mediterranean environment

A detailed description of the Tunisian environment is given in Sadok *et al*. (2019). Tunisia is a North-African Mediterranean nation situated in the transition zone between the arid Saharan climate and the Mediterranean subhumid climate, and covers three main pedo-climatic subdivisions illustrated in Fig. 1. In the northern region, yearly precipitation ranges from 400 mm to over 1500 mm, and the central region typically receives 200–400 mm of precipitation. The southern-most region is essentially arid (<200 mm precipitation) where local wheat varieties are grown under 150 mm of precipitation or less (Perrot 1909; Émile 1950; Abaza 2012). Rainfed durum wheat (*Triticum durum*) is the most widely cultivated cereal in Tunisia and this crop's production typically decreases along the north–south precipitation gradient (Latiri *et al*. 2010).

General model description

The simple simulation model, adapted for wheat (SSM-Wheat) was used to simulate crop development, growth and yield. This model, originally developed by Sinclair and Amir (1992) is described in detail by Soltani and Sinclair (2012) and Soltani *et al*. (2013). The SSM model has been applied to geospatial analyses in the Middle-East (Schoppach *et al*. 2017) and its adaptation for wheat grown in Tunisia is fully described in Sadok *et al*. (2019). The model was successfully used for evaluating physiological traits as a basis for releasing drought-tolerant germplasm in soybean (Sinclair *et al*. 2010; Carter

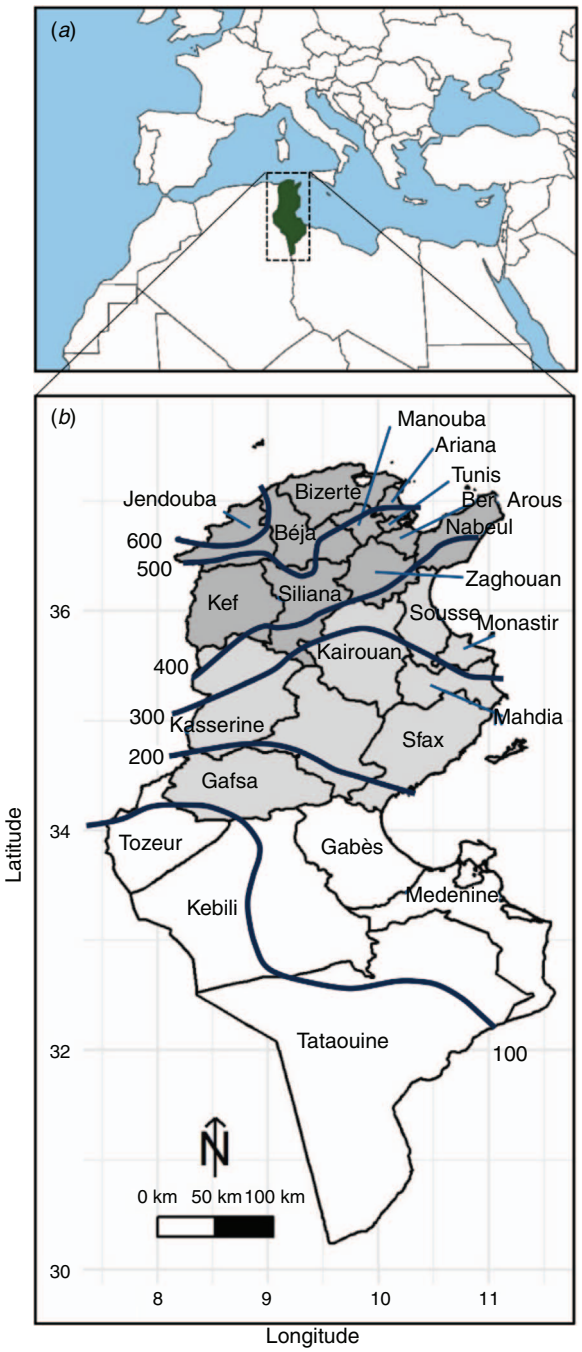

Fig. 1. General location of Tunisia in (*a*) and map of the country (*b*) highlighting its 24 governorates, the three main regional subdivisions (Latiri *et al*. 2010) and average yearly (1909–1996) precipitation (mm) isohyets (Benzarti 2003).

et al. 2016) and maize (Gaffney *et al*. 2015; Messina *et al*. 2015).

In the model, plant leaf-area development is calculated as a function of temperature accumulation expressed as cumulative temperature units (°C). To obtain daily increase in leaf area index (LAI), plant leaf area was multiplied by plant density, which in these simulations was held constant 300 plants m^{-2} (Soltani and Hoogenboom 2007; Schoppach *et al*. 2017). Daily crop growth is a function of the quantity of photosynthetically active radiation intercepted calculated from LAI, multiplied by photosynthetically active radiation use efficiency (RUE), which was set to 2.2 g MJ^{-1} (Sadok *et al*. 2019). The development of soil water deficit, as simulated by fraction of transpirable soil water (FTSW), results in decreased daily growth. Grain yield is simulated by assuming a constant linear increase in harvest index (HI) through the seed filling period (0.014 g $g^{-1}day^{-1}$). Therefore, any impacts on crop mass accumulation will have a direct influence on seed growth rate (Schoppach *et al*. 2017; Sadok *et al*. 2019).

Daytime and night-time weather data and adaptation to an hourly time step

For these simulations, a grid pattern of 0.312 × 0.312° latitude and longitude (~29 × 29 km) was established for the wheat-production areas of Tunisia, resulting in a total of 183 locations. At each grid location, daily weather data (minimum and maximum temperature, solar radiation, and precipitation) were generated for a 33-year period from 1979 to 2008 (Sadok *et al*. 2019). Because the available weather dataset did not include VPD values, these were determined based on daily minimum and hourly temperature values. Briefly, VPD calculated using daily minimum temperature is subtracted from the VPD calculated with hourly average temperature as outlined by Tanner and Sinclair (1983). As minimal (i.e. nocturnal) VPD was also needed, the daily minimum temperature of the day after was used. The following equation was applied in order to compute nocturnal VPD (VPD_N) transitions for each night.

$$VPD_N = ([24 - H]/10) \times VPD_{N,i} + ([H - 24]/10) \times VPN_{N,i+1}, \quad (1)$$

where H is the hour of the day (0–24) and $VPD_{N,i}$ and $VPD_{N,i+1}$ are the VPDs calculated using the minimum temperature of the current day (i) and the following day ($i + 1$) respectively.

To take into account the genetic variability in the transpiration time courses and response curves of TR to VPD through the daily cycle, the model was modified to function at an hourly time step (Sadok *et al*. 2019) while taking into account daytime and night-time VPD. This was necessary since SSM-Wheat originally operated on a daily time step where daily TR (TR_{Daily}) was calculated as follows:

$$TR_{Daily} = DMP_{Daily} \times (VPD_{Daily}/TEC), \quad (2)$$

where DMP_{Daily} is the daily dry mass production, VPD_{Daily} is the weighted daily VPD and TEC is a constant transpiration efficiency coefficient set to 5.8 Pa for wheat (Schoppach *et al*. 2017). This conversion from daily to hourly weather data input was successfully used previously in SSM models for sorghum (Sinclair *et al*. 2005), soybean (Sinclair *et al*. 2010), maize (Messina *et al*. 2015) and wheat (Sadok *et al*. 2019).

Soil data

The model treats soil as a bucket represented by the volume of soil colonised by the roots that expands with increasing depth

of water extraction front as described in Sadok *et al*. (2019). Briefly, the fractions of sand, silt, clay, rock fragments and bulk density were obtained for each grid location using the Harmonised World Soil Database (HWSD 2012), and these data were used to define curve number for runoff, volumetric soil water content at saturation and at drained upper limit, extractable soil water and drainage factor (Gijsman *et al*. 2002). Similarly, root growth and water extraction parameters were set as in Sadok *et al*. (2019). Maximal soil depth was assumed not to exceed 1.2 m across all simulated locations and the maximum increase per biological day in depth for water extraction was set at 3 cm. The extraction zone was set at 0.2 m depth at plant emergence. Water used by the plant is located in the root system extraction zone, between the soil surface and the root system water extraction front. Rainfall was the only source of water input to the soil and soil water loss was considered to occur via plant uptake, soil evaporation, runoff and drainage below the root extraction depth (Sadok *et al*. 2019).

Integration of nocturnal variables in SSM-Wheat

Given the goals of this study, the simulation required integrating new variables reflecting nocturnal canopy conductance (G_{sN}, described in following section) and hourly TR_N values. This is a new development that was not part of previous uses of the model SSM-Wheat. Here, TR_N was calculated as:

$$TR_N = G_{sN} \times LAI \times VPD_N \times WSF, \quad (3)$$

where VPD_N is the hourly night-time VPD and WSF is a water stress factor. Since SSM-Wheat originally did not take into account TR_N, other adjustments in the code were necessary. Because SSM calculates daytime TR based on crop mass accumulation, it can result in abrupt transitions in TR between days and nights. In order to obtain more realistic transitions, we allowed the model to operate in a 'hybrid mode' during the transition, but not during the day where the model runs as detailed in the literature (e.g. Soltani *et al*. 2013). That is, to calculate hourly TR during the three last hours before sunset using the formalism for calculating TR_N. The resulting TR transitions (Fig. 2) are consistent with observed diel VPD time courses (e.g. Bazié *et al*. 2018) and the resulting water losses are counted as part of daytime TR in our simulations.

Simulations

Simulations of optimum sowing dates and daytime water use traits were done exactly as in Sadok *et al*. (2019). These were done initially to determine the optimum sowing date at each location based on maximum yield calculated using the 'standard' traits model. A single optimal sowing date for each location was then calculated by averaging the best sowing dates from each year. In the analysis, arid and Saharan regions in the south of the country were excluded as wheat yields did not exceed 0.3 t ha^{-1} over 50% of the years, and such low yields were not considered to be economically viable. No nitrogen limitation was simulated in the present study so that the yield results reflected only the environmental limitation of water availability and no irrigation was implemented in these simulations. An initial set of simulations were made for a 'standard' genotype with a

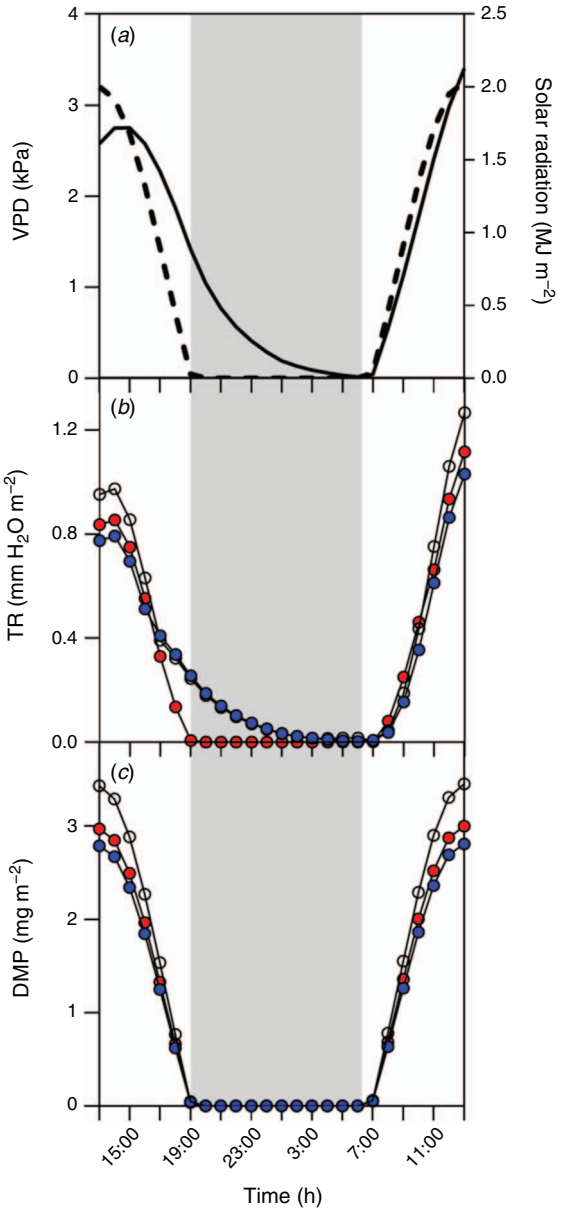

Fig. 2. Simulated hourly time courses of vapour pressure deficit (VPD, continuous line, *a*) and photosynthetically active solar radiation (dashed line, *a*), whole-plant transpiration rate (TR, *b*) and dry matter production (DMP, *c*) over a 24-h period centred on the night-time period (grey area). The simulated genotypes represent the lowest and highest levels of night-time canopy conductance (G_{sN}) examined. In each panel, red, blue, and open dots represent respectively genotypes with: (i) G_{sN} = 0 mm H_2O h^{-1} kPa^{-1}, (ii) G_{sN} = 0.107 mm H_2O h^{-1} kPa^{-1}, (iii) same G_{sN} as (ii) but with a circadian increase during the last 3 h of the night resulting in 25% increase in radiation use efficiency (RUE). See 'Materials and methods' for details.

null G_{sN}, leading to a null TR_N, a standard RUE set at 2.2 g MJ^{-1} and a threshold in fraction of transpirable soil water ($FTSW_{Th}$) at which partial stomata closure started to cause a decrease in daytime TR that was set at a typical value of 0.5 (Schoppach and Sadok 2012; Sadok *et al*. 2019).

Another set of simulations examined the effects on yield stemming from different nocturnal traits. The first series of

simulations examined the effects of imposing a non-zero G_{sN} (set at two average nightly levels: 0.033 and 0.107 mm H_2O h^{-1} kPa^{-1}). The lowest and highest G_{sN} values were determined based on experimental data gathered on wheat by Rawson and Clarke (1988) and Schoppach *et al*. (2014) respectively. The lowest G_{sN} values were estimated based on a TR_N of 0.5 mm H_2O per night measured in the field under a night-time VPD of 1.5 kPa for a LAI of 1. The highest G_{sN} values were determined by calculating the slope of TR response to nocturnal VPD measured by Schoppach *et al*. (2014), but excluding the two extreme TR_N measurements that were measured under night-time VPDs exceeding 1.5 kPa. TR_N sensitivity to soil drying was simulated by making TR_N responsive to progressive soil drying by decreasing TR_N at the typical $FTSW_{Th}$ of 0.5

Finally, a third series of simulations examined the yield responses arising from imposing a pre-dawn, circadian increase in TR_N over the last 3 h of the night, which was positively correlated with an increase in the slope of daytime TR response to VPD (Tamang *et al*. 2019). Based on that study, a genotype with a G_{sN} of 0.033 mm H_2O h^{-1} kPa^{-1} and 25% increase in pre-dawn TR_N over the last hours was simulated to exhibit 25% increase in RUE relative to the standard genotype (G_{sN} = 0 mm H_2O h^{-1} kPa^{-1} and no increase in pre-dawn TR_N or RUE). In order to simulate a 25% increase in TR_N over the last hours of the night despite the typical decrease in pre-dawn VPD conditions, G_{sN} was simulated to increase according to the following exponential function during the three last night-time hours:

$$G_{sN} = G_{sN} \times 14.092e^{-0.754H}, \quad (4)$$

where H represents the number of hours before sunrise. Despite these increases in late night G_{sN}, a 25% increase in TR_N was hardly achieved because of the steep decrease in VPD conditions and TR_N time course was usually more akin to alleviate a decrease than a clear increase (Fig. 2). In order to isolate the effect of the circadian increase coupled with a 25% increased RUE from the base effect of TR_N, the genotype expressing the trait (G_{sN} of 0.033 mm H_2O h^{-1} kPa^{-1} and 25% increase in pre-dawn TR_N and RUE) was also compared with a 'standard' genotype displaying only a G_{sN} of 0.033 mm H_2O h^{-1} kPa^{-1} with no circadian increase and unchanged RUE.

Data analysis

In each location and year, the effects of the night-time traits were evaluated through three main indexes; (i) the yield ratio between the average yield of the standard genotype and the yield resulting from the same genotype expressing the evaluated night-time traits; (ii) the probability of getting a yield advantage from the considered trait over the 33 simulated years; and (iii) the average decrease in yield variability coefficient due to the introduction of the trait of interest. For each location, the yield variability coefficient (CV_{Yield}) was calculated as a function of the standard deviation of the yield (SD_{Yield}) and the average yield (μ_{Yield}) as follows:

$$CV_{Yield} = (SD_{Yield}/\mu_{Yield}) \times 100, \quad (5)$$

Yield variability was defined as the value of the coefficient with the trait minus the value of the coefficient without the trait. Negative values therefore indicate that the considered trait reduces the year-to-year yield variability. All the statistical analyses were done using Microsoft Excel 2016 macros. The maps were generated using R scripts (ver. 3.4.2, R Core Team 2017) using a kriging method to extrapolate data between simulated points (Schoppach *et al*. 2017; Sadok *et al*. 2019).

Results

The average TR_N resulting from the two imposed G_{sN} levels of 0.033 and 0.107 mm H_2O h^{-1} kPa^{-1} was, respectively, 15 and 44% of daytime TR. These values are consistent with the range reported on wheat by Richards *et al*. (1986), Rawson and Clarke (1988) and Tamang *et al*. (2019), but are below the more extreme value of 50% of the average daytime TR identified by Schoppach *et al*. (2014).

Irrespective of the level of night-time water loss, imposing a non-zero TR_N resulted in yield decreases across all of Tunisia, with near-zero probabilities for yield increase and higher variability in wheat yields (Fig. 3). The yield decreases were more pronounced in the more arid central and southern regions of the country, while the northern part was relatively less affected. Not surprisingly, the highest yield penalties were attributed to the highest G_{sN} regime (0.107 mmH_2O h^{-1} kPa^{-1}, representing on average 44% of daily TR). This level of nocturnal water use resulted in the most extreme yield decreases, reaching up to 60% in the food-insecure central and southern regions of the country, and was accompanied with substantial increases in yield variability (Fig. 3*d–f*). Further, those decreases were quite significant even when imposing a less aggressive TR_N (G_{sN} = 0.033 mmH_2O h^{-1} kPa^{-1}), which represents a night-time water use equivalent to 15% of daytime TR (Fig. 3*a–c*). In this case, the yield decreases incurred in the north, central and south regions of Tunisia averaged 10, 15 and 20%, with smaller increases in yield variability of up to 10%, particularly observed in the north (Fig. 3*c*).

As illustrated in Fig. 4, those decreases were slightly amplified relative to the previous scenario when TR_N became insensitive to progressive soil drying, with penalties averaging 2 and 8% over the whole country for the low and high G_{sN} scenarios respectively. Under this condition, and similar to the first case (i.e. sensitivity to FTSW), the severity of yield penalties gets larger along a north–south gradient, particularly for the most water-consumptive TR_N regime (G_{sN} = 0.107 mm H_2O h^{-1} kPa^{-1}). Under that scenario, potential yield losses reached 70% in the central and southern regions, which were associated with substantial increases in yield variability, to up to 35–40% in the north-central regions (Fig. 4). For the less aggressive TR_N regime (G_{sN} = 0.033 mm H_2O h^{-1} kPa^{-1}), yield losses in the north, central and southern regions averaged respectively 10, 20 and 25%, with the same level of variability as in the previous scenario.

In sharp contrast to the above, simulating a predawn increase in TR_N leading to an increased RUE compared with a standard genotype with a zero TR_N not only alleviated some of the yield penalties reported, but resulted in yield gains in the northern part of the country, despite imposing a G_{sN} of 0.033 mm H_2O h^{-1} kPa^{-1}, (Fig. 5*a–c*).

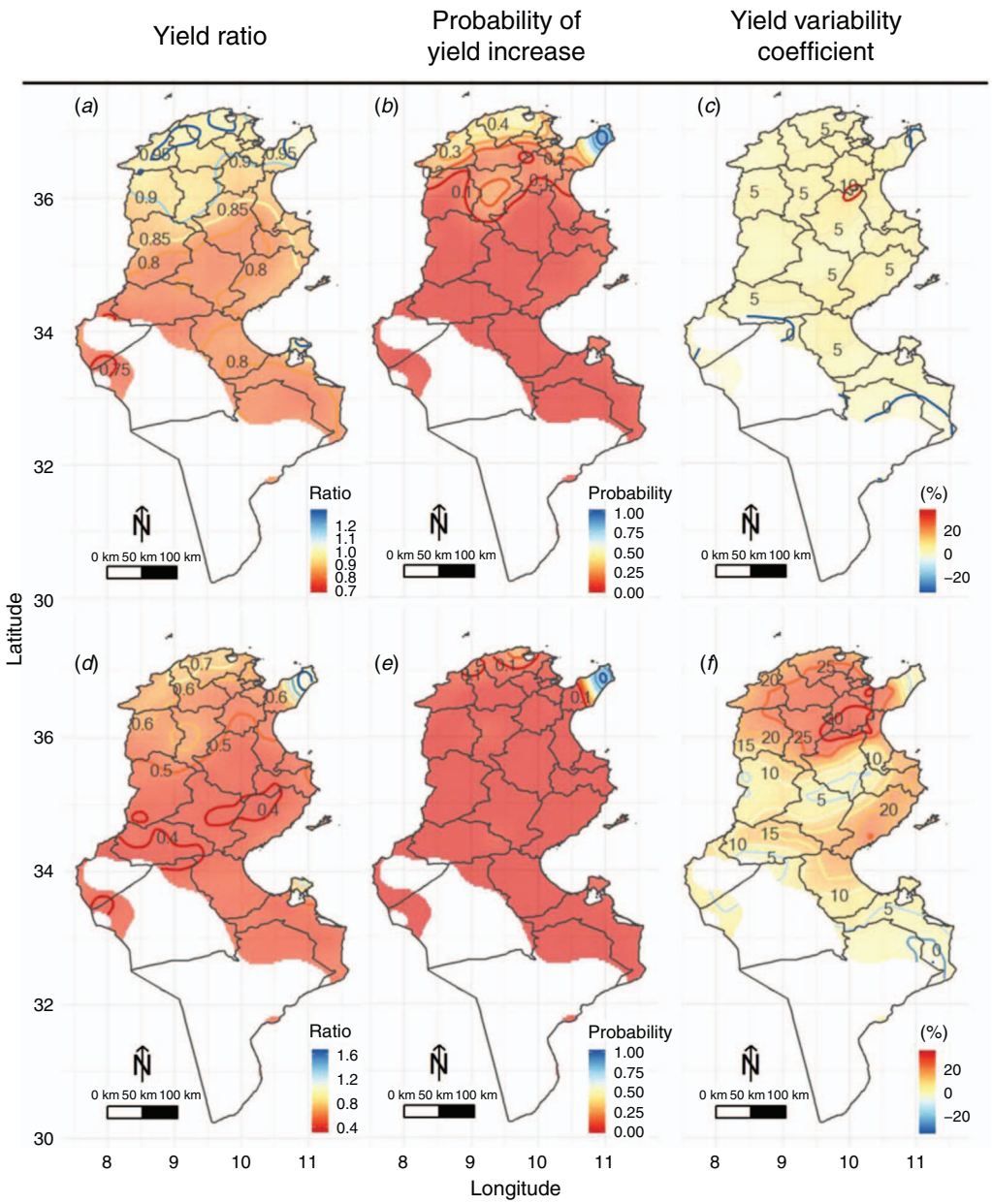

Fig. 3. Yield ratios, probabilities for yield increase and yield variability coefficients resulting from deploying wheat genotypes exhibiting nighttime canopy conductance (G_{sN}) of 0.033 mm H_2O h^{-1} kPa^{-1} (*a–c*) and 0.107 mm H_2O h^{-1} kPa^{-1} (*d–f*), respectively, reflecting night-time transpiration rate (TR_N) values representing 15 and 44% of daytime transpiration rate (TR). (*a*, *d*) Ratio between the yields of the standard wheat genotype and those resulting from increasing TR_N. (*b*, *e*) Probabilities of yield increase from increasing TR_N. (*c*, *f*) Variability coefficients where positive and negative values indicate increases or decreases in yield variability respectively. The internal subdivisions represent the 24 governorates of the country. The blank area represents desert regions not suited for growing wheat. In the colour scale of all panels, blue and red refer to favourable and unfavourable outcomes respectively.

Although yields decreased by 5–10% in most of the central and southern regions, the yield losses due to TR_N representing 15% of daytime TR (Fig. 4*a–c*) were alleviated by pre-dawn TR_N increase enabling RUE to raise (Fig. 5*a–c*). Gains of up to 5% were observed in the north, with marginal effects on yield variability. An exceptional 25% increase was also simulated at the tip of Nabeul's Governate (extreme north of the country), but this result was strongly influenced by the impact of the surrounding sea areas on the VPD estimates and should be interpreted carefully. Such gains, however, were relatively uncertain, as the probability for yield increase is never >0.6 for the northern regions.

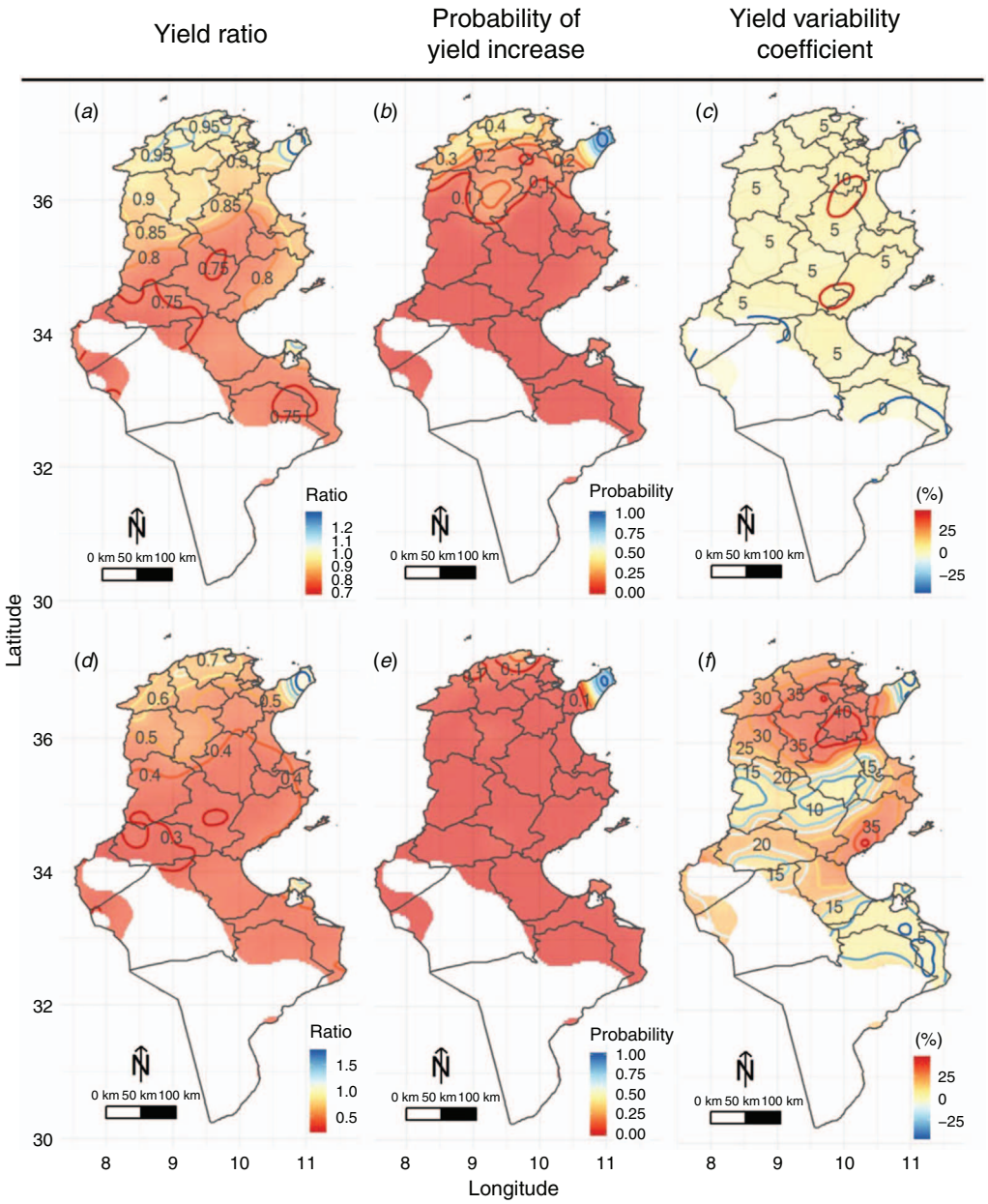

Fig. 4. Yield ratios, probabilities for yield increase and yield variability coefficients resulting from deploying wheat genotypes exhibiting night-time transpiration rate (TR_N) values representing 15 and 44% of daytime transpiration rate (TR), which are insensitive to progressive soil drying. The components of the figure are as described in the caption for Fig. 3.

On the opposite, simulating the same behaviour (i.e. a predawn increase in TR_N leading to an increased RUE) but compared with a standard genotype exhibiting a G_{sN} = 0.033 mm H_2O h^{-1} kPa^{-1} without circadian increase in TR_N nor effect on RUE revealed more substantial and systemic yield increases. In this case, yield gains ranging from 10 to 15 and 25% (Fig. 5*d*) were found for the central, northern and southern regions, respectively, with a high probability (>0.7). Such gains were associated with a marginal effect on yield variability, which ranged from –5% to +5% (Fig. 5*e*, *f*). The increases in yield observed were the result of higher daytime TR and carbon acquisition enabling rapid use of water by the crop so that a greater proportion of the soil water was used to support transpiration and crop growth, rather than lost in soil evaporation.

Discussion

Wheat production is key to Tunisian food security and is regularly threatened by drought events that often generate yield losses of nearly 50%, as happened multiple times during the last decade (USDA-FAS 2005, 2007; 2009,

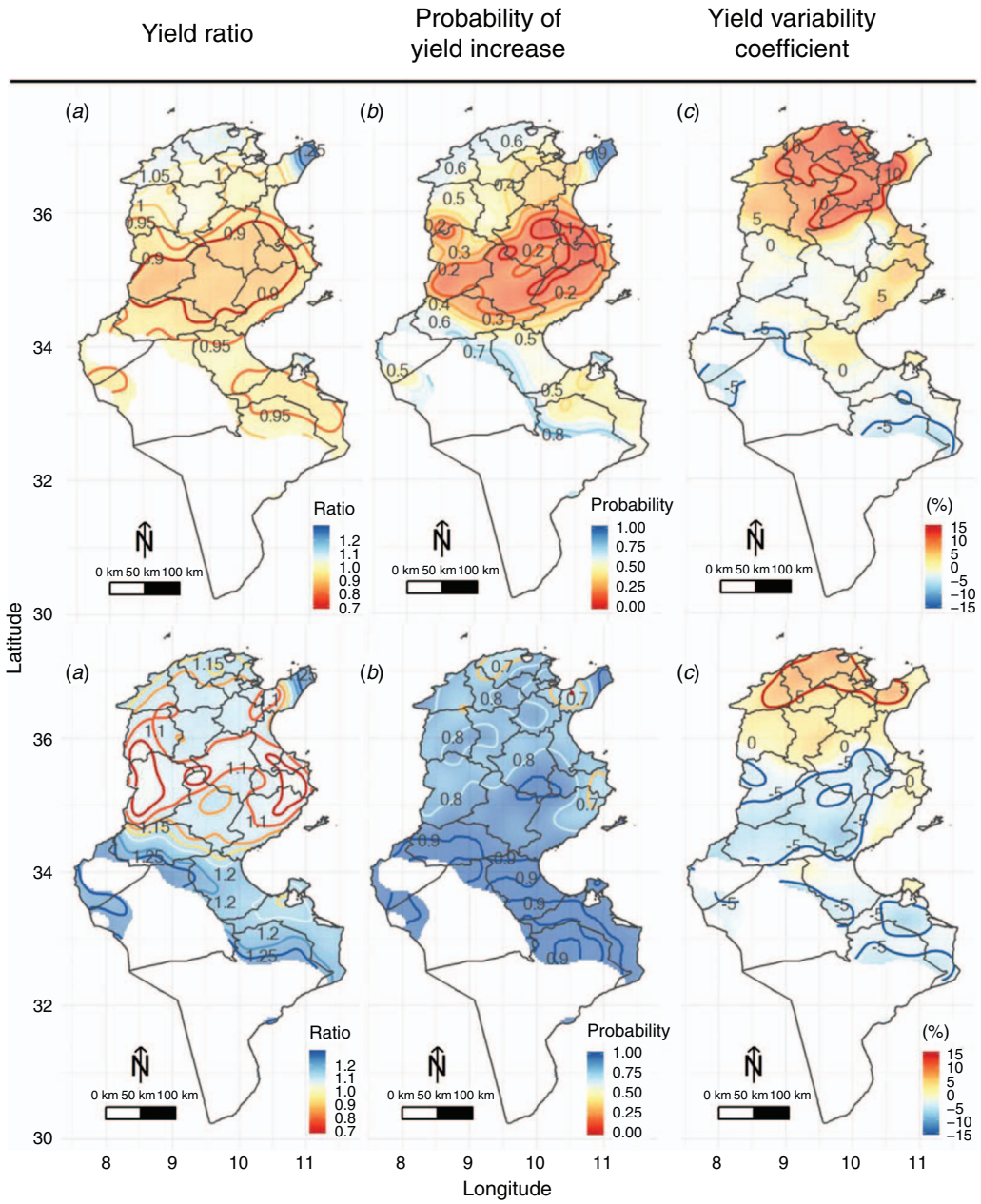

Fig. 5. Yield ratios (*a*, *d*), probabilities for yield increase (*b*, *e*) and yield variability coefficients (*c*, *f*) resulting from deploying wheat genotypes exhibiting a pre-dawn increase (25%) in night-time transpiration rate (TR_N) over the last 3 h of the night, leading to a 25% increase radiation use efficiency (RUE). The simulations were carried out for G_{sN} equal to 0.033 mm H_2O h^{-1} kPa^{-1}. Yields are compared with a standard genotype expressing a G_{sN} equal to zero (*a*–*c*) and 0.033 mm H_2O h^{-1} kPa^{-1}, but without circadian increase (*d*–*f*). The components of the figure are as described in the caption for Fig. 3.

2011, 2013, 2016; WFP 2011). Therefore, the deployment of cultivars equipped with drought tolerance traits is particularly desirable for the country. Recently, Sadok *et al*. (2019) identified daytime TR-based traits resulting in water conservation that led to yield increases in major wheat growing areas across the country. However, while such traits were shown to be effective in other crops (e.g. Sinclair *et al*. 2005, 2010; Messina *et al*. 2015; Guiguitant *et al*. 2017), there was no attempt at examining the possible yield benefits or decreases resulting from variation in nocturnal water use. Here, based on published research documenting significant diversity in TR_N in wheat, along with evidence for its circadian, pre-dawn control and its relationship with daytime gas exchange, we leveraged crop simulation modelling to examine the potential of such traits for further increasing wheat yield in Tunisia.

Reducing whole-night TR_N may be a good breeding target to enhance water conservation

In wheat, all of the studies examining night-time water use concluded that TR_N or G_{sN} is not null (Richards *et al*. 1986; Rawson and Clarke 1988; Schoppach *et al*. 2014; Tamang *et al*. 2019). In fact, none of the 92 genotypes examined in all of these studies combined exhibited a TR_N of zero, with values representing 8–17% of daytime water use reported under night-time VPD ranging from 0.37 to 1.4 kPa. The VPD range tested in these studies is consistent with field conditions, particularly in semiarid environments during the summer, where overnight nocturnal VPD averages often exceed 1 kPa, sometimes reaching 2 kPa or higher on certain nights (Rawson and Clarke 1988; Tolk *et al*. 2006; Alvarado-Barrientos *et al*. 2015; Bazié *et al*. 2018). For the night-time VPD conditions in Tunisia and G_{sN} = 0.033 $mmH_2O\ h^{-1}\ kPa^{-1}$, the simulation analysis indicated that wheat had TR_N values in the range of 15% of the daily water use. This additional water loss at loss resulted in non-marginal yield penalties of 5–20% in all wheat production environments encountered in Tunisia (Fig. 3). Furthermore, the analysis points to even more severe yield penalties (up to 25%, Fig. 4) if TR_N is not sensitive or less sensitive to soil drying than simulated for daytime TR as found in Richards *et al*. (1986) and Claverie *et al*. (2018).

Because TR_N and its response to drought are typically never considered in breeding programs, these results point to an additional, untapped potential to ameliorate wheat drought tolerance in Tunisia. This could be done by breeding for genotypes with the lowest possible TR_N that are additionally highly sensitive in response to soil drying (i.e. TR_N decreasing at high $FTSW_{Th}$). Although there are obvious logistical costs associated with screening TR_N response curves to FTSW, a phenotypic screen based on whole-plant TR_N of potted plants would be a feasible and cost-effective approach for local breeding programs.

Maximising pre-dawn increase in TR_N as a breeding target to enhance radiation use efficiency

When simulating a late-night, circadian increase in TR_N, the analysis confirmed the hypothesis that productivity gains may arise from pre-dawn increases in stomata conductance if that increase is associated with an increase in RUE as suggested by Resco de Dios *et al*. (2016) and Tamang *et al*. (2019). Despite imposing such increase for a relatively 'profligate' genotype exhibiting a TR_N that was 15% of daytime TR, the net result was a yield increase (of up to 5%) in the northern regions (Fig. 5*a*). Relative to a standard genotype with a TR_N of 15% of daytime TR but with no pre-dawn TR_N increase, those gains increased from 10 to 25% with high probability across the entire country (Fig. 5*d–f*). Such gains stemmed from the positive relationship between pre-dawn increases in TR_N and higher daytime TR and dry matter production (DMP). Therefore, increasing its conductance in return for a higher DMP appears to be a worthwhile trade-off in regions with sufficient rainfall. Finally, the simulations showed that an extreme level of TR_N, as high as 44% of daytime TR, generated by the highest simulated G_{sN} would result in dramatic decreases in yield. These decreases cannot be, in any case, compensated by any daytime trade-off (data not shown).

Taken together, the above findings indicate that conservative genotypes with low TR_N but with a strong circadian, pre-dawn increase in TR_N would maximise the yield benefits under water-limited conditions in the Mediterranean environment of Tunisia. Based on this simulation, the expected yield gains appear to be significant, and may be even more pronounced if nocturnal traits are coupled with other daytime water-saving traits. From a modelling perspective, the results of this investigation point to the need for a more accurate estimation of nocturnal water use and its environmental, physiological and genetic drivers.

Caveats and recommendations for future research

This simulation analysis is to our knowledge the first effort attempting at evaluating the quantitative impacts on yields resulting from variation in nocturnal transpiration in any crop. In this effort, we used data from a relatively limited set of observations that were consistent despite differences in methodologies used for estimating TR_N (gas exchange, Richards *et al*. 1986; Rawson and Clarke 1988: lysimetric, Schoppach *et al*. 2014; Tamang *et al*. 2019) combined with a specific crop model (SSM-Wheat). Therefore, further investigations are critically needed to assess those values and their range over a wider spectrum of experimental conditions and genotypes and to quantify their yield impacts using different crop models. This implies (i) modifying crop models such that they capture non-zero stomata conductance during the night; (ii) relying on directly measured daytime and night-time VPD values (which we had to simulate in this study); while (iii) taking into account seasonal and climatic variation of weighted VPD for transpiration estimation (Ghanem *et al*. 2020). Furthermore, more basic research is needed in the eco-physiology of night-time transpiration to better understand and model its implications not only in terms of water use, but also with respect to key processes such as hydraulic redistribution in the soil, dark respiration, nutrient uptake and patterns of growth (Neumann *et al*. 2014; Yu *et al*. 2019; Fricke 2019).

Conflicts of interest

The authors declare no conflicts of interest.

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References

Abaza K (2012) Dynamique de l'occupation des sols et risques environnementaux en milieux montagneux arides: le cas de la région de Ghomrassen et ses environs (Sud- Est de la Tunisie). *Revue des Regions Arides* **29**, 77–101.

Abdel-Aziz MH, Taylor SA, Ashcroft GL (1964) Influence of advective energy on transpiration. *Agronomy Journal* **56**, 139–142. doi:10.2134/agronj1964.00021962005600020005x

Alvarado-Barrientos MS, Holwerda F, Geissert DR, Muñoz-Villers LE, Gotsch SG, Asbjornsen H, Dawson TE (2015) Nighttime transpiration in a seasonally dry tropical montane cloud forest environment. *Trees* **29**, 259–274. doi:10.1007/s00468-014-1111-1

Bazié HR, Sanou J, Bayala J, Bargués-Tobella A, Zombré G, Ilstedt U (2018) Temporal variations in transpiration of *Vitellaria paradoxa* in West African agroforestry parklands. *Agroforestry Systems* **92**, 1673–1686. doi:10.1007/s10457-017-0115-4

Benzarti Z (2003) La pluviométrie en Tunisie. Analyse Des Années Très Pluvieuses. ENS Editions, 2003.

Caird MA, Richards JH, Donovan LA (2007*a*) Night-time stomatal conductance and transpiration in C_3 and C_4 plants. *Plant Physiology* **143**, 4–10. doi:10.1104/pp.106.092940

Caird MA, Richards JH, Hsiao TC (2007*b*) Significant transpirational water loss occurs throughout the night in field-grown tomato. *Functional Plant Biology* **34**, 172–177. doi:10.1071/FP06264

Carter TE, Todd SM, Gillen AM (2016) Registration of 'USDA-N8002' soybean cultivar with high yield and abiotic stress resistance traits. *Journal of Plant Registrations* **10**, 238–245. doi:10.3198/jpr2015.09.0057crc

Claverie E, Meunier F, Javaux M, Sadok W (2018) Increased contribution of wheat nocturnal transpiration to daily water use under drought. *Physiologia Plantarum* **162**, 290–300. doi:10.1111/ppl.12623

Coupel-Ledru A, Lebon E, Christophe A, Gallo A, Gago P, Pantin F, Doligez A, Simonneau T (2016) Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 8963–8968. doi:10.1073/pnas.1600826113

Duursma RA, Blackman CJ, Lopéz R, Martin-StPaul NK, Cochard H, Medlyn BE (2019) On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. *New Phytologist* **221**, 693–705. doi:10.1111/nph.15395

Émile M (1950) Les principales espèces et variétés de Blé cultivées en Afrique du Nord. In Revue internationale de botanique appliquée et d'agriculture tropicale, 30e année. Bulletin no. 327–328. Janvier-février 1950. pp. 16–38. (Muséum national d'histoire naturelle, Laboratoire d'agronomie colonial: Paris)

Even M, Sabo M, Meng D, Kreszies T, Schreiber L, Fricke W (2018) Night-time transpiration in barley (*Hordeum vulgare*) facilitates respiratory carbon dioxide release and is regulated during salt stress. *Annals of Botany* **122**, 569–582. doi:10.1093/aob/mcy084

Fricke W (2019) Nighttime transpiration – favoring growth? *Trends in Plant Science* **24**, 311–317. doi:10.1016/j.tplants.2019.01.007

Fuentes S, Mahadevan M, Bonada M, Skewes MA, Cox JW (2013) Night-time sap flow is parabolically linked to midday water potential for field-grown almond trees. *Irrigation Science* **31**, 1265–1276. doi:10.1007/s00271-013-0403-3

Fuentes S, De Bei R, Collins MJ, Escalona JM, Medrano H, Tyerman S (2014) Night-time responses to water supply in grapevines (*Vitis vinifera* L.) under deficit irrigation and partial root-zone drying. *Agricultural Water Management* **138**, 1–9. doi:10.1016/j.agwat.2014.02.015

Gaffney J, Schussler J, Löffler C, Cai W, Paszkiewicz S, Messina C, Groeteke J, Keaschall J, Cooper M (2015) Industry scale evaluation of maize hybrids selected for increased yield in drought stress conditions of the US Corn Belt. *Crop Science* **55**, 1608–1618. doi:10.2135/cropsci2014.09.0654

Ghanem ME, Kehel Z, Marrou H, Sinclair TR (2020) Seasonal and climatic variation of weighted VPD for transpiration T estimation. *European Journal of Agronomy* **113**, 125966. doi:10.1016/j.eja.2019.125966

Gijsman AJ, Jagtap SS, Jones JW (2002) Wading through a swamp of complete confusion: how to choose a method for estimating soil water retention parameters for crop models. *European Journal of Agronomy* **18**, 77–106. doi:10.1016/S1161-0301(02)00098-9

Green SR, McNaughton KG, Clothier BE (1989) Observations of night-time water use in kiwifruit vines and apple trees. *Agricultural and Forest Meteorology* **48**, 251–261. doi:10.1016/0168-1923(89)90072-5

Guiguitant J, Marrou H, Vadez V, Gupta P, Kumar S, Soltani A, Sinclair TR, Ghanem ME (2017) Relevance of limited-transpiration trait for lentil (*Lens culinaris* Medik.) in South Asia. *Field Crops Research* **209**, 96–107. doi:10.1016/j.fcr.2017.04.013

HWSD (2012) HWSD ver. 1.2. FAO 2012. Available at https://daac.ornl.gov/SOILS/guides/HWSD.html [Verified 26 June 2020]

Latiri K, Lhomme JP, Annabi M, Setter TL (2010) Wheat production in Tunisia: progress, inter-annual variability and relation to rainfall. *European Journal of Agronomy* **33**, 33–42. doi:10.1016/j.eja.2010.02.004

Marks CO, Lechowicz MJ (2007) The ecological and functional correlates of nocturnal transpiration. *Tree Physiology* **27**, 577–584. doi:10.1093/treephys/27.4.577

Messina CD, Sinclair TR, Hammer GL, Curan D, Thompson J, Oler Z, Gho C, Cooper M (2015) Limited-transpiration trait may increase maize drought tolerance in the US corn belt. *Agronomy Journal* **107**, 1978–1986. doi:10.2134/agronj15.0016

Neumann RB, Cardon ZG, Teshera-Levye J, Rockwell FE, Zwieniecki MA, Holbrook NM (2014) Modelled hydraulic redistribution by sunflower (*Helianthus annuus* L.) matches observed data only after including night-time transpiration. *Plant, Cell & Environment* **37**, 899–910. doi:10.1111/pce.12206

Perrot E (1909) Les productions végétales de la Tunisie (Notes de botanique économique) *Bulletin de la Société Botanique de France* **56**(9), CCCXVII–CCCLII. doi:10.1080/00378941.1909.10832150

R Core Team (2017) R: A language and environment for statistical computing. (R Foundation for Statistical Computing, Vienna, Austria) Available at http://www.R-project.org/ [Verified 26 June 2020]

Rawson HM, Clarke JM (1988) Nocturnal transpiration in wheat. *Australian Journal of Plant Physiology* **15**, 397–406. doi:10.1071/PP9880397

Resco de Dios V, Roy J, Ferrio JP, Alday JG, Landais D, Milcu A, Gessler A (2015) Processes driving nocturnal transpiration and implications for estimating land evapotranspiration. *Scientific Reports* **5**, 10975. doi:10.1038/srep10975

Resco de Dios V, Loik ME, Smith R, Aspinwall MJ, Tissue DT (2016) Genetic variation in circadian regulation of nocturnal stomatal conductance enhances carbon assimilation and growth. *Plant, Cell & Environment* **39**, 3–11. doi:10.1111/pce.12598

Resco de Dios V, Chowdhury FI, Granda E, Yao Y, Tissue DT (2019) Assessing the potential functions of nocturnal stomatal conductance in C_3 and C_4 plants. *New Phytologist* **223**, 1696–1706. doi:10.1111/nph.15881

Richards RA, Rawson HM, Johnson DA (1986) Glaucousness in wheat: its development and effect on water-use efficiency, gas exchange and photosynthetic tissue temperatures. *Australian Journal of Plant Physiology* **13**, 465–473. doi:10.1071/PP9860465

Rogiers SY, Greer DH, Hutton RJ, Landsberg JJ (2009) Does night-time transpiration contribute to anisohydric behaviour in a *Vitis vinifera* cultivar? *Journal of Experimental Botany* **60**, 3751–3763. doi:10.1093/jxb/erp217

Rogiers SY, Greer DH, Hatfield JM, Hutton RJ, Clarke SJ, Hutchinson PA, Somers A (2012) Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid. *Tree Physiology* **32**, 249–261. doi:10.1093/treephys/tpr131

Sadok W, Jagadish SVK (2020) The hidden costs of nighttime warming on yields. *Trends in Plant Science* doi:10.1016/j.tplants.2020.02.003In press

Sadok W, Tamang BG (2019) Diversity in daytime and night-time transpiration dynamics in barley indicates adaptation to drought regimes across the Middle-East. *Journal Agronomy & Crop Science* **205**, 372–384. doi:10.1111/jac.12331

Sadok W, Schoppach R, Ghanem ME, Zucca C, Sinclair TR (2019) Wheat drought-tolerance to enhance food security in Tunisia, birthplace of the Arab Spring. *European Journal of Agronomy* **107**, 1–9. doi:10.1016/j.eja.2019.03.009

Schoppach R, Sadok W (2012) Differential sensitivities of transpiration to evaporative demand and soil water deficit among wheat elite cultivars indicate different strategies for drought tolerance. *Environmental and Experimental Botany* **84**, 1–10. doi:10.1016/j.envexpbot.2012.04.016

Schoppach R, Claverie E, Sadok W (2014) Genotype-dependent influence of night-time vapour pressure deficit on night-time transpiration and daytime gas exchange in wheat. *Functional Plant Biology* **41**, 963–971. doi:10.1071/FP14067

Schoppach R, Soltani A, Sinclair TR, Sadok W (2017) Yield comparison of simulated rainfed wheat and barley across Middle-East. *Agricultural Systems* **153**, 101–108. doi:10.1016/j.agsy.2016.12.017

Sinclair TR, Amir J (1992) A model to assess nitrogen limitations on the growth and yield of spring wheat. *Field Crops Research* **30**, 1–11. doi:10.1016/0378-4290(92)90052-B

Sinclair TR, Hammer GL, van Oosterom EJ (2005) Potential yield and water – use efficiency benefits in sorghum from limited maximum transpiration rate. *Functional Plant Biology* **32**, 945–952. doi:10.1071/FP05047

Sinclair TR, Messina CD, Beatty A, Samples M (2010) Assessment across the United States of the benefits of altered soybean drought traits. *Agronomy Journal* **102**, 475–482. doi:10.2134/agronj2009.0195

Sinclair TR, Marrou H, Soltani A, Vadez V, Chandolou KC (2014) Soybean production potential in Africa. *Global Food Security* **3**(1), 31–40. doi:10.1016/j.gfs.2013.12.001

Soltani A, Hoogenboom G (2007) Assessing crop management options with crop simulation models based on generated weather data. *Field Crops Research* **103**, 198–207. doi:10.1016/j.fcr.2007.06.003

Soltani A, Sinclair TR (2012) 'Modeling physiology of crop development, growth and yield.' (CAB International: Wallingford, UK)

Soltani A, Maddaha V, Sinclair TR (2013) SSM-wheat: a simulation model for wheat development, growth and yield. *International Journal of Plant Production* **7**, 714

Tamang BG, Schoppach R, Monnens D, Steffenson BJ, Anderson JA, Sadok W (2019) Variability in temperature-independent transpiration responses to evaporative demand correlate with nighttime water use and its circadian control across diverse wheat populations. *Planta* **250**, 115–127. doi:10.1007/s00425-019-03151-0

Tanner CB, Sinclair TR (1983) Efficient water use in crop production: research or re- search? In 'Limitations to efficient water use in crop production'. (Eds HM Taylor, JR Wayne, SR Thomas) pp. 1–27. (American Society of Agronomy, Crop Science Society of America, Soil Science Society of America: Madison, WI, USA)

Tolk JA, Howell TA, Evett SR (2006) Night-time evapotranspiration from alfalfa and cotton in a semiarid climate. *Agronomy Journal* **98**, 730–736. doi:10.2134/agronj2005.0276

USDA-FAS (2005) Tunisia Grain and Feed Annual, 2005. GAIN Report Number: TS5004.

USDA-FAS (2007) Tunisia Grain and Feed Annual, 2007. GAIN Report Number: TS7006.

USDA-FAS (2009) Tunisia Grain and Feed Annual, 2009. GAIN Report Number: TS9003.

USDA-FAS (2011) Tunisia Grain and Feed Annual, 2011. GAIN Report Number: TS1102.

USDA-FAS (2013) Tunisia Grain and Feed Annual, 2013. GAIN Report Number: TS1303.

USDA-FAS (2016) Tunisia Grain and Feed Annual, 2016. GAIN Report Number: TS1604.

World Food Programme (WFP) (2011) Secondary data analysis of the food security situation in Tunisia. Available at https://documents.wfp.org/stellent/groups/public/documents/ena/wfp236106.pdf [Verified 2 July 2020]

Yu K, Goldsmith GR, Wang Y, Anderegg WRL (2019) Phylogenetic and biogeographic controls of plant nighttime stomatal conductance. *New Phytologist* **222**, 1778–1788. doi:10.1111/nph.15755

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