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RFLP mapping of quantitative trait loci controlling abscisic acid concentration in leaves of drought-stressed maize (*Zea mays* L.)

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Abstract Abscisic acid (ABA) concentration in leaves of drought-stressed plants is a quantitatively inherited trait. In order to identify quantitative trait loci (QTLs) controlling leaf ABA concentration (L-ABA) in maize, leaf samples were collected from 80 $F_{3:4}$ families of the cross Os420 (high L-ABA) × IABO78 (low L-ABA) tested under drought conditions in field trials conducted over 2 years. In each year, leaf samples were collected at stem elongation and near anthesis. The genetic map obtained with 106 restriction fragment length polymorphism (RFLP) loci covered 1370 cM, which represented approximately 85% of the UMC maize map. Sixteen different QTLs with a LOD > 2.0 were revealed in at least one sampling. Across samplings, only four QTLs significantly influenced L-ABA, accounting for 66% of the phenotypic variation and 76% of the genetic variation among families. At these QTLs, the alleles which increased L-ABA were contributed by Os420. The two most important QTLs were mapped on chromosome 2 near *csu133* and *csu109a*. The effects associated with the QTL near *csu133* were more pronounced near anthesis. The support intervals of the four primary QTLs for L-ABA did not overlap the presumed map position of mutants impaired in ABA biosynthesis.

Key words Abscisic acid (ABA) · Drought stress · Quantitative trait loci (QTLs) · Maize (*Zea mays* L.) · RFLPs

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Introduction

Drought tolerance of crop species is influenced by a wealth of constitutively and adaptively expressed traits, for the most part poorly understood in terms of their underlying genetic basis and their associated effects on yield. The concentration of abscisic acid (ABA) in leaves and other organs is one of the traits most universally affected by a water deficit (Hartung and Davies 1991). In cereals, several studies have indicated that the increase in ABA concentration observed during a drought episode plays a pivotal role in the adaptive response of the plant to reduced water availability (for a review see Quarrie 1991). For this reason, it has been proposed that leaf ABA concentration (L-ABA) may represent a physiological trait of potential value to improve yield under drought conditions (Innes et al. 1984; Quarrie 1987). A detailed knowledge of the genetic basis of a trait represents an essential factor in evaluating the feasibility of its use for an indirect selection for yield.

In maize (*Zea mays* L.) the inheritance of L-ABA is complex. Following the pioneering work of Larqué-Saavedra and Wain (1974, 1976), more recent work based on a generation-means analysis estimated that a few independent genes controlled L-ABA in the same cross examined (Sanguineti et al. 1996). This type of estimate is based on assumptions which are seldom met in practice, so that the number of genes is usually underestimated. Furthermore, generation analysis does not provide information on the map position of the genes controlling L-ABA and thus precludes us from ascertaining which genes might influence L-ABA in different genetic backgrounds and might, either by linkage and/or pleiotropy, affect other traits.

The advent of mapping techniques based on the use of molecular markers allows for the identification of the chromosomal regions harboring quantitative trait loci (QTLs) for the trait of interest, thus permitting the comparative analysis of each QTL in different crosses

(Paterson et al. 1991; Lin et al. 1995). More importantly, it is also possible to estimate the relative effect of each QTL on the trait in question and other characteristics for which sufficient genetic variation is present in the mapping population. Finally, it is possible to compare the localization of QTLs with that of mutants and/or cDNA and EST (expressed sequence tagged) clones of known function; this information provides important clues on possible candidate genes underlying the expression of the investigated trait (Paterson 1996). According to the hypothesis formulated by Robertson (1985), many QTLs could represent weaker allelic variants at loci corresponding to mutants showing a drastic effect on the phenotype for the same quantitative trait. Several mutants impaired in ABA biosynthesis have been described and mapped in maize (Neuffer et al. 1997; Schwartz et al. 1997); provided that QTLs for L-ABA were identified, the map information available for the ABA mutants could allow for the verification of Robertson's hypothesis for this trait.

QTLs for L-ABA have been identified in barley (Sanguineti et al. 1994; Noli et al. 1996), maize (Lebreton et al. 1995), and wheat (Quarrie et al. 1994). In maize, six QTLs were found to control L-ABA in a population of 81 F_2 plants grown in a glasshouse experiment (Lebreton et al. 1995); a number of these QTLs mapped within the clusters of developmental genes identified in maize by Khavkin and Coe (1997). Due to the rather low heritability of L-ABA on a single-plant basis (Ivanovic et al. 1992; Sanguineti et al. 1996), it is important that the phenotypic data for QTL analysis are obtained using mapping populations which allow for replication in time and space (e.g. F_3 families, RILs, DH lines, etc.). In the present research, we studied 80 $F_{3:4}$ families derived from a cross between two maize inbreds differing in L-ABA in order to construct a genetic map based on restriction fragment length polymorphism (RFLP) marker loci and to characterize the relative importance and stability of QTLs for L-ABA under field conditions of drought stress.

Materials and methods

Plant material

The experimental material consisted of 80 $F_{3:4}$ random families derived from a cross between Os420, a US maize inbred line, and IABO78, an inbred line derived from Italian germplasm. Based on the results of a 2-year investigation (Conti et al. 1994), Os420 was chosen as the high L-ABA parent and IABO78 as the low L-ABA parent. The 80 $F_{3:4}$ families were derived as follows: 80 F_2 plants were chosen at random among 480 F_2 plants and were selfed. For each $F_{2:3}$ family, two plants were grown and selfed in order to avoid the loss of families in case the $F_{2:3}$ plants were characterized by a poor seed set. In three $F_{2:3}$ families one of the plants did not set seed. In the remainder of the $F_{2:3}$ families one selfed plant was then chosen at random.

The $F_{3:4}$ families and the parent lines were tested in field experiments conducted in 1994 and 1995 at Cadriano near Bologna, Italy, according to a randomized complete block design with three replications. The parent lines were separated from the $F_{3:4}$ families by border rows. Plants were grown according to the field practices usually adopted for maize in our region, except for planting date and irrigation. Planting was delayed to have anthesis during the driest time of summer in our region. Each plot was over-seeded and thinned to include 12 plants (4.0 plants m^{-2}). To achieve an adequate level of water stress, irrigation volumes corresponded to approximately 50% of the actual evapotranspiration, after accounting for rainfall.

Samples to determine L-ABA were collected on the eight central plants of each plot at growth stages 3 and 4 (described in Hanway 1963), i.e. at rapid stem elongation and about 2–3 days before anthesis, respectively; the fifth and the third leaf from the top was considered at stages 3 and 4, respectively. Leaf samples (third distal portion of the leaf lamina) were immediately placed in plastic bags and stored at $-30°C$ until they were used.

Measurement of L-ABA

The concentration of physiologically active, unconjugated ABA [2-*cis*(+)-ABA] was determined by a radioimmunoassay using an ABA-specific monoclonal antibody (Quarrie et al. 1988) on samples of crude aqueous extracts of leaf disks (approximately 150 mg fresh weight; dilution in distilled water 1:10 w/v) and following the procedures described in Tuberosa et al. (1994). Determination of ABA concentration was carried out with duplicate assays. Values of L-ABA are reported as ng ABA g^{-1} dry weight (d.w.). Henceforth, L-ABA at growth stages 3 and 4 in 1994 and 1995 will be abbreviated as S3/94, S4/94, S3/95, and S4/95, respectively.

Analysis of variance

The analysis of variance (ANOVA) was first carried out separately for each of the four 'growth stage × year' combinations (samplings). Then, a combined ANOVA was computed across samplings. The effects due to $F_{3:4}$ families were considered random. Analogously, the effects due to years and growth stages were also considered random because previous work (Conti et al. 1994; Landi et al. 1995) has indicated that L-ABA differences between years and growth stages in maize were largely related to water availability, hence to the rainfall pattern, and other partially unpredictable meteorological factors influencing the water balance of the plant (e.g. temperature, relative humidity, etc.). Broad-sense heritability (h^2) was estimated on a family mean basis for each sampling as well as across the four samplings; confidence intervals of the estimates were calculated according to Knapp et al. (1985).

DNA extraction and RFLP analysis

For each $F_{3:4}$ family, one leaf was harvested from each of the 16 plants which were grown for 7 weeks in the greenhouse; the leaves of each family were bulked and freeze-dried. The isolation of total DNA was performed following the procedures described by Saghai-Maroof et al. (1984). Purified DNA was re-suspended in a 1 × TE buffer. DNA restriction digests included 6 μg of total DNA and 18 U of each of three restriction enzymes (*Bam*HI, *Eco*RI, and *Hin*dIII). DNA fragments were separated by electrophoresis on 0.8% agarose (IBI, Eastman Kodak) and transferred overnight by capillarity onto charged nylon membranes (Hybond N^+, Amersham International) using a 0.4 M NaOH solution. Membranes were air-dried at room temperature and stored at 4°C. Pre-hybridization, hybridization, and washings were carried out following the procedures described in

Sharp et al. (1988). Membranes were hybridized with 133 RFLP probes kindly provided by the University of Missouri (Columbia, USA). Probes were labelled with [^{32}P]-dCTP using the random-hexamer procedure. Membranes were exposed to autoradiographic film with one intensifying screen at -80°C for as long as needed (from 3 to 6 days).

Construction of the linkage map

The software package JOINMAP in the MS-DOS version 1.4 (Stam 1993) was used for linkage analysis among the data obtained with the 80 $F_{3:4}$ families scored with 106 RFLP marker loci and for the construction of the genetic map. The critical LOD (logarithm of odd value) score for the test of independence of pairs of markers was set at 3.0. Using Haldane's mapping function, the recombination frequency between linked loci was transformed into centimorgan (cM) distances. The χ^2 test was applied to identify markers with a distorted ($P < 0.01$) segregation from the expected ratios.

QTL analysis

The recently proposed procedure of composite interval mapping (CIM) was used to identify QTLs and to estimate their effects (Jansen and Stam 1994; Zeng 1994). The main advantage of CIM over interval mapping is that it incorporates co-variate effects of other markers. QTL analysis was performed separately on the data of the four individual samplings, as well as on their mean, utilizing the software package PLABQTL (Utz and Melchinger 1995) which is based on interval mapping by the regression approach (Haley and Knott 1992), and, using selected markers as co-factors, allows for the reduction of possible bias in the estimation of QTL positions and effects. More details on the underlying model are reported in Bohn et al. (1996). Co-factors were chosen for each trait by a stepwise regression procedure (F to enter: 3.5; F to drop: 3.5) according to the suggestions of Utz and Melchinger (1995); in this way markers adjacent to most of both the important and the minor QTLs were selected as co-factors.

A putative QTL was declared significant when the LOD score was > 2.0. According to simulation studies (Jansen 1994; Zeng 1994) on samples larger than ours, we utilized the $\chi^2_{(3)}$ approximation to compute the comparison-wise Type-I error probability. With a LOD threshold of 2.0 and 96 marker intervals, the comparison-wise Type-I error probability is <0.027. Other factors (e.g. sample size, genome size, genetic map density, missing data, etc.) can determine the exact probability of a Type-I or Type-II error associated with a particular LOD value (Churchill and Doerge 1994). We chose a threshold value of 2.0 because: (1) it allowed for a comparison with the results of Lebreton et al. (1995) which were also based on a LOD threshold of 2.0; and (2) simulation work indicated that Type-II errors (i.e. not declaring the presence of a real QTL) represent a greater problem than Type-I errors, particularly when a population of small size is considered (Beavis 1994). QTL positions were assigned at the point of maximum LOD score in the regions under consideration. The support interval of each QTL expressed in cM represents the region comprised within a LOD fall off = 1.0 from the QTL peak. The proportion of phenotypic variance (σ^2_p) accounted for by each individual QTL was computed by the square of the partial correlation coefficient (R^2). For each sampling, a multiple regression model including all the corresponding putative QTLs was fitted in order to estimate additive (a_i) and dominance (d_i) effects of each ith QTL, the total proportion of σ^2_p and genotypic variance ($\sigma^2_g = R^2/h^2$) explained by the fitted model (Schön et al. 1993). The presence of significant additive and dominance effects at each ith QTL (H_0: $a_i = 0$ or H_0: $d_i = 0$) was tested using the F ratio = (partial sum of squares of the single QTL effects)/(residual mean squares of the model fitting all detected QTLs simultaneously). Because $F_{3:4}$ families were phenotypically evaluated, the difference between the mean value of the segregating families (with an expected 50% frequency of heterozygotes) and that of the homozygous families represents one half of the dominance effects; therefore, the dominance effects calculated with PLABQTL were doubled. The average level of dominance at each ith QTL was computed according to the criteria defined by Stuber et al. (1987) as the dominance ratio (DR): $|d_i|/|a_i|$. Gene action was classified as follows: additive for DR <0.2; partially dominant for $0.2 \leq$ DR < 0.8; dominant for $0.8 \leq$ DR $<$ 1.2; overdominant for DR ≥ 1.2.

In order to compute the total phenotypic variance explained by the QTLs declared significant in the analysis of the mean values, each sampling was also analysed using a model considering only such QTLs. The ANOVA to determine the presence of 'QTL × sampling' interaction included only those QTLs detected in the analysis of means across samplings.

Results

Construction of the linkage map

The percentage of missing marker data amounted to 2.0% and was mainly due to the poor signal of the corresponding bands. Among the 133 RFLP probes tested, 97 detected scorable polymorphisms with at least one of the three restriction enzymes utilized. Because nine probes (bnl7.49, csu16, csu17, csu25, csu26, csu36, csu58, umc39, and umc193) each identified two polymorphic loci, the total number of scored loci was equal to 106 (96 co-dominant and ten dominant markers). The parental genomes were represented in almost equal amounts (49.8% for IABO78 and 50.2% for Os420); the proportion of the parent line genome represented in the 80 $F_{3:4}$ families was normally distributed (from 25.0 to 73.2% for IABO78). The level of homozygosity of the parental F_3 plants ranged from 50.5 to 90.6% and showed a significant ($P < 0.01$) negative skewness, though its mean (74.4%) was very close to the expected value (75%).

A total of 101 RFLP markers were grouped in 12 linkage groups, each of which was assigned to a specific chromosome (Fig. 1) based on the information of the UMC maize map (Davis et al. 1996). Five markers (*bnl6.32*, *csu54b*, *csu95a*, *csu48*, and *umc169*) remained unlinked (LOD < 3.0). Also in this case, the assignment to specific chromosomes was possible using the information of the UMC map; in all cases the indications of the LOD values supported the chromosome assignment of the loci. Including the gaps with a LOD <3.0, the map covers 1370 cM. The average distance between adjacent markers is 14.3 cM.

Nine marker loci (*umc121* and *bnl13.05b* on chr. 3; *csu134* on chr. 5; *umc85* on chr. 6; *csu13*, *bnl15.40*, and *umc168* on chr. 7; *umc89a* on chr. 8; *csu145a* on chr. 9) representing eight different regions (Fig. 1) showed a distorted ($P < 0.01$, χ^2 test) segregation from the expected ratios (30 : 20 : 30 and 50 : 30 for co-dominant and dominant markers, respectively). At these eight regions, the prevailing alleles were equally contributed by Os420 and IABO78.

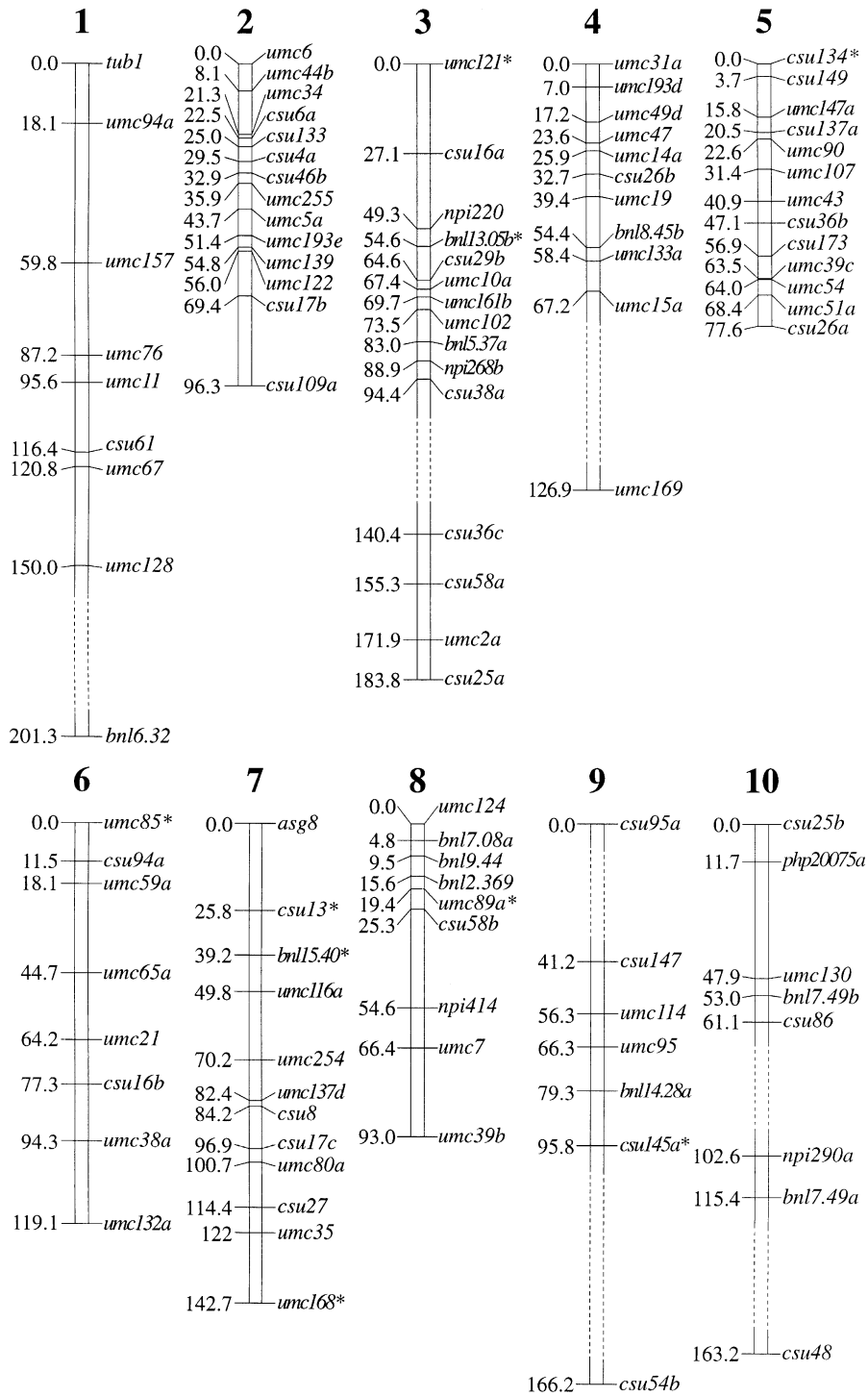

Fig. 1 RFLP linkage map of the maixe cross Os420 × IABO78 obtained with 80 $F_{3:4}$ families and 106 RFLP markers. *Dashed areas* between markers indicate that linkage was not significant (LOD < 3.0). Chromosomes have been aligned with the short arm toward the top of the page. The *asterisks* indicate the RFLP markers with a distorted segregation ($P < 0.01$) from the expected ratios

Analysis of variance

The ANOVA (data not reported) for the trait L-ABA showed significant ($P < 0.01$) effects due to parent lines and due to $F_{3:4}$ families, while no significant differences were found between growth stages and years. Moreover, significant 'family × year' ($P < 0.05$), 'growth stage × year' ($P < 0.01$), and 'family × growth stage × year' ($P < 0.01$) interactions were detected. Due to the significance of the higher-order interaction, the four 'growth stage × year' combinations were considered as separate samplings.

Table 1 reports the mean values for L-ABA of the parent lines and the $F_{3:4}$ families. The range in values

Table 1 Means of the parent lines, means and range of the derived 80 $F_{3:4}$ families, and heritabilities on a family mean basis for leaf ABA concentration at four samplings (S3/94, S4/94, S3/95, and S4/95) and across samplings

Sampling	Parent lines			$F_{3:4}$ families					
	(ng ABA g^{-1} d.w.)			(ng ABA g^{-1} d.w.)				h^2	90% CI[b]
	IABO78	Os420		Mean	Min	Max			
S3/94	425	771	**[a]	587	427	983	**	0.79	(0.71–0.85)
S4/94	223	678	**	367	225	790	**	0.88	(0.84–0.92)
S3/95	228	471	**	328	213	605	**	0.68	(0.56–0.77)
S4/95	178	801	**	348	186	648	**	0.88	(0.84–0.91)
Across samplings	264	680	**	408	264	680	**	0.87	(0.83–0.90)

[a] **Significant at the 0.01 probability level
[b] Confidence intervals of h^2

among $F_{3:4}$ families varied from a minimum of 2.3-fold for S3/94 (from 427 to 983 ng ABA g^{-1} d.w.) to a maximum of 3.5-fold for both S4/94 (from 225 to 790 ng ABA g^{-1} d.w.) and S4/95 (from 186 to 648 ng ABA g^{-1} d.w.). Across samplings, the values of the $F_{3:4}$ families characterized by the lowest and highest L-ABA coincided with those of the low- and high-L-ABA parent lines, respectively, whereas the mean value of the 80 $F_{3:4}$ families (408 ng ABA g^{-1} d.w.) was significantly ($P < 0.01$) lower than the mid-parent value (472 ng ABA g^{-1} d.w.). Among samplings, S3/94 showed the highest mean value (587 ng ABA g^{-1} d.w.), most likely due to a higher level of drought stress consequent on the low rainfall in the month preceeding this sampling (data not shown).

Heritability on a family mean basis was quite high, ranging from 0.68 (S3/95) up to 0.88 (S4/94 and S4/95). The mean value of S3/94 was 60% higher than that of S4/94 (587 vs 367 ng ABA g^{-1} d.w.) but the heritability of S3/94 ($h^2 = 0.79$) was slightly lower than that of S4/94 ($h^2 = 0.88$). Accordingly, the heritability of S3/95 ($h^2 = 0.68$) was lower than that of S4/95 ($h^2 = 0.88$). Accordingly, the heritability of S3/95 ($h^2 = 0.68$) was lower than that of S4/95 ($h^2 = 0.88$) although the mean values were quite similar (328 and 348 ng ABA g^{-1} d.w., respectively).

A significant, albeit low, positive correlation was evidenced between the average level of homozygosity of the F_3 mother plants as estimated with RFLP data and the L-ABA values of the derived F_4 progenies at S3/94 ($r = 0.26$; $P < 0.05$), S4/94 ($r = 0.29$; $P < 0.05$), and S4/95 ($r = 0.31$; $P < 0.01$).

Identification of QTLs

Individual samplings

In order to assess the consistency of QTL effects under different developmental and environmental conditions, QTL analysis was first carried out separately for each individual sampling. Considering each sampling individually, the LOD profiles exceeded 2.0 in 28 cases (Fig. 2). In order to reduce the probability of a Type-I error for the QTLs with a LOD from 2.0 to 2.5, we further considered only those QTLs with similar LOD profiles in at least two samplings or in one sampling and in the mean, even if they did not reach a LOD of 2.0 in more than one sampling. Therefore, the QTL on chr. 6 (peak at 20 cM for S4/95) and two QTLs on chr. 8 (peaks at 18 cM for S4/94 and at 38 cM for S4/95) were discarded. Conversely, one QTL on chr. 2 (peak at 58 cM for S3/94) and one QTL on chr. 4 (peak at 34 cM for S4/94) were retained.

The approximate map position of known mutants impaired in ABA biosynthesis (Neuffer et al. 1997) was not localized within the support intervals of the QTLs; the position of ABA mutants in our map (Fig. 2) was estimated based on the position of the flanking RFLP markers common to our map and the UMC maize map. The most interesting ABA mutant is *VP14* which controls what appears to be the key rate-limiting step for ABA biosynthesis, namely the oxidative cleavage of epoxy carotenoids to C_{25} epoxy apo-aldehyde and xanthoxin, the precursor of ABA (Schwartz et al. 1997).

QTLs characterized by overlapping support intervals in different samplings were considered as coincident. Following this criterion, the number of QTLs revealed at each sampling varied from five (S3/94) to seven (S3/95 and S4/94; Table 2). Among the 16 different QTLs, ten showed significant effects in only one sampling, three (one on chr. 2 near *umc6*, one on chr. 6 between *umc38a* and *umc132a*, and one on chr. 9 near *bnl14.28a*) in two samplings, and three (one on chr. 1 near *umc11* and two on chr. 2 between *csu133* and *csu6a* and near *csu109a*) in three samplings. The proportion of phenotypic variance explained by a single QTL ranged from 11.7 to 31.0%. Fitting a multiple regression model to each sampling accounted for a sizeable portion of the genetic variance among $F_{3:4}$ families (67.6% in S3/94, 80.8% in S4/94, 83.8% in S3/95, and 85.4% in S4/95; data not shown). At nine of the 16 candidate QTLs, Os420 contributed the alleles increasing L-ABA, while IABO78 contributed the L-ABA increasing alleles at six QTLs; in only one case (QTL on chr. 6) the effect of the parent alleles showed an

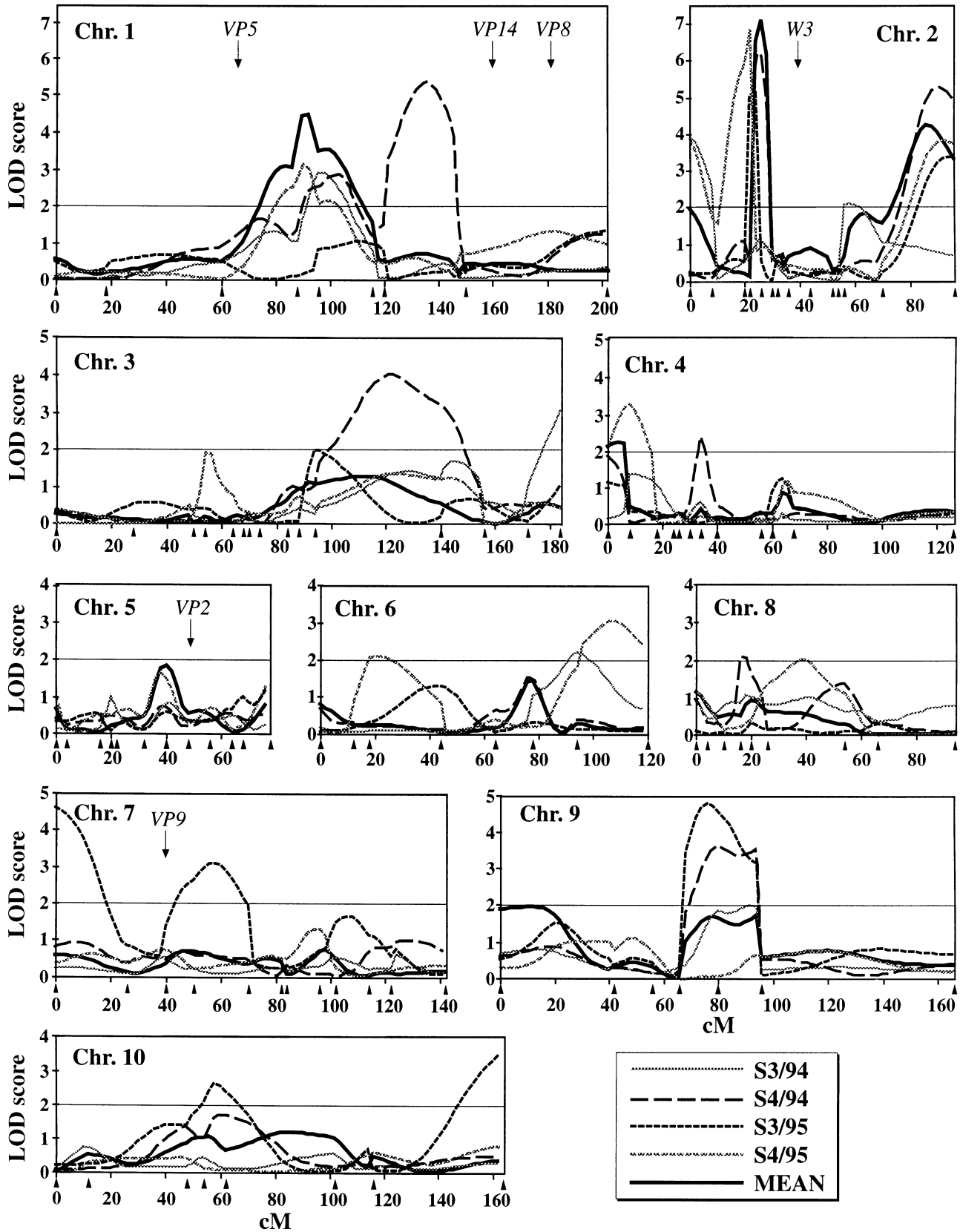

Fig. 2 Profiles of the LOD scores for leaf ABA concentration (L-ABA) of the cross Os420 × IABO78. The five profiles represent L-ABA at growth stage 3 in 1994 (S3/94; ·····), growth stage 4 in 1994 (S4/94; – – –), growth stage 3 in 1995 (S3/95; - - - - -), growth stage 4 in 1995 (S4/95; ~~~~~), and their mean (——). *Triangles* (▲) indicate the RFLP marker loci and the six *arrows* indicate the approximate position (as presumed on the basis of the UMC map) of mutants impaired in ABA biosynthesis

Table 2 Main characteristics and effects of the QTLs with a LOD > 2.0 for leaf ABA concentration in the individual samplings (S3/94, S4/94, S3/95, and S4/95) and in the combined analysis across samplings (in bold). QTL parameters have been estimated based on the phenotypic means of 80 $F_{3:4}$ families

Sampling[a]	Chromosome		Nearest flanking marker	Maximum LOD	R^2 value (%)	Additive[c] effect (*a*) (ng ABA g^{-1} d.w.)	Dominance effect (*d*)	Gene[d] action
	Number	Position[b] (cM)						
S3/94	1	90-96-108	*umc11*	2.89	15.6	40.2	−95.0	OD
S4/94	1	88-102-112	*umc11*	2.88	16.1	17.7	−110.6	OD
S4/95	1	80-90-96	*umc11*	3.15	17.4	2.8	−216.9	OD
Mean	**1**	**86-92-102**	***umc11***	**4.46**	**22.9**	**26.0**	**−148.0**	**OD**
S4/94	1	126-136-146	*umc128*	5.36	26.1	41.4	29.8	PD
S3/94	2	0-0-8	*umc6*	3.87	20.1	38.9	−82.2	OD
S4/95	2	0-0-6	*umc6*	3.86	20.5	3.8	−62.5	OD
S4/94	2	22-26-28	*csu133*	6.30	29.3	29.2	−8.4	PD
S3/95	2	20-24-26	*csu133*	5.16	25.6	26.5	21.5	D
S4/95	2	18-22-24	*csu6a*	6.83	31.0	85.7	−62.4	PD
Mean	**2**	**22-26-30**	***csu133***	**7.08**	**32.1**	**49.2**	**−33.5**	**PD**
S3/94	2	54-58-70	*umc122*	2.07	11.7	41.7	−62.3	OD
S4/94	2	82-90-96	*csu109a*	5.26	26.9	36.2	−104.9	OD
S3/95	2	84-96-96	*csu109a*	3.36	19.3	27.7	−14.2	PD
S4/95	2	82-92-96	*csu109a*	3.83	21.1	50.0	−8.7	A
Mean	**2**	**78-86-96**	***csu109a***	**4.20**	**22.7**	**49.6**	**−52.1**	**D**
S4/94	3	108-122-144	*csu36c*	3.99	20.8	41.9	30.8	PD
S3/94	3	176-182-182	*csu25a*	2.67	14.6	−32.6	43.9	OD
S4/95	4	0-8-16	*umc193d*	3.28	17.8	35.6	−77.1	OD
Mean	**4**	**0-4-8**	***umc193d***	**2.20**	**12.8**	**22.0**	**11.9**	**PD**
S4/94	4	30-34-38	*csu26b*	2.39	13.6	−12.2	−31.2	OD
S3/94	6	82-94-108	*umc38a*	2.20	12.4	34.3	−36.7	D
S4/95	6	94-108-118	*umc132a*	3.06	16.7	−30.4	−26.3	PD
S3/95	7	0-0-12	*asg8*	4.58	23.4	−26.2	−16.6	PD
S3/95	7	44-58-70	*umc116a*	3.07	17.0	18.1	−41.3	OD
S4/94	9	72-80-96	*bnl14.28a*	3.62	19.2	−17.7	119.3	OD
S3/95	9	68-76-88	*bnl14.28a*	4.76	24.1	−28.2	12.7	PD
S3/95	10	48-58-72	*csu86*	2.59	14.9	−8.7	83.4	OD
S3/95	10	150-162-162	*csu48*	3.43	19.5	−26.1	−12.4	PD

[a] Marker used as cofactors (chosen by stepwise regression) in the order of selection for:
S3/94: *umc11*, *umc6*, *umc122*, *csu36c*, *csu25a*, and *umc38a*
S4/94: *umc128*, *csu6a*, *csu109a*, *csu36c*, *umc31*, *csu26b*, *umc89a*, and *bnl14.28a*
S3/95: *csu61*, *umc34*, *csu109a*, *csu38a*, *asg8*, *umc116a*, *bnl14.28a*, *umc15a*, and *csu48*
S4/95: *umc6*, *csu133*, *csu109a*, *bnl13.05b*, *csu36c*, *umc193d*, *umc59a*, and *umc132a*
Mean: *umc11*, *umc6*, *csu6a*, *csu17b*, *csu109a*, *umc31a*, *csu95a*, *bnl14.28a*, and *npi290a*
[b] The central value indicates the QTL peak; flanking values represent the 1.0 LOD support interval
[c] Computed as (Os420 − IABO78)/2; estimates were obtained with a simultaneous fit of all putative QTLs
[d] Computed as *d*/*a*; A: additive gene action; PD: partial dominance; D: dominance; OD: overdominance

important change in direction, which may indicate the presence of two distinct QTLs with overlapping support intervals in different samplings. At four of the six QTLs with significant effects in more than one sampling, the plus alleles were contributed by Os420; additionally, the direction of the effect was consistent across samplings even when the LOD score did not reach 2.0 (data not shown). The average LOD score was lower for the QTLs detected in one sampling (LOD = 3.34) compared to the QTLs detected in two and three samplings (LOD = 3.56 and 4.41, respectively). The strongest additive effect was contributed by the QTL on chr. 2 near *csu6a* ($a = 85.7$ ng ABA g^{-1} d.w. for S4/95). Dominance effects (*d*) showed considerable fluctuations with very high absolute values. Considering the ratio between dominance and additive effects (*d*/*a*) at the QTLs observed in the individual samplings, gene action was additive in only one case, partially dominant in nine cases, dominant in two cases, and overdominant in 13 cases (Table 2).

Across samplings

When QTL analysis was performed on the mean values of the four samplings, only four QTLs (one on chr. 1 near *umc11*, two on chr. 2 near *csu133* and *csu109a*, and one on chr. 4 near *umc193d*) of the 16 QTLs revealed in the individual samplings showed a LOD value >2.0 (Table 2 and Fig. 2). The QTL near *csu133*

Table 3 QTL position, significance of the F ratio of the 'QTL × sampling' interaction, and values of the additive (a) and dominance (d) effects of the QTLs for leaf ABA concentration with a LOD > 2.0 in the analysis across samplings. Values are reported for each individual sampling (S3/94, S4/94, S3/95, and S4/95) and for their mean

Chromosome	Position (cM)	Nearest probe	F[a]	Genetic effects (ng ABA g^{-1} d.w.)					
				Action	S3/94	S4/94	S3/95	S4/95	Mean
1	92	*umc11*	**	*a*	45.1	32.1	16.2	10.5	26.0
			ns	*d*	−166.6	−156.7	−93.2	−175.6	−148.0
2	26	*csu133*	**	*a*	42.0	43.7	27.8	83.2	49.2
			*	*d*	−84.6	−22.0	36.4	−63.8	−33.5
2	86	*csu109a*	ns	*a*	60.6	51.8	33.3	52.6	49.6
			ns	*d*	−97.8	−79.3	−77.0	45.6	−52.1
4	4	*umc193d*	ns	*a*	21.6	16.7	17.9	31.6	22.0
			ns	*d*	21.8	−10.0	81.6	−45.8	11.9
R^2 (%)					49.0	54.6	45.3	65.4	66.3

[a] ns, *, **: not significant, significant at the 0.05 and 0.01 probability level, respectively

accounted for the largest portion of phenotypic variance ($R^2 = 32.1\%$; Table 2). The other QTL on chr. 2 and the QTL on chr. 1 also accounted for a substantial portion of phenotypic variance ($R^2 = 22.7$ and 22.9%, respectively). The multiple regression model fitting the four putative QTLs simultaneously accounted for 66.3% of the phenotypic variance and 76.2% of the genetic variance. Interestingly, the sum of the estimated additive effects for the mean values amounted to 147 ng ABA g^{-1} d.w., a value quite close to that expected (158 ng ABA g^{-1} d.w.) based on the difference between parent lines (416 ng ABA g^{-1} d.w.) and the percentage of genetic variance explained by the model. At these QTLs, gene action was partially dominant in two cases, dominant in one case, and overdominant in one case. The direction of dominance was prevailingly negative.

'*QTL × sampling*' *interaction*

In order to more accurately evaluate the consistency of the effects of the four QTLs declared significant across samplings, the analysis of the 'QTL × sampling' interaction was performed disregarding all remaining QTLs. The ANOVA detected the presence of a significant ($P < 0.01$) 'QTL × sampling' interaction (data not reported). A more detailed analysis of the genetic effects at each individual sampling (Table 3) revealed that the interaction was caused ($P < 0.01$) by the additive components of the QTL on chr. 1 and the QTL on chr. 2 near *csu133*. In both cases, the interaction was determined by fluctuations in the magnitude of the effects. In all 'QTL × sampling' combinations, the allele for high L-ABA was contributed by Os420, the parent line characterized by high L-ABA. A significant ($P < 0.05$) 'QTL × sampling' interaction was also detected for the dominance component at the QTL on chr. 2 near *csu133*. In this case, the interaction was caused by a change in direction of the dominance component for S3/95. Changes in the direction of the dominance effects were also observed for the other QTL on chr. 2 and the QTL on chr. 4; however, in these cases, the corresponding interactions were not significant. In the individual samplings, the R^2 values of the multiple regression fitting the four QTLs varied from 45.3 (S3/95) to 65.4% (S4/95).

Discussion

Genetic map

The high level of polymorphism (73% of RFLP probes) detected between parent lines is most likely due to their rather different origins. Two probes (csu17 and umc193) detected duplicated loci which, to our knowledge, have not been previously reported. In each case, the map position of the duplicated loci is in keeping with the information available on duplications in the maize genome (Helentjaris 1995). The two loci evidenced with csu17 were mapped on chr. 2 (*csu17b*, stronger signal) and on chr. 7 (*csu17c*, weaker signal). As expected from the information retrieved from the maize genome data base (http://www.agron.missouri.edu), a rather complex profile was revealed by umc193. Although the strongest bands were not polymorphic, we were able to map two weaker polymorphisms. One marker locus was assigned to chr. 4, where *umc193d* had been previously mapped, and one marker locus (*umc193e*) was assigned to chr. 2.

According to the position of the RFLP markers herein scored on the UMC map (Davis et al. 1996), we estimate that approximately 85% of the maize genome was surveyed in our study. After excluding the five unlinked markers, the percent difference between the length of our map and the UMC map showed sizeable fluctuations among chromosomes (from +40.2% of chr. 3 to −34.8% of chr. 5). Notably, the two most important QTLs in terms of additive effects and R^2 values were identified on chr. 2 which is 24.4% shorter than chr. 2 in the UMC map. A lower rate of

recombination implies a higher level of linkage disequilibrium which, in turn, improves the probability of detecting the presence of QTLs and allows for a more accurate estimate of their effects.

Interpretation of QTL analysis

The absolute values and the relative differences in L-ABA reported in this study are within the range of those reported in previous experiments evaluating field-grown maize undergoing water stress (Pekic and Quarrie 1988; Ivanovic et al. 1992; Conti et al. 1994; Tuberosa et al. 1994; Landi et al. 1995), thus indicating that the intensity of drought stress was adequate for our objectives. In accordance with a previous study investigating L-ABA in a set of maize inbred lines (Conti et al. 1994), heritability was higher at stage 4 in both years, thus confirming that variation in L-ABA is more tightly regulated by genetic effects near anthesis, regardless of the level of expression of the trait.

Among the QTLs revealed in the individual samplings, only a small fraction (4 out of 16) was also revealed across samplings. This result could be related to one or more of the following reasons: (1) different genetic factors influenced L-ABA at the four samplings; (2) differences in the dynamic (e.g. severity and timing) of the water-stress episodes before each sampling; or (3) the number of families herein evaluated was not sufficiently large to identify, at each individual sampling, the QTLs revealed across samplings. As to the last reason, simulation work carried out by Beavis (1994) revealed that the size of the populations commonly considered in QTL studies is often inadequate for a consistent identification of QTLs not only across environments but also in a single environment. The effectiveness of detecting QTLs using the values of single environments and their mean has been evaluated in a number of studies. Jansen et al. (1995) concluded that the chances of detecting a QTL in several environments is small even if no 'QTL × environment' interaction is present. Other authors have reported the inconsistency of QTL detection across environments (Paterson et al. 1991; Bubeck et al. 1993; Tinker et al. 1996; Mather et al. 1997).

The partial dispersion of the plus and minus alleles for L-ABA in the parent lines is in contrast with the lack of transgressive values of the $F_{3:4}$ families. The high level of negative dominance accounts, at least in part, for the lower than expected frequency of transgressive families at high L-ABA values. Furthermore, the size of the population was not large enough to include all possible genotypes combining a sufficiently high number of plus or minus alleles at the QTLs for L-ABA. The high level of negative dominance and overdominance could be the result of the greater vigour of families with a higher level of heterozygosity which, in turn, may have favoured a more positive water balance. Additionally, the pronounced overdominance could also be attributed to pseudo-overdominance of tightly linked loci in repulsion phase (Edwards et al. 1987). A recent paper by Graham et al. (1997) reported the results of a fine-mapping experiment and demonstrated that an overdominant QTL consisted of at least two QTLs each showing dominance in repulsion-phase linkage. In one case (QTL on chr. 1 near *umc11*), the LOD profiles (Fig. 2) and the corresponding profiles of the effects due to additivity and dominance (data not reported) suggest the presence of two closely linked QTLs in repulsion phase. The values of *d* should, however, be considered with great caution, since they were estimated with an expected low average number of 20 $F_{3:4}$ heterozygous families. Furthermore, when QTL effects are estimated in the same experiment in which they are detected, it is likely that the estimates are upwardly biased (Lande and Thompson 1990). Recent work in maize has also shown that a substantial portion of QTLs for yield and other agronomic traits were characterized by dominance and overdominance gene action (Veldboom and Lee 1996 a, b).

Adopting the terminology of Tinker et al. (1996), the four regions influencing L-ABA across samplings will be referred to as primary QTLs. Notably, based on Wright's formula, it was estimated that four genes controlled L-ABA in Os420 × IABO78 evaluated in a previous trial conducted at the same location and at the growth stages considered herein (Sanguineti et al. 1996). One of the assumptions underlying the application of Wright's formula is that the plus and minus alleles for the investigated trait are not dispersed in the parent lines. Indeed, the alleles increasing L-ABA at the four primary QTLs were all contributed by Os420. The direction of the additive effects of these QTLs in the individual samplings was fairly consistent, a result in keeping with the definition of primary QTLs (Tinker et al. 1996). Similar results have also been shown for QTLs influencing agronomic traits in maize grown in different environments (Stuber et al. 1992; Schön et al. 1994; Bohn et al. 1996; Veldboom and Lee 1996 a, b). QTL detection on the basis of means across environments and/or samplings is more reliable due to the decrease in the standard error of the values which, in turn, should increase the chances of detecting QTLs significantly affecting the investigated trait (Tinker et al. 1996; Veldboom and Lee 1996 b).

Based on the percentage of genotypic variance explained by the four primary QTLs and the genome coverage of our map, the presence of other major QTLs for L-ABA seems unlikely, unless a sizeable portion of the effects associated with the four QTLs is due to epistatic interactions and/or to non-random assortment between the pairs of marker alleles flanking each QTL region. The size of our population does not allow for a meaningful test for the presence of epistasis; however, the analysis of the frequency of the genotypic classes for the six pair-wise combinations between the

markers closest to the four primary QTLs evidenced a noticeable, but not significant, imbalance only in one case (a combination between the QTL on chr. 1 and the QTL on chr. 6; data not reported).

The strongest additive effects on L-ABA were contributed by the two QTLs on chr. 2. It is worth mentioning that the QTL near *csu133* maps almost exactly where a QTL for L-ABA was reported by Lebreton et al. (1995) in a different maize cross. The same authors also showed a significant effect of this chromosomal region on root-pulling strength, with a positive association between this trait and L-ABA. This region has also shown an important effect on plant height and grain yield in tropical maize (Bohn et al. 1996). Recent work reviewing a vast set of maize data indicates that at least seven QTLs and five naked-eye polymorphisms for developmental traits map in the chromosomal bin which includes *csu133* (Khavkin and Coe 1997). The QTL near *csu133* showed a more pronounced effect at stage 4 in both years, although in 1994 L-ABA was considerably higher (+60%) at stage 3. Therefore, the action of this QTL does not seem to be influenced to a great extent by water stress, assuming that L-ABA represents, in our study, a general indicator of the level of water stress experienced by the plant. Indeed, the hypothesis for this role of L-ABA is supported by the significant ($P < 0.05$) and positive correlations of L-ABA with a visually scored drought-stress index and leaf temperature and by the negative associations of L-ABA with stomatal conductance and grain yield (data not reported; manuscript in preparation). In order to more accurately assess the effects of the QTL region near *csu133* on L-ABA and to ascertain its effects on physiological and agronomic traits, we are developing F_7 families homozygous for the IABO78 alleles and F_7 families homozygous for the Os420 alleles at this chromosome region.

Several factors could account for the pronounced effect of the growth stage on the expression of the QTL near *csu133*. Among such factors, plant perception of water availability may change as the root system develops and water distribution in the soil profile varies accordingly. In a recent study in rice, profound developmental effects were reported for QTLs influencing root characteristics (Price and Tomos 1997). Additionally, the pool of ABA in the leaf at the two growth stages considered herein may have been influenced to a varying extent by different ABA sources, such as the ABA produced by the roots and transported to the leaf in the xylem sap (Tardieu et al. 1992). However, the QTLs controlling the concentration of ABA in the leaf and in the xylem sap have shown a poor overlap in drought-stressed maize (Lebreton et al. 1995).

The comparative analysis of our results with those of Lebreton et al. (1995) indicated that, besides the QTL on chr. 2 near *csu133*, a region on chr. 3 near *csu36c* and a region on chr. 1 between *umc67* and *umc128* also influenced L-ABA in both populations.

Interpreting the nature of the QTLs for L-ABA

Although Robertson's hypothesis has been validated by a number of QTL studies on different traits (Beavis et al. 1991; Doebley et al. 1995; Lin et al. 1995), the support intervals of the four primary QTLs for L-ABA evidenced in our work did not overlap with the regions containing the structural genes presently known to encode for the enzymes involved in ABA biosynthesis, particularly *VP14*, the key rate-limiting step for ABA biosynthesis (Schwartz et al. 1997). Presently, it is not possible to elucidate the mode of action at the physiological level of the QTLs controlling L-ABA. The QTLs detected in our study could represent genes influencing ABA biosynthesis through the regulation of the intensity of the transduction signal associated with turgor loss, a major determinant in the increase of ABA concentration, and/or genes controlling morphological traits (e.g. root size and morphology, leaf area, leaf angle, etc.) affecting the water balance of the plant. In maize seedlings, ABA has been shown to influence root growth under artificially induced conditions of water deficit (Saab et al. 1990). A manuscript analyzing the overlap between the QTLs for L-ABA and the QTLs for morpho-physiological traits involved in the response to drought stress is in preparation.

Conclusions

Our study confirms the quantitative inheritance of L-ABA in maize and does not provide evidence that the loci for qualitative mutants for ABA biosynthesis underscore the QTLs for L-ABA. The complex control of L-ABA is revealed by the 16 QTLs which were identified at two vegetative growth stages during 2 years. A greater number of QTLs and higher heritability values were observed in the samplings carried out near anthesis. Across samplings, only four primary QTLs were revealed, a number which is in accordance with that estimated in a previous study. At these QTLs the direction of the parent alleles was consistent when analyzed in each individual sampling. The QTL on chr. 2 near *csu133* appears to be under a pronounced developmental control with a stronger effect, both as shown by R^2 and additive values, just prior to anthesis. Because the region near *csu133* has also been shown to control L-ABA as well as root pulling strength in another maize cross and grain yield in tropical maize, we are developing isolines to better characterize the effects associated with genetic variation in L-ABA at this QTL region.

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