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Are source and sink strengths genetically linked in maize plants subjected to water deficit? A QTL study of the responses of leaf growth and of Anthesis-Silking Interval to water deficit

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Abstract

Leaf growth and Anthesis–Silking Interval (ASI) are the main determinants of source and sink strengths of maize via their relations with light interception and yield, respectively. They depend on the abilities of leaves and silks to expand under fluctuating environmental conditions, so the possibility is raised that they may have a partly common genetic determinism. This possibility was tested in a mapping population which segregates for ASI. Maximum leaf elongation rate per unit thermal time (parameter *a*) and the slopes of its responses to evaporative demand and soil water status (parameters *b* and *c*) were measured in greenhouse and growth chamber experiments, in two series of 120 recombinant inbred lines (RILs) studied in 2004 and 2005 with 33 RILs in common both years. ASI was measured in three and five fields under well-watered conditions and water deficit, respectively. For each RIL, the maximum elongation rate per unit thermal time was reproducible over several experiments in well-watered plants. It was accounted for by five QTLs, among which three co-localized with QTLs of ASI of well-watered plants. The alleles conferring high leaf elongation rate conferred a low ASI (high silk elongation rate). The responses of leaf elongation rate to evaporative demand and to predawn leaf water potential were linear, allowing each RIL to be characterized by the slopes of these response curves. These slopes had three QTLs in common with ASI of plants under water deficit. The allele for leaf growth maintenance was, in all cases, that for shorter ASI (maintained silk elongation rate). By contrast, other regions influencing ASI had no influence on leaf growth. These results may have profound consequences for modelling the genotype×environment interaction and for designing drought-tolerant ideotypes.

Key words: Anthesis–Silking Interval, ASI, evaporative demand, growth, leaf, QTL, silk, temperature, tropical lines, water deficit, *Zea mays* L.

Introduction

Maize is one of the most sensitive species to water deficit, in spite of the fact that its C_4 metabolism confers a high photosynthetic rate combined with a relatively low transpiration rate. In addition, maize has an interesting isohydric behaviour which maintains leaf water potential at high values under water deficit via the fine-tuning of stomatal control, thereby avoiding leaf dehydration (Tardieu and Simonneau, 1998). The cause of high sensitivity probably involves reproductive development which determines sink strength, especially over the period of time from about one week before to one week after flowering (Claasen and Shaw, 1970; Zinselmeyer *et al*., 1999). Extensive breeding

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programmes have been carried out to overcome this limitation, and have led to select against the increase in Anthesis–Silking Interval (ASI) with water deficit. ASI is associated with a low yield maintenance (Bolaños and Edmeades, 1996; Ribaut *et al*., 2004). This breeding strategy has been highly efficient, for instance, in a CIMMYT programme where yield under water deficit increased by 3% per cycle over eight cycles of selection (Bolaños and Edmeades, 1993). As a consequence, it has been suggested that the flowering-time sensitivity to water deficit has decreased in recently released hybrids compared with older ones (Bruce *et al*., 2002). In a series of experiments, the period during which a water deficit had a maximum effect on yield was late vegetative development in new hybrids, versus flowering time in older hybrids.

It is therefore necessary now to examine jointly the sensitivities of vegetative and reproductive development to water deficit. Leaf expansion is a good candidate for vegetative growth, because (i) it is one of the most sensitive physiological processes to water deficit (Wesgate and Boyer, 1985), (ii) it determines the ability of plants to intercept light and convert it into biomass, and (iii) early closure of the canopy avoids direct evaporation from the soil and, therefore, a waste of water (Condon *et al*., 2004). Because the timing of anthesis is largely unaffected by water deficit, ASI is linked to the date of silk appearance. It can be interpreted as a delay in the growth and development of young reproductive organs, namely ovules and silks, subjected to stressing conditions (Edmeades *et al*., 1993). The possibility is therefore raised that the mechanisms governing the maintenance of ASI and of leaf growth under water deficit are partly common.

The study presented here aimed at evaluating to what extent the maintenance of ASI and of leaf growth under water deficit are linked to common loci of the genome in a population of recombinant inbred lines (RILs) known to segregate for ASI (Ribaut *et al*., 1996). (i) The maintenance of leaf expansion rate under water deficit was analysed via its responses to soil water deficit and to evaporative demand (Reymond *et al*., 2003, 2004). Response curves were established for each RIL over several experiments, and QTLs of the parameters of response curve were identified. These QTLs therefore take into account the genotype×environment interaction. (ii) The maintenance of ASI was evaluated via a reanalysis of field experiments in which ASI of all RILs was measured in well-watered conditions or under water deficit (Ribaut *et al*., 1996, 2002; Sawkins *et al*., 2004).

Materials and methods

Genetic material

The study was carried out on a mapping population of 200 RILs developed at CIMMYT at the sixth inbreeding level from the cross between two tropical maize inbred lines, Ac7643 and Ac7729/TZSR W (referred to as P1 and P2; Ribaut *et al*., 1996, 1997). P1 and P2 belong to Tuxpeno germplasm and contrast for ASI and yield in dry conditions (P1, tolerant parent). Both have similar plant cycle length (97±1 d in the two experiments in well-watered conditions described here). In the whole RIL population, flowering occurred at 97±7 d, but the anthesis of two-thirds of RILs occurred at 97±3 d. RILs were mapped with 132 RFLP probes. Two sets of 120 RILs were randomly selected for the experiments in 2004 and 2005, with 33 RILs in common in the two years.

Experimental set-up

Plants were grown in PVC columns (0.15 m diameter and 0.4 m height) containing a 40:60 (v/v) mixture of loamy soil (particles diameter ranging from 0.1 mm to 4 mm) and an organic compost. Columns were filled with 6.8 kg of soil and sampled for measurement of water content at filling time. It was checked that soil water content was similar in all columns and homogeneous within each column (not shown). Seeds were sown at 2.5 cm depth and thinned when leaf 3 emerged.

The experimental set-up in the greenhouse consisted of 122 balances which measured changes in soil water status, 366 displacement transducers which continuously measured leaf elongation rate, and a set of climatic sensors. A companion set-up was placed in a growth chamber, with the same sensors for the measurement of 63 plants simultaneously. Leaf elongation rate of the sixth leaf was measured with rotational displacement transducers every 15 min (RDTs, 601-1045 Full 360° Smart Position Sensor, Spectrol Electronics, LTD. Wiltshire, England). Leaf elongation was transmitted to the sensor via a pulley attached to it, which carried a thread attached to the leaf tip and to a 20 g counterweight. In all experiments, measurements began when the tip of the sixth leaf appeared above the whorl and lasted until the appearance of leaf 8. This period corresponds to a plateau during which leaf elongation rate is stable (Sadok *et al*., 2007). Final length and width of leaf 6 were measured in all plants at the end of the experiment.

Soil water content was determined by weighing columns every day, after that the thread connected to RDTs was detached from the leaves. Differences in weight were attributed to changes in soil water content, after correction for the increase in mean plant biomass as a function of phenological stage. It was checked that this procedure generated errors smaller than 3 g, i.e. an error in soil water content of about 6×10^{-4} g g^{-1}. In a companion experiment, predawn leaf water potential was measured in leaves 4–7 at contrasting water contents on parental lines and on the reference hybrid Dea. At any water content, predawn leaf water potential did not differ significantly between genotypes (not shown). A water release curve relating soil water content to predawn leaf water potential was then fitted to the equation of Van Genuchten (1980) independently of the RIL. Air temperature and relative humidity were measured at plant level every 30 s with two sensors (HMP35A, Vaisala Oy, Helsinki, Finland). The temperature of the meristematic zone was measured with fine copper-constantan thermocouples (0.2 mm diameter), inserted between the sheaths of leaves 2 and 3 of 10 plants per experiment. Photosynthetic Photon Flux Density (*PPFD*) was measured every 30 s using two sensors (LI-190SB, Li-Cor Quantum PAR, Lincoln, NE USA or SOLEMS 01/012/012, Palaiseau, France). All climatic data were averaged and stored every 15 min in a data logger (Campbell Scientific, LTD-CR10X Wiring Panel, Shepshed, Leicestershire, UK).

Experiments

Two sets of experiments were carried out in 2004 and 2005 (Table 1). Experiments 1–3 and 5–7 aimed at establishing the responses of leaf elongation rate to meristem temperature and to evaporative demand. They were carried out in both the growth chamber and in the greenhouse, at a soil water content close to retention capacity. One plant per RIL was sown at each sowing date, with two weeks

Table 1. *Characteristics of the environmental conditions during experiments*

Name	Sowing date	Conditions[a]	Night T[b] (°C)	Day T[c] (°C)	*PPFD*[d] (mol m^{-2} d^{-1})	*VPD*[e] (kPa)	Ψ_s[f] (MPa)
1	9/01/2004	Gh+GC	14–28	14–32	15–32	0.6–1.9	–0.05
2	23/01/2004	Gh+GC	14–29	16–31	18–35	0.7–1.8	–0.05
3	5/02/2004	Gh+GC	14–28	16–31	7–38	0.5–2.0	–0.05
4	18/05/2004	Gh	18–25	24–30	5–23	0.2–2.5	–0.05 to –1.8
5	16/02/2005	Gh+GC	10–28	10–31	13–26	0.8–2.8	–0.09
6	1/03/2005	Gh+GC	12–28	12–31	14–34	0.7–2.4	–0.09
7	16/03/2005	Gh+GC	12–28	11–30	12–34	0.4–2.4	–0.09
8	13/05/2005	Gh	19–25	23–32	18–34	0.6–3.3	–0.05 to –1.8

[a] Gh, Greenhouse; GC, growth chamber.
[b] Range of mean night meristem temperatures.
[c] Range of mean day meristem temperatures.
[d] Range of photosynthetic photon flux density in the greenhouse.
[e] Range of mean vapour pressure difference of water between leaf and air.
[f] Range of predawn leaf water potential during the experiment.

between each sowing date. Columns contained three different RILs and were distributed randomly. Each RIL was therefore studied for three growing periods per year, considered as blocks in the data analysis. Plants were first grown and analysed in the greenhouse in which environmental conditions fluctuated naturally. Batches of 21 plants were transferred to the growth chamber in which plants were subjected to a stable climatic scenario for 5 d. The night temperature varied in three steps. It was maintained at 28 °C for the first 4 h, 22 °C for the next 3 h, and 14 °C for the final 3 h. Air vapour pressure deficit (*VPD*) was maintained constant at 0.8 kPa. The day-time temperature was kept constant at 28 °C, while the *VPD* was varied in three steps (1, 2, and 2.7 kPa) during 6, 4, and 4 h, respectively. Light was provided by a bank of cool-white fluorescent tubes and incandescent bulbs. The *PPFD* was 450–550 μmol m^{-2} s^{-1} at leaf level depending on experiments.

Experiments 4 and 8 aimed at analysing the response of leaf elongation rate to soil water deficit in the absence of evaporative demand (night periods). They were entirely carried out in the greenhouse. Each column contained three different RILs and each RIL was sown in three columns distributed randomly in a block design. Watering was withdrawn two days before emergence of leaf 6. Within 2 weeks, soil water was progressively depleted to water potentials of about –1.8 MPa. Plants were then rewatered and a second cycle of dehydration was initiated. Both *PPFD* and *VPD* were higher in 2005 than in 2004, while temperatures were kept in similar ranges (Table 1).

ASI was measured (i) in five fields with water deficit in Tlatizapan, Mexico in 1996, 1999, and 2001 (short growing season) and in Zimbabwe in 2002, (ii) in three well-watered fields at Tlatizapan in 1996 and 2004 (main growing season) and in 1999 (short growing season).

Data processing

Growth and environmental data were checked for errors and stored in a database (MySQL, www.mysql.com). The R language (R Development Core Team, 2005) was used for data computing and for most statistical analyses. The relationships between leaf elongation rate and meristem temperature obtained during the night were established for each individual RIL, considering all data points originating from the three growing periods carried out one year, in the greenhouse and in the growth chamber. This relationship was fitted to a linear regression,

$$LER = a(T - T_0) \quad (1)$$

where *LER* is leaf elongation rate per unit clock time, T is temperature, a and T_0 are the slope and x-intercept of the relationship between leaf elongation rate and temperature. Leaf elongation rate was averaged during 3-h periods of the night with a stable temperature in the growth chamber, and during 4-h periods from 00.00 h to 04.00 h (solar time) in the greenhouse. Mean values of *LER* were then plotted against the mean temperature during the same periods. Values of a and T_0 were calculated individually for each plant, and then calculated over the three plants together. Because a and T_0 were correlated, hampering a genetic analysis of both parameters, a single value of T_0 was considered for the whole mapping population. It was checked that this process generated small errors in the calculation of thermal time (Sadok *et al*., 2007). The slope a corresponding to one plant was then recalculated by forcing the x-intercept at the common value of T_0. Regression coefficients were calculated by considering the common range of temperature in 2004 and 2005.

Response curves of leaf elongation rate to evaporative demand were established in the same experiments, considering all datapoints originating from the three growing periods of each year, in the greenhouse and in the growth chamber. Datapoints in the greenhouse corresponded to a period of 6 h from 10.00 h to 16.00 h of each day, during which evaporative demand was maximum. Those in the growth chamber corresponded to one 3-h period with stable evaporative demand during the day. Leaf elongation rate was expressed per unit thermal time at each 15 min step, and averaged during the considered period of 3–6 h. Evaporative demand was estimated by the difference in water vapour pressure between the leaf and the air, corrected for the effect of light as in Reymond *et al*. (2003), and averaged for the same period as leaf elongation rate. Parameters taken into account in the analysis were b and b_0 the slope and x-intercept of the relationship corresponding to each RIL. They were first calculated for individual plants, and then calculated for each RIL over the three growing periods. Because of a technical problem in 2004, only data obtained in 2005 are considered here.

Response curves of leaf elongation rate to predawn leaf water potential were obtained each year by considering all datapoints during drying periods, including those obtained after rewatering. Leaf elongation rate was expressed per unit thermal time and was averaged during 4 h periods each night, from 00.00 h to 04.00 h (solar time). It was plotted against the mean predawn leaf water potential corresponding to the same period. Parameters taken into account in the analysis were c and c_0 the slope and x-intercept of the relationship corresponding to each RIL. They were first calculated for individual plants, and then calculated for each RIL over the three replicates of the experiment.

Analyses of variance were performed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA) with a model including a replication effect in analyses carried out in individual years, and

a year effect in analyses carried out over the two years. For parameters a and b, each replica therefore corresponded to a plant grown during one growing period. For parameter c, each replica corresponded to a plant grown in one block. The genetic effect and the genotype×year-experiment interaction effects of the statistical model were considered as random. For each trait, the broad-sense heritability was estimated according to Knapp *et al*. (1985) on the whole data set and within each year-experiment.

QTLs were detected by composite interval mapping with the use of cofactors, using the Plab QTL software (Utz and Melchinger, 2000). The choice of cofactors was carried out using a step-wise regression between the studied trait and the allele value at each marker. The retained marker number in this study was conditioned by the Fisher's test of analysis of variance, fixed at 6. Presence of main effect QTL was tested every 2 cM using a multiple regression with cofactors. The threshold value of LOD was determined by 1000 permutations (Churchill and Doerge, 1994) and was close to 2.35 in our analyses. The QTL model was established by a multiple regression analysis of phenotypic values against allelic values at all QTL locations. QTL for ASI were detected with methods presented elsewhere (Ribaut *et al*., 1996, 2002). LOD ratios of ASI under different environments, of final leaf length and of parameters a, b, b_0, c, and c_0 obtained each year were visually aligned using the Comparative Map and Trait Viewer tool (CMTV; Sawkins *et al*., 2004).

Results

Robust QTLs were identified for leaf elongation rate in the absence of water deficit and co-located with QTLs of leaf length

In each of the two subpopulations of RILs grown in 2004 and 2005, the response of leaf elongation rate to temperature was linear and applied to experiments in the growth chamber and in the greenhouse, with common responses during the three growing periods each year (Fig. 1). Values of r^2 ranged from 0.80 to 0.97, heritabilities were 0.75 and 0.80 in 2004 and 2005. The most frequent x-intercept of regression lines was 9 °C, and was considered as the common value of T_0 in the calculation of thermal time (equation 1). The mean elongation rate during the night, expressed per unit thermal time, was deduced from the slope of relationships and termed 'maximum elongation rate' hereafter (parameter a).

A large variability in parameter a was observed in each experiment, with an appreciable transgression beyond the rates measured in parental lines (Fig. 2). Mean values of a significantly differed between experiments of 2004 and 2005 in the set of 33 common RILs (4.78 versus 4.06 mm $°Cd^{-1}$), but they were correlated between both years (0.74; Table 2) and the rank of genotypes was essentially conserved. This difference between years was also observed in the two subpopulations of 120 RILs (4.88 versus 4.17 mm $°Cd^{-1}$) and translated into a difference in final leaf length (Fig. 3). A possible explanation for the difference in *LER*s between 2004 and 2005 was a slight difference in soil water status (predawn leaf water potentials of –0.05 and –0.09 MPa, respectively). Unlimited irrigation usually causes hypoxia which reduces leaf elongation rate. A target soil water content corresponding to retention capacity was therefore aimed at, and was not reached precisely enough in 2005. For the joint analysis of the 200 RILs over both years, the data of the 120 RILs studied in 2005 were corrected either for the mean difference in elongation rate between years in the set of 33 common RILs, or for the mean difference in predawn leaf water potential multiplied by the sensitivity of each individual RIL (parameter c, see below). These corrections yielded similar results, and the overall heritability was 0.74. Only the results of the second option are presented hereafter.

Five QTLs were identified for parameter a over the whole set of data, accounting for 32% and 43% of the phenotypic and genetic variances, respectively (Table 3). They are presented in Fig. 4, with their positions on chromosomes (bins, i.e. consensus segments of chromosomes). Hereafter, QTLs are referred to by their chromosome and bin number s (e.g. bin 2.04 states for chromosome 2, bin 4). Three QTLs, detected on the

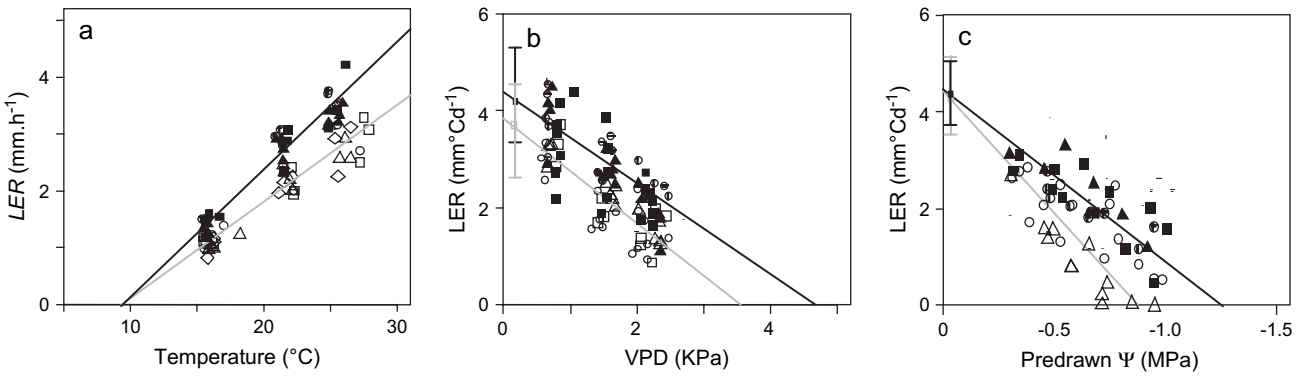

Fig. 1. Response curves of leaf elongation rate (*LER*) to (a) temperature, (b) difference in vapour pressure deficit of water between leaf and air (*VPD*) and (c) predawn leaf water potential (Ψ) in two recombinant inbred lines (filled and empty symbols). Each point represents the mean leaf growth rate plotted against the correspondent environmental conditions averaged for the same period of time. Each symbol (filled squares, filled diamonds, filled circles), one plant from three different experiments for (a) and (b). Straight lines, linear regressions.

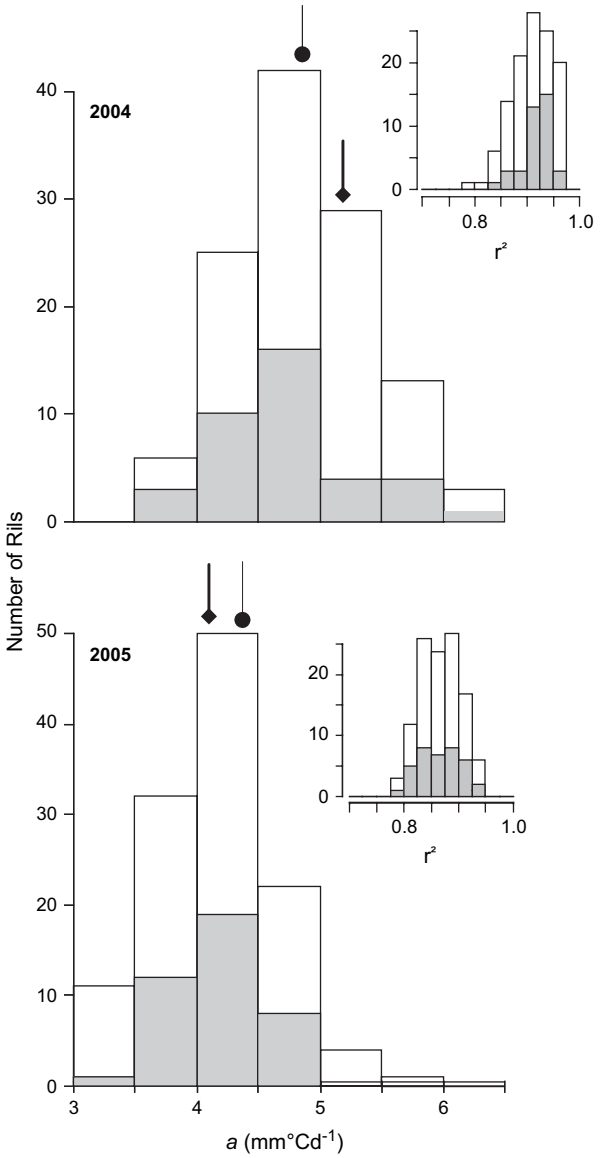

Fig. 2. Distributions of maximum leaf elongation rate per unit thermal time (parameter *a*) in 2004 and 2005. Insets, frequency distributions of r^2. The values of parents are indicated by arrows (arrow with filled circles, P1; arrow with filled diamonds, P2).

whole set of data, were also detected in both individual experiments of 2004 and 2005 (bins 2.04, 8.03, and 9.02, with positive effects of alleles P1, P2, and P1, respectively). The QTL on bin 8.03 was significant in all cases, while those on bins 2.04 and 9.02 were significant in 2005 and on the whole set of data, but were not detected as significant by the QTL analysis in 2004, although they were significant in the multiple regression analysis. Two observations reinforce the validity of the QTL on bin 2.04 in spite of its relatively low value of LOD score. (i) This QTL was significant in 2004 (LOD=3.3, r^2=0.12) if parameter *a* was calculated in the whole range of temperatures instead of the range which was common to both

Table 2. *Phenotypic correlation between leaf growth parameters evaluated in 2004 (above diagonal), in 2005 (below diagonal), and between the two years (on diagonal, italics)*

Lw, final leaf length in well-watered plants. *Ls*, final leaf length under water deficit. *a*, maximum leaf elongation rate per unit thermal time; *c*, c_0, slope and *x*-intercept of the relationship between leaf elongation rate and predawn leaf water potential; *b*, b_0 slope, and *x*-intercept of the relationship between leaf elongation rate and evaporative demand (see Fig. 1).

	Lw	*Ls*	*a*	*c*	c_0	
Lw	*0.71*	0.65	0.70	0.42	0.02	
Ls	0.65	*0.64*	0.47	0.18	0.30	
a	0.72	0.43	*0.74*	0.74	0.19	
c	0.45	0.12	0.77	*0.69*	0.74	
c_0	0.05	0.28	0.20	0.72	*0.60*	
b	0.48	0.26	0.75	0.70	0.24	*b*
b_0	0.01	0.02	0.22	0.30	0.15	0.77

years. (ii) Final leaf length of well-watered plants, which was well correlated to parameter *a* (Table 2), had significant QTLs on bin 2.04 in both 2004 and 2005 (Fig. 4). The other QTLs presented in Table 3 had a lower effect on the whole set of data, and were observed in one individual year only. The QTL on bin 3.04 co-localized with a QTL of leaf length in 2004.

A large genetic variability in the responses of leaf elongation rate to evaporative demand and soil water deficit translates into a set of QTLs of model parameters

Response curves to evaporative demand were established in 120 RILs over three experiments in which well-watered plants experienced a range of *VPD* from 0.4–2.8 kPa during the day (Fig. 1). High r^2 were observed for each individual response curve (Fig. 5, inset), and the overall heritability was high (h^2=0.71). A large variability in slope was observed (parameter *b*, from –0.77 to –1.56 mm °Cd^{-1} kPa^{-1}). The *x*-intercepts of the relationships, interpreted as the *VPD* which would stop leaf elongation, ranged from 2.9–4.8 kPa (parameter b_0). Both distributions showed an appreciable transgression beyond the responses of the two parents. Seven QTLs were identified for parameter *b*, which accounted together for 51% and 72% of the phenotypic and genetic variances (Table 3; Fig. 4). Those on bins 1.06 and 1.11 co-localized with QTLs of parameter b_0 (Fig. 4).

The responses of leaf elongation rate to predawn leaf water potential were linear (Fig. 1), with median r^2 of 0.81 and 0.80 in 2004 and 2005 (Fig. 5, inset). It is noteworthy that elongation rate fully recovered after rewatering, and that the cloud of points corresponding to the second drying period after rewatering was indistinguishable from that of the first drying period. Reductions in elongation rate over time were therefore due to soil water deficit and not to leaf ageing. Heritabilites of the slope of

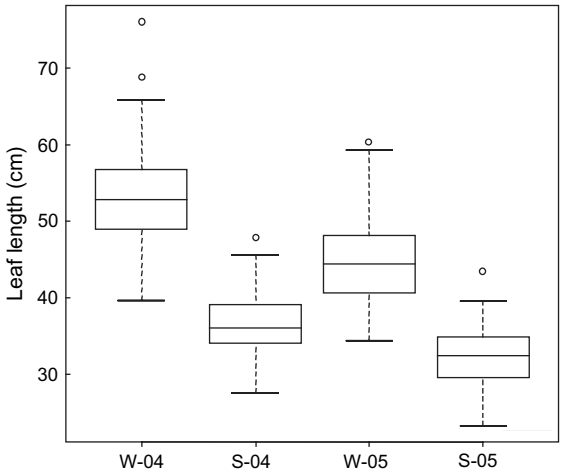

Fig. 3. Mean and quartile of the final length of the sixth leaf in the 33 Rils studied in all experiments. W, well-watered; S, water deficit in 2004 and 2005.

regression lines (parameter *c*) were 0.79 and 0.80. The mean values of *c* did not vary significantly between 2004 and 2005 (–4.43 and –4.30 mm °C^{-1} MPa^{-1}) in the set of 33 RILs analysed both years, and values were well correlated (0.69, Table 2). As a consequence, the heritability calculated over the whole set of data was high (h^2=0.69) on the whole set of values of parameter *c*. A large variability was observed, from 2.8 to 6 mm °C^{-1} MPa^{-1}, corresponding to a range of *x*-intercepts (c_0, the predawn water potential which stops leaf elongation) of –0.8 to –1.4 MPa. Leaf length in water deficit treatments was reduced by 33% and 28% on average in 2004 and 2005 (Fig. 2). Six QTLs of parameter *c* were detected over the whole set of data, accounting for 29% and 42% of the phenotypic and genetic variances respectively (Table 3; Fig. 4). Among those, one QTL on bin 8.03 was detected both years and on the whole set of data, with a positive contribution of allele P1 in the three cases. QTLs with co-locations of parameters *c* and c_0 were observed on bin 2.08

Table 3. *QTL for parameters of leaf elongation rate:maximum leaf elongation rate (*a*), response to evaporative demand (*b*) and response to soil water deficit (*c*)*

Results are presented for individual experiments in 2004 and 2005, and for the joint analysis of both years. The QTL detection was first carried out with the PlabQTL package. All QTLs detected in 2004 or 2005 were then placed in a multiple regression analysis. Lines with normal characters correspond to QTLs identified by PlabQTL, lines in italics correspond to QTLs with LOD scores between 2.00 and 2.35 but confirmed by the multiple regression analysis at P <0.001, **, QTL non identified by PlabQTL but significant at P <0.01 in the multiple regression analysis, *, QTL non identified by PlabQTL but significant at at P <0.05 in the multiple regression analysis.

Trait	Bin[a]	Pos[b]	Marker[c]	Al[d]	2005			2004			Common analysis 2004 and 2005		
					LOD[e]	r^2	Add[f]	LOD	r^2	Add	LOD	r^2	Add
a	1.10	264	bnl6.29	P1	3.84	13.7	–0.168	–	–	–	*	–	*–0.088*
	2.04	84	umc34	P1	2.41	8.8	–0.125	*	–	*–0.090*	2.85	6.7	–0.129
	3.04	68	npi114	P2	**	–	*0.108*	–	–	–	2.71	6.4	0.124
	3.09	250	umc96	P2	–	–	–	2.40	8.9	0.155	*	–	*0.116*
	5.05	130	bnl5.71	P2	–	–	–	*2.15*	*8.0*	*0.202*	2.50	5.9	0.186
	5.06	186	umc51	P2	2.95	10.7	0.144	–	–	–	–	–	–
	8.03	90	umc120	P2	3.24	11.7	0.150	3.80	13.7	0.208	2.51	5.9	0.115
	9.02	72	bnl3.06	P1	4.08	14.5	0.176	**	–	*0.144*	4.33	10.0	0.169
c	2.08	174	umc137	P1	–	–	–	2.71	10.4	0.200	–	–	–
	3.04	64	umc50	P1	*2.11*	*8.0*	*0.170*	–	–	–	*2.31*	*5.4*	*0.179*
	4.08	242	umc326	P2	2.48	10.0	–0.192	–	–	–	–	–	–
	5.03	80	umc27	P1	*2.29*	*8.7*	*0.216*	–	–	–	*	–	*0.181*
	5.05	138	bnl6–22	P1	–	–	–	2.75	10.6	0.303	–	–	–
	5.06	186	umc51	P1	*2.00*	*7.5*	*0.165*	–	–	–	*2.18*	*5.1*	*0.179*
	8.03	88	umc152	P1	2.59	9.8	0.192	*2.02*	*7.5*	*0.172*	3.93	9.0	0.209
	9.02	62	umc182	P1	–	–	–	–	–	–	3.52	8.1	0.214
	10.01	0	bnl3.04	P1	2.51	9.5	0.178	–	–	–	*	–	*0.094*
b	1.06	154	bnl5.59	P1	3.61	12.9	–0.069						
	1.11	276	umc147	P2	4.16	14.8	0.071						
	3.04	68	npi114	P1	2.81	10.2	–0.055						
	5.03	78	umc27	P1	2.82	10.3	–0.057						
	5.06	184	umc48	P1	2.82	10.3	–0.056						
	8.03	98	umc120	P1	*2.08*	*8.8*	*–0.045*						
	10.01	2	bnl3.04	P1	2.74	10.0	–0.053						

[a] Bin in which the QTL was detected.
[b] Position of the QTL in cM from the top of the chromosome.
[c] Left marker.
[d] Allele which favours elongation rate (high *a* or flat responses for *b* and *c*).
[e] LOD score.
[f] Additive value of the QTL.

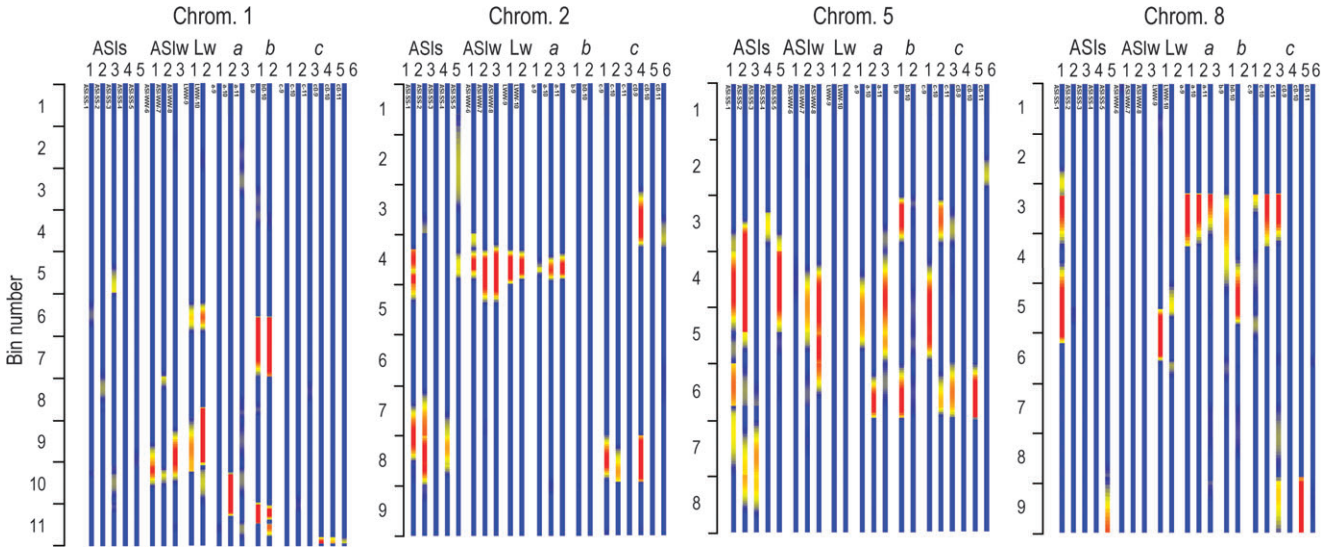

Fig. 4. Comparative viewing of QTLs across experiments and traits in chromosomes 1, 2, 5, and 8 for identification of consensus regions for ASI and leaf growth, and their responses to water deficit. The bin positions are presented in the left of each panel. Each strip represents a combination of chromosome, experiment and trait. Blue, no or non-significant QTL, yellow, LODscore >2.4. Red, LODscore >3. ASIs, QTLs detected on the anthesis–silking interval in field studies with water deficit. ASIw, QTLs detected on the anthesis–silking interval in field studies with well-watered conditions. Lw, final leaf length in 2004 (1) and 2005 (2). *a*, QTLs detected on the maximum leaf elongation rate per unit thermal time in 2004 (1) and 2005 (2) and over the whole set of data (3). *b*, QTLs detected on the slope of the response of leaf elongation rate to evaporative demand in 2005 (1). The *x*-intercept of the same relationship (b_0) is presented in (2). *c*, QTLs detected on the slope of the responses of leaf elongation rate to predawn leaf water potential in 2004 (1), 2005 (2), and over the whole set of data (3). The *x*-intercepts of the same relationship (c_0) are presented in (4) to (6) for 2004, 2005, and the whole set of data. Field studies ASIs at TL01A (1), TL96A (2), TL96A (3), TL99A (4), ZW02B (5); ASIw at TL04B (6), TL96B (7), TL99A (8).

in 2004 (with a non-significant QTL in this bin in 2005), and on bins 4.08, 5.06, and 8.08 in 2005 (Fig. 4).

Consistent with the high correlation between parameters *b* and *c* (Table 2), six QTLs concomitantly influenced the responses to soil water deficit and to evaporative demand (Fig. 4). Those on bins 3.04, 5.03, 5.06, 8.03, and 10.01 had the allele P1 favouring growth maintenance under stressing conditions, while that on bin 1.11 (*b* and c_0) had the parent P2 favouring growth maintenance. Among those, two QTL on bins 5.03 and 10.01 were not observed in parameter *a*. The other four QTLs (bins 1.11, 3.04, 5.06, and 8.03) were common to parameter *a*, consistent with the correlation between all three parameters (Table 2). In all cases the allele conferring low sensitivity to evaporative demand and soil water deficit (P1 in all cases except one) also conferred low maximum elongation rate.

Several QTLs of ASI in well-watered fields co-locate with QTLs of maximum elongation rate, QTLs of ASI in water deficit co-locate with QTLs of response of leaf elongation rate to water deficit

A total of 12 QTLs for ASI was determined, among which eight were detected in well-watered plants and six in water deficit.

A first group of three QTLs involved ASI and leaf growth under well-watered conditions (Fig. 4). The most consistent of them was observed on bin 2.04 and involved QTLs for leaf length and for parameter *a* in 2004 and 2005, for ASI in well-watered conditions in 1996, 1999, and 2004, and for ASI in water deficit in 2001 and 2002. The favourable allele was in all cases P1 for both leaf growth and low ASI. Another overlap between QTLs of parameter *a*, leaf length and ASI in well-watered conditions was observed on bin 1.10, with a favourable allele brought by P1 for all variables. The third one was observed on bin 5.05/06, and involved parameter *a*, ASI in well-watered conditions and in water deficit, with a favourable allele brought by P2 for all variables.

A second group of two QTLs involved ASI under water deficit and parameters of responses to water deficit or evaporative demand. The region in bin 2.08 influenced both ASI *c* and c_0. The region on bin 8.03 influenced parameters *b* and *c*, and ASI in one experiment with water deficit (but it was consistently observed for ASI in water deficit in the same P1×P2 population at the F_3 inbreeding level; Vargas *et al*., 2006).

A third group of QTLs on bins 5.05 and 5.06 influenced ASI and parameters of response of leaf growth in both well-watered and stressing conditions. The signs of the corresponding additive effects matched in all cases, with an increase in ASI associated with a higher sensitivity of leaf growth rate to soil water deficit or to evaporative demand.

By contrast, several genomic regions having an appreciable contribution to ASI did not match with QTLs for

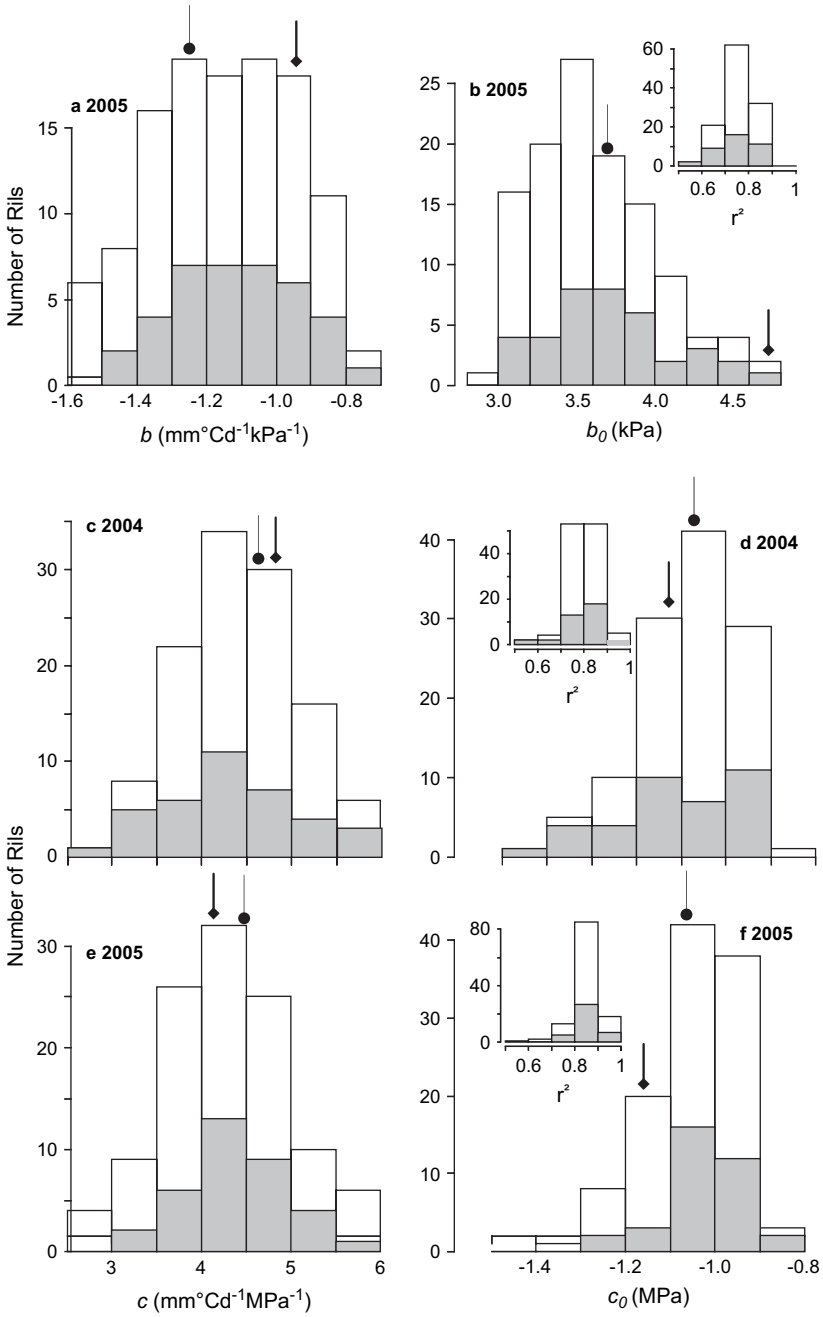

Fig. 5. Distributions of (a) the slope of the relationship between leaf elongation rate and meristem to air vapour pressure difference (parameter *b*), (b) the *x*-intercept of the same relationship b_0), (c, e) the slope of the relationships between leaf elongation rate and predawn leaf water potential (parameter *c*) and (d, f) the *x*-intercept of the same relationships (parameter c_0) in 2004 and 2005. Insets, frequency distributions of r^2. The values of the parental lines are indicated by allows (arrow above filled circles, P1; arrow above filled diamonds, P2).

leaf growth (on chromosomes 6 and 7), while zones of chromosomes 1 and 3 involved in the response of leaf growth did not match with those of ASI. QTLs for anthesis time were essentially independent of those of ASI, and were located on bins 4.05 and 6.05 for the more robust of them (LOD >3). Less robust QTLs for anthesis (LOD <2.3) or QTLs observed in one year only were detected in bins 2.04, 2.07, 4.01, 4.06, and 9.06.

Discussion

The model of leaf growth applies to a tropical genetic material and allows genetic analysis of parameters

A first issue, at the beginning of the study presented here, was whether the model developed for European and North American genetic materials (Ben-Haj-Salah and Tardieu, 1997; Reymond *et al*., 2003, 2004) applied to tropical

genetic material. Because the responses of leaf elongation rate to meristem temperature, evaporative demand, and soil water status were linear and repeatable (high heritabilities of slopes), the model could be considered as adequate. The *x*-intercept of the response to meristem temperature (T_0, equation 1), interpreted as the temperature which would stop elongation, was similar to that in European material, although it could have been expected that tropical genetic material has a higher T_0 than temperate material (Bonhomme *et al*., 1994; Giauffret *et al*., 1995). Maximum leaf elongation rates, (parameter *a*) were significantly higher than those observed in two mapping populations with a temperate origin (Reymond *et al*., 2003; Sadok *et al*., 2007), reflecting a higher early vigour in the genetic material considered.

QTLs detected here were partly common with those in the two temperate populations (IoxF2 and F2×F252) studied by Reymond *et al*. (2003) and Sadok *et al*. (2007). Bin 2.04 harboured a stable QTL of parameter *a* and of leaf length in the population P1×P2, and stable QTLs of parameter *a* in the two temperate mapping populations. Two regions of chromosome 8 (bins 8.03 and 8.09) were observed in the two temperate populations and in the present study for the responses to evaporative demand and soil water status.

Co-locations of QTLs suggest common mechanisms of growth and growth maintenance of leaves and silks under water deficit

The results presented here suggest that the controls of leaf elongation rate and of ASI may have common genetic determinisms, both in well-watered conditions and under water deficit. The mapping population P1×P2 segregates for ASI in both well-watered and water deficit (Ribaut *et al*., 1996) and for the response of leaf elongation rate to environmental conditions (this study), with several QTLs in common. Anthesis occurs almost synchronously in well-watered and stressing conditions. ASI is therefore essentially linked to the silking date which is largely affected by stresses (Bänziger and Lafitte, 1997; Betrán *et al*., 2003; Welcker *et al*., 2005). A possible mechanism for a partly common genetic determinism could be that ASI depends on the growing ability of silks, inversely related to the time for a silk tip to grow from the ovule to the end of husks. In this view, common sets of mechanisms would determine, on the one hand the intrinsic ability of both leaves and silks to grow under well-watered conditions (parameter *a* for leaves, ASI in well-watered conditions for silks), on the other hand the growth maintenance of leaves and silks under soil water deficit or high evaporative demand (parameters *b* and *c* for leaves; ASI in water deficit for silks).

The ability for a tissue to expand under well-watered conditions is essentially determined by cell-wall properties in the growing region of each organ in particular under the influence of expansins (Cosgrove, 2005). Common expansins have been identified in leaves and silks (Exp. A1, B2, and B8; Wu *et al*., 2001). There is therefore a good possibility that alleles associated with a high expansin activity act in both leaves and silks. The same possibility applies to cell division rate which is also associated with tissue elongation rate and its changes with temperature or developmental stages (Granier *et al*., 2000), possibly via co-ordination between cell division and cell elongation (Fleming, 2005). In our data, the involved regions would be located on bins 2.04, 1.10, and 5.06. Bin 2.04 harbours QTLs for leaf growth in other maize mapping populations (see above). It harbours QTLs of constitutive root characteristics, suggesting that it may be also involved in the constitutive control of root growth (Lebreton *et al*., 1995; Tuberosa *et al*., 2002)

The genetic variability of responses of tissue expansion to water deficit may have different origins. A first hypothesis involves hydraulic mechanisms. Wesgate and Boyer (1985) have shown that silks have a low ability for osmotic adjustment, while leaves maintained turgor under water deficit. However, Bouchabke *et al*. (2006) recently found that turgor, as measured by a pressure probe, is not maintained in cells of the growing zone of maize plants subjected to high evaporative demand. Furthermore, they observed an appreciable variability in turgor maintenance among five maize genotypes. A good possibility therefore exists that turgor maintenance could be a common mechanism for growth maintenance in silks and leaves. It is noteworthy that bin 8.03, on which was found a co-location of QTLs of ASI and responses of leaf growth, also harbours a QTL of osmotic adjustment in mature leaves determined in the same mapping population by Ribaut *et al*. (2004). Other hypotheses may involve the change with water deficit in cell-wall properties (Wu and Cosgrove, 2000) or in cell division rate (Granier *et al*., 2000), but they remain to be investigated. Consensus regions identified here apply to ASI in other genetic backgrounds, especially that on bin 8.03 for ASI under both Low N and water-limited conditions (Vargas *et al*., 2006) and on bins 2.08 and 8.05 (Stuber *et al*., 1992; Veldboom and Lee, 1996; Sari-Gorla *et al*., 1999; Bertin and Gallais, 2001; Moreau *et al*., 2004).

Conclusion

These results raise the possibility that the growths of leaves and silks, and their degrees of maintenance under water deficit, may be genetically linked. In this case, the genetic determinisms of ‘sources’ and ‘sinks’ could be partly common. If this result is confirmed by further studies, it may have profound consequences both for the modelling of the genotype×environment interaction and for designing drought-tolerant ideotypes.

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