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[1]

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Ecophysiological models predict quantitative traits of one genotype in any environment, whereas quantitative trait locus (QTL) models predict the contribution of alleles to quantitative traits under a limited number of environments. We have combined both approaches by dissecting into effects of QTLs the parameters of a model of maize (*Zea mays*) leaf elongation rate (LER; H. Ben Haj Salah, F. Tardieu [1997] Plant Physiol 114: 893–900). Response curves of LER to meristem temperature, water vapor pressure difference, and soil water status were established in 100 recombinant inbred lines (RILs) of maize in six experiments carried out in the field or in the greenhouse. All responses were linear and common to different experiments, consistent with the model. A QTL analysis was carried out on the slopes of these responses by composite interval mapping confirmed by bootstrap analysis. Most QTLs were specific of one response only. QTLs of abscisic acid concentration in the xylem sap colocalized with QTLs of response to soil water deficit and conferred a low response. Each parameter of the ecophysiological model was computed as the sum of QTL effects, allowing calculation of parameters for 11 new RILs and two parental lines. LERs were simulated and compared with measurements in a growth chamber experiment. The combined model accounted for 74% of the variability of LER, suggesting that it has a general value for any RIL under any environment.

In an agricultural context, a plant that tolerates water deficit can produce a maximum harvested biomass under moderate water deficits. Involved mechanisms are not necessarily common with those underlying the ability of cells to survive tissue dehydration (e.g. Cushman and Bohnert, 2000; Seki et al., 2001). In maize (*Zea mays*), moderate water deficits usually cause no appreciable decrease in leaf water status because of an efficient stomatal control combining hydraulic and chemical messages (Tardieu and Davies, 1993; Wilkinson et al., 1998). A similar combination of messages allows maize plants to dramatically reduce leaf elongation rate (LER) under moderate water deficits sensed either in the soil or in the air, before that leaf water status is appreciably altered (Sharp et al., 2000; Tardieu et al., 2000). Reductions in stomatal conductance and in leaf expansion decrease transpiration rate, thereby saving soil water and maintaining leaf water potential at high values. They also reduce photosynthesis, growth, and yield, so optimum tolerance strategies cannot be common to different climatic scenarios. Plants with steepest responses might be most adapted to scenarios with most severe water deficits, whereas maintenance of growth and photosynthesis under deficit might be appropriate for scenarios with milder deficits. Therefore, identification of sources of variability in the responses to water deficit is necessary for designing plants adapted to a given climatic scenario.

We aimed to identify and analyze the genetic variability of responses of leaf elongation to water deficits caused either by partial soil water depletion or by high evaporative demand. We did not adopt the method consisting of comparing quantitative trait loci (QTLs) of a trait in control and stressed treatments (e.g. Sanguineti et al., 1999; Theulat et al., 1998; Hirel et al., 2001). Because water deficit and climatic conditions fluctuate in natural conditions, it is impossible to reproduce experiments with exactly similar environmental scenario in terms of temperature, soil water status, and water vapor pressure deficit (VPD) in the air. This may result in non-stable QTLs caused by the difference in climatic scenarios between experiments (e.g. Ribaut et al., 1997; Simko et al., 1999).

An alternative consists in using an ecophysiological model that relates quantitative traits to environmental conditions. Ben Haj Salah and Tardieu (1995, 1997) proposed such a model, which combines response curves of maize LER to environmental conditions. Response curves are based on experimental relationships common to several experiments in the field, greenhouse, and growth chamber, and valid over a large range of environmental conditions for a given genotype. Therefore, they can be considered as a stable characteristic of a genotype. The model combines response curves, and dissects LER observed at a given time into: (a) an intrinsic elongation rate that is a characteristic of the genotype at a given temperature, and (b) two additive negative effects, one of evaporative demand (characterized by meristem to air water VPD) and one of soil water deficit characterized by soil water potential (Ψ). These two effects were linear over the studied ranges.

$$\mathrm{d}L/\mathrm{d}t = (T - T_0)(a + b\ VPD + c\Psi) \qquad (1)$$

where $\mathrm{d}L/\mathrm{d}t$ is LER, T is meristem temperature, a and T_0 are the slope and the x intercept of the relationship between meristem temperature and LER, b is the slope of the relationships between LER (corrected for temperature) and VPD, and c is the slope of the relationship between LER (corrected for temperature) and soil water potential. The combined model also applied locally to the spatial distribution of elongation rate in the leaf (Tardieu et al., 2000).

The study presented here is a genetic analysis of the parameters of the model presented in Equation 1. It was carried out on a population of recombinant inbred lines (RILs) presented earlier (Causse et al., 1996). Each parameter of the model presented in Equation 1 was dissected into a sum of QTLs, so a genetic model could predict the value of each of these parameters for all RILs of the studied population. If both the genetic and the ecophysiological models are correct, their combination should be able to predict elongation rate of any RIL of the mapping population, even not taken into account in the QTL study, under any climatic scenario. This possibility was tested successfully, suggesting that the resulting model is valid.

RESULTS

Intrinsic LER and Response of LER to Evaporative Demand in Well-Watered Plants

LER was first analyzed during night periods, in the absence of evaporative demand, and then during day periods with evaporative demands that varied between consecutive days. An example of this analysis is presented in Figure 1, A through D, for two RILs.

The response to temperature was analyzed in a series of nights with mean meristem temperatures ranging from 14°C to 28°C (Fig. 1A; Table I). Common
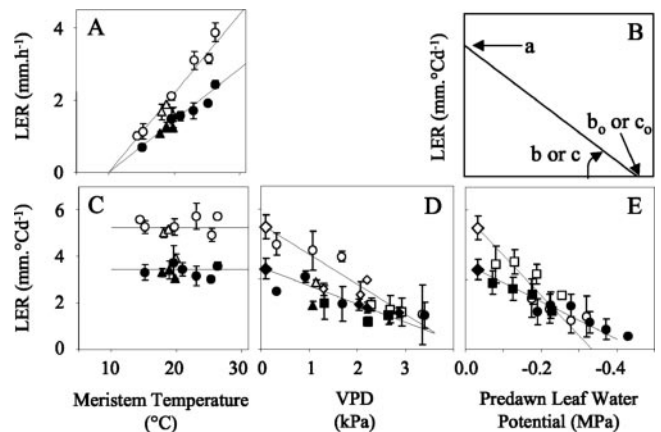

Figure 1. Dissection of the responses of LER to temperature, evaporative demand, and soil water status in two typical RILs (white and black symbols). A, LER per unit clock time, plotted against meristem temperature. C, LER per unit thermal time, plotted against meristem temperature. The mean LER is an estimate of parameter a of Equation 1. D, LER per unit thermal time, plotted against meristem to air water vapor pressure difference (VPD) in well-watered plants. E, LER per unit thermal time during night periods, plotted against predawn leaf water potential. B, Graphical representations of parameters a, b, b_0, c and c_0 of Equation 1. A through D, experiments GC1 ($\triangle$), GC2 ($\bigcirc$), FC1 ($\square$), and FC2 ($\triangledown$). E, Experiments GS1 ($\square$), GS2 ($\bigcirc$), and mean values of LER in experiments GC1 and GC2 in the absence of evaporative demand and water deficit ($\Diamond$).

linear relationships applied, in this range, to experiments GC1 and GC2 in each RIL ($r^2 = 0.86$ and 0.93) with an x intercept close to 10°C in both cases (nonsignificant difference). Slopes significantly differed, indicating that one RIL grew more rapidly than the other at any temperature. If LER of an RIL ($\mathrm{d}L/\mathrm{d}t$) is proportional to meristem temperature minus 10°C ($T - 10$), the ratio (a) of both values is a temperature-independent expression of the ability of the RIL to elongate (Fig. 1C). This ratio was named the intrinsic elongation rate (parameter a).

$$\mathrm{d}L/\mathrm{d}t = a(T - 10) \qquad (2)$$

This is equivalent to expressing elongation rates per unit thermal time where, a is expressed in mm °C d^{-1} (see "Materials and Methods").

The sensitivity to evaporative demand was estimated via the response curve of LER (expressed per unit thermal time) to evaporative demand estimated by leaf to air water vapor pressure difference corrected for the effect of light (VPD_{eq}; see "Materials and Methods"). Night values were taken into account in regressions, with the corresponding VPD_{eq}. In the examples presented in Figure 1D, common linear relationships applied to the four experiments in the field and in the greenhouse without water deficit ($r^2 = 0.79$ and 0.68 for the two RILs). For each RIL, sensitivity to evaporative demand, therefore, was common to four experiments in the studied range of VPD_{eq}. It was estimated either by the slope of the

Table I. *Experiments performed in this study*

Experiment	Sowing Date	Treatment[a]	Night Temperature[b]	Day VPD$_{eq}$[c]	Leaf Ψ_w[d]
			°C	kPa	MPa
FC1 (field)	May 20, 1999	T,VPD	15.8–20.8	1.5–2.8	−0.03 to −0.05
FC2 (field)	July 5, 1999	T,VPD	16.3–22.8	1.0–2.5	−0.03 to −0.05
GC1 (greenhouse)	March 10, 2000	T,VPD	17.4–19.8	0.6–1.5	−0.03 to −0.05
GC2 (greenhouse)	June 10, 2000	T,VPD	14.7–27.8	0.2–3.2	−0.03 to −0.05
GS1 (greenhouse)	March 10, 2000	Ψ	17.4–19.8	0.6–1.5	−0.03 to −0.25
GS2 (greenhouse)	October 5, 2000	Ψ	16.7–19.7	0.2–1.3	−0.10 to −0.50
GcC (growth chamber)	April 25, 2001 and July 1, 2002	T,VPD	16.0–28.0	1.0–2.2	−0.03 to −0.05

[a]Environmental conditions that varied in the considered experiment. T, Temperature; VPD, evaporative demand; Ψ, soil water status.   [b]Range of mean night temperatures.   [c]Range of mean day VPD$_{eq}$.   [d]Range of predawn leaf water potential.

relationship between LER and VPD$_{eq}$ (*b*, Fig. 1B), or by the *VPD* at which elongation would cease (*b$_0$*, Fig. 1B). Because VPDs as high as 4 to 7 kPa (Fig. 2) cannot usually be observed together with temperatures lower than 35°C, compatible with maize leaf growth, *b$_0$* should be considered in a statistical way. It represents the *x* intercept of a linear relationship, rather than the actual value of *VPD* at which leaf elongation ceases. Although the response to evaporative demand was calculated in all experiments, the response to temperature was calculated in the greenhouse experiments only. The putative "night" periods in field experiments comprised evening hours with unexpectedly high evaporative demand. The analysis of the response of LER to meristem temperature, therefore, was restricted to the greenhouse experiments GC1 and GC2. However, the fact that values were common to all experiments at a given VPD during day periods (Fig. 1D) suggests that in-
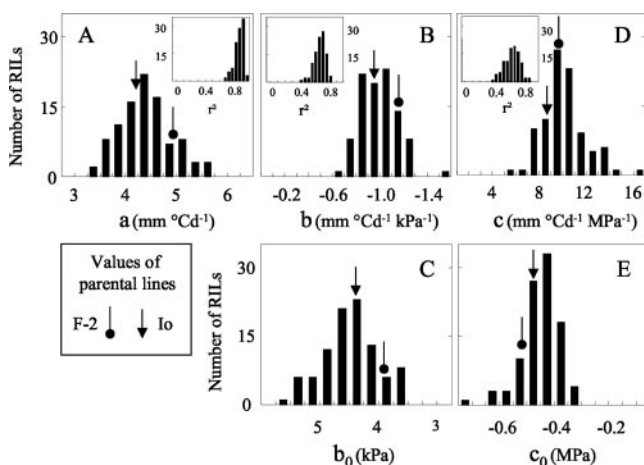

Figure 2. Frequency distributions of parameters of the response curves in the 100 studied RILs. A, Intrinsic LER (parameter *a*). B, Slope of the relationship between LER and meristem to air vapor pressure difference (parameter *b*). C, *x* Intercept of the same relationships (parameter *b$_0$*). D, Slope of the relationship between LER and predawn leaf water potential (parameter *c*). C, *x* Intercept of the same relationships (parameter *c$_0$*). The values corresponding to parental lines (PLs) are also shown. Insets in A, B, and D, Frequency distributions of *r^2* corresponding to each RIL.

trinsic elongation rates per unit thermal time were common to field and greenhouse experiments.

QTLs of Intrinsic LER

The responses to meristem temperature were estimated in the 100 RILs with data originating from the two experiments as in Figure 1, A and C. All relationships were linear in the studied range, with individual *r^2* ranging from 0.69 to 0.99 (Fig. 2A, inset). None of the *x* intercepts significantly differed from 10°C. Therefore, responses of LER to meristem temperature differed between RILs by one parameter only, the slope of response curves (parameter *a*). The genetic variability of this character was appreciable, ranging from 3.3 to 5.7 mm °C d^{-1} (Fig. 2A). Its heritability was 0.81 over the whole set of data.

The QTL detection yielded a genetic model that explained 55% of the total phenotypic variability of parameter *a*, analyzed jointly in experiments GC1 and GC2 (Table II). This model comprised nine significant QTLs with high LOD scores, among which three were detected as main effect QTLs and six were detected as epistatic interactions. Several QTLs detected in epistatic interactions had high bootstrap values (e.g. that on chromosome 7 with a value of 57%, meaning that a QTL was detected at that position in 57% of the 1,000 studied cases), although bootstrap analysis was carried out, taking into account the main effect of QTLs. Three QTLs corresponded to high bootstrap values and two others had lower values because two QTLs coexisted on one chromosome (Table II).

QTLs of Response of LER to VPD

The responses to evaporative demand, estimated in the 100 RILs, were linear in the studied range and applied to the four experiments (*r^2* from 0.6–0.8 except in five RILs, Fig. 2D, inset). The heritability of the slope of relationships was 0.47, with a 3-fold phenotypic variation (*b* from −1.50 to −0.56 mm °C d^{-1} kPa^{-1}, Fig. 2D). For instance, elongation rates measured at 2.5 kPa ranged from 1.05 to 4.55 mm °C d^{-1}. RILs with greatest slopes had highest intrinsic

Table II. *QTLs detected by composite interval mapping for each of the parameters of the ecophysiological model and for concentration of ABA in the xylem sap*

a, Intrinsic elongation rate; *b*, slope of the response of elongation rate to meristem to air VPD; *c* , slope of the response of elongation rate to predawn leaf water potential; b_0 and c_0, *x* intercept of the LER to VPD and LER to predawn water potential relationships, respectively (see Fig. 1B).

Trait	r^2p[a]	Type	Chr[b]	Marker[c]	Distance[d]	Effect[e]	Log of the Odds (LOD) Score	r^2_{indiv}[f]	Bootstrap[g]
									%
a	0.55	Main	2	sc348c	30.0	−0.046	4.65	0.108	30∼
		Main	2	sc348c	45.0	+0.057	6.18	0.149	54∼
		Main	4	sc431pd	58.7	+0.028	3.19	0.072	52∼
		Epistatic	8	sc179_cs	49.5	−0.060	6.88	0.169	36
			9	sc143_c	119.3	–	–	–	13∼
		Epistatic	6	sc236_ag	229.5	+0.032	3.33	0.075	26
			7	sc155_cs	9.7	–	–	–	57
		Epistatic	4	umc66	148.9	+0.059	4.87	0.114	14∼
			9	bnl1428	159.4	–	–	–	39∼
b	0.47	Main	1	sc19_kn1	192.7	−0.008	2.21*	0.058	92
		Main	8	umc36a	171.7	+0.009	2.90	0.077	72
		Epistatic	4	sc431pd	58.7	−0.019	6.35	0.183	60
			5	umc43	84.8	–	–	–	19
		Epistatic	2	umc44a	13.6	−0.013	3.96	0.107	41
			7	sc345_o2	0.0	–	–	–	37
c	0.43	Main	2	umc6	5.5	−0.013	3.75	0.094	76
		Epistatic	1	adh1_iso	198.5	−0.018	4.34	0.271	41
			6	umc85c	58.4	–	–	–	9
		Epistatic	5	umc104a	235.6	+0.015	3.26	0.133	55∼
			9	sc66_sh1	16.9	–	–	–	71
		Epistatic	4	sc431_pd	58.7	−0.017	3.34	0.121	47
			5	sc343b_1	119.9	–	–	–	2∼
b_0	0.35	Epistatic	7	umc110	55.5	+0.49	4.32	0.158	20
			9	sc330td	160.2	–	–	–	54
		Epistatic	8	umc36a	171.7	+0.56	3.96	0.141	14
			10	bnl304	10.0	–	–	–	9
		Epistatic	1	sc19_kn1	192.7	−0.59	2.69*	0.084	64
			5	sc343b_1	104.9	–	–	–	56
c_0	0.39	Main	10	sc329_pp	58.7	−0.066	4.93	0.154	94
		Epistatic	7	umc116	26.4	−0.108	6.47	0.210	90
			8	sc224b_s	150.6	–	–	–	54
		Epistatic	4	sc419_at	129.9	−0.073	4.84	0.151	40∼
			5	umc104a	235.6	–	–	–	78
		Epistatic	4	sc59c_bt	113.9	+0.076	3.65	0.111	23∼
			8	umc32a	182.2	–	–	–	38∼
Concentration of ABA in the xylem sap	0.38	Main	5	sc403_pa	132.4	−46.2	4.02	0.134	50
		Main	9	sc143a_c	109.3	−39.7	4.43	0.150	80∼
		Epistatic	7	sc155_cs	14.7	+52.1	3.90	0.131	60
			9	sc183_cs	65.0	–	–	–	8∼
		Epistatic	4	sc315_su	100.5	−36.7	3.00	0.098	40
			6	umc65	88.9	–	–	–	49

[a]Percentage of the phenotypic variance explained by the genetic model.   [b]Chromosome no.   [c]Closest left marker.   [d]Genetic distance of the QTL from the top of the chromosome.   [e]Additive effect of parent F_2 allele.   [f]Individual r^2 of the QTL.   [g]Proportion of cases in which a QTL was detected in 1,000 random samplings. ∼, More than one QTL was detected in the considered chromosome, thereby reducing the boostrap value; *, QTL was detected in simple interval mapping with an LOD score higher than 3, although the LOD of the composite interval mapping was lower.

elongation rates ($r^2 = 0.53$, Table III). The use of parameter b_0 (*x* intercept of the relationships presented in Fig. 2D) avoided possible confusion of effects because it was independent of intrinsic elongation rate ($r^2 = 0.0$), but was well related to the slope *b* ($r^2 = 0.46$, Table III).

A genetic model of response to evaporative demand (parameter *b*) applied to the four presented experiments, in the field as well as in the greenhouse, and accounted for 47% of the total phenotypic variability (Table II). This model comprised six significant QTLs, two detected as the main effect and four

Table III. *Determination coefficients (r²) between parameters of the ecophysiological model*

a, Intrinsic elongation rate; *b*, slope of the response of elongation rate to meristem to air VPD; *c*, slope of the response of elongation rate to predawn leaf water potential; b_0 and c_0, *x* intercept of the LER to VPD and LER to predawn water potential relationships, respectively (see Fig. 1B).

Parameter	r² of Correlation between Parameters			
	a	*b*	*c*	b_0
b	0.53	–	–	–
c	0.43	0.26	–	–
b_0	0.00	0.46	0.00	–
c_0	0.00	0.00	0.56	0.00

detected as epistatic interaction. LOD values were high except in one QTL (chromosome 1), which had an LOD larger than 3 in simple interval mapping and had the highest bootstrap value, and, therefore, was conserved in the analysis. Bootstrap values were very high in the main effect QTLs (72% and 92%). Three other QTLs detected as epistatic interaction had still high bootstrap values, from 37% to 60%. Two QTLs were common to characters *a* and *b*, consistent with the correlation between them (Table III). One was located on chromosome 4 at 59 cM, and the second was on chromosome 7 between 0 and 7 cM (Fig. 3). In contrast, the genetic model of the character b_0 (Fig. 1B) was completely independent of intrinsic elongation rate (Table III). This model accounted for 35% of the phenotypic variability. It had two QTLs in common with those of *b* on chromosome 1 at 192.7 cM and chromosome 8 at 171.7 cM (Table II).

The QTLs of *a*, *b*, and b_0 presented in Table II and Figure 3 were detected on measurements carried out on leaf 6, over the set of data originating from all experiments considered together. However, the models detected in this way also applied to leaf 7 (r^2 = 0.45, 0.19, and 0.15 for parameters *a*, *b*, and b_0, respectively). They also applied to individual experiments. The genetic model of parameter *a* applied to Exp GC2 alone (r^2 = 0.53), and the model of parameter *b* explained 28% to 35% of the phenotypic variability of individual experiments.

QTLs of Response of LER to Soil Water Deficit

The response of LER to soil water status was studied in the absence of evaporative demand during nights of two greenhouse experiments (Table I). Soil water status, estimated by the predawn leaf water potential, ranged from −0.5 (severe deficit) to −0.03 MPa (well-watered plants). Two experiments with well-watered plants in the greenhouse were also considered in the analysis, with corresponding predawn leaf water potentials. Responses are presented in Figure 1E, for the same two RILs shown in Figure 1, A through D. For each RIL, a common linear relationship applied to the four experiments, with high regression coefficients (r^2 = 0.65 and 0.66). As in the

case of evaporative demand, two estimates of the response to soil water potential were considered (Fig. 1B): the slope of the relationship between LER and soil water potential (parameter *c*), and the *x* intercept of this relationship (parameter c_0), an estimate of the soil water potential at which leaf elongation ceases.

Linear relationships were observed in the 100 studied RILs, with r^2 ranging from 0.50 to 0.87 in 91% of cases and a peak r^2 of 0.64 (Fig. 2D, inset). The slope of this relationship (parameter *c*) had a 3-fold variability, from 5.6 to 18 mm °C d^{-1} MPa^{-1} (Fig. 2D). Its heritability was 0.42. Parameter c_0 ranged from −0.575 to −0.350 MPa (Fig. 2E). As in the case of evaporative demand, a high correlation was observed between parameters *c* and *a* (r^2 = 0.43, Table III). The parameter c_0 was independent of *a* (r^2 = 0.0), but was well related to *c* (r^2 = 0.56).

The QTL detection yielded a genetic model explaining 43% of the phenotypic variation of the slope of the response to soil water potential (parameter *c*), with seven QTLs, among which one was detected in the main effect and six were detected as epistatic interaction (Table II). These QTLs had high LOD scores and QTLs found in epistatic interactions had high bootstrap values as main effect QTLs. Seven QTLs were found for parameter c_0, which explained 39% of its phenotypic variability. One QTL colocalized for *c* and c_0 (Fig. 3).
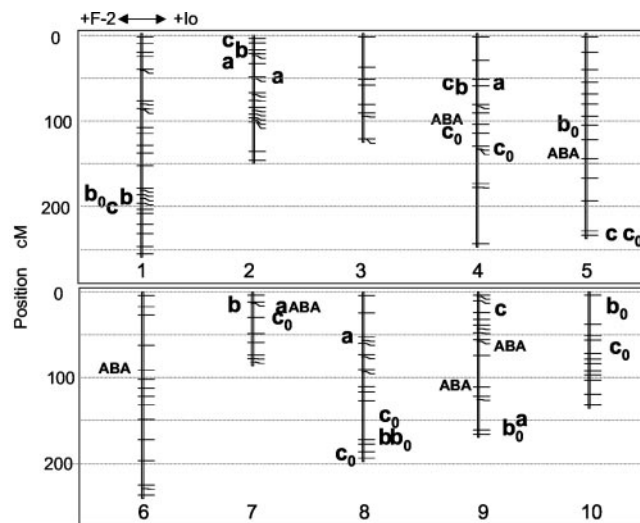

Figure 3. Positions of the most relevant QTLs detected. *a*, Intrinsic elongation rate; *b*, slope of the response of elongation rate to meristem to air VPD; *c*, slope of the response of elongation rate to soil water potential; b_0 and c_0, *x* intercept of the same relationships (see Fig. 1B). QTLs of concentration of abscisic acid (ABA) in the xylem sap in plants grown at a predawn leaf water potential of −0.20 MPa. Only QTLs with highest *F* and high bootstrap values are presented, for better legibility (see Table II for other QTLs). QTLs that decrease the value of the trait in the PL F-2 are on the left side of the chromosomes and those that increase it are on the right.
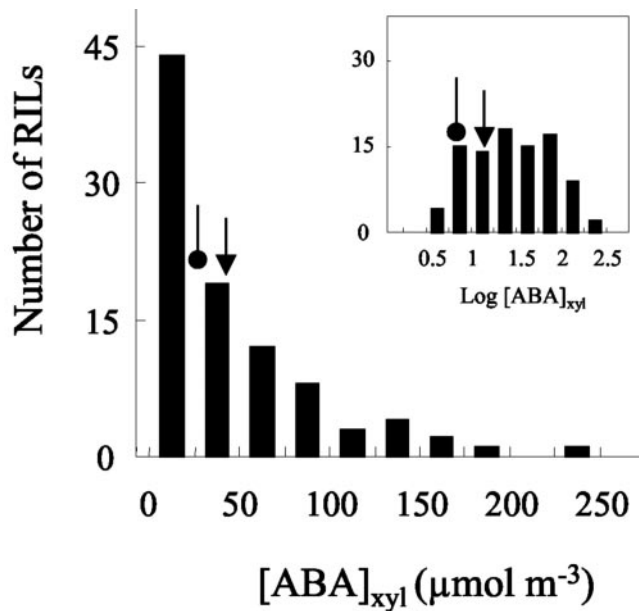
rates calculated from Equation 1 and measured meristem temperature, evaporative demand, and soil water potential. Parameters *a*, *b*, and *c* of Equation 1 were estimated in two ways, either by individual regression for each RIL, as in Figure 1, or by using the QTL models of Table II. To do so, we estimated for each RIL the allelic probability at QTL positions, given the information at flanking markers, and then used them in the QTL model. LER was accurately predicted in both ways, with r^2 of 0.83 and 0.80, respectively, for the first and second method of parameter estimation (Fig. 5, A and B). Although the QTL models of *a*, *b*, and *c* accounted for only part of the genetic variance of each parameter, their use, therefore, generated a very small decrease in the r^2 of the regression in comparison with the use of individual estimates of *a*, *b*, and *c* for each RIL.

The combination of ecophysiological and genetic models was then tested on 13 lines not involved in the construction of the QTL models. These were the two PLs and 11 additional RILs of the population, chosen to maximize the expected differences in responses to temperature and evaporative demand. These responses were predicted using the QTL models of Table II and the allelic probabilities at QTL positions. LERs measured in a growth chamber experiment were compared with those predicted by the model, using measured temperature, VPD, and soil water potential as inputs. 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. 6A). Examples of predicted and measured time courses of LERs of five RILs are presented in Figure 6, B and C (two RILs with similar predicted and measured LERs are presented via average values). LER had similar time courses in modeled and observed data. It decreased in three steps during the night, simultaneously with temperature (periods 1–3). It decreased steeply when lights were turned on and temperature was returned to 28°C. It partly recovered and stabilized under the low VPD (period 4). It decreased afterward in two steps simultaneously with 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 (thin line) had a low response compared with the others, consistent with predicted values. The effect of evaporative demand tended to be slightly overestimated by the model in all RILs. In addition, the transient decrease in elongation rate just after illumination was not predicted by the model.

A regression analysis was carried out on the whole set of data, for the 13 studied lines over the five experiments in growth chamber, together with the results of one experiment with water deficit in the greenhouse (Fig. 5C). Data taken into account corresponded to periods with stable environmental con-
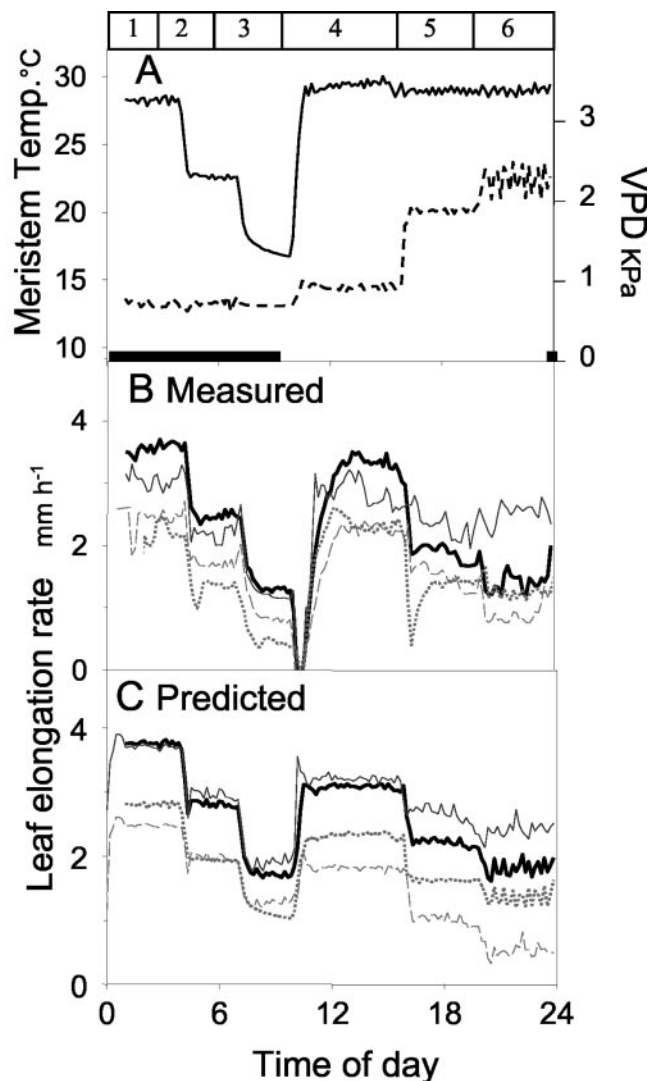

Figure 6. Time courses of measured and modeled LERs during a climatic scenario in the growth chamber. Five RILs are presented, which were not taken into account in the detection of QTLs. Values corresponding to two RILs with similar predicted and measured values were averaged for better legibility. The modeled values were obtained from the ecophysiological model (Eq. 1) whose parameters were calculated as a sum of QTL effects with the genetic models (Table II). A, Change with time of meristem temperature (plain line) and VPD (dotted line). Numbers on the top of the panel represent periods, identified for better legibility in the text. Black bars on the bottom indicate the night periods. B, LER measured with linear variable displacement transducers (LVDTs), averaged on two or more plants of each RIL. Each line style represents a RIL. C, Modeled LER for the same RILs.

ditions and elongation rates, i.e. the transient changes in elongation rate associated with changes in light were not taken into account. Predicted elongation rates were closely related to observed elongation rates ($r^2 = 0.74$). In 10 RILs of 13, the model was accurate with a ranking between RILs conserved in measured and modeled data. The most sensitive RILs according to the genetic model were also the most

sensitive in measured data. Three RILs had systematic bias toward either higher (two cases) or lower (one case) elongation rates in the model compared with observed values, probably because of extra QTLs that were not detected in this study.

DISCUSSION

A Genetic Analysis of the Response of a Trait to Environmental Conditions Allows Modeling the Genotype x Environment Interaction

A major difficulty in the use of QTLs of traits depending on environmental conditions is their instability in different experiments. For instance, Ribaut et al. (1996, 1997), found 13 QTLs in a study of flowering dates of maize, but only three were common to three experiments with different levels of water deficit. Keller et al. (1999) found 16 QTLs of lodging in wheat (*Triticum aestivum*) but only five were common to three experiments. Tuberosa et al. (1998) found 16 QTLs involved in leaf ABA concentration but only four were common to four experiments. Such an instability is not surprising because these traits strongly depend on environmental conditions. To take this dependence into account, a genotype x environment interaction term can be used in the genetic analysis (Epinat-Le Signor et al., 2001) but QTLs of interaction are not easy to interpret. Here, we used a different approach because the trait analyzed was itself a response to an environmental condition, which can be interpreted per se. This approach implies that the response is stable among experiments and does not depend on additional terms. This was the case here in several experiments in the field and in the greenhouse. The test of the method with independent RILs and new climatic scenarios in the growth chamber suggests that modeling the genotype x environment interaction is feasible.

Dissection and Integration of Elementary Processes

Dissecting a phenotype into elementary responses is associated with several risks. First, this dissection may be inadequate, thereby resulting in non-stable elemental traits. Following a reasoning similar to that presented here, Yin et al. (1999a , 1999b) dissected plant growth following the models SUCROS (Goudriaan and Van Laar, 1994) and ORYZA1 (Kropff et al., 1994). Leaf expansion rate was considered to depend on the carbon import by the leaf and on the specific leaf area (ratio of leaf area to leaf mass). In contrast with QTLs of flowering date that were found to be relatively stable (Yin et al., 1999b), QTLs for a specific leaf area were particularly nonstable. This result could be because of the fact that leaf expansion rate is not related to the plant carbon status on short timescales, so specific leaf area is highly variable with environmental conditions and

cannot be considered as a characteristic of a genotype (Tardieu et al., 1999). This is in contrast with the stability of the dissection proposed here for each RIL over several experiments, resulting in common QTLs.

Second, the dissection into elementary processes may generate noise that results in low heritability, impeding the detection of QTLs. For instance, an inadequate separation of night and day periods in our field experiments generated "night" periods with appreciable evaporative demand, thereby lowering mean elongation rate. Therefore, these data had to be discarded to recover an acceptable heritability. Obtaining a large number of data with high heritability was helped by the fact that maize LER is stable for 4 to 7 d (Muller et al., 2001), so measurements during successive days can be considered as replicates.

Finally, the combination of ecophysiological and genetic models is not necessarily robust enough to allow reconstruction of the time course of the analyzed trait. The combined model accounted for observed data on the same RILs (Fig. 5B), but the real test involved a genetic material independent of the QTL detection and climatic scenarios in the growth chamber that differed from those in the experiments used to establish response curves. Therefore, this test cumulated three sources of error: (a) the experimental error linked to LVDT measurements, (b) the error linked to the model applied to one RIL, and (c) the error of the genetic models, which accounted for about one-half of the phenotypic variability of the responses to temperature and evaporative demand. Despite that, the overall precision of the combined model was still high ($r^2 = 0.74$, Fig. 5C). A first explanation is that the three sources of error were not statistically independent, because errors in the determination of slopes contribute to the global error of the genetic model. Second, the correlation between expected and observed values was carried out on actual values of LER and not on parameters of response curves as in the QTL model. Because the range of environmental conditions was wide, this has generated high values of r^2.

Interpretation of QTLs of Responses to Environmental Conditions

One of the interests of the method presented here is that each series of QTLs corresponds to relatively well-defined functions. Most QTLs of intrinsic elongation rate, response to evaporative demand, and response to soil water deficit did not coincide on the genome (Fig. 3), suggesting that these traits are not regulated by the same genes.

QTLs of intrinsic elongation rate are linked to the ability of leaves of a given RIL to elongate with a near-zero water flux in the absence of water or nutrient deficits. It is expected that they could be associated to a genetic variability of the rheological char-
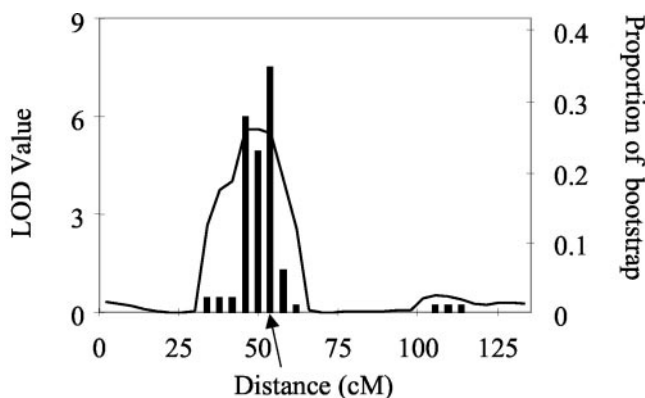
Figure 7. Example of output of the bootstrap analysis for a QTL located on chromosome 10. Each vertical bar indicates the proportion of cases in which a QTL was detected at the considered position in a series of 1,000 random samplings. Positions were 5 cM apart. In the case considered here, a QTL was found in 94% of cases in an interval of 20 cM encompassing the QTL. The trace of LOD values in the composite interval mapping on the 100 RILs is also shown.

acteristics of cell walls, depending, for instance, on genes that code for expansins (Cosgrove, 2000), endoglucanases (Yuan et al., 2001), or xyloglucan endotransglycosylase (Reidy et al., 2001).

QTLs of the response to evaporative demand are linked to the ability of a RIL to elongate in the presence of a water flux, but in the absence of soil water deficit. We have shown previously that the concentration of ABA in the xylem sap was very low and root water potential was close to zero in this case (Ben Haj Salah and Tardieu, 1997). These QTLs might be linked to differences in osmotic adjustment in elongating cells, associated with differences in turgor at low leaf water potential because of high water flux. Alternatively, they might be linked to any plant characteristic affecting the resistance to water flux in the plant, such as stomatal aperture, aquaporins, xylem, or apoplast characteristics. It is noteworthy that two QTLs associated with stomatal conductance, found by Lebreton et al. (1995) on chromosome 7 and 8, are close to QTLs of response to evaporative demand found in the present study.

QTLs of response to soil water deficit, determined in the absence of water flux, can be expected to be linked to differences in any step in the signaling cascade of water deficit such as synthesis of ABA or ethylene (Milborrow, 2001) or sensitivity to these hormones (Leung and Giraudat, 1998). The QTL of response of leaf elongation to soil water potential located on chromosome 10 is close to the gene ASR, a protein expressed in drought conditions and responsive to ABA (de Vienne et al., 1999). Two QTLs of the same character coincided with QTLs of concentration of ABA in the xylem sap (chromosomes 4 and 7, Fig. 3). One QTL, located on chromosome 1, was close to the gene coding for the 9-cis-epoxycarotenoid dioxygenase (Schwartz et al., 1997), which was proposed to be a water responsive limit-

ing step in ABA biosynthesis (Qin and Zeevaart, 1999; Iuchi et al., 2001). The QTL located on chromosome 2 was close to a robust QTL of leaf ABA found by Tuberosa et al. (1998). Although no QTLs of ABA were found on these last two positions, these results might argue in favor of an involvement of ABA in the response of LER to soil water deficit. The alleles conferring higher xylem ABA concentration were associated with lower response to soil water deficit, consistent with an hypothesis recently raised that a high ABA concentration could prevent rather than favor the reduction of elongation under water deficit (Sharp and LeNoble, 2002).

MATERIALS AND METHODS

Genetic Material

The mapping population used in this study consisted of RILs with six generations of self pollination, derived from a cross between two PLs, maize (*Zea mays*) F-2 (an early French flint) and Io (a late North American semident). A total of 145 RILs was produced from this cross and 152 RFLP probes were used for mapping these RILs (Causse et al., 1996). A first subset of 100 RILs of this cross (the same in all experiments) was used for QTL identification and a second subset of 11 others RILs plus the two PLs was used for testing the combination of the ecophysiological and QTL models.

Field Experiments

Two field experiments were carried out near Montpellier, southern France (FC1 and FC2, Table I). Sowing dates were May 20, 1999, and July 5, 1999. Soil was watered twice a week, with water amounts larger than Penman evapotranspiration during the same periods so plants experienced no water deficit. In experiment FC1, 20 seeds per RIL were sown in pair. Plants were thinned to one when leaf 3 emerged. Analysis of leaf elongation was carried out on four plants per RIL, chosen for homogeneity among the 10 plants. The same procedure was used in experiment FC2, but 30 seeds were sown and five plants were used for analysis of leaf growth. In experiment FC2, plants were sown under a mobile shelter allowing us to manipulate temperature and VPD.

Air temperature and relative humidity were measured every 20 s (HMP35A, Vaisala Oy, Helsinki). Temperature of the meristematic zone of 10 plants was measured with a fine copper-constantan thermocouple (0.4-mm diameter) located inside the stem in the meristematic zone. Light was measured continuously using a photosynthetic photon flux density (PPFD) sensor (LI-190SB, LI-COR, Lincoln, NE). All temperatures referred to hereafter are meristem temperatures. All data of temperature, PPFD, and relative humidity were averaged and stored every 600 s in a data logger (Campbell Scientific, LTD-CR10 Wiring Panel, Shepshed, Leicestershire, UK). Water vapor pressure difference between meristem and air (VPD in kPa) was calculated as the difference between saturation vapor pressures at meristem temperature and at air dew point temperature. The water flux cumulated over the day period (J, kg m^{-2} d^{-1}) was approximated as:

$$J = \sum_{i=1}^{144} (M \times VPD_i \times g_{si}/R \times T_{ai}) \times 600 \qquad (3)$$

where M is the molar weight of water (kg mol^{-1}) and R is the gas constant (Pa m^3 mol^{-1} K^{-1}). VPD$_i$ (Pa) is the mean leaf to air VPD during the considered time step of 600 s, and g_{si} and T_{ai} are the mean stomatal conductance (m s^{-1}) and air temperature (K) during the same time step (144 time steps in a day). Consistent with this calculation, we calculated an equivalent VPD for a 1-d period by averaging measured VPDs corrected for changes in stomatal conductance because of diurnal variations of PPFD. For that, we multiplied mean VPDs at each time step of 600 s by a coefficient (k_i) that was 0 and 1, respectively, at PPFDs of 0 and 500 mmol m^{-2} s^{-1} and proportional to PPFD between these two values.

$$VPD_{eq} = \sum_{i=1}^{n} (VPD_i \times k_i)/n \qquad (4)$$

where VPD_{eq} is the equivalent VPD corresponding to the considered period and n is the number of 600-s time steps during this period. During the night, VPD_{eq} was close to 0 kPa (PPFD close to 0 μmol m^{-2} s^{-1}).

Thermal time (t_{th}; °C d) elapsed during a period was calculated by cumulating and integrating, at each time step, the differences between the mean meristem temperature (T_i) and the x intercept of the relationship between meristem temperature and LER (T_0, Eq. 1 and Fig. 1).

$$t_{th} = \sum_{i=1}^{n} (T_i - T_0) \times 600/(3,600 \times 24) \qquad (5)$$

where n is the number of 600-s time steps during the considered period.

Meristem temperatures averaged during night periods ranged from 15.8°C to 21°C in experiment FC1. They reached 23°C in experiment FC2 during nights when the mobile shelter was placed above plants. Daytime temperatures ranged form 19°C to 35°C in experiment FC1 and from 17°C to 34°C in experiment FC2. VPD_{eq}, estimated as in Equation 4, ranged between 1.5 and 2.8 kPa. During experiment FC2, the use of the mobile shelter combined with spraying water on the soil allowed us to get lower VPDs during some days (1 kPa).

The vertical position of the tip of the sixth and seventh leaves was measured twice a day, in the morning (6 ± 1 AM solar time) and in the evening (5 ± 1 PM) during the period from appearance of the leaf tip above the whorl until the end of the period with linear elongation, checked a posteriori (Muller et al., 2001). 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 in the soil (fixed reference). Leaf elongation was calculated as the rate of displacement of the leaf tip either during the night (5 PM–6 AM) or during the day (6 AM–5 PM).

Greenhouse Experiments and Well-Watered Plants

Two experiments were carried out in the greenhouse in well-watered conditions (experiments GC1 and GC2, Table I). On March 10 and June 10, 2000, seeds were placed at 0.025-m depth in columns (0.15-m diameter and 0.4-m height) containing a 40:60 (v/v) mixture of a loamy soil and an organic compost. RILs were sown in pairs and thinned to one when leaf 3 emerged. Each column contained three different RILs and each RIL was sown in three columns. Soil was maintained at retention capacity by daily watering with a modified one-tenth-strength Hoagland solution corrected with minor nutrients. Columns were individually weighted every 3rd d to check that the soil water content was between 35% and 40% of dry soil. Leaf water potential of 10 plants was measured before dawn every week to check that plants experienced no water deficit.

Meristem temperature was measured and VPD_{eq} (Eq. 4) was estimated as in the field experiment. To get a large range of climatic conditions, plants were covered on two nights of experiment GC2 with a 4.0- × 4.7-m plastic shelter and air temperature was lowered by two air conditioners, allowing meristem temperature to reach 18.7°C. The same shelter was used on other two nights but air was heated so meristem temperature reached 26.1°C and 27.4°C. Finally, all plants were moved into a growth chamber at 15.1°C and 14.4°C on two nonconsecutive nights to measure LER at low meristem temperature. Daytime VPD was varied either by turning off the water of air cooling (2–3.5 kPa), or leaving the water circulation in the air cooling system (0.6–1.5 kPa). Low VPDs were obtained by spraying continually water on the soil (0.2–1 kPa).

Measurement of LER was carried out as in the field. The positions of the tips of leaves 6 and 7 were measured twice a day (6 ± 1 AM and 5 ± 1 PM solar time) on three plants per RIL during the period with steady LER. A ruler fixed on the top of the columns was used for that, providing night and day elongation rates.

Greenhouse Experiments and Water-Deficient Plants

Two experiments with the 100 RILs were carried out with soil water deficit (experiments GS1 and GS2). Plants were sown with the same procedure as in other experiments on March 10 and October 6, 2000. While filling columns, a soil sample was taken every second column to determine the initial soil water content. It was checked that soil water content was similar

in all columns and homogenous within each column (not shown). Soil water content was determined afterward by weighing columns every day. Differences in weight were attributed to changes in soil water content. In a preliminary experiment, predawn leaf water potential was measured in leaves 4 and 5 at contrasting water contents. At any water content, predawn leaf water potential did not differ significantly between RILs (not shown). A water release curve relating soil water content to predawn leaf water potential, therefore, was built irrespective of the RIL.

Irrigation was stopped when leaf 5 appeared. A predawn leaf water potential of −0.3 MPa was reached in 3 to 6 d, depending on the leaf area of the considered RIL. Soil water status was then controlled by daily irrigation, in such a way that each plant experienced a range of predawn leaf water potential from −0.03 to −0.5 MPa (Table I). Light, meristem, or air temperatures and meristem to air VPD were measured as in well-watered experiments. The concentration of ABA in the xylem sap, extracted by pressurization, was measured on 95 RILs in experiment GS1. Soil water potential was first set to −0.20 MPa, corresponding to a soil water content of 0.23 g g^{-1}. On the following morning, 100 mm^3 of sap was collected in each RIL before dawn with a pressure of 0.5 MPa above the balancing pressure. The sap of three plants of the same RIL was pooled and stored at −80°C for subsequent ABA analysis. The same sequence of measurements was carried out on three successive nights. The concentration of ABA in crude sap was analyzed by radio-immunoassay (Quarrie et al., 1988).

Test Experiments

Experiments were carried out in the growth chamber and in the greenhouse to test the combination of ecophysiological and QTL models (Figs. 5 and 6C). Eleven RILs that were not used in the former experiments were sown in the greenhouse on April 25, 2001 (six RILs) and July 1, 2002 (five RILs and the two PLs). Upon the emergence of leaf 6, plants were moved into the growth chamber and watered twice a day during the experiment. Air temperature and VPD were varied as shown in Figure 6. This experiment was repeated for consecutive days, with four RILs (eight plants) in the growth chamber on each day. LER was measured continuously with a linear variable differential transducer (LVDT-L100, Chauvin Arnoux, Paris). The LVDT was attached to the tip of each sixth leaf and connected to the data logger. At least two leaves were measured on each date for each RIL. A LVDT was fixed on a metal bar to measure changes in string length with temperature or VPD treatments. In addition, the two PLs were sown in the greenhouse and managed as in the experiments with water deficit. LER was measured using LVDTs.

Genetic Analysis

Genetic analysis were performed on Statistical Analysis System software (SAS Institute Inc., Cary, NC). QTLs were detected by composite interval mapping (Jansen, 1993; Zeng, 1994) using the linear regression (Haley and Knott, 1992), with research of epistatic interactions (Holland et al., 1997). We tested the presence of a QTL taking into account the effect of cofactors, which are putative QTLs located at the marker locations. The choice of cofactors was first carried out using a step-wise regression between the studied trait and the allele value at each marker. At each step, we retained the marker that best explained the phenotypic variability of the quantitative character, with cofactors already accepted in previous steps. The percentage of phenotypic variability explained by all cofactors was not allowed to be higher than the heritability of the studied character. To refine this first selection, a backward regression was carried out in each chromosome between the studied trait and the allele value at each marker, including all the markers of the considered chromosome and the cofactors retained by the step-wise regression on the others chromosomes. At each step, we removed the marker that less explained the phenotypic variability of the trait. This allowed determination of the best model involving the same number of cofactors as that determined in the step-wise analysis.

Presence of main effect QTLs was tested every 5 cM between the 152 markers (445 positions on the genome) using a multiple regression with the cofactors. For that, the allele value was determined every 5 cM as the probability of occurrence of allele F_2 at this position according the genotype at flanking markers (Martinez and Curnow, 1994). When the tested position on the genome was close to a cofactor (±10 cM), the effect of this cofactor was removed. Because the theoretical distribution of the test statistics (the F

of the linear regression) was unknown for multiple regression with cofactors, the threshold value was determined by 1,000 permutations (Churchill and Doerge, 1994). The empirical distribution of the statistical test allowed definition of a threshold value corresponding to a type I error of 5%. The corresponding F value was close to 14, resulting in threshold LOD scores ranging from 2.98 to 3.04 depending on QTL number (Haley and Knott, 1992). In the last step, a regression was carried out between the studied trait and all combinations of two positions on the genome, taking into account the main effect QTLs previously determined. This allowed determination of epistatic QTLs.

The final formalism of the genetic model was:

$$Y = \Sigma \; QTLi + \Sigma \; EPIj \qquad (6)$$

where Y is the studied trait, QTLi are the effects of each main effect QTL, and EPI is the effect of each epistatic interaction. The total phenotypic variability explained by the model (r^2_P) was also estimated. The partial r^2 of each QTL (main or interaction) was also estimated after accounting for the effects of all other QTLs found for the same trait.

Because some QTLs can be linked to a few number of RILs generating significant but non-stable QTLs, we detected QTLs on subpopulations of the studied RIL population (bootstrap). Thousand random sampling of 100 RILs with replacements were carried out among the 100 studied RILs. A QTL detection was carried out for each sample, using the same cofactors as in the original population. The most significant QTL was detected in each chromosome (Visscher et al., 1996). The proportion of cases in which a QTL was detected in a given position was recorded for the 445 positions on the genome (Fig. 7). Bootstrap proportions were also estimated at both loci of epistatic interactions.

Heritability was estimated according to Gallais (1990):

$$h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_E) \qquad (7)$$

Where σ^2_G is the genetic variance and σ^{2E} is the environmental variance, estimated using mean square expectations of a classical ANOVA model. Observations in this ANOVA correspond to parameter estimates for each individual plant and genotype effect to that of the RIL.

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