Linking physiological and genetic analyses of the control of leaf growth under changing environmental conditions

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Abstract. Decrease in leaf growth rate under water deficit can be seen as an adaptive process. The analysis of its genetic variability is therefore important in the context of drought tolerance. Several mechanisms are widely believed to drive the reduction in leaf growth rate under water deficit, namely leaf carbon balance, incomplete turgor maintenance, and decrease in cell wall plasticity or cell division rate, with contributions from hormones such as abscisic acid or ethylene. Each of these mechanisms is still controversial, and involves several families of genes. It is argued that gene regulatory networks are not feasible for modelling such complex systems. Leaf growth can be modelled via response curves to environmental conditions, which are considered as ‘meta-mechanisms’ at a higher degree of organisation. Response curves of leaf elongation rate to meristem temperature, atmospheric vapour pressure deficit, and soil water status were established in recombinant inbred lines (RILs) of maize in experiments carried out in the field and in the greenhouse. A quantitative trait locus (QTL) analysis was conducted on the slopes of these responses. Each parameter of the ecophysiological model could then be computed as the sum of QTL effects, allowing calculation of parameters of new RILs, either virtual or existing. Leaf elongation rates of new RILs were simulated and were similar to measurements in a growth chamber experiment. This opens the way to the simulation of virtual genotypes, known only by their alleles, in any climatic scenario. Each genotype is therefore represented by a set of response parameters, valid in a large range of conditions and deduced from the alleles at QTLs.

Additional keywords: water deficit, tolerance, modelling, QTL, temperature.

Introduction

Leaf area is the main determinant of photosynthesis, via the well-documented relationship between light interception and biomass production (Monteith 1977). Rapidly reaching a high proportion of absorbed light is therefore an important trait for crop productivity. However, leaf area is also an important determinant of plant transpiration. A common adaptive trait for drought tolerance is a reduced leaf growth under water deficit. Reduction in leaf expansion rate usually occurs before any reduction in photosynthesis (Boyer 1970; Saab and Sharp 1989), or in the growth of other plant organs (Westgate and Boyer 1985; Saab et al. 1990). Reduction in leaf growth rate occurs in response to both air and soil water deficits (Ben Haj Salah and Tardieu 1997; Serpe and Matthews 2000). The resulting reduction in transpiration contributes to avoidance of cell water stress. On short time-scales, reduction in leaf area has a similar role to stomatal closure in reducing the water flow through the plant, thereby decreasing the gradient of water potential between the soil and leaves. This avoids deleterious leaf water potentials in leaves. On longer time-scales, a reduced leaf area saves soil water for later stages of plant development via reduction in transpiration.

Breeding for tolerance to water deficit can therefore involve 2 opposite strategies with regard to leaf growth: either selecting plants that reduce leaf area under water deficit in order to avoid plant water stress, or selecting plants that can maintain leaf area to maximise light interception. Several genetic manipulations are based on the first strategy and increase plant survival under water deficit. Arabidopsis plants that over-express a gene involved in the synthesis of abscisic acid (ABA) stay green longer and die after control plants (Iuchi et al. 2001). This is probably due to the fact that an overproduction of ABA causes reductions in leaf area and stomatal conductance, and therefore in transpiration. The same sequence of events allows tomato plants that over-express the transcriptional activator CBF1, DREB1B to stay green longer than wild-type plants.
(Hsieh et al. 2002). The second strategy involves the maintenance of growth, photosynthesis, and development during the whole cycle under dry conditions. Maize plants, obtained in a divergent selection for yield under dry conditions, have these characteristics and also maintain kernel number under water deficit (Bruce et al. 2002). This allows plants to accumulate maximal biomass during dry periods, but with the risk that the elevated transpiration associated with high photosynthesis causes total water depletion and plant death before the end of the crop cycle.

Which of these strategies best contributes to tolerance to water deficits? The answer probably depends on the climatic scenario. The first, conservative, strategy is suited to environments with severe water deficits in which saving water is essential. The second strategy is adapted to scenarios with shorter water deficits, in which it is not crucial to save water but rather to maximise growth at any stage of development and hence provide the best chance of a high yield. Tolerance to water deficit therefore involves, at least in part, a balance between reduction in risk of severe stress, obtained by stomatal closure and reduction in leaf area, and conservation of the ability of the plant to accumulate biomass, which involves leaf growth maintenance. Because rainfall patterns vary from year to year, typically more years than the number required to complete a breeding program cycle are necessary to explore the most likely climatic scenarios for a region (Chapman et al. 2000). This suggests that an exclusively experimental procedure is not feasible. Simulation of the behaviour of genotypes in multiple climatic scenarios could be a useful alternative to test the effects of gene transformation or allele diversity on the tolerance to water deficit.

Can we model the genetic variability of leaf response to water deficit from known mechanisms and genes?

A conceptually simple strategy would consist of placing the effects of all involved genes in a regulatory network that simulates the effects of environmental conditions on transcript levels, on the amount and activities of corresponding proteins, and finally on the resulting phenotype. This strategy has been used for simulating relatively simple systems such as the behaviour of prokaryotes as a function of their environment (Gilman and Arkin 2002), and flowering time in Arabidopsis thaliana (Welch et al. 2005). This approach has been considered as suitable to study the effects of water deficit on plants (Cushland and Bonhert 2000). However, is our knowledge of the regulatory processes involved in the control of leaf growth precise enough for such a prediction? Leaf expansion depends on several mechanisms, which have all been shown to have a driving effect under water deficit. This results in an over-determination of leaf growth and in several possible models.

Leaf growth as determined by the carbon available to leaves

Leaf expansion can be considered to depend on carbon availability. The resulting equation relates the increase in plant leaf area on a given day ($\Delta A/\Delta t$, mm$^2$/day) to carbon gain on the same day (difference between photosynthesis, $J$, and respiration, $R$, g/day), to the proportion of carbon allocated to leaves on the same day ($p_l$, g/g), and to specific leaf area (SLA, leaf area per unit dry weight, mm$^2$/g):

$$\Delta A/\Delta t = (J - R) \times p_l + SLA$$  (1)

This approach is used in many current simulation models. It implies either that $p_l$ and SLA are constant for a genotype, or that their changes with plant age and with environmental conditions can be predicted in a straightforward way.

A rapid analysis could lead to the conclusion that Eqn 1 is acceptable because a change in the carbon balance of the plant causes a reduction in leaf area (Wilson 1966; Dengler 1980). However, an analysis of the time course of expansion does not support the idea that the expansion rate of a leaf on one day is related to the carbon balance on the same day. Covering 40% of plant leaf area with aluminium foil does not affect the expansion rate of sunflower leaves during the period with maximum absolute expansion rate (i.e. while carbon demand of the leaf is maximum), whereas it does during the early period of leaf development (Granier and Tardieu 1999a). In the same way, leaf elongation rate of maize is unaffected by changes in incident photosynthetic photon flux density (PPFD) during the period when elongation rate is maximum, for PPFD in the range 8–50 mol/m$^2$.day (Ben Haj Salah and Tardieu 1996). Leaf expansion rate on one day is, therefore, not linked to plant carbon balance on the same day during the period with maximum absolute expansion rate. During this period, leaves are autotrophic so growing tissues are close to a carbon source, resulting in a high sink priority (Mcinisin et al. 1993) so leaf expansion can be sustained even with low PPFD. In contrast, root elongation rate is closely dependent on intercepted PPFD because roots have a lower sink priority as they are further from a carbon source (Aguiarreabal et al. 1994; Muller et al. 1998). The same reasoning probably applies to young heterotrophic leaves whose expansion is reduced by a decrease in intercepted PPFD.

Leaf growth as determined by changes in cell wall plasticity

Leaf expansion can be considered as linked to the deformation of epidermal cell walls, the less plastic part of leaves during a deformation (Kutschera 1992). In the equation of Lockhart (1965), the relative expansion rate (RER) is linked to epidermal cell turgor ($P$, Pa) and to the theological properties of epidermal cells, namely wall extensibility.
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(φ, mm²/mm².day.Pa) and minimum turgor allowing epidermal cell expansion (Y, Pa): RER = ΔA/AΔt = φ(P - Y) (2)

Water deficit affects the plasticity of cell walls, thereby reducing leaf expansion rate (Matthews et al. 1984). The opposite occurs in roots, thereby allowing roots to partly maintain growth under water deficit in spite of a reduced turgor (Spoelen and Sharp 1991; Wu and Cosgrove 2000). However, the molecular processes are not understood in a way comprehensive enough to allow modelling. Three gene families are the main molecular candidates for changes in cell wall properties. Expansins, which can induce cell wall extension in vitro, are believed to disrupt the hydrogen bonds between cell wall polymers (Cosgrove 1999). Their expression is appreciably affected in the case of water deficit (Wu and Cosgrove 2000) but, because it is a large multigenic family, the relationship between transcript levels and expansion rate is not straightforward (Caderas et al. 2000; Choi et al. 2003). It is hypothesised that the enzyme XET cuts and rejoins xyloglucan molecules, which form tethers between cellulose microfibrils, but controversy remains on the exact role of this family of enzymes and even on their effects on growth (Cosgrove 1999). Finally, cell wall peroxidases act as a stiffening agent by catalysing cross-links of phenolic groups in the cell wall. The cell-wall-associated peroxidase activity is increased in loliol leaves subjected to water deficit, but without clear demonstration of their role (Bacon et al. 1997). Other families of proteins may also be involved, such as endoglucanases (Yuan et al. 2001). Each of these families can involve several tens of genes whose individual effects are not known, and the interaction among families of genes is still less known.

Leaf growth as determined by cell turgor and osmotic adjustment

Reduction in leaf turgor has long been considered as a straightforward interpretation of the reduction in leaf growth under water deficit, following Eqn 2 (Zhang et al. 1999). This is observed in experiments carried out on very short time-scales (Pritchard et al. 1991). It implies that osmotic adjustment in growing tissues is not complete enough to compensate for the decrease in total water potential, so turgor would decrease in cells of droughted plants. The theory of a central role of turgor in the control of leaf growth has been discussed over the last 30 years (Green et al. 1971). Reductions in leaf elongation rate were observed in response to soil water deficit in spite of an unchanged turgor pressure (Matthews et al. 1984; Westgate and Boyer 1985; Tang and Boyer 2002). There is, therefore, a contradiction between 2 sets of papers, which originate from different scientific communities and were obtained by different approaches to calculating turgor and osmotic adjustment. Ideally, these 2 views could be challenged by direct measurements of cell turgor with a cell pressure probe, in growing zones of plants subjected to soil or air water deficits. However, few data are available, with contrasting results. Shackel (1987), Shackel et al. (1987) and Serpe and Matthews (2000) observed a marked change in turgor of dictor leaves following a change in evaporative demand, with small changes in solute potential. In the same way, Spollen and Sharp (1991) observed a turgor decrease in maize roots subjected to a severe water stress (~1.5 MPa), contrasting with results of Pritchard et al. (1991) in barley. If the importance of osmotic adjustment is still controversial, it is unlikely that one can predict in the short-term the effect of genes involved in osmotic adjustment.

Leaf growth as determined by cell division rate

Leaf expansion can finally be considered as the result of both epidermal cell division and epidermal cell expansion. Leaf area is the product of epidermal cell number (N) and mean area of epidermal cells (A, mm²) so, at each time step (dt):

RER = dA/A dt = dN/N dt + dAc/Ac dt (3)

The 2 terms in the right part of the equation are frequently considered as independent, so any increase in epidermal cell division rate should result in an increase in leaf expansion rate. Consistently, manipulation of enzymes of the cell cycle affects leaf area (Hemerly et al. 1995) and root length (Doerner et al. 1996).

Cell division rate in leaves of several species is clearly affected by water deficit in both dicot (Yegappan et al. 1982; Granier and Tardieu 1999b) and monocot species (Schuppler et al. 1998; Tardieu et al. 2000). In maize, the reduction in cell division rate due to several environmental conditions, especially temperature and water deficit, is clearly linked to the activity of a key enzyme of the cell cycle, the p34cdc2kinase (Granier et al. 2000). However, it is not linked to the amount of this enzyme, but probably to inhibitors of its activity (Schuppler et al. 1998). Again, the precise role of individual genes in the response to water deficit is insufficiently known to allow a predictive approach.

Hormone signalling

Abscisic acid (ABA) is widely believed to be a major contributor in the signalling pathway of water deficit and in the controls of plant transpiration and leaf growth. This view is reinforced by experiments in which the ABA biosynthesis pathway was affected (Boreel et al. 2001; Iuchi et al. 2001) and by the use of feeding with artificial ABA (Zhang and Davies 1990; Ben Haj Salah and Tardieu 1997). Models involving ABA have been proposed for the controls of transpiration (Tardieu and Davies 1993; Dewar 2002) and leaf expansion rate (Ben Haj Salah and Tardieu 1997).

However, the picture is more complex when the effect of
ABA is dissected genetically. The signalling pathways of ABA and ethylene overlap, because mutants affected in sensitivity to ABA are allelic with mutants of ethylene sensitivity (Beaudoin et al. 2000). A similar overlapping is observed between the signalling pathways of ABA and sucrose (Leon and Sheen 2003). Furthermore, recent studies show that the respective roles of ABA and ethylene have to be reconsidered in relation to water deficit, in that ABA might promote growth instead of inhibiting it under water deficit (Sharp 2002).

Can gene regulatory networks be used for predicting the response of leaf growth to water deficit?

Each of the mechanisms considered above is widely believed to be involved in the response of leaf growth to water deficit. However, in each case, fundamental debate remains on the functional and genetic basis of the effects. Instead of simplifying the picture, the molecular dissection of each mechanism has tended to increase its complexity, generate new debates, and increase the number of genes involved because several families of up to 30 genes are implicated for each single mechanism. Transcriptome analyses should help to analyse this complexity but they provide no avenues for modelling plant behaviour. Another difficulty concerns phenotypic analysis. When plant survival is analysed, the phenotype is qualitative with 2 possibilities (alive/dead), as in the gene regulatory network analysed by Gilman and Arkin (2002). When leaf growth is analysed, the phenotype is quantitative, i.e. an infinite number of values of each variable and of combinations of variables is possible. The analysis of plant phenotype is necessarily based upon a theory, even implicit, which leads one to choose the variables to be measured, the time to measure them, and the chosen levels of stress in experiments.

Overall, the above paragraphs suggest that we cannot build a sensible gene network model that would encapsulate all the gene regulations leading to reduced leaf growth under water deficit. The primary reason for that is our lack of knowledge for establishing a hierarchy, or common regulations, between the above-mentioned mechanisms believed to drive leaf growth. Even if our knowledge significantly improved, it is still possible that the resulting gene network would be too complex to be dealt with. If models of behaviours of genotypes are to be developed, they will therefore be based on principles that differ from the gene regulatory networks, at least in the next years or decades.

‘Meta-mechanisms’ can be identified at plant or organ levels

Crop modellers have long used another way of expressing plant controls. They express a phenotypic trait at a given time (e.g. transpiration rate, expansion rate of organs, or biomass accumulation) as a function of environmental inputs, such as organ temperature, light irradiance, or soil water potential, using relatively simple equations, some of which are straightforward because they represent a physical process. For example, physical equations describing mass or heat fluxes, such as water transfer in the plant or energy balance of the leaf, have a known formalism. The parameters of equations have a physical value, which characterises an environment (e.g. soil hydraulic properties) or a genotype (e.g. leaf albedo, hydraulic conductivity of the xylem). The difficulty in the use of these equations is to define the degree of simplification that is acceptable, and to experimentally determine their parameters. However, the formalism of equations is usually non-controversial.

Control equations have another status: they describe, for example, the response of growth to an environmental condition or the progression of development of the plant. These equations are based neither on physical concepts nor on the knowledge of gene action. They are therefore theoretically fragile and can be misleading. However, several examples suggest that control equations may have a value per se, such as that presented in Fig. 1 for the response of leaf elongation rate to meristem temperature. Although the combination of molecular mechanisms leading to the response to temperature is not known, leaf elongation rate is linearly related to meristem temperature, and the same response curve applies to plants grown over several years in the field, in the greenhouse, and in the growth chamber, provided that the plant experiences no stress and a near-zero evaporative demand during the night or during days with very low vapour pressure deficit (VPD) (Fig. 1a, c). The slope of this relationship is therefore a stable characteristic of the genotype, and differs between genotypes (Fig. 1d). In this example, it would be impossible to establish the gene regulatory network that controls the response of leaf elongation rate to temperature, but the quantitative analysis of the phenotype allows prediction of the response of a genotype and comparison of genotypes.

‘Meta-mechanisms’ are therefore control equations that can seem empirical at first sight. They are based on 2 sensible assumptions: (i) that the network of gene regulation is coordinated in such a way that a plant reacts in a predictable way to a given environmental condition, giving way to a mechanism at a higher level of organization; (ii) that a finite number of combinations of molecular mechanisms has been selected by evolution, so it is possible to analyse the responses to environmental conditions at the plant level. The legitimacy of these mechanisms at the plant level rests on their stability over contrasting environmental scenarios, in addition to their compatibility with current physiological knowledge. Any experimental relationship obtained during an experiment does not provide a control equation. In the example of Fig. 1, the relationship between night-time leaf elongation rate and air (instead of meristem) temperature is experimentally unstable. It is not stable during the day either if the effect of evaporative demand is not taken into account.
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Predawn leaf water potential (MPa)

VPD (kPa)

0          1 2          3

0          1 2 3 4

Meristem temperature (°C)

LER (mm/degree-day) (mm/h)

Fig. 1. Dissection of the responses of leaf elongation rate (LER) to temperature, evaporative demand, and soil water status in 2 typical RILs (open and closed symbols). (a) LER per unit clock time, plotted against meristem temperature. (b) LER per unit thermal time, plotted against meristem temperature. The mean LER is an estimate of parameter a of Eqs 4 and 7. (c) LER per unit thermal time, plotted against meristem to air water vapour pressure deficit (VPD) in well-watered plants. (d) LER per unit thermal time during night periods, plotted against pre-dawn leaf water potential. Each symbol, one experiment in the field (○) or in the greenhouse (△), or in the greenhouse (□). Empty and filled symbol correspond to two maize lines. Redrawn from Reymond et al. (2003).

(Ben Haj Salah and Tardieu 1996). Once a control equation (or a combination of control equations) is proposed, its validity is tested with conditions and genotypes different from those on which it was established (Reymond et al. 2003). The consistency of a putative model with kinematic analyses of growth and development (Granier et al. 2000, Tardieu et al. 2000), of transcript levels (Pic et al. 2002), or of protein activity (Granier et al. 2000) can also be checked. Several potentially acceptable models are usually discarded during this process. Obtaining such models is time consuming but provides a generic characterization of a series of genotypes in any environmental condition.

Bases for modelling leaf expansion rate and its response to environmental conditions

Expressing elongation rates per unit thermal time

Thermal time is used in crop modelling to take into account the effect of temperature on plant development. We have proposed (Granier and Tardieu 1998) that its use can be extended to the calculation of rates with a sound mathematical basis. Leaf expansion rate and cell division rate respond linearly to organ temperature (Fig. 2), with an X-intercept that is most often common to several processes. For instance in sunflower, the X-intercepts for cell division rate, tissue expansion rate, duration of expansion, duration of cell division, and leaf appearance rate have a common X-intercept of 5°C (Granier and Tardieu 1998). The same applies to maize (Ben Haj Salah and Tardieu 1996) and Arabidopsis thaliana (Granier et al. 2002) but with different X-intercepts. This leads to 2 equations. The first relates temperature to the rates of processes involved in leaf growth:

\[
dL/dt = a(T - T_0) \quad (4)
\]

where \( L \) is leaf length (or surface, or cell number), \( T \) is current temperature, and \( a \) and \( T_0 \) are the slope and the \( X \)-intercept of the relationship between \( dL/dt \) and \( T \). The second relationship involves the reciprocal of the duration of the studied process:

\[
1/d = b(T - T_0) \quad (5)
\]

where \( d \) is the time during which expansion or cell division occurs in a given leaf, or the time during which leaf initiation occurs on the apex. Because these equations account for relationships that have a broad value and apply to fluctuating as well as stable conditions (Fig. 2), they can be integrated and used for expression of leaf elongation rate per unit thermal time. At time \( d \):

\[
L = a \int_0^d (T - T_0) dt \quad (6)
\]

\[
\int_0^d (T - T_0) dt \text{ is thermal time (unit: degree-day when calculated with a daily time-step). This implies that time,}
\]
can be visualised in Fig. 1 that both durations and rates as sensed by plants, elapses more rapidly at high than at low temperature but that both durations and rates are independent of temperature if expressed in thermal time. This can be visualised in Fig. 1b in which maize leaf elongation rate becomes independent of meristem temperature if expressed per unit thermal time. This way of expressing rates is a powerful tool to analyse processes in naturally fluctuating conditions.

High evaporative demand decreases leaf elongation rate of several species even in moist soil

A clear effect of evaporative demand on leaf expansion rate has been observed for maize (Ben Haj Salah and Tardieu 1996) and begonia (Serpe and Matthews 2000). This effect occurs in addition to the effect of soil water deficit, but cannot be observed in common growth chambers or greenhouses where water VPD and light intensity are low. It is very clear in Mediterranean climates where VPD undergoes large variations (Ben Haj Salah and Tardieu 1996). A high and constant evaporative demand without soil water deficit caused approximately the same effect on the spatial distribution of leaf elongation rate as a change in soil water status between consecutive days. This was in spite of the fact that predawn leaf water potential and the concentration of ABA in the xylem sap were close to 0 (Ben Haj Salah and Tardieu 1997). This effect is species-dependent, for instance it was not observed in sunflower. It is also genotype-dependent in maize (Reymond et al. 2003).

**Response to soil water deficit**

Leaf expansion rate decreases with soil water potential in nearly all species. In dicot plants such as sunflower or pea, the effect of soil water deficit is greatest during the early stages of leaf development, during which both relative expansion rate and cell division rate are maximum (Lecoer et al. 1995; Granier and Tardieu 1999b). In monocot plants such as maize, this effect seems largely independent of the stage of leaf development as leaf elongation rate is affected in a reproducible way in several experiments at different stages. The spatial distribution is affected, also in a reproducible way, at all distances from the leaf insertion point, resulting in a reduction of the elongating zone of the leaf. Water deficit affects both tissue expansion and cell division; however, expansion is slightly more affected than cell division, resulting in smaller cells in the mature zone of the leaf. Large genetic variability in the sensitivity of leaf expansion rate to soil water deficit was observed in maize (Reymond et al. 2003).

**Analysis of the genetic variability of responses of leaf elongation rate to water deficit**

Final leaf length changes with environmental conditions, but the responses of leaf elongation rate to temperature, evaporative demand, and soil water status are stable for a given genotype and apply to field as well as to controlled conditions (Figs 1, 2). They can therefore be considered as intrinsic characteristics of the specific genotype, and can be used in a genetic analysis.

We have proposed recently that a quantitative trait locus (QTL) analysis can be carried out on parameters of the response curves of maize leaf elongation rate to environmental conditions, thereby dealing with an adaptive trait with an explicit treatment of the genotype × environment interaction (Reymond et al. 2003). QTLs were determined for sensitivities of leaf elongation rate to meristem temperature, leaf to air vapour pressure deficit, and soil water status. It was shown earlier that light intensity has no direct effect on maize leaf elongation rate at this timescale, and essentially acts via its effect on leaf-to-air VPD (Ben Haj Salah and Tardieu 1996). Each slope of the response curves presented in Fig. 1 was established in the following series of experiments. (i) The response of leaf elongation rate to meristem temperature was considered during night periods in experiments without soil water deficit, when the evaporative demand was null and the leaf elongation rate only depended on meristem temperature. (ii) The response to evaporative demand was considered during day periods without soil water deficit, while meristem temperature, light intensity, and evaporative demand fluctuated with time. The sensitivity to evaporative demand was then estimated via the slope of the response curve of leaf elongation rate to meristem-to-air VPD. (iii) The response to soil water deficit...
was analysed during night periods. The slope of the response curve of leaf elongation rate to pre-dawn leaf water potential was an estimate of the sensitivity to soil water status. The 3 responses can be combined in a model with 3 parameters (Tardieu et al. 2000):

\[
\frac{dT}{dt} = (T - T_0)(a + b \cdot VPD - c \cdot \Delta)
\]

where \( \frac{dT}{dt} \) is leaf elongation rate, \( T \) is meristem temperature, \( a \) and \( T_0 \) are the slope and the \( X \)-intercept of the relationship between meristem temperature and leaf elongation rate, \( b \) (negative value) is the slope of the relationship between leaf elongation rate (mm/degree-day) and VPD, and \( c \) (negative value) is the slope of the relationship between leaf elongation rate (mm/degree-day) and soil water potential.

Each parameter of the response curves was analysed in a QTL analysis. Each parameter of Eqn 7 was then expressed as a sum of QTL effects. The resulting model, which combined QTL analysis and ecophysiological modelling, allowed the prediction of the behaviour of new recombinant inbred lines (RILs) under a new climatic scenario, although these new RILs were only known by their alleles at QTL positions. This combination of models was tested on 13 lines not involved in the construction of the QTL models and chosen to maximise the expected differences. Leaf elongation rates measured in a growth chamber experiment were compared with those predicted by the model, using measured temperature, VPD and soil water potential as inputs (Fig. 3). Plants were subjected to a near-zero evaporative demand during the night, and to varying evaporative demands at a constant meristem temperature during the day (Fig. 3a). Examples of predicted and measured time courses of leaf elongation rates of 4 RILs are presented in Fig. 3b, c. Leaf elongation rates had similar time courses in modelled and observed data. It decreased in 3 steps during the night, simultaneously with temperature (Periods 1, 2, 3). It decreased steeply when lights were turned on and temperature was returned to 28°C. It partly recovered and stabilised under the low VPD (Period 4). It decreased afterwards in 2 steps simultaneously with stepped increase in VPD (Periods 5 and 6). The model predicted differences in elongation rates observed between RILs during the night at all temperatures. It also predicted the difference in response to evaporative demand during the day: one RIL had a low response compared with the others, consistent with predicted values.

Concluding remarks

How can one link the physiological and genetic analyses of the control of leaf growth under varying environmental conditions? The view presented here suggests that aggregating all the available knowledge about action of contributing genes into a model is not feasible for the time being, and that this may well be the case for a long time. This conclusion may seem contradictory with the successful study of Welch et al. (2005), who modelled flowering time of Arabidopsis thaliana with a gene network. The difference between the studies, however, lies in the degree of understanding of processes and of gene action in the system studied. Flowering is controlled by a limited set of genes whose action is increasingly understood, thereby making possible a modelling approach of the gene network. In contrast, the first part of this paper suggests that a very large number of genes with different actions may be involved in the control of leaf growth under fluctuating environmental conditions, and that the controlling processes themselves are still poorly understood.

We therefore propose a different approach in which the phenotype of a given genetic line is ‘footprinted’ via a vector of parameters of models. The genetic analysis of these parameters can be a useful avenue for modelling the genotype × environment interaction, but also to identify the
genes involved in these controls. This view and that of genetic networks present the classical opposition between ‘top-down’ and ‘bottom-up’ approaches (Hammer et al. 2004). They might be reconciled by identification of subsystems that could be modelled taking into account individual gene action, and then assembling these subsystems into a quantitative phenotype via a macroscopic approach. In this view, parameters of an equation could be calculated by using a genetic network; for instance, parameter \( a \) of Eqs 0.4 and 7, which describes the elongation rate under optimum conditions, may be predicted for a given genotype by a network of genes involved in tissue expansion and cell division. This possibility remains to be explored.

It must be recognised that the exercise presented here for coupling genetic and ecophysiological analyses applies to a relatively simple case. Firstly, it was carried out on a single phenotypic character, leaf elongation rate, which will have to be combined with many other characters in order to predict the plant architecture, transpiration, and biomass production. However, the combination of approaches proposed by Hammer et al. (2005) suggests that such an integration of mechanisms is possible and might allow one to evaluate plant-breeding strategies with crop models. Secondly, the ‘virtual genotype’ exercise was carried out in a single mapping population of recombinant inbred lines, although one would expect the method to apply to a wider genetic range. However, we have suggested elsewhere (Tardieu 2003) that the method can also apply to transgenic plants. We are presently applying it to other mapping populations (W. Sadok, C. Welcker, and F. Tardieu, unpublished data, 2005) Improved methods to detect QTLs in several populations and several environments will assist in this endeavour (van Eeuwijk et al. 2005). It is possible to imagine that association genetics studies carried out on large panels of genetically unrelated lines could allow one to reconstruct the phenotype of plants in climatic scenarios for a large range of genotypes.

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