CHAPTER 7

A MODELLING APPROACH TO GENOTYPE × ENVIRONMENT INTERACTION

Genetic analysis of the response of maize growth to environmental conditions

W. SADOK, B. BOUSSUGE, C. WELCKER AND F. TARDIEU

INRA – ENSAM, Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, Montpellier, France.
E-mail: ftardieu@ensam.inra.fr

Abstract. Expansive growth of organs has a very large genotype × environment (G×E) interaction. Maximum leaf expansion rate observed in the absence of stress and of evaporative demand has a genetic variability which is usually smaller than environmental effects. The mechanisms driving the reduction in leaf growth rate under stress, namely changes in cell division rate, in cell-wall mechanical properties and in turgor, and their signalling pathways, interact in such a way that a bottom-up approach from genes to the G×E interaction cannot be envisaged. We propose an approach combining modelling and genetic dissection of model parameters. Three genotype-dependent parameters are considered for analysing the G×E interaction for leaf elongation rate of maize. The maximum leaf elongation rate per unit thermal time is stable during the night and over several nights, and it is repeatable for each genotype over several experiments. The responses of leaf elongation rate to evaporative demand and soil water status are linear and their slopes are reproducible over several experiments. Maximum elongation rate and slopes of the responses to evaporative demand and to soil water potential have been analysed genetically in three mapping populations. QTLs of maximum leaf elongation rate tended to co-localize with QTLs of leaf length under well-watered conditions, but also under water deficit. They also co-localized with QTLs of the Anthesis Silking Interval (ASI). In contrast, QTLs of response parameters did not co-localize with QTLs of length under water deficit. They are therefore 'adaptive' traits which cannot be identified otherwise. Each parameter of the ecophysiological model was computed as the sum of QTL effects, allowing calculation of parameters of new RILs known by their allelic values only. Leaf elongation rates of these 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.

J.H.J. Spiertz, P.C. Struik and H.H. van Laar (eds.), Scale and Complexity in Plant Systems Research:Gene-Plant-Crop Relations, 77-91.

INTRODUCTION

Expansive growth occurs from plant emergence to flowering. It determines the plant architecture and, indirectly, fundamental characteristics of plant functioning such as water and nutrient uptake by roots or light interception by leaves. In contrast to biomass accumulation, which is the result of numerous processes, expansive growth is the direct consequence of two main processes only, namely cell division and tissue expansion, which are largely synchronized (Fleming 2005). While the ability of genotypes to grow under favourable conditions has an appreciable genetic variability (e.g., 'intrinsic leaf elongation rate', Reymond et al. 2003; 'early vigour', Condon et al. 2004), environmental conditions usually have an overriding effect. Expansive growth is therefore one of the plant processes with highest genotype × environment interaction. Light availability determines tissue expansion in sink tissues such as roots or young leaves, in close relation with the local sugar concentration (Granier and Tardieu 1999; Freixes et al. 2002; Walter and Schurr 2005). In case of water deficit, a reduction in leaf expansion rate usually occurs before any reduction in photosynthesis (Boyer 1970; Saab and Sharp 1989). Numerous mechanisms can account for the changes in growth rate with environmental conditions, but all of them are still the object of contradictory experimental results and of some degree of controversy.

Cell division rate in leaves and roots is affected by restrictions in light availability (Muller et al. 1998; Granier and Tardieu 1999; Cookson and Granier 2006), probably with a signalling pathway involving local sugar concentrations. It is also affected by water deficit (Sacks et al. 1997; Granier and Tardieu 1999), possibly with a signalling involving abscisic acid (Wang et al. 1998). The reduction in cell division rate because of several environmental conditions is linked to the activity of a key enzyme of the cell cycle, the p34cdc2kinase (Schuppler et al. 1998; Granier et al. 2000), but the precise role of individual genes in the response to water deficit is insufficiently known to allow a predictive approach.

Cell wall stiffening is a major cause of the reduction in leaf growth in case of water deficit (Tang and Boyer 2002; Cosgrove 2005). Two gene families are the main molecular candidates for changes in cell wall properties with environmental conditions, namely expansins (Wu and Cosgrove 2000) and cell-wall-associated peroxidase (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 dozens of genes whose individual effects are not known, and the interaction between families of genes is still less known.

Reduction in cell turgor has long been considered the cause of the decrease in leaf growth with water deficit (Zhang et al. 1999), implying that osmotic adjustment in growing tissues is not complete. This has been discussed in the last thirty years (Green et al. 1971) and, indeed, reductions in leaf elongation rate have been 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). However, turgor decreases in response to soil water deficit or evaporative demand have been observed in roots and leaves (Spollen and Sharp 1991; Bouchabke et al. 2006).

Abscisic acid (ABA) is widely believed to be a major contributor in the controls

of plant transpiration and leaf growth, consistent with experiments in which the ABA biosynthesis pathway was affected (Iuchi et al. 2001; Borel et al. 2001) or in which artificial ABA was fed to plants (Zhang and Davies 1990; 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 (Beaudoin et al. 2000), and the same applies to ABA and sugars (Leon and Sheen 2003). Furthermore, ABA might promote the growth of droughted plants by restricting the biosynthesis of ethylene, instead of decreasing it as formerly believed (Sharp 2002).

In each of the four mechanisms presented above, the current state of knowledge appreciably differs from that widely accepted ten years ago. Both the categories of genes involved in the control of growth under fluctuating environmental conditions and the hierarchy of candidate mechanisms are the object of controversy. It seems therefore difficult to identify candidate genes from the literature. It is still more premature to elaborate a gene network model which would encapsulate all the gene regulations leading to reduced leaf growth under water deficit. If models of behaviour of genotypes are to be developed, they will be based on principles that differ from the gene-regulatory networks, at least in the next years or decades. The object of the following paragraphs is to present methods to deal with the genetic variability of the response of growth to environmental conditions.

GENETIC ANALYSIS OF RAW PHENOTYPIC TRAITS IN CONTRASTING ENVIRONMENTAL CONDITIONS

A different approach, which does not suppose that mechanisms are known, has been used by geneticists for the past 15 years. It consists in associating statistically gene alleles to phenotypes under abiotic stresses via quantitative trait loci (QTLs) identification in mapping populations (Prioul et al. 1997). This strategy has allowed identification of a large number of QTLs involved in the maintenance of yield or of related plant traits under abiotic stresses, and has had practical consequences in the elaboration of new genetic materials that tolerate water deficit (Bruce et al. 2002; Ribaut et al. 2002; Condon et al. 2004). A major interest of this strategy is that it helps interpreting correlations between traits and establishing a hierarchy of candidate mechanisms.

- Some associations between traits could be expected, such as the co-location of QTLs of maize yield and of those of the anthesis-silking interval (ASI) in case of water deficit (Ribaut et al. 1996; 2002), because ASI is phenotypically well correlated to yield. Expected associations between a complex trait in stressing conditions and enzyme activities have also been detected (Hirel et al. 2001; Consoli et al. 2002).
- Some co-locations were less expected, and may provide indications on the conditions in which experiments were carried out, rather than on genetic association *per se*. For example, Tuberosa et al. (2002b) found co-location of QTLs of field-measured yield under water deficit with QTLs of root growth in hydroponic conditions. This co-location suggests that deep rooting was a highly

favourable trait in the considered field, which is not always the case. Such results are only observed when there is a soil water reserve that is not exploited by roots (e.g., water table or deep soil). In cases where plants grow on a limited amount of water (e.g., shallow soil), improving the root system's ability to take up water is of little interest or even counter-productive (Richards and Passioura 1989). In the case presented by Tuberosa et al. (2002b), co-location of QTLs therefore provides an indication on the soil characteristics of the considered field rather than a widely valid association between root traits and yield.

- In some cases, the genetic dissection of traits provides results that could hardly have been expected. This is the case for a QTL of water use efficiency identified by measuring the leaf carbon-isotopic discrimination in a mapping population of *Arabidopsis thaliana* (Masle et al. 2005). When the underlining gene was cloned, it was found to be involved in the development of the inflorescence and not in the controls of stomatal conductance or photosynthesis.

Quantitative genetics is therefore an efficient way to identify mechanisms involved in the responses to environmental conditions and to propose a hierarchy of them. In all examples presented above, the phenotypic variables were analysed in individual experiments with or without the considered stress, and then the QTL × environment interaction was studied as in Van Eeuwijk et al. (2005). This method is efficient but faces conceptual problems. Each genotype senses differently its environment (e.g., genotypes with contrasting root systems or leaf area), and because each genotype affects its environment in its own way (e.g., they deplete soil water or nutrients at different rates), so treatments are not always well-defined. An alternative approach is developed by several groups, consisting of a dissection of the phenotype before any genetic analysis, in such a way that phenotypic measurements are stable characteristics of each studied genotype. This can be obtained either by fine-tuning environmental conditions during experiments in such a way that all studied genotypes sense the same environmental conditions (Granier et al. 2006), or by designing phenotypic variables that encapsulate the genotype × environment interaction (Tardieu 2003; Yin et al. 2004).

GENETIC ANALYSIS OF THE PARAMETERS OF A GROWTH MODEL

Metamechanisms at organ level can characterize a genotype

Crop modellers have long expressed phenotypic traits as a function of environmental inputs such as organ temperature, light intensity or soil water potential. Relatively simple equations are used, some of which are straightforward because they represent a physical process and have a known formalism (e.g., water or heat transfer). Other equations describe plant processes, e.g., the response of growth to an environmental condition or the progression of development of the plant. These control equations have no clear theoretical background but are based on reproducible behaviours such as that presented in Figure 1 for the response of leaf elongation rate to meristem temperature. Although the combination of molecular mechanisms which leads 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 in different experiments 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). The slope of this relationship is therefore a stable characteristic of the genotype and differs between genotypes (Figure 1C). In this example, it would be impossible to establish the gene regulatory network which controls the response of leaf elongation rate to temperature, but the quantitative analysis of the phenotype allows prediction of the response of a genotype in different environments and comparison of genotypes.

We have proposed that response curves, which are reproducible under different environments for each genotype, can be considered a 'metamechanism' at organ level, although we do not know all their genetic bases (Tardieu 2003). They can be dissected genetically, thereby allowing one to discover *a posteriori* their genetic determinisms, rather than *a priori*. As a 'proof of concept', we have proposed a method based on the genetic analysis of the parameters of response curves to environmental conditions (Reymond et al. 2003, Sadok et al. unpublished).

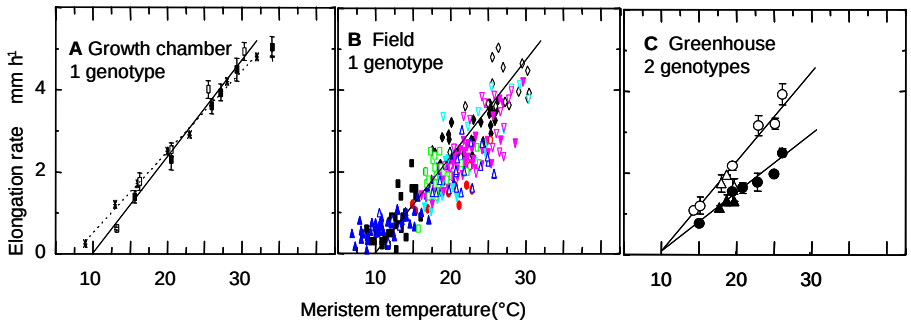

Figure 1. *Relationship between meristem temperature and maize leaf elongation rate under low evaporative demand (night).* (**A** and **B**) *In a genotype (hybrid Dea), a single relationship applied to three experiments in the growth chamber and to 15 experiments in the field when plants were grown in the absence of evaporative demand (redrawn from Ben-Haj-Salah and Tardieu 1995). Each symbol, one coupled value temperature – elongation rate. Each type of symbol represents one experiment.* (**C**) *Two inbred lines with marked differences in slopes in two experiments each (redrawn from Reymond et al. 2003)*

Leaf elongation rate per unit thermal time has a genotype-dependent maximum value which is consistently observed in the absence of stress and of evaporative demand

Thermal time is used in crop modelling to take into account the effect of temperature on plant development (Bonhomme 2000). It is based on a linear formalism between rate and temperature, identified for instance for the rates of germination (Steinmaus et al. 2000), leaf development (Granier and Tardieu 1999) or leaf expansion (Ong 1983; Ben-Haj-Salah and Tardieu 1995). For monocot leaves, which have an

essentially unidirectional expansion, this results in the linear relationship between elongation rate *LER* and meristem temperature *(T)* presented in Figure 1:

$$LER = \mathrm{d}L/\mathrm{d}t = a\,(T - T_0) \tag{1}$$

where L is leaf length, a and T_0 are the slope and x-intercept of the relationship between leaf elongation rate and temperature. If Equation 1 is acceptable, as suggested by Figure 1, 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,

$$L = a \int_0^t (T(t) - T_0)\,\mathrm{d}t \tag{2}$$

where $\int_0^t (T(t) - T_0)\,\mathrm{d}t$ is thermal time (°Cd), termed t_{th} hereafter. The time course of leaf elongation rate can be expressed per unit thermal time (LER_{th}) which is temperature-independent if elongation is only limited by temperature (e.g., during the night, without water deficit and with a low evaporative demand):

$$LER_{th} = \mathrm{d}L / \mathrm{d}t_{th} = a. \tag{3}$$

Equation 3 implies that leaf elongation rate should be stable and characteristic of a genotype 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. Examples of temperature-independence of leaf elongation rate per unit thermal time are presented in Figure 2A for two maize genotypes.

To test the formalism of Equation 3, recombinant inbred lines (RILs) of three mapping populations were grown on a phenotyping set-up allowing one to measure the leaf elongation rate, the soil water status and the transpiration of 360 plants simultaneously, together with micro-meteorological conditions (Figure 3). A night plateau of leaf elongation rate was observed over a large number of time courses in the greenhouse and in the growth chamber. Although temperature fluctuated in the greenhouse, leaf elongation rate per unit thermal time was stable during the night and over up to 8 successive nights, corresponding to two phyllochrons (Figure 4). This plateau value was similar in the greenhouse (fluctuating conditions) and in the growth chamber (stable conditions) for each genotype, but differed between genotypes. Its heritability was 0.5 to 0.6 in three mapping populations (Reymond et al. 2003, Sadok et al. unpublished, Welcker et al. unpublished).

Genetic analyses of the maximum leaf elongation rate were carried out in three mapping populations, two with temperate and one with tropical origins (Reymond et al. 2004, Sadok et al. unpublished, Welcker et al. unpublished). In the three cases, QTLs were identified (Figure 5) and the QTL models accounted for about 50% of the genetic variance of parameter a in the three mapping populations. It was, therefore, possible to identify alleles associated with high or low maximum

elongation rate in these three genetic backgrounds, either from temperate or tropical origin.

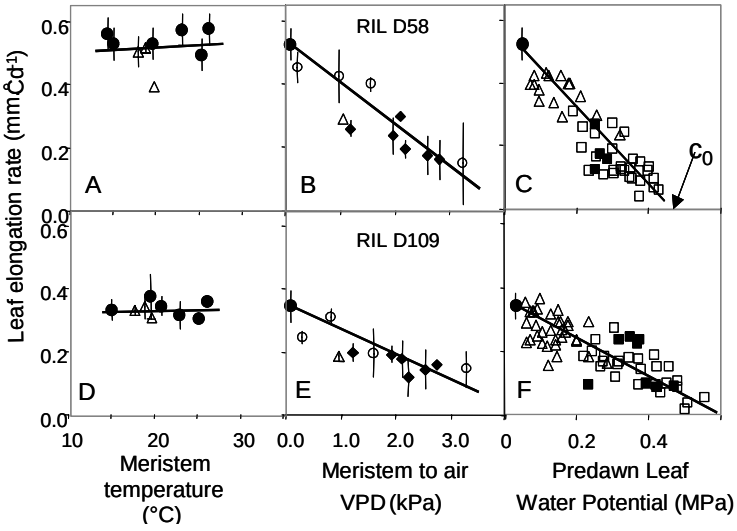

Figure 2. *Responses of leaf elongation rate per unit thermal time to meristem temperature* **(A, D)**, *evaporative demand* **(B, E)** *and soil water deficit* **(C, F)** *in two different RILs.* **(A and D)** *Leaf elongation rate measured in the absence of evaporative demand plotted against meristem temperature. Individual results are pooled for better legibility.* **(B and E)** *Leaf elongation rate during day periods plotted against meristem to air VPD in well-watered plants. Night periods are regarded as having a VPD of 0, and individual results are pooled for better legibility.* **(C and F)** *Leaf elongation rate of night periods plotted against predawn leaf water potential. Individual values are presented.* ○ *Exp GC2 day values,* ● *Exp GC2 night values,*♦ *Exp FC2,* Δ *Exp GS1,* □ *Exp GS2,* ● *Exp GS2, second cycle of dehydration after rewatering*

Figure 3*. Phenotyping platform for continuous measurement of leaf elongation rate, soil water status and micrometeorological variables (up to 366 plants). Plants are grown in PVC columns and placed on balances. Each leaf is attached to a rotative displacement transducer. Environmental sensors (PPFD, vapour-pressure deficit, meristem temperature) are placed at plant level. All sensors are connected to data loggers with a time resolution of 15 min.*

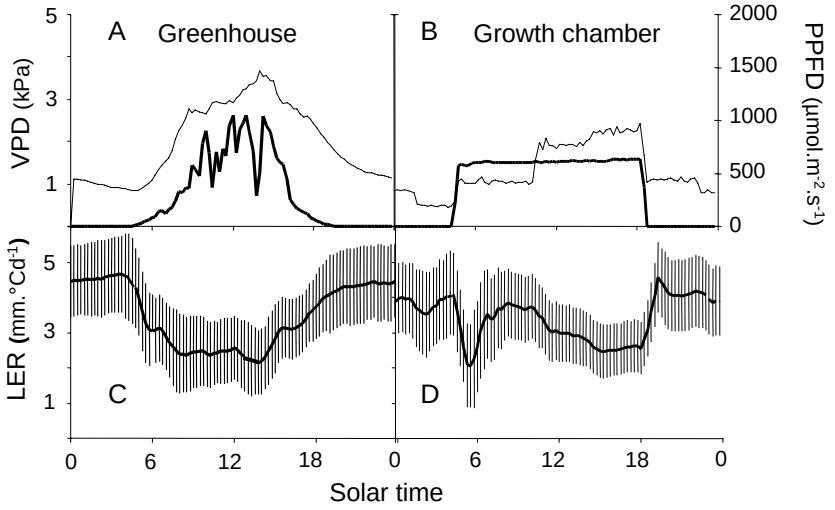

Figure 4. *Meta-analysis of time courses of leaf elongation rate (LER) in the greenhouse (516 24-h time courses) and in the growth chamber (373 24-h time courses) for several days of experiments with well-watered plants subjected to fluctuating temperatures (greenhouse) or stable temperatures (growth chamber).* **(A, B)** *Leaf-to-air VPD (thin lines) and PPFD (thick lines) in the greenhouse* **(A)** *and the growth chamber* **(B)**. **(C, D)** *Mean and standard deviation of leaf elongation rate averaged over all time*

Responses of leaf elongation rate to evaporative demand and soil water status are characteristic of a genotype and can be dissected genetically

A clear effect of evaporative demand has been observed on leaf expansion rate of well-watered maize plants. A high and constant evaporative demand without soil water deficit reduced elongation rate, although predawn leaf water potential and the concentration of ABA in the xylem sap were close to 0 (Ben-Haj-Salah and Tardieu 1997). Consistently, a day-time depression of leaf elongation rate was observed every day in the meta-analyses presented in Figure 4, in which successive days had different temperatures but similar VPDs. LER_{th} decreased with increasing evaporative demand, closely following the transpiration rate. The morning decrease in LER_{th} occurred in less than 15 min. in the growth-chamber experiment, recovered in 1 h and followed afterwards the step changes in VPD. The morning decrease in LER_{th} was also rapid in the greenhouse, following a model with a negative linear effect of transpiration rate on elongation rate:

$$LER_{th} = \mathrm{d}L / \mathrm{d}t_{th} = a\,(1 - \mathrm{d}\,J_w) \qquad (4)$$

where J_w is the transpiration rate per unit leaf area. Because transpiration cannot be measured in all experiments, we have proposed a simplified formalism (Reymond et al. 2003):

$$LER_{th} = dL / dt_{th} = a\ (1 - b\ VPD_{eq}) \quad (5)$$

in which VPD_{eq} is the water vapour-pressure difference between leaves and air, corrected for the effect of light intensity. Relationships corresponding to different experiments analysed jointly are presented in Figure 2B,E. Equation 5 was applied to data of all RILs of three mapping populations (Reymond et al. 2003, Welcker et al. unpublished), and the slope *b* was calculated for each RIL by taking into account several experiments analysed jointly, some of them in the field, some in the greenhouse and some in the growth chamber. In spite of that, heritabilities of parameter *b* were high and QTLs were identified (Figure 5), accounting for about 40 to 50% of the phenotypic variance.

Soil water status affects leaf elongation rate in a reproducible way, in the same way as for evaporative demand. Reproducibility was only observed in the absence of evaporative demand, i.e., during the night. Common relationships applied to different experiments in the growth chamber and in the greenhouse, carried out over different years (Figure 2C,F):

$$LER_{th} = dL / dt_{th} = a\ (1 - c\ \Psi_{predawn}) \quad (6)$$

where $\Psi_{predawn}$ is the predawn leaf water potential, an indicator of soil water status. The latter was indirectly estimated from soil water status, itself deduced from the weight of soil columns. Equation 6 was applied to all RILs of three mapping populations (Reymond et al. 2003, Welcker et al. unpublished). The slope *c* calculated for each RIL had high heritabilities and QTLs were identified (Figure 5), accounting for about 30 to 40% of the phenotypic variance.

DO GENETIC ANALYSES OF MODEL PARAMETERS PROVIDE DIFFERENT RESULTS COMPARED WITH QTL × ENVIRONMENT ANALYSES OF RAW PHENOTYPIC TRAITS?

It is commonly assumed that QTLs of constitutive traits are those which are observed in both well-watered and stressed conditions, while QTLs of adaptive traits are those observed in stressed treatments only (Prioul et al. 1997; Ribaut et al. 1996). The approach presented above provides another way of identifying constitutive versus adaptive traits. By definition, the maximum leaf elongation rate (parameter *a*) is a constitutive trait while the responses of leaf elongation rate to evaporative demand and to soil water status (parameters *b* and *c*) are adaptive traits. We have, therefore, compared both approaches by considering the co-locations between model parameters and the final leaf lengths measured either in well-watered conditions or in water deficit in the same sets of experiments (Reymond et al. 2004).

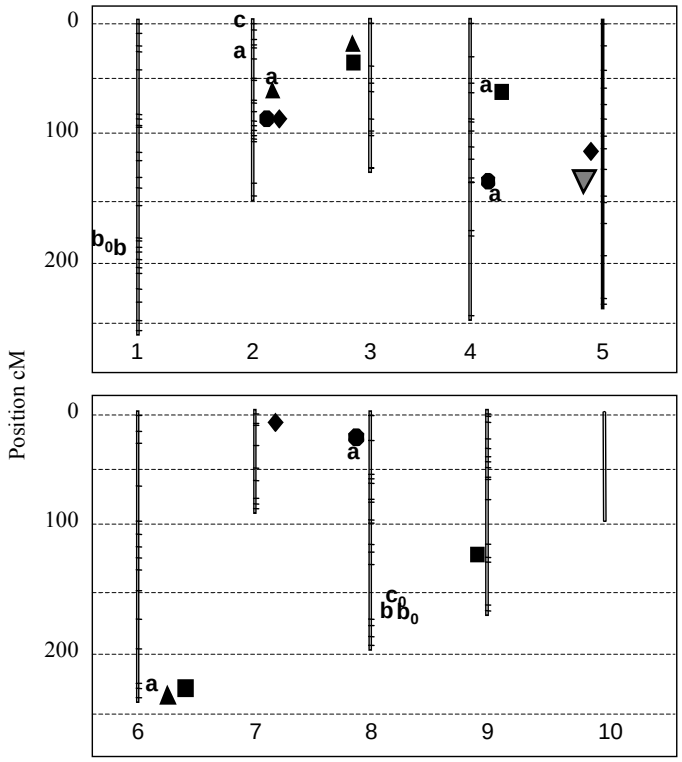

Figure 5. *QTLs of final leaf length (○, ♦, Δ and □ for 4 experiments) and QTLs of parameters of leaf elongation model (a, intrinsic elongation rate; b, slope of the response of leaf elongation rate to meristem to air VPD; b_0, x-intercept of the same relationship; c, slope of the response of leaf elongation rate to predawn leaf water potential; c_0, x-intercept of the same relationship). The QTL of leaf width common to the four experiments is also presented (●). Bars on chromosome indicate positions of markers. For leaf length and parameter a, symbols are located on the right-hand side of the chromosome if the allele F-2 increases the value of the trait. For parameters b and c, symbols are located on the right-hand side of the chromosome if the allele F-2 decreases the sensitivity of leaf elongation rate to the considered environmental condition (redrawn from Reymond et al. 2004)*

One QTL detection was carried out on length and width of leaf 6 in four experiments with either well-watered or stressing conditions in the field or in the greenhouse. The second QTL detection was carried out on parameters of response curves, following the method presented above. QTL of leaf length differed between experiments, but co-localized in 7 cases out of 13 with QTLs of the maximum leaf elongation rate, even in experiments with stressing conditions (Figure 5). QTLs of leaf length under water deficit were either alone or co-localized with those of maximum elongation rate (parameter *a*). They never co-localized with QTLs of responses to air or soil water conditions (parameters *b* and *c*). The same study was

repeated in a mapping population with tropical parents (Welcker et al. unpublished), with similar conclusions. Several QTLs of leaf length under well-watered conditions and the most reliable QTL of leaf length in water deficit co-localized with QTLs of maximum elongation rate, while no QTL of leaf length under water deficit co-localized with QTLs of responses to water deficit or to evaporative demand (C. Welcker, unpublished).

A first interpretation of this result could be that we failed to detect QTL of parameters of response curves in loci where QTLs of leaf length of stressed plants were identified. However, (i) the clusters of QTLs of responses to soil water status or to evaporative demand did not correspond to QTLs of leaf length in stressed experiments; (ii) QTL detection on leaf length under water deficit often provided weak QTLs, in particular in the tropical mapping population in which no QTL of length under water deficit was detected in one year out of two, while QTLs of response to water deficit were detected. This may be due to the fact that each studied plant underwent slightly different scenarios of soil drying, which reduced the heritability of final leaf length but not of parameters of response. The classical method to identify QTLs of constitutive versus adaptive traits therefore did not apply to the experiments presented here. We suggest that identification of QTLs of parameters of response curves provide a promising alternative to deal with the genetic variability of adaptive traits.

HAVE THE EXPANSIVE GROWTHS OF DIFFERENT ORGANS A PARTLY COMMON GENETIC DETERMINISM?

The mechanisms which control the changes in tissue expansive growth with environmental conditions are essentially the same for several organs of the plant (see Introduction). The possibility is therefore raised that their genetic determinisms may be partly common. This possibility can be studied by considering co-locations of QTLs of growth of several organs. For instance, the QTL of parameter *a* on chromosome 2 (bin 2.04, Figure 4), which was also observed in the other two mapping populations (Sadok et al. unpublished; Welcker et al. unpublished), harbours a QTL of constitutive root characteristics (Lebreton et al. 1995; Tuberosa et al. 2002a). However, co-location of QTLs may be misleading because of the high probability of fortuitous co-locations when a large number of QTLs are considered.

We have considered the possibility that leaf and silk growth have common QTLs by analysing jointly QTLs of leaf growth parameters with QTLs of anthesis-silking interval (ASI), which depends on the growth rate of silks (A. Fuad and O. Turc unpublished data). ASI was measured in three and five fields under well-watered and water-deficit conditions, respectively, and QTLs of parameters of response curves were identified as presented above (Welcker et al. unpublished). The maximum elongation rate per unit thermal time (parameter *a*) was accounted for by five QTLs, among which three co-localized with QTLs of ASI in well-watered conditions. The responses of leaf elongation rate to evaporative demand and to predawn leaf water potential had partly common QTLs with ASI in water deficit. In all cases, the alleles conferring either high growth rate under favourable conditions

or growth maintenance under water deficit were the same as those which conferred rapid silk growth (short ASI). This study therefore raises the possibility that different organs, involved in vegetative and reproductive developments, respectively, have partly common genetic determinisms.

TOWARDS VIRTUAL GENOTYPES WHOSE BEHAVIOURS COULD BE ANALYSED *IN SILICO* IN A VERY LARGE NUMBER OF CLIMATIC SCENARIOS

The QTL analysis of parameters presented above allows combining Equations 5 and 6 with expression of parameters as a sum of QTL effects:

$$LER_{th} = \mathrm{d}L / \mathrm{d}t_{th} = a\,(1 - b\,VPD_{eq} - c\,\Psi_{predawn}) \tag{7}$$

$$a = \mu_a + \Sigma\,\mathrm{QTLs}_a;\;\; b = \mu_b + \Sigma\,\mathrm{QTLs}_b;\;\; c = \mu_c + \Sigma\,\mathrm{QTLs}_c \tag{8}$$

If Equations 7 and 8 apply, it should be possible to predict the behaviour of any RIL known by its alleles at QTLs, in any climatic scenario combining fluctuating temperatures, evaporative demand and soil water status. This possibility was tested on lines not involved in the construction of the QTL models and chosen to maximize the expected differences (Reymond et al. 2003). 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. Leaf elongation rate had similar time courses in modelled and observed data, and expected differences between RILs were observed.

CONCLUDING REMARKS

We propose that aggregating all the available knowledge about gene actions into a model is not feasible at the time being, and that this may well be the case for a long time. 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 its controls.

The coupling of genetic and ecophysiological models presented here has now been tested in three mapping populations of maize with different origins, including tropical genetic material that could have been expected to have different behaviour compared with temperate material. The common analysis of anthesis-silking interval and of leaf growth parameters suggests that this approach could apply to different organs of a plant, with partly common genetic determinism across organs.

Three challenges are ahead of us. (i) The method presented in Equations 1 to 8 does not fully take advantage of the kinetic analysis presented in Figures 3 and 4. It is based on the use of averaged values over several hours, while kinetic parameters may provide new insights into the genetic variability. (ii) Three mapping

populations have been used for testing the method. It is necessary now to deal with more complex genetic material, for instance collections of accessions. (iii) The phenotypic traits presented here were relatively simple, and will have to be combined with many others 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.

REFERENCES

Bacon, M.A., Thompson, D.S. and Davies, W.J., 1997. Can cell wall peroxidase activity explain the leaf growth response of *Lolium temulentum* L. during drought? *Journal of Experimental Botany,* 48 (317), 2075-2085.

Beaudoin, N., Serizet, C., Gosti, F., et al., 2000. Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell,* 12 (7), 1103-1115.

Ben-Haj-Salah, H. and Tardieu, F., 1995. Temperature affects expansion rate of maize leaves without change in spatial distribution of cell length: analysis of the coordination between cell division and cell expansion. *Plant Physiology,* 109 (3), 861-870.

Ben-Haj-Salah, H. and Tardieu, F., 1997. Control of leaf expansion rate of droughted maize plants under fluctuating evaporative demand: a superposition of hydraulic and chemical messages? *Plant Physiology,* 114 (3), 893-900.

Bonhomme, R., 2000. Bases and limits to using 'degree.day' units. *European Journal of Agronomy,* 13 (1), 1-10.

Borel, C., Frey, A., Marion-Poll, A., et al., 2001. Does engineering abscisic acid biosynthesis in *Nicotiana plumbaginifolia* modify stomatal response to drought? *Plant, Cell and Environment,* 24 (5), 477-489.

Bouchabke, O., Tardieu, F. and Simonneau, T., 2006. Leaf growth and turgor in growing cells of maize (*Zea mays* L.) respond to evaporative demand under moderate irrigation but not in water-saturated soil. *Plant, Cell and Environment,* 29 (6), 1138-1148.

Boyer, J.S., 1970. Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. *Plant Physiology,* 46 (2), 233-235.

Bruce, W.B., Edmeades, G.O. and Barker, T.C., 2002. Molecular and physiological approaches to maize improvement for drought tolerance. *Journal of Experimental Botany,* 53 (366), 13-25.

Condon, A.G., Richards, R.A., Rebetzke, G.J., et al., 2004. Breeding for high water-use efficiency. *Journal of Experimental Botany,* 55 (407), 2447-2460.

Consoli, L., Lefevre, A., Zivy, M., et al., 2002. QTL analysis of proteome and transcriptome variations for dissecting the genetic architecture of complex traits in maize. *Plant Molecular Biology,* 48 (5/6), 575-581.

Cookson, S.J. and Granier, C., 2006. A dynamic analysis of the shade-induced plasticity in *Arabidopsis thaliana* Rosette leaf development reveals new components of the shade-adaptative response. *Annals of Botany,* 97 (3), 443-452.

Cosgrove, D.J., 2005. Growth of the plant cell wall. *Nature Reviews. Molecular Cell Biology,* 6 (11), 850-861.

Fleming, A.J., 2005. The control of leaf development. *New Phytologist,* 166 (1), 9-20.

Freixes, S., Thibaud, M.C., Tardieu, F., et al., 2002. Root elongation and branching is related to local hexose concentration in *Arabidopsis thaliana* seedlings. *Plant, Cell and Environment,* 25 (10), 1357-1366.

Granier, C. and Tardieu, F., 1999. Leaf expansion and cell division are affected by reducing absorbed light before but not after the decline in cell division rate in the sunflower leaf. *Plant, Cell and Environment,* 22 (11), 1365-1376.

Granier, C., Inzé, D. and Tardieu, F., 2000. Spatial distribution of cell division rate can be deduced from that of P34cdc2 kinase activity in maize leaves grown at contrasting temperatures and soil water conditions. *Plant Physiology,* 124 (3), 1393-1402.

Granier, C., Aguirrezabal, L., Chenu, K., et al., 2006. PHENOPSIS: an automated platform for reproducible phenotyping of plant responses to soil water deficit in *Arabidopsis thaliana* permitted the identification of an accession with low sensitivity to soil water deficit. *New Phytologist,* 169 (3), 623-635.

Green, P.B., Erickson, R.O. and Buggy, J., 1971. Metabolic and physical control of cell elongation rate: in vivo studies in *Nitella*. *Plant Physiology,* 47 (3), 423-430.

Hammer, G.L., Chapman, S., Van Oosterom, E., et al., 2005. Trait physiology and crop modelling as a framework to link phenotypic complexity to underlying genetic systems. *Australian Journal of Agricultural Research,* 56 (9), 947-960.

Hirel, B., Bertin, P., Quilleré, I., et al., 2001. Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. *Plant Physiology,* 125 (3), 1258-1270.

Iuchi, S., Kobayashi, M., Taji, T., et al., 2001. Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. *Plant Journal,* 27 (4), 325-333.

Lebreton, C., Lazic-Jancic, V., Steed, A., et al., 1995. Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. *Journal of Experimental Botany,* 46 (288), 853-865.

Leon, P. and Sheen, J., 2003. Sugar and hormone connections. *Trends in Plant Science,* 8 (3), 110-116.

Masle, J., Gilmore, S.R. and Farquhar, G.D., 2005. The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. *Nature,* 436 (7052), 866-870.

Matthews, M.A., Van Volkenburgh, E. and Boyer, J.S., 1984. Acclimation of leaf growth to low water potentials in sunflower. *Plant, Cell and Environment,* 7 (3), 199-206.

Muller, B., Stosser, M. and Tardieu, F., 1998. Spatial distributions of tissue expansion and cell division rates are related to irradiance and to sugar content in the growing zone of maize roots. *Plant, Cell and Environment,* 21 (2), 149-158.

Ong, C.K., 1983. Response to temperature in a stand of pearl millet (*Pennisetum typhoides* S. & H.). I. Vegetative development. *Journal of Experimental Botany,* 34 (140), 322-336.

Prioul, J.L., Quarrie, S., Causse, M., et al., 1997. Dissecting complex physiological functions through the use of molecular quantitative genetics. *Journal of Experimental Botany,* 48 (311), 1151-1163.

Reymond, M., Muller, B., Leonardi, A., et al., 2003. Combining quantitative trait loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. *Plant Physiology,* 131 (2), 664-675.

Reymond, M., Muller, B. and Tardieu, F., 2004. Dealing with the genotype x environment interaction via a modelling approach: a comparison of QTLs of maize leaf length or width with QTLs of model parameters. *Journal of Experimental Botany,* 55 (407), 2461-2472.

Ribaut, J.M., Hoisington, D.A., Deutsch, J.A., et al., 1996. Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. *Theoretical and Applied Genetics,* 92 (7), 905-914.

Ribaut, J.M., Bänziger, M., Betran, J.A., et al., 2002. Use of molecular markers in plant breeding: drought tolerance improvement in tropical maize. *In:* Kang, M.S. ed. *Quantitative genetics, genomics, and plant breeding*. CAB International, Wallingford, 85-99.

Richards, R.A. and Passioura, J.B., 1989. A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain yield in rain-fed environments. *Australian Journal of Agricultural Research,* 40 (5), 943-950.

Saab, I.N. and Sharp, R.E., 1989. Non-hydraulic signals from maize roots in drying soil: inhibition of leaf elongation but not stomatal conductance. *Planta,* 179 (4), 466-474.

Sacks, M.M., Silk, W.K. and Burman, P., 1997. Effect of water stress on cortical cell division rates within the apical meristem of primary roots of maize. *Plant Physiology,* 114 (2), 519-527.

Schuppler, U., He, P.H., John, P.C.L., et al., 1998. Effect of water stress on cell division and cell-division-cycle 2-like cell-cycle kinase activity in wheat leaves. *Plant Physiology,* 117 (2), 667-678.

Sharp, R.E., 2002. Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. *Plant, Cell and Environment,* 25 (2), 211-222.

Spollen, W.G. and Sharp, R.E., 1991. Spatial distribution of turgor and root growth at low water potentials. *Plant Physiology,* 96 (2), 438-443.

Steinmaus, S.J., Prather, T.S. and Holt, J.S., 2000. Estimation of base temperatures for nine weed species. *Journal of Experimental Botany,* 51 (343), 275-286.

Tang, A.C. and Boyer, J.S., 2002. Growth-induced water potentials and the growth of maize leaves. *Journal of Experimental Botany,* 53 (368), 489-503.

Tardieu, F., 2003. Virtual plants: modelling as a tool for the genomics of tolerance to water deficit. *Trends in Plant Science,* 8 (1), 9-14.

Tuberosa, R., Salvi, S., Sanguineti, M.C., et al., 2002a. Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize. *Annals of Botany,* 89 (special issue), 941-963.

Tuberosa, R., Sanguineti, M.C., Landi, P., et al., 2002b. Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. *Plant Molecular Biology,* 48 (5/6), 697-712.

Van Eeuwijk, F.A., Malosetti, M., Yin, X., et al., 2005. Statistical models for genotype by environment data: from conventional ANOVA models to eco-physiological QTL models. *Australian Journal of Agricultural Research,* 56 (9), 883-894.

Walter, A. and Schurr, U., 2005. Dynamics of leaf and root growth: endogenous control versus environmental impact. *Annals of Botany,* 95 (6), 891-900.

Wang, H., Qi, Q.G., Schorr, P., et al., 1998. ICK1, a cyclin-dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and CycD3, and its expression is induced by abscisic acid. *Plant Journal,* 15 (4), 501-510.

Westgate, M.E. and Boyer, J.S., 1985. Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water potentials in maize. *Planta,* 164 (4), 540-549.

Wu, Y.J. and Cosgrove, D.J., 2000. Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins. *Journal of Experimental Botany,* 51 (350), 1543-1553.

Yin, X., Struik, P.C. and Kropff, M.J., 2004. Role of crop physiology in predicting gene-to-phenotype relationships. *Trends in Plant Science,* 9 (9), 426-432.

Yuan, S., Wu, Y.J. and Cosgrove, D.J., 2001. A fungal endoglucanase with plant cell wall extension activity. *Plant Physiology,* 127 (1), 324-333.

Zhang, J., Nguyen, H.T. and Blum, A., 1999. Genetic analysis of osmotic adjustment in crop plants. *Journal of Experimental Botany,* 50 (332), 291-302.

Zhang, J.H. and Davies, W.J., 1990. Does ABA in the xylem control the rate of leaf growth in soil-dried maize and sunflower plants. *Journal of Experimental Botany,* 41 (230), 1125-1132.