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Leaf growth rate per unit thermal time follows QTL-dependent daily patterns in hundreds of maize lines under naturally fluctuating conditions

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ABSTRACT

We have analysed daily patterns of leaf elongation rate (*LER*) in large data sets with 318 genotypes placed in naturally fluctuating temperature and evaporative demand, and examined the effect of targeted alleles on these patterns. The method consisted, firstly, in expressing elongation rate per unit thermal time, so it became temperature independent; secondly, in a joint analysis of diurnal fluctuations of elongation rate and of micrometeorological conditions in several experiments, and finally, in a comparison of daily patterns between groups of genotypes possessing targeted alleles. (1) Conditions for using thermal time at a time step of 15 min were first tested successfully in 318 recombinant inbred lines (RILs) of three mapping populations. (2) An analysis of 1989 time courses revealed a robust daily pattern of *LER* per unit thermal time (LER_{th}) over several experiments. LER_{th} was constant during the night and was reproducible (for a given RIL) over up to 10 consecutive nights in different experiments. It declined rapidly during the early morning, closely following the daily pattern of transpiration rate. (3) Groups of RILs carrying alleles conferring a high response to temperature had markedly higher night-time plateau of *LER* than those with low responses. Groups of RILs with high response to evaporative demand had rapid decreases in elongation rate at the transition between night and day, while this decrease was slower in groups of RILs with low response. These results open the way for using kinetics of responses to environmental stimuli as a phenotyping tool in genetic analyses.

Key-words: evaporative demand; phenotyping; temperature; time course; transpiration.

INTRODUCTION

Kinetic analyses are essential tools for analysing the responses of plant growth to the environment. In particular, they allow identification of the phenotypic variables that first respond to an environmental stimulus (Boyer 1970; Saab & Sharp 1989; Tang & Boyer 2002). This helps hypothesize the most likely mechanisms, or at least exclude mechanisms whose time characteristics do not match with observed time courses of growth (Ben Haj Salah & Tardieu 1997; Munns *et al.* 2000). However, time courses are most often analysed individually for each experiment, day and genotype when micrometeorological conditions are not strictly stable because of the overriding effect of temperature on most physiological processes. This hampers the possibilities to analyse jointly several time courses in different days or experiments, and thus to distinguish which temporal variations can be attributed to 'noise' and which are reproducible. This also hampers the genetic analysis of temporal patterns of responses to environmental stimuli.

This difficulty would be overcome if rates can be expressed in a temperature-independent way, allowing one to work in fluctuating temperature as in stable temperature. Thermal time has been used for a long time in this purpose (Bonhomme 2000). It is used by most crop models to simulate the occurrence of phenological stages (e.g. number of leaves or flowering time) (Keating *et al.* 2003; Lizaso, Batchelor & Westgate 2003). Our group has also used it for expressing tissue expansion rate or cell division rate with a temporal resolution of 1 d (Granier & Tardieu 1998; Tardieu *et al.* 2000). However, expressing rates per unit thermal time with a temporal resolution of minutes has never been attempted to our knowledge. The objective of this study was to propose such a method, and to test its validity for analysing the genetic and environmental variabilities of daily time courses of *LER* over large data sets.

The first step was to check whether the conditions for a rigorous use of thermal time at short time steps were fulfilled for an analysis of large numbers of recombinant inbred lines (RILs) in several experiments. This examination was carried out in 318 RILs belonging to three mapping populations with different geographical origins (Europe, North America and tropical Mexico) and in different climatic scenarios in the growth chamber and in the greenhouse.

The second step consisted in analysing the commonality of daily patterns in different experiments and RILs.

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Because *LER* per unit thermal time (LER_{th}) was stable during the night and over several successive nights for each RIL, we could interpret its diurnal variations around this baseline as responses to environmental conditions and transpiration rate. We then identified common temporal patterns of responses.

The third step consisted in the identification of differences in time courses in groups of RILs with different allelic combinations previously identified as affecting the responses of *LER* to temperature or to evaporative demand. This was carried out in an independent experiment.

THEORY

Three equations are currently used in the literature for the expression of rates of physiological processes as a function of temperature.

The first one is the Arrhenius equation, which applies to a large range of metabolic processes (Gillooly *et al.* 2001),

$$J = A\exp[-E_i/(RT_{\circ K})], \quad (1)$$

where J is the considered flux; $T_{\circ K}$ is the temperature in °K, E_i represents an average activation energy for the considered flux; R is the gas constant, and A is a proportionality constant. Equation 1 is usually expressed and tested in a modified way:

$$\ln(LER) = \alpha + (\beta/T_{\circ K}), \quad (2)$$

where $\alpha = \ln(A)$ and $\beta = -E_i/k$.

The second one is a linear relationship between rate and temperature, identified for instance for the rates of germination (Steinmaus, Prather & Holt 2000), leaf development (Granier & Tardieu 1998) or leaf expansion (Ong 1983; Ben Haj Salah & Tardieu 1995). For monocot leaves, which have an essentially unidirectional expansion, this results in

$$LER = dL/dt = a(T - T_0), \quad (3)$$

where *LER* is leaf elongation rate; L is leaf length; T (°C) is temperature, and a and T_0 are the slope and x-intercept of the relationship between *LER* and temperature (T_0 is termed threshold temperature hereafter).

The third one, the most used in crop models (Keating *et al.* 2003; Lizaso *et al.* 2003), is a bilinear model with three critical temperatures. Growth is supposed to increase linearly with temperature from T_0 (as defined earlier) to an optimum temperature, and to decrease linearly from the optimum to a maximum temperature. In contrast to the models of Eqns 1 and 2, this model has three parameters. Because a bilinear model with a discontinuity at the optimum temperature poses statistical problems, we tested instead a quadratic model that approximately follows the same pattern and also comprises three parameters:

$$LER = dL/dt = a_0 + a_1T + a_2T^2, \quad (4)$$

where a_0, a_1 and a_2 are fitted parameters. a_2 is negative if high temperatures tend to have a negative effect, as in the bilinear model, and positive if high temperatures tend to act in an exponential fashion as in the Arrhenius model.

The correct use of thermal time involves several conditions: (1) the relationship between temperature and *LER* should be linear (Eqn 2) in the set of data, or corrected by taking into account 'optimum' and 'maximum' temperatures; (2) the threshold temperature (T_0) should not vary between experiments for a given RIL; and (3) if different RILs are to be compared, their threshold temperature should ideally be similar, thereby allowing common calculation of thermal time. Because it has been expected that these conditions would not be fulfilled to the same extent in all studied RILs, the crucial question is whether the use of thermal time in whole mapping populations generates large bias in the comparison of RILs and of environmental conditions.

If Eqn 3 is acceptable, it can be integrated to express leaf length at any time (t) as a function of the cumulated temperature above the threshold temperature T_0,

$$L = a\int_0^t [T(t) - T_0]\,dt \quad (5)$$

where $\int_0^t [T(t) - T_0]\,dt$ is thermal time (°Cd) termed t_{th} hereafter. The time course of *LER* can therefore be expressed per unit thermal time (LER_{th})

$$LER_{th} = dL/dt_{th} = a. \quad (6)$$

MATERIALS AND METHODS

Genetic material and experiments

Three mapping populations (plus their parents) were analysed. The first population, called population D hereafter, derives from the cross of the parental line F-2 (an early French flint) and Io (a late north American semi-dent) after six cycles of self-pollination. It consists of 145 RILs mapped with 152 RFLP probes (Causse *et al.* 1996). A subset of 100 RILs was used in this study. The second population, called population E hereafter, derives from a cross between the lines F-2 (common with the former population) and F252 (an American early dent line) after six cycles of self-pollination, and consisted of 98 RILs mapped with 173 RFLP probes (Charcosset, personal communication). The third population (called P1 × P2) developed at sixth inbreeding levels at CIMMYT consists of 120 RILs from the cross between the tropical parents Ac7643 and Ac7729/TZSRW (referred to as P1 and P2, respectively). These RILs were mapped with 132 RFLP probes (Ribaut, personal communication).

Nine experiments were carried out, four entirely in the greenhouse, and five in which plants were first grown in the greenhouse until the sixth leaf emerged and were then transferred to a growth chamber for short periods of time. Five experiments comprised all RILs of a population (two

for populations D and E, and one for population P1 × P2, Table 1), while the other aimed at testing the repeatability of results under different experimental conditions. In total, 127 RILs were analysed in one experiment, 105 in two experiments, 63 in three experiments, 24 in four experiments, 3 in five experiments and the F-2 line in six experiments.

Experimental set-up

The experimental set-up in the greenhouse is presented in Fig. 1. It consists of 122 balances that continuously measure the changes in soil water status, 366 displacement transducers that measure *LER*s and a set of environmental sensors. A companion set-up was placed in a growth chamber, with the same sensors for the simultaneous measurement of 60 plants. Measurements of leaf elongation were carried out with rotating displacement transducers (RDTs, 601–1045 Full 360 Smart Position Sensor; Spectrol Electronics, Ltd, Wiltshire, England). Leaf elongation was transmitted to the sensor via a pulley attached to it, which carried a thread attached to the leaf tip and to a 20 g counterweight. Measurements (every 15 min) began when the tip of the sixth leaf appeared above the whorl and lasted until the end of the period with linear elongation. The latter was checked a posteriori and coincided with the appearance of leaf 8. We tested whether temperature and vapour pressure deficit (VPD) had an effect on the thread extensibility by connecting the RDT sensors to a thread fixed on a static support in different environments (greenhouse and growth chamber), with conditions similar to those imposed to the plants in the experiment. To test whether the counterweights had an appreciable effect on elongation rate (Cramer & Bowman 1991; Walter, Feil & Schurr 2002), *LER* was measured in 10 plants (maize hybrid Dea) with counterweights of 10, 20 and 30 g, and no significant effect was observed. Elongation rate was measured in non-attached leaves with a ruler and with RDTs in 52 RILs for 5 d in well-watered conditions. A tight relationship was observed between RDT and manual measurements ($r^2 = 0.72$), without appreciable bias. In Exps G1, G2, G3 and G4, the vertical position of the tip of leaf 6 was measured twice a day, in the morning at a time when the mean photosynthetic photon flux density (PPFD) measured over several days was still lower than 10 μmol m^{-2} s^{-1}, and in the evening when mean PPFD was lower than the same value. The position of the leaf tip was measured using a ruler attached to a 2.5 m horizontal bar fixed on vertical metal sticks permanently left on the soil.

The plants were grown in polyvinyl chloride (PVC) columns (0.15 m diameter and 0.4 m height) containing 7.5 kg of a 40:60 (v/v) mixture of loamy soil (aggregate diameter ranging from 0.1–4.0 mm) and an organic compost. Seeds were sown at 0.025 m depth by triplets and were thinned to one when leaf 3 emerged. Each column contained three different RILs, and each RIL was sown in three columns distributed randomly.

Table 1. Characteristics of experiments

Name	Environment[a]	Sowing date	No. of genotypes[a]	Population[b]	No. of time courses[c]	Technique[d]	Night T (°C)[e]	PPFD (mol·day^{-1})[f]	Day VPD_{eq} (kPa)[g]
G1	Gh	10/04/2000	102	D	306	Manual	17.4, 19.8	5.0, 29.9	0.6, 1.5
G2	Gh + GC	10/06/2000	102	D	306	Manual	14.7, 27.8	8.0, 31.5	0.2, 3.2
G3	Gh + GC	26/06/2001	100	E	300	Manual	14.5, 27.8	8.0, 30.3	0.2, 2.8
G4	Gh	18/02/2002	100	E	300	Manual + RDT	15.0, 20.1	4.7, 29.7	0.2, 1.7
G5	Gh	16/05/2002	32	E	96	RDT	15.8, 22.9	4.0, 24.3	0.1, 1.5
G6	Gh	11/07/2003	10	D	30	RDT	24.3, 25.9	10.6, 24.8	1.7, 2.5
G7	Gh + GC	17/06/2004	33	50% D–50% E	99	RDT	14.9, 27.7	9.2, 26.0	0.7, 3.1
G8	Gh + GC	9/01/2004, 23/01/2004 and 5/02/2004	122	P1 × P2	366	RDT	14.9, 27.7	5.1, 28.2	0.3, 3
C1	Gh + GC	14/03 and 25/04/2003	62	E	186	RDT	14.7, 28.5	13.8, 33.7	0.3, 3.0

[a]Number of genotypes in the experiment.
[b]Mapping population in the experiment.
[c]Number of time courses obtained in the experiment.
[d]Technique for leaf elongation rate (*LER*) measurements.
[e]Range of mean night temperature (T).
[f]Range of day photosynthetic photon flux density (PPFD) .
[g]Range of mean day vapour pressure difference between leaves and air (VPD_{eq}).
RDT, rotating displacement transducer; Gh, greenhouse; GC, growth chamber; D and E, temperate; P1 × P2, tropical.

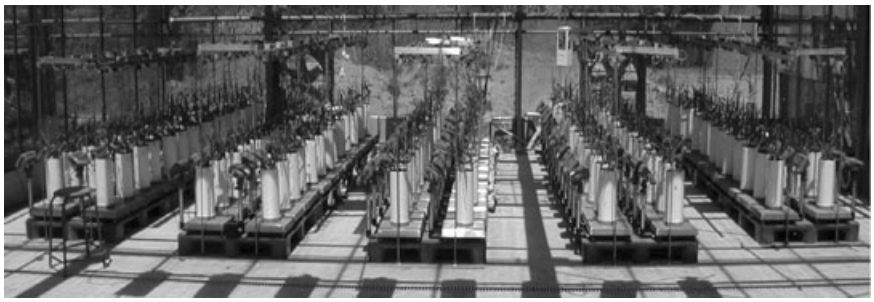

Figure 1. The *Phenodyn* phenotyping platform for continuous measurement of leaf elongation rate, soil water status and micrometeorological variables (up to 366 plants). Plants were grown in polyvinyl chloride (PVC) columns and placed on balances. Each leaf was attached to a rotating displacement transducer (RDT). Environmental sensors [photosynthetic photon flux density (PPFD), vapour pressure deficit (VPD) and meristem temperature] were placed at plant level. All sensors were connected to data loggers with a time resolution of 15 min.

Air temperature and relative humidity were measured every minute at plant level (HMP35A; Vaisala Oy, Helsinki, Finland). The temperature of the meristematic zone of 10 plants per experiment was also measured with a fine copper-constantan thermocouple (0.2 mm diameter) located between the sheaths of leaves 1 and 2 at meristem height. It was checked that temperature measured in this way was close to that measured by thermocouples inserted in the growing zone itself. Leaf-to-air vapour pressure difference was estimated at each time step as the difference in water vapour pressure between saturation at meristem temperature and the current vapour pressure in the air. Light was measured every minute using two PPFD sensors [LI-190SB, Li-Cor quantum photosynthetically active radiation (PAR); Li-Cor Inc., Lincoln, NE, USA or SOLEMS 01/012/012; SOLEMS, Palaiseau, France]. All data of temperature, PPFD and relative humidity were averaged and stored every 15 min in a data logger (LTD-CR10X wiring panel; Campbell Scientific, Leicestershire, UK). Soil water content was determined by weighing columns everyday; after that, the thread connected to RDTs was detached from leaves. Differences in weight were attributed to changes in soil water content, after correction for the increase in mean plant biomass as a function of phenological stage. It was checked that this procedure generated errors smaller than 3 g (i.e. an error in soil water content of about $6\ 10^{-4}$ g g^{-1}). The soil was maintained at retention capacity by daily watering with a modified one-tenth-strength Hoagland solution.

Plant transpiration was estimated in Exps G6–G8 from the weight loss of each column every 15 min. Direct evaporation from the soil was estimated by measuring the weight loss of columns without plants, whose upper layer was watered at the same time as other columns. Plant transpiration was divided by an average leaf area of 20 plants, estimated by measurement of leaf length and width every third day. This measurement provides an estimate of the evaporative demand as sensed by plants, taking into account the diurnal changes in stomatal conductance, but was not precise enough to compare the transpiration rates of RILs.

Manipulation of environmental conditions

In Exps G2 and G3, the night-time air temperature was varied by covering plants with a 4.0×4.7 m plastic shelter that allowed one to either lower the air temperature down to 16.7 °C by using commercial air conditioners, or to increase it to 28 °C by using heaters. In addition, the plants were moved into a growth chamber for two nights during which meristem temperature was 14.5 ± 0.6 °C. The temperature was fluctuating naturally during other nights. The daytime VPD was varied by either turning off the water of air cooling or by leaving the water circulation in the air cooling system. Low VPDs were obtained by spraying water continually on the floor of the greenhouse. In the remaining greenhouse experiments (G1, G4, G5 and G6), the plants were followed under naturally fluctuating conditions (Table 1).

In Exps C1, G7 and G8, the plants were grown in the greenhouse but were transferred for 2–3 d periods into the growth chamber. The night temperature was 28 °C for the first 4 h, 22 °C for the next 3 h and 14 °C for the final 3 h, while air VPD was 0.8 kPa. A similar protocol was applied to the hybrid Dea in the range 9–32 °C. The daytime temperature was kept constant at 28 °C, while the VPD was varied in three steps (1.0, 2.0 and 2.7 kPa) during 6, 4 and 4 h, respectively. Light (450–550 μmol m^{-2} s^{-1} at leaf level) was provided by a bank of cool-white fluorescent tubes and incandescent bulbs.

Data analysis

Computer programmes were developed for data analysis in the R language (R Development Core Team 2005). The first procedure consisted in a daily identification of the problems associated with sensor functioning, inevitable when 382 sensors (RDT + environment) were used together. Data were then stored in a database (MySQL, http://www.mysql.com). Another set of procedures was developed to analyse growth and environmental data jointly, firstly for the analyses of time courses on short time steps and

secondly for regression analyses over several hours. In the greenhouse, elongation rates and environmental data were averaged during night and day periods with a PPFD threshold of 10 μmol m^{-2} s^{-1}. In the growth chamber experiments, data were averaged for each period with constant temperature and VPD.

Statistical analyses were carried out with the R-language 'stat' package (http://www.R-project.org), which allowed us to easily interface the calculations of phenotypic and climatic values with statistical analyses within the same loops of calculation for each RIL. Regression analyses were performed by fitting a linear model on data corresponding to each RIL, either with two parameters for Eqns 2 and 3, or with three parameters for Eqn 4. The function used for that is the equivalent of the 'lm' function of the S package (Chambers 1992), which calculates regression parameters, SEs and significance of each individual parameter of each RIL. Quantitative trait loci (QTLs) were detected by composite interval mapping with the use of cofactors, using the PlabQTL software (Utz & Melchinger 2000). The choice of cofactors was first carried out using a stepwise regression between the studied trait and the allele value at each marker. The retained marker number in this study was conditioned by Fisher's analysis of variance (ANOVA) test, fixed at 7. Presence of main effect QTL was tested every 2 cM using a multiple regression with the retained cofactors. The threshold value of likelihood of odds (LOD) was determined by 1000 permutations (Churchill & Doerge 1994), and was 2.3 and 3.0 for 0.25 and 0.10 risks.

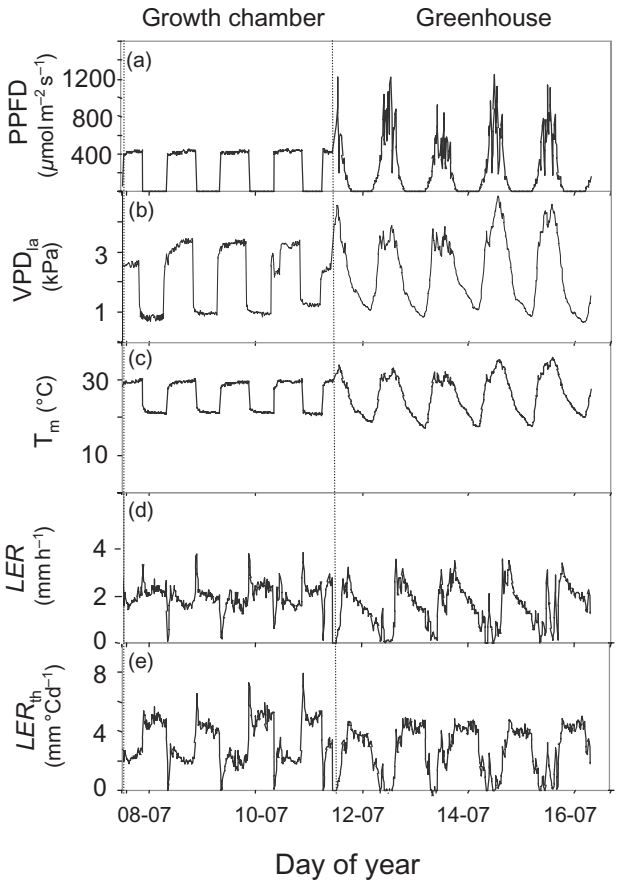

Figure 2. Time courses of environmental variables (a–c) and of leaf elongation rate expressed per unit clock time (d) or thermal time (e), with a time resolution of 15 min. The plant [population D, recombinant inbred line (RIL) 124] was first placed for 4 d in the growth chamber, and was then moved to the greenhouse for 5 d. PPFD, photosynthetic photon flux density; VPD_{la}, difference in vapour pressure of water from leaf to air; T_m meristem temperature; *LER* leaf elongation rate per unit clock time; LER_{th}, leaf elongation rate per unit thermal time.

RESULTS

An example of a 9 d long time course is presented in Fig. 2 for a plant measured first in the growth chamber and then in the greenhouse. The time course of *LER* was difficult to interpret if expressed per unit clock time, with different patterns in the growth chamber and in the greenhouse (no day–night difference in the growth chamber versus a day–night pattern in the greenhouse). Conversely, a clear day–night pattern appeared if *LER* was expressed per unit thermal time.

Are the conditions required for the use of thermal time sufficiently fulfilled in the 318 RILs of the three mapping populations?

The first condition for expressing rates per unit thermal time is a linear relationship between *LER* and meristem temperature during the night (i.e. in the absence of evaporative demand) (Eqn 3). In the experiment with an extended range of temperature (Fig. 3a), the relationship between night-time *LER* and meristem temperature was linear in the range 9–32 °C ($r^2 = 0.99$), while that between ln(*LER*) and $1/T_{°K}$ departed from linearity (Fig. 3a, inset). Regression analyses were then carried out for the 318 RILs of the three mapping populations in the range 14–28 °C by considering experiments in the growth chamber and in the greenhouse. Four examples are presented in Fig. 3b–e. All RILs of the three populations showed significant linear regressions for Eqn 3 (median $r^2 = 0.83$, Fig. 4 inset). Regressions corresponding to Eqn 2 were also significant (median $r^2 = 0.81$), and provided response curves whose values were close to those of the linear regression. The quadratic equation (Eqn 4) had r^2 similar to those of the linear equation, increased by 0.01 in average, but its quadratic term was significant in only 18, 11 and 26% of RILs in populations D, E and P1 × P2, respectively. The other parameters were non-significant in 76% of cases, suggesting an over-parameterization of the quadratic model. It is concluded that the relationships between temperature and *LER* can be considered as linear in the considered range of temperature. Because regressions were applied to several experiments, these relationships could be considered as unique for each RIL (second condition stated in Theory).

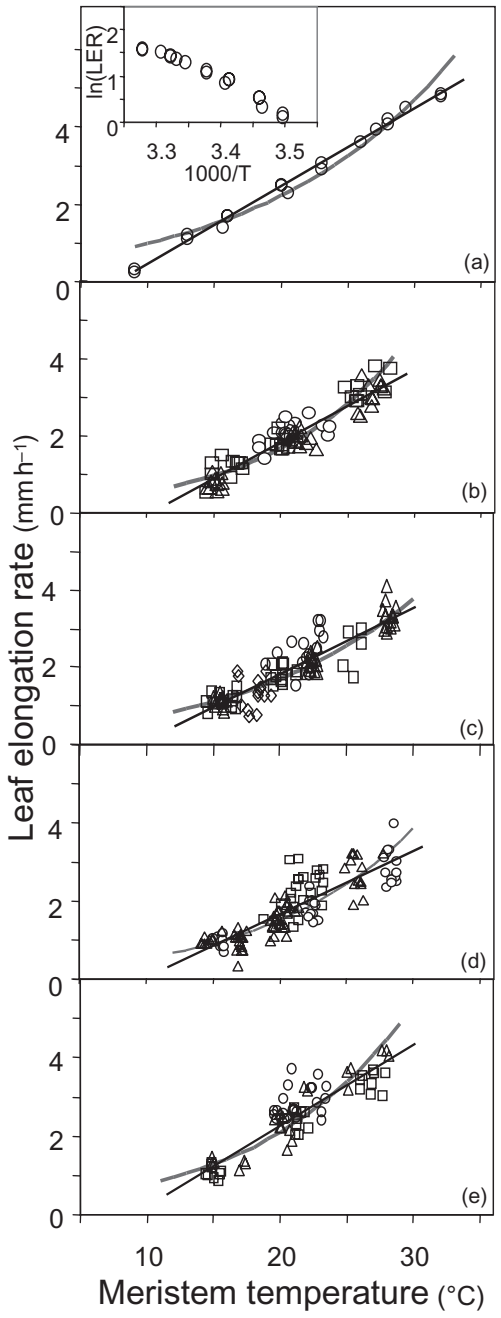

Figure 3. Relationships between meristem temperature and leaf elongation rate (*LER*). Each symbol represents one night (greenhouse) or a fraction of night with constant temperature (growth chamber). Straight black lines, linear regression (Eqn 3). Curvilinear lines, exponential regression (Eqn 1). (a) Growth chamber experiments, (b–e) combination of experiments in the greenhouse and in the growth chamber for one parental line (F252, d), and three recombinant inbred lines (RILs) of populations D and E. Panel (a), inset, Arrhenius equation (Eqn 2). Squares, Exp G3 (greenhouse and growth chamber); diamonds, Exp G4 (greenhouse); triangles, Exp C1 (greenhouse and growth chamber); circles, Exp G7 (greenhouse and growth chamber).

The last condition for the use of thermal time in all RILs of a mapping population is the commonality of threshold temperature (T_0, Eqn 3). In each population, the variability of T_0 was larger than intervals of confidence of T_0, about $\pm$ 1 °C (Fig. 4). Furthermore, significant QTLs for T_0 were identified (one per population, not shown), suggesting that the genetic variability of T_0 was structured in the three populations.

The question was therefore whether LER_{th} (Eqn 6), calculated with a common T_0, remains temperature independent even in RILs whose individual T_0 differ from the mean value of the population. A temperature independence was expected in RILs whose individual T_0 is close to the mean value of the population, and a temperature dependence in the opposite case (Fig. 5, upper panels). A sensitivity analysis was carried out by calculating the derivative of LER_{th} with respect to meristem temperature in all RILs for threshold temperatures ranging from 8 to 12 °C, with a 0.5 °C step (Fig. 5). In each mapping population, the proportions of RILs with a significant effect of temperature decreased when the tested threshold temperature got closer to the mean value of the mapping population. Minimum proportions were 10, 22 and 20%, respectively, in populations D, E and P1 × P2, with small variations in proportions

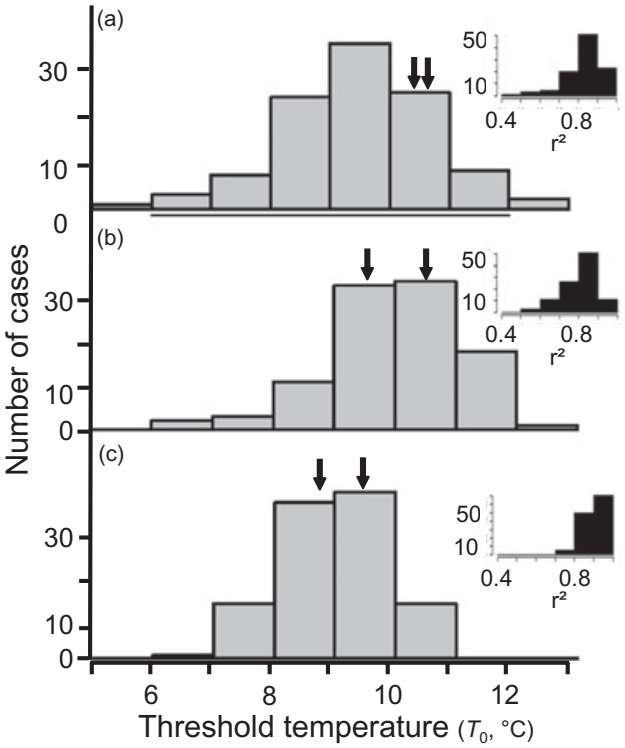

Figure 4. Distributions of the threshold temperature (T_0, Eqn 3) estimated by linear regression in the three mapping populations. (a) Population D [temperate, F-2 × Io, 100 recombinant inbred lines (RILs)], (b) population E (temperate, F-2 × F252, 98 RILs), (c) population P1 × P2 (tropical, 120 RILs). The values of the parental inbred lines are shown as arrows. Insets, frequency distributions of r^2 calculated for individual RILs in each mapping population.

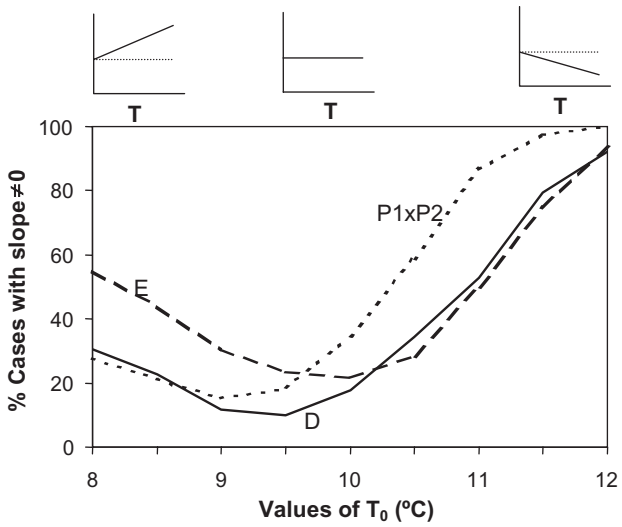

Figure 5. Proportion of cases in which leaf elongation rate per unit thermal time (LER_{th}) was not independent of meristem temperature, for threshold temperatures (T_0) from 8 to 12 °C. LER_{th} was calculated with threshold temperatures from 8 to 12 °C with a step of 0.5 °C for the 318 recombinant inbred lines (RILs) of the three mapping populations. For each threshold temperature and RIL, the regression was calculated between LER_{th} and meristem temperature. The percentage of cases in which the slopes were significantly different from zero was recorded. Plain line, population D; dashed line, population E; dotted line, population P1 × P2.

around the minima values. The bias generated by imposing a unique threshold temperature, estimated by the difference in LER_{th} at 14 and 28 °C, was smaller than one SD in 74, 86 and 80% of RILs in populations D and E and P1 × P2 (median bias, 0.1, 0.5 and 0.2 mm °Cd^{-1}). As a consequence, the error linked to the choice of a common threshold temperature was small. This resulted in the fact that change in threshold temperature in the range 8–12 °C had no effect on major QTLs of *LER* published earlier for population D (Reymond *et al.* 2003; Reymond, Muller & Tardieu 2004). The same result applied to the other two mapping populations (not shown). It was therefore considered as acceptable to express LER_{th} with a common threshold temperature for each mapping population.

LER_{th} is stable during the night and over several nights

Equation 6 implies that LER_{th} should be constant, at the value a, when plants are subjected to changes in temperature but to no other environmental constraint. In particular, this should be the case during the night in well-watered plants, as it was for one plant in Fig. 2e. Stability of *LER* during the night was observed in the analyses of 150, 373 and 516 24 h time courses, respectively, in experiments in the greenhouse (Figs 6c & 7b) or in the growth chamber (Fig. 6d). The existence of a night plateau of LER_{th} was tested individually over 1633 one-night time courses of all experiments. For that, the slope of the relationship between time and LER_{th} was calculated each night for each plant (Fig. 8). Slopes ranged from –0.4 mm °Cd^{-1} h^{-1} (decrease with time during the night) to +0.5 mm °Cd^{-1} h^{-1} (increase during the night), with a median value of 0 mm °Cd^{-1} h^{-1} (constant). They significantly differed from zero in 20% of cases but with small differences between the beginning and the end of the night (lower than 10% in 86% of cases). No QTLs were detected on this slope. There was no relation

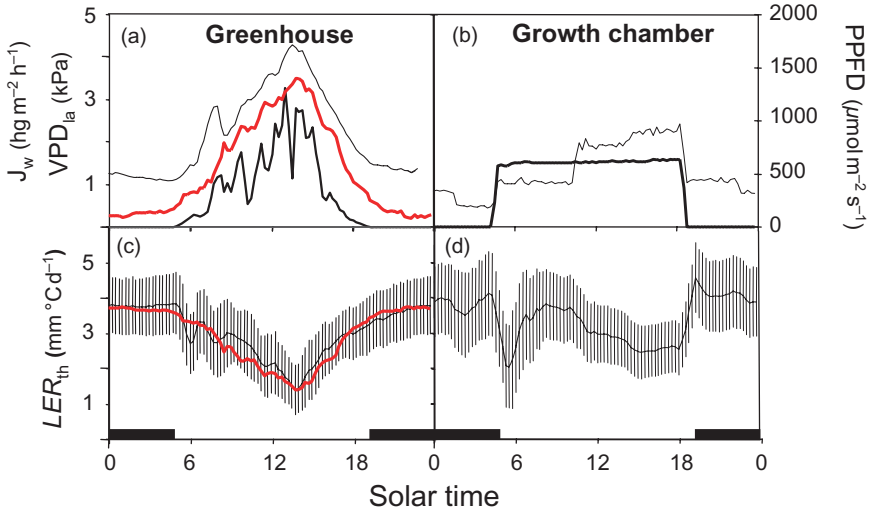

Figure 6. Time courses of leaf elongation rate per unit thermal time (LER_{th}) of groups of recombinant inbred lines (RILs) in the greenhouse and in the growth chamber. (a,b) Leaf-to-air vapour pressure deficit (VPD) (thin lines), photosynthetic photon flux density (PPFD) (thick lines) and transpiration rate (J_w, red line) every 15 min in the greenhouse, Exp G6 (a) and the growth chamber, Exp C1 (b). (c,d) mean and SD of LER_{th} averaged over all RILs and days (black), and model corresponding to Eqn 7 with a linear and negative effect of transpiration rate (red line). Number of 24 h time courses in panels (c) and (d), 150 and 373, respectively. RILs belonged to populations D and E. Horizontal black lines, night.

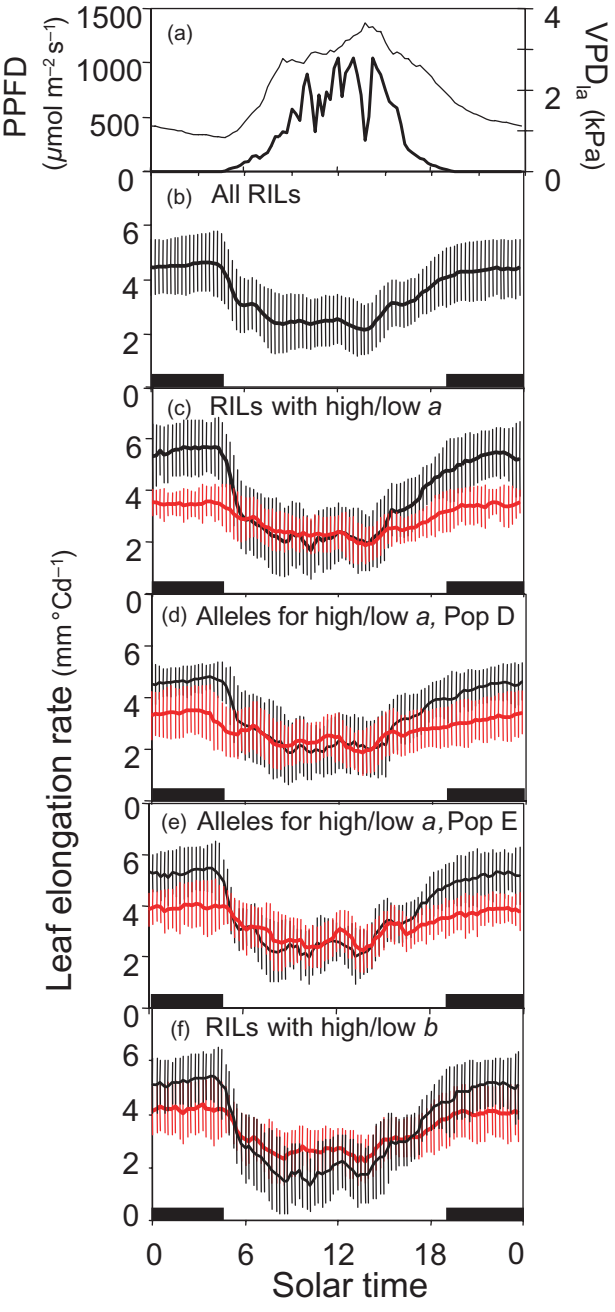

Figure 7. Time course of leaf elongation rate per unit thermal time (LER_{th}) for different groups of recombinant inbred lines (RILs) in the greenhouse Exp G7. (a) Leaf-to-air vapour pressure deficit (VPD) (thin lines), photosynthetic photon flux density (PPFD) (thick lines). (b) All RILs considered together, mean and SDs every 15 min time step (RILs of populations D and E). (c) Groups of RILs differing in parameter *a*, Eqn 3, either high (black) or low (red). (d,e) Groups of RILs differing in allelic values at quantitative trait loci (QTLs) which confer them high (black) or low (red) values of parameter *a*, in mapping populations D and E, respectively. (f) Groups of RILs with high (black) or low (red) values of parameter *b*. Slope of the response of LER_{th} to difference in vapour pressure of water from leaf to air (VPD_{la}) (Eqn 8). Number of 24 h time courses in panels (b–f): 516, 59 and 61, 39 and 34, 34 and 42, and 61 and 59. Horizontal black lines, night.

either between the tendency towards increasing or decreasing LER_{th} during the night and the PPFD during the preceding day, as every class of slope in Fig. 8 corresponded to plants receiving PPFDs ranging from 10 to 35 mol m^{-2} d^{-1}. The only consistent effects of PPFD were transient changes in LER_{th} at each day–night transition.

The next question was whether this plateau value of LER_{th} is stable over successive nights, as suggested by Fig. 2e for one RIL. We have tested this stability on 611 time courses lasting from 4 to 10 d between the appearances of leaves 6 and 8 (two phyllochrons). In 97% of RILs, the slope of the regression between time and mean LER_{th} did not differ from 0. The mean slope was 0.05 mm $°Cd^{-1}$ $night^{-1}$ (slight increase with time) and ranged from –0.2 and +0.2 mm $°Cd^{-1}$ $night^{-1}$ in 73% of cases. As a consequence, there was no general trend towards a decrease or increase in night-time LER_{th} during leaf development. The mean LER_{th} measured on one night was independent of the light, of the evaporative demand and of the LER_{th} of the preceding day. In contrast, a slight tendency existed towards higher values of night LER_{th} in the growth chamber than in the greenhouse, as in Fig. 2e. This may be due to non-negligible transpiration rate in the greenhouse during the night (30 g m^{-2} h^{-1}, Fig. 6a) that was much higher than in the growth chamber (near zero, too low for measurement). Overall, these results show that LER_{th} follows a plateau each night, whose value is conserved over two phyllochrons. This plateau value can therefore be considered as an intrinsic characteristic of a plant.

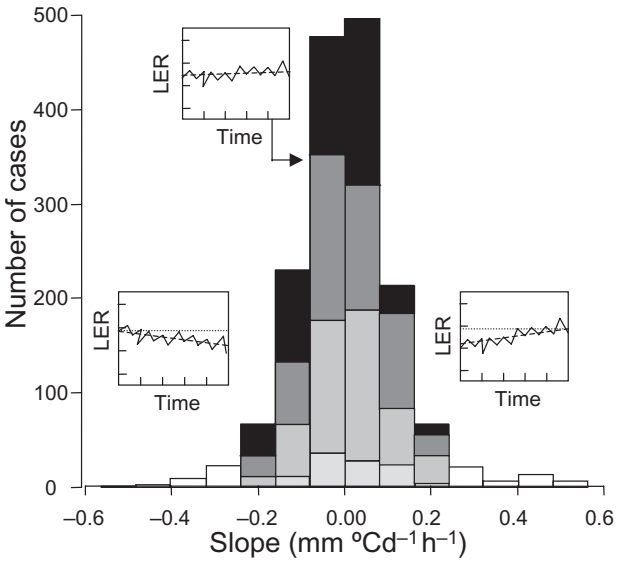

Figure 8. Distribution of the slope of the change in leaf elongation rate per unit thermal time (LER_{th}) during nights. Negative and positive values represent an increase (respectively, decrease) in leaf elongation rate (LER) during the night. Grey levels in bars represent the photosynthetic photon flux density (PPFD) values during the day preceding the considered night. Black, 28–35 mol m^{-2} d^{-1}; dark grey, 21–27 mol m^{-2} d^{-1}; medium grey, 16–20 mol m^{-2} d^{-1}; light grey, 10–15 mol m^{-2} d^{-1}; white, too small population.

Table 2. Quantitative trait loci (QTLs) of parameter *a* detected by composite interval mapping in population E

Chr[a]	Pos[b]	Marker[c]	IC[d]	LOD[e]	r^{2f}	Additive effect[g]
1	102	gsy324_G	80–132	2.45	0.11	0.027
1	144	umc128	142–148	3.06	0.13	0.023
2	76	umc34	70–82	3.25	0.14	–0.017
7	50	bnl1540	40–60	3.57	0.16	–0.016
8	8	gsy172b	0–12	3.06	0.15	0.016

[a]Chromosome number.
[b]QTL position from the top of the chromosome.
[c]Closest marker.
[d]Confidence interval of the QTL.
[e]Log base 10 of the likelihood odds ratio.
[f]Coefficient of determination.
[g]Additive effect of the parent F252 allele.

Groups of lines with high responses to temperature have high plateaux of night-time *LER*

The daily patterns presented in Figs 6 and 7 group all the studied RILs of an experiment. A further step is to compare daily patterns of groups of RILs with contrasting parameters of growth, or with contrasting QTLs of these parameters. Two groups of about 60 time courses with either highest or lowest values of parameter *a* were selected among the 516 time courses of Exp G7 (Fig. 7c). The values of *a* were determined for each RIL over all experiments except G7. They were therefore independent of phenotypic data presented in Fig. 7c. Groups of RILs that differed in parameter *a* had a clear difference in night behaviour, with higher night values for RILs with high *a* than for those with low *a* (Fig. 7c). This difference disappeared during the day under higher evaporative demand.

The behaviours of groups of RILs with different allelic values at QTLs of parameter *a* were then analysed in the same experiment. QTLs taken into account were those presented earlier for population D (Reymond *et al.* 2003, 2004), and were determined in the same way for population E (Table 2) in experiments presented in Table 1 but excluding Exp G7 presented in Fig. 7. QTL positions and effects were therefore calculated independently of the experiment presented in Fig. 7. The allelic values taken into account were those at QTLs on chromosomes 1, 2, 4 and 8 for population D and on chromosomes 1, 2, 7 and 8 for population E. In both mapping populations, groups of RILs with different allelic values at QTLs had strikingly different behaviours, with higher night values of LER_{th} in groups of RILs with QTLs which conferred them high values of parameter *a* (Fig. 7d,e).

The pattern of *LER* during the day follows that of transpiration rate

Because night values of LER_{th} were stable and reproducible over several nights and experiments, they can be considered as the baseline from which elongation rate diverges during the day depending on environmental conditions. A daytime depression of *LER* per unit thermal time was observed every day in the time course presented in Fig. 2. It was also observed in the three daily patterns presented in Figs 6c,d and 7b, in which successive days had different temperatures but similar VPDs. In both cases, LER_{th} decreased when evaporative demand increased. The diurnal change in elongation rate closely followed that of transpiration rate in the greenhouse experiment presented in Fig. 6, with a negative linear effect of transpiration rate (Fig. 6c, thick line):

$$LER_{th} = dL/dt_{th} = a(1 - dJ_w), \quad (7)$$

where J_w is the transpiration rate per unit leaf area. The decrease in LER_{th} was rapid in the two greenhouse experiments presented in Figs 6c and 7b, beginning at 0500 and 0430 h, respectively (i.e. when PPFD reached 10 μmol m^{-2} s^{-1}) (Figs 6a,c & 7a,b). LER_{th} recovered transiently in Exp G6 before decreasing again, which was not observed in Exp G7 where the decrease in elongation rate was very rapid (33% in 90 min). The morning decrease in LER_{th} also occurred in less than 15 min in the growth chamber experiment in which the PPFD increased suddenly from 0 to 500 μmol m^{-2} s^{-1}. LER_{th} first sharply decreased, as in most time courses of *LER* reported in the literature, then recovered in 1 h and reached a lower plateau. Subsequently, it followed the step changes in VPD (Fig. 6d).

Daytime patterns of *LER* are smoother in groups of lines with low response to evaporative demand than in lines with high responses

The patterns of daytime *LER* was analysed in groups of RILs with contrasting responses to evaporative demand (Fig. 7f). Because transpiration was not measured in all experiments, the equation for studying the diurnal time course of elongation rate was the approximation proposed by Reymond *et al.* (2003):

$$LER_{th} = dL/dt_{th} = a(1 - bVPD_{eq}), \quad (8)$$

in which VPD_{eq} is the vapour pressure difference between leaves and air, corrected for the effect of PPFD. Groups of RILs with low response to evaporative demand (low absolute values of b) had slower day–night transitions than those with high absolute values of b, and slightly higher daytime LER_{th}. They also had different night-time elongation rates, consistent with the correlation usually observed between parameters a and b (Reymond *et al.* 2003).

DISCUSSION

The method presented here fulfils the conditions for a rigorous use of thermal time

We observed only small divergences from these conditions, which had no serious consequences on the calculation of *LER* per unit thermal time.

Surprisingly, the linear equation representing the effect of temperature was as good as, or better than, the exponential equations generally considered as the most correct theoretically (Miedema 1982; Gillooly *et al.* 2001). The same applies to other processes or other species (Ong 1983; Granier & Tardieu 1998; Steinmaus *et al.* 2000). However, it must be recognized that this is not necessarily a general case and that the threshold temperature is an extrapolation of the linear part of the response curve to temperature. The use of a linear equation reduces the number of parameters compared to bilinear equations used in crop models, but it also reduces the range of validity of the method. In maize, this range is limited, for low temperatures, to some degrees above the threshold temperature and, for high temperatures, to non-stressing temperatures (i.e. some degrees above 30 °C for both leaf elongation) (Ben Haj Salah & Tardieu 1995) and photosynthesis (Crafts-Brandner & Salvucci 2002).

Night-time *LER* (slope of regression lines) was common to several experiments or growing conditions for a given RIL. It is noteworthy that it was independent of the carbon gain during the former day, in opposition to several models (Lizaso *et al.* 2003) and to the case of root growth (Aguirrezabal, Deléens & Tardieu 1994). It was also independent of the elongation rate during the former day, in opposition to the 'stored growth' theory (Hohl & Schopfer 1992), which would suppose that elongation rate would be faster on a night that follows a day with slow elongation rate. Conversely, we detected a tendency towards higher night-time elongation rates in the growth chamber than in the greenhouse, consistent with observations of Donovan, Linton & Richards (2001). This did not generate large errors in the calculation of LER_{th}.

The choice of a common threshold temperature for a whole mapping population is not an absolute condition, but it would be counter-intuitive to compare LER_{th} of RILs having different threshold temperatures, because one elongation rate per unit thermal time would correspond to different rates per unit clock time. In spite of the observed variability of T_0 within each population, we suggest that a common T_0 can be considered in all RILs of a population because it caused small errors in the calculation of LER_{th} when temperature fluctuated in the studied range.

Towards the modelling of leaf growth in large collections of genotypes in fluctuating environment

This modelling requires the identification of genotype-dependent parameters that account both for the ability of a genotype to grow and for its responses to environmental conditions. The calculation of these parameters is not possible when time courses are considered individually. If the behaviour of one plant in Fig. 2d is apparently erratic, that of several hundreds of plants is impossible to analyse without a unifying method. A method can be the fine tuning of environmental conditions in such a way that all studied plants sense the same environmental conditions, so response curves can be calculated for different genotypes (Granier *et al.* 2006). We used here another method, the design of phenotypic variables that encapsulate daily variations of temperature and the genotype x environment interaction (Tardieu 2003; Yin, Struik & Kropff 2004).

The night plateau of *LER* per unit thermal time is one of these variables because it is observed for a genotype over several nights of several experiments. Parameter a, which was determined statistically in previous studies (Reymond *et al.* 2003, 2004), has therefore a kinetic interpretation that can be used in modelling. The value of this parameter can be dissected genetically via QTLs which accounted for the night behaviour of RILs of two mapping populations in an independent experiment, not taken into account in QTL identification. Each RIL is therefore characterized by a baseline LER_{th}, which can be deduced from allelic values at QTLs. Because similar values of parameter a were observed for different leaves of a plant (Muller, Reymond & Tardieu 2001; Lafarge & Tardieu 2002), a genotype-dependent night-time *LER* can be considered for the whole plant in architectural models (Fournier & Andrieu 1998). The daytime elongation rate is deduced from the baseline LER_{th}, with a negative effect of transpiration rate. The response to evaporative demand, stable for a genotype and QTL dependent, is therefore a second parameter which characterizes a genotype and allows calculation of *LER*.

The depression of *LER* during the day is associated with transpiration and with the response to evaporative demand, suggesting a hydraulic origin

Several arguments are in favour of a hydraulic origin of the daytime depression of LER_{th}: (1) The time course of daytime elongation rate was linked with that of transpiration rate in the greenhouse experiment, consistent with the observations of Clifton-Brown & Jones (1999). This makes unlikely the hypothesis that the day–night alternations of leaf expansion are due to a circadian clock in this set of

data, in opposition to results of Harmer *et al.* (2000). Furthermore, plants experiencing stable light and evaporative demand for several days showed no oscillation of *LER* (Ben Haj Salah & Tardieu 1995). (2) Time courses observed in independent experiments with natural variations of light in the greenhouse showed a very rapid decrease in *LER* in the early morning, for low values of PPFD regardless of the photoperiod (about 10 μmol m^{-2} s^{-1} in all experiments). In contrast, transitions were slower in field conditions, with higher soil hydraulic conductivity than in greenhouse potting compost (Ben Haj Salah & Tardieu 1995). The effect of evaporative demand even disappeared when roots were in contact with saturating water, thereby nullifying the resistance to water flow at the soil–root interface (Bouchabke, Tardieu & Simonneau 2006). This suggests again a predominant hydraulic control of the day depression, reinforced by the fact that night transpiration probably had a negative effect on night *LER*, consistent with a lower night-time leaf water potential under high evaporative demand (Donovan *et al.* 2001).

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