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Root-ABA1 QTL affects root lodging, grain yield, and other agronomic traits in maize grown under well-watered and water-stressed conditions

P. Landi[1],*, M. C. Sanguineti[1], C. Liu[2], Y. Li[3], T. Y. Wang[3], S. Giuliani[1], M. Bellotti[1], S. Salvi[1] and R. Tuberosa[1]

[1] *Department of Agroenvironmental Science and Technology (DISTA), University of Bologna, Viale Fanin 44, I-40127 Bologna, Italy*

[2] *Institute of Food Crops, Xinjiang Academy of Agricultural Sciences, Urumqi 830000, China*

[3] *Institute of Crop Science, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Beijing 100081, China*

Abstract

A major QTL affecting root traits and leaf ABA concentration was identified in maize (*Zea mays* L.) and named *root-ABA1*. For this QTL, back-cross-derived lines (BDLs) homozygous either for the (+) or for the (−) allele increasing or decreasing, respectively, root size and leaf ABA concentration, were developed. This study was conducted to evaluate the QTL effects in various genetic backgrounds and at different water regimes. The (+/+) and (−/−) BDLs were crossed with five or 13 inbred tester lines of different origin, thus producing two sets of test-crosses that were evaluated in Italy and China, respectively. Testing was conducted under both well-watered and water-stressed conditions. In Italy, the test-crosses derived from (+/+) BDLs, as compared with those derived from (−/−) BDLs, showed, across both water regimes, higher leaf ABA concentration (on average 384 versus 351 ng g^{-1} DW) and lower root lodging (28.0 versus 52.5%), and lower grain yield under water-stressed conditions (4.88 versus 6.27 Mg ha^{-1}). In China, where root lodging did not occur, the test-crosses derived from (+/+) BDLs were less productive at both water regimes (on average, 6.83 versus 7.49 Mg ha^{-1}). The lower grain yield of the test-crosses derived from (+/+) BDLs was due to a lower number of ears per plant and to lower kernel weight. The results indicate that the (+) *root-ABA1* allele confers not only a consistently lower susceptibility to root lodging but also a lower grain yield, especially when root lodging does not occur.

Key words: Abscisic acid, back-cross-derived lines, QTL, root lodging, *Zea mays*.

Introduction

The application of QTL (quantitative trait locus) analysis and other genomics approaches provides new opportunities with which to identify the chromosome regions controlling variation in the adaptive response to water stress and, eventually, to clone the sequence/s responsible for such variation. In addition, the availability of near-isogenic lines (NILs) for a specific QTL allows for a more in-depth and accurate characterization of the QTL effects which, in turn, facilitates the elaboration of models and hypotheses on the causal relationships of the QTL effects on different traits (Tuberosa and Salvi, 2006).

Among the quantitative traits affecting the adaptive response of crops to drought, the concentration of abscisic

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acid (ABA) has received particular attention in view of the pivotal role played by ABA in modulating important molecular and morpho-physiological processes at the cellular, organ and whole plant levels (Quarrie, 1991; Pekic *et al.*, 1995; Tuberosa *et al.*, 2002; Sharp *et al.*, 2004), including also reproductive fertility (Saini and Westgate, 2000; Wang *et al.*, 2002; Boyer and Westgate, 2004). Previous QTL studies in maize (*Zea mays* L.) indicated that the concentration of ABA in the leaf (L-ABA) is controlled by several QTLs (Lebreton *et al.*, 1995; Prioul *et al.*, 1997; Tuberosa *et al.*, 1998; Pelleschi *et al.*, 2006). In particular, Tuberosa *et al.* (1998) detected 16 QTLs for L-ABA by analysing 80 $F_{3:4}$ families derived from the cross between Os420, the high (+) L-ABA parental line, and IABO78, the low (−) L-ABA parental line. The most important QTL, identified near the RFLP marker *csu133* on chromosome 2 (bin 2.04), accounted for 32% of the phenotypic variation for L-ABA. The important role of this QTL was also confirmed by divergent selection for L-ABA conducted on the F_2 population derived from Os420×IABO78 (Landi *et al.*, 2001). In order to gain a better understanding of the effects of the QTL in question on L-ABA and other drought-related traits, sets of NILs at this QTL were developed from Os420×IABO78, following a marker-assisted back-cross procedure. Therefore, these NILs are hereafter indicated as back-cross-derived lines (BDLs). Two pairs of BDLs were obtained for each parental inbred: Os420.1 (+/+) and (−/−), Os420.2 (+/+) and (−/−), IABO78.1 (+/+) and (−/−), and IABO78.2 (+/+) and (−/−). These BDLs were field tested in well-watered (WW) and water-stressed (WS) trials, both *per se* (Landi *et al.*, 2005) and in hybrid combination, obtained as factorial crosses among the four Os420 BDLs and the four IABO78 BDLs (Giuliani *et al.*, 2005). Such evaluations validated the effect of the QTL on L-ABA and revealed a significant effect of the QTL on root traits. Based upon such results, a tentative model accounting for the effects of the QTL on L-ABA and other traits was suggested (Giuliani *et al.*, 2005; Landi *et al.*, 2005). This model postulates that the primary action of this major QTL is on root architecture and size, which, in turn, affect L-ABA and other traits according to water availability. For this reason, the QTL has been named *root-ABA1* (Giuliani *et al.*, 2005).

A major limitation for the applications of QTL studies is the lack of consistency of QTL effects across environments and/or different genetic backgrounds because of epistatic interaction. This is particularly relevant when evaluating QTL effects on agronomic traits (especially grain yield) largely affected by many loci as well as by several environmental factors. An accurate evaluation of the effects of a QTL is also beneficial before embarking upon its cloning, a resource-demanding undertaking which can be facilitated when QTL effects show limited interactions with the environment (Salvi and Tuberosa, 2005). In addition, a QTL with consistent effects across genetic backgrounds has a greater breeding value, particularly when the agronomically favourable allele is not present in the elite germplasm. In view of these considerations, the present study was undertaken in order to evaluate the effects of *root-ABA1* on L-ABA and agronomic traits in various genetic backgrounds and at different water regimes.

Materials and methods

The genetic backgrounds in which the QTL effects were evaluated were represented by two different sets of test-crosses (TCs), obtained by crossing pairs of the above-cited (+/+) and (−/−) BDLs with several inbred line testers of different origin. One set of TCs was tested in Italy and the other was tested in China.

Test-crosses evaluated in Italy

The two pairs of BDLs, Os420.1 (+/+) and (−/−) and IABO78.2 (+/+) and (−/−), were crossed, according to the factorial scheme, with five tester lines: A632, B98, B99, B103, and K55. These testers were chosen because they are well known to maize geneticists across the world and represent different genetic backgrounds of the US Corn Belt germplasm (A632 is related to Iowa Stiff Stalk Synthetic; B98 to Pioneer Two-Ear Composite; B99 to Iowa Corn Borer Synthetic; B103 to Pool 41 of CIMMYT; K55 to Pride of Saline).

The 20 TCs were evaluated in 2003 at Cadriano (11° 24′ E, 44° 33′ N; Po Valley, northern Italy) in two trials conducted in the same field. These two trials were separated by three border rows on each side and by a 4-m-wide alley, and differed only in the irrigation volumes, which corresponded to either *c.* 120% (well-watered, WW) or to *c.* 40% (water-stressed, WS) of the evapotranspiration after accounting for rainfall. For each trial, the field layout was a randomized complete block design with three replications. Each plot included a single 3.65-m-long row and was separated from adjacent rows by 0.80 m. Trials were sown on 5 May 2003, and 5 weeks later plots were thinned to 16 plants per plot, corresponding to 5.5 plants m^{-2}. Fertilizer rates were 200 kg ha^{-1} of N (half applied before sowing and half after thinning) and 45 kg ha^{-1} of P (applied before sowing); K was not applied because of its high availability in the soil. Weeds were controlled by hand and mechanically. Plots were irrigated from the mid–end of stem elongation to the end of silking (i.e. from the V14–V15 to the R1 stage, according to Ritchie *et al.*, 1997). On the whole, the irrigation volumes corresponded to 90 mm and 30 mm of water for the WW and WS trials, respectively. Total rainfall from sowing to harvest was 177 mm. The two trials were hand-harvested on 3 September 2003. Ears were air-dried and shelled after reaching constant moisture.

The following 13 traits were measured: (i) root lodging, following a heavy windstorm occurred at mid-stem elongation (V9–V10 stage) (plants were counted as root lodged when leaning >30° from the vertical); (ii) L-ABA, measured at pollen shedding (VT) on the third leaf from the top, following the procedure described in Tuberosa *et al.* (1998) (due to sampling problems, the four TCs of the B98 tester were not measured); (iii) leaf relative water content (RWC), measured at pollen shedding on the third leaf from the top following the procedure described in Giuliani *et al.* (2005); (iv) pollen shedding date, assessed when 50% of the plants had extruded anthers; (v) silking date, assessed when 50% of the plants had extruded silks; (vi) anthesis-silking interval (ASI), as the difference between silking and pollen shedding dates; (vii) plant height, measured at the flag leaf collar; (viii) number of ears per plant; (ix) grain yield, adjusted to 15.5% moisture; (x) kernel weight, adjusted to 15.5% moisture (as a mean of 200 kernels); (xi) number of kernels per ear, calculated as the ratio between grain yield per plant and the product between number of ears per plant and kernel weight; (xii) weight of the above-ground biomass, including ears (plants were cut at the soil level, then

were chopped, dried and weighed); and (xiii) harvest index, as ratio between traits (ix) and (xii) adjusted to the same moisture level. Traits were measured on the central plants of each plot; in particular, 10 plants were sampled for root lodging and flowering traits, seven for grain yield and its components, and five for RWC and weight of the above-ground biomass. Silking date and weight of the above-ground plants are not presented because they were used to calculate ASI and harvest index, respectively.

The analysis of variance (ANOVA) was first conducted on plot mean values separately for each trial; subsequently, a combined analysis across the two trials was performed. Prior to ANOVA, the root lodging data were subjected to angular transformation. The partitioning of the whole variation into its main sources and the corresponding degrees of freedom (df) are summarized for both trials in Table 1. The whole variation was first partitioned into 'between trials' (i.e. WW versus WS; 1 df), 'among TCs' (19 df), and '(WW versus WS)×TCs interaction' (19 df). The 'among TCs' component was then partitioned into 'among testers' (4 df), 'among BDLs' (3 df), and 'testers×BDLs interaction' (12 df). The 'among testers' and the 'among BDLs' indicate effects of general combining ability (gca) of the two groups of lines, while the 'testers×BDLs' interaction indicates the effects of specific combining ability (sca). The gca effect of a line is related to its mean performance across its TCs and is mainly due to additive gene action. The sca effect of a TC is related to the deviation between its observed and expected performance based on the gca effects of the two parental lines; this effect is due to non-additive gene actions (e.g. dominance). The 'among BDLs' was partitioned into 'between families of BDLs', i.e. Os420.1 versus IABO78.2 (1 df), '(+/+) versus (−/−)' (1 df), and 'families×[(+/+) versus (−/−)] interaction' (1 df). The interaction 'testers×BDLs' was partitioned analogously to the BDLs; the interaction '(WW versus WS)×TCs' was partitioned similarly to the TCs. For the *F*-test, a fixed model was used having considered as fixed both irrigation levels and lines.

Test-crosses evaluated in China

Only the two BDLs of the IABO78.2 family were considered in this experiment. The (+/+) and (−/−) BDLs were crossed to the following 13 inbred line testers: Cai11-8, Dan340, Dan3301, Fu123, HuangC, L042, Lo1142, Mo17, Moqun17, Nongda178, Qi318, Xin10-513, and Zongxi241. While Lo1142 and Mo17 were selected in Italy and in the USA, respectively, and are both related to the Lancaster

breeding group, the other 11 inbred lines were selected in China from different sources and are representative of the germplasm used locally for breeding purposes. In particular, testers Dan340 and Dan3301 belong to the LDHG breeding group, Cai11-8 and Zongxi241 to the Zi330 group, HuangC to the Reid group, Moqun17 to the Lancaster group, Nongda178 and Qi318 to the P group, while Fu123, L042 and Xin10-513 have unknown pedigrees. The 26 TCs were evaluated in 2004, in two trials conducted in the same field, at the experimental farm of Xinjiang Academy of Agricultural Sciences in Urumqi (87°36′ E, 43°48′ N; north-western China). Analogously to the investigation conducted in Italy, the two trials were separated by border rows and differed only for the irrigation volumes, i.e. well-watered (WW) and water-stressed (WS). WW and WS corresponded to *c*. 120% or to *c*. 30%, respectively, of the evapotranspiration after accounting for rainfall. In the WW trial, plots were irrigated from the beginning of stem elongation (V7–V8) to the blister-milk stage of kernel development (R2–R3), attaining a total volume of *c*. 100 mm of water; in the WS trial, plots were irrigated from V7–V8 to the beginning of pollen shed (VT) with *c*. 25 mm of water. Total rainfall from sowing to harvest was 171 mm. For both trials, the field layout was a randomized complete block design with three replications. Plots were 4.25-m-long single rows separated by 0.70 m from adjacent rows. The trials were sown on 28 April 2004 and then thinned to 17 plants per plot (5.7 plants m^{-2}). The local field practices were adopted; in particular, fertilizer rates were 296 kg ha^{-1} of N (half applied before sowing and half after thinning) and 64 kg ha^{-1} of P (applied before sowing). Weeds were controlled by hand and mechanically. Trials were hand-harvested on 3 September 2004.

The following ten traits were investigated: (i) pollen shedding date; (ii) silking date; (iii) anthesis-silking interval (ASI); (iv) plant height; (v) number of ears per plant; (vi) grain yield; (vii) kernel weight; (viii) number of kernels per ear; (ix) weight of the above-ground biomass; and (x) harvest index. The procedures followed to measure these traits were similar to those described for the trials conducted in Italy, except that plant height was measured from the soil surface to the top of the tassel.

The statistical analysis was carried out following the procedure previously described (Table 1). In particular, the TC degrees of freedom (25 df) were partitioned into 'among testers' (12 df), 'between IABO78.2 BDLs', i.e. (+/+) versus (−/−) (1 df), and 'testers×[(+/+) versus (−/−)] interaction' (12 df); the '(WW versus WS)×TCs interaction' (25 df) was partitioned as the TCs. The model used for the *F*-test was fixed.

Results

Test-crosses evaluated in Italy

The ANOVA indicated that the difference between mean values of the WW and WS trials was significant ($P \leqslant 0.05$) for RWC, ASI, number of ears per plant, number of kernels per ear, kernel weight, and harvest index, and was highly significant ($P \leqslant 0.01$) for L-ABA and grain yield (Table 2). As expected, the WS condition, in comparison with the WW, reduced all the previously cited traits, with the exception of L-ABA and ASI, which showed an increase. The most notable changes were observed for L-ABA (319 versus 416 ng g^{-1} DW, corresponding to an increase of 30.4%) and for grain yield (9.62 versus 5.57 Mg ha^{-1}, i.e. −42.1%); this latter change was due to a reduction in all yield components (−23.1% for number of ears per plant, −18.0% for number of kernels per ear, and −8.1% for kernel weight). The difference between WW and WS trials

Table 1. *Main sources of variation and degrees of freedom in the combined ANOVA of the trials conducted in Italy and in China*

Main sources of variation	Degrees of freedom			
	Italy		China	
WW versus WS[a]	1		1	
Test-crosses (TCs)	19		25	
Inbred line testers		4		12
Back-cross derived lines (BDLs)	3		—	
Families[b]		1		—
(+/+) versus (−/−)		1		1
Families×[(+/+) versus (−/−)]		1		—
Testers×BDLs	12		—	
Testers×families		4		—
Testers×[(+/+) versus (−/−)]		4		12
Testers×[families×(+/+) versus (−/−)]		4		—
(WW versus WS)×TCs	19		25	

[a] WW and WS, well-watered and water-stressed trials, respectively.
[b] Families of BDLs, i.e. Os420.1 and IABO78.2.

Table 2. *Test-crosses evaluated in Italy: mean values of the well-watered (WW) and water-stressed (WS) trials across 20 TCs*

Trait	WW	WS	[a]
Root lodging (%)[b]	37.0	43.5	(†)
L-ABA (ng g^{-1} DW)[c]	319	416	**
RWC (%)	91.8	90.2	*
Pollen shedding (d)[d]	4.2	4.6	ns
ASI (d)	4.1	5.0	*
Plant height (cm)	165	167	ns
Grain yield (Mg ha^{-1})	9.62	5.57	**
Ears/plant (no.)	1.08	0.83	*
Kernels/ear (no.)	545	447	*
Kernel weight (mg)	297	273	*
Harvest index (%)	46.0	37.3	*

[a] Comparison between WW and WS trials: (†), meaningless comparison because root lodging occurred before starting the irrigation treatments; * and **, significant at $P \leqslant 0.05$ and $P \leqslant 0.01$, respectively; ns, not significant.
[b] Mean values refer to untransformed data.
[c] Mean values across 16 TCs (as the TCs with B98 tester were not included).
[d] The values given indicate the dates of pollen shedding in July with 1 refers to 1 July.

for root lodging cannot be related to the irrigation treatments because lodging occurred prior to the starting of irrigation; most likely, such a difference could be related to the fact that the WS trial was more exposed to wind action.

The (WW versus WS)×TCs interaction and its components were not significant for any trait, except the component (WW versus WS)×[(+/+) versus (−/−)], which was significant for grain yield, number of ears per plant, and number of kernels per ear.

The differences among TCs across trials were significant for all traits except RWC and were due to gca effects of testers and/or of BDLs. Table 3 shows the mean values of the five testers in combination with the (+/+) and (−/−) BDLs across the two families and the water regimes. For all traits, except harvest index, the relative mean performance of the testers did not significantly vary from the (+/+) to the (−/−) BDL groups, as also indicated by the lack of significance of the interaction testers×BDLs. These findings thus reveal that sca effects were not important. For this reason, the whole information provided by the 20 TCs is hereafter presented and discussed mainly in terms of mean performance of their parental lines, i.e. testers and BDLs.

When averaged across (+/+) and (−/−) BDLs (data not shown), the five testers significantly differed for root lodging, L-ABA, pollen shedding date, ASI, grain yield, number of ears per plant, number of kernels per ear, and harvest index. As to the best performing testers, the lowest mean value across BDLs for root lodging was shown by K55 (25.9%, as compared with an overall mean of 40.3%), while the highest mean value for grain yield was shown by B99 (8.25 Mg ha^{-1} versus an overall mean of 7.60 Mg ha^{-1}); for L-ABA, the highest and the lowest mean values were shown by A632 and K55, respectively.

Table 3. *Test-crosses evaluated in Italy: mean values of the inbred line testers in combination with the (+/+) and (−/−) BDLs across two families (Os420.1 and IABO78.2) and well-watered and water-stressed trials*

Trait	BDLs	A632	B98	B99	B103	K55	[a]
Root lodging (%)[b]	(+/+)	24.4	38.6	25.3	35.8	15.9	
	(−/−)	50.1	52.3	60.7	63.6	35.8	ns
L-ABA (ng g^{-1} DW)	(+/+)	427	–	389	380	342	
	(−/−)	375	–	374	349	307	ns
RWC (%)	(+/+)	90.8	90.9	90.6	91.9	91.1	
	(−/−)	90.9	89.0	89.9	92.4	92.3	ns
Pollen shedding (d)[c]	(+/+)	3.5	4.4	4.8	5.1	7.6	
	(−/−)	1.9	4.3	3.8	4.0	4.8	ns
ASI (d)	(+/+)	3.9	5.5	4.8	4.1	4.5	
	(−/−)	3.1	5.8	4.9	4.9	4.4	ns
Plant height (cm)	(+/+)	166	162	163	163	156	
	(−/−)	178	167	170	167	170	ns
Grain yield (Mg ha^{-1})	(+/+)	7.72	7.08	8.19	6.72	6.50	
	(−/−)	7.98	7.44	8.30	8.00	8.04	ns
Ears per plant (no.)	(+/+)	0.93	0.91	0.98	0.90	0.92	
	(−/−)	0.96	0.90	1.03	1.01	1.01	ns
Kernels per ear (no.)	(+/+)	547	541	556	478	440	
	(−/−)	513	472	516	459	489	ns
Kernel weight (mg)	(+/+)	271	273	268	282	284	
	(−/−)	290	301	281	311	292	ns
Harvest index (%)	(+/+)	47.3	39.9	39.0	41.4	37.1	
	(−/−)	46.1	38.8	41.4	40.6	45.1	*

[a] Significance level of the interaction testers×[(+/+) versus (−/−)]: *, significant at $P \leqslant 0.05$; ns, not significant.
[b] Mean values refer to untransformed data.
[c] The values given indicate the dates of pollen shedding in July with 1 refers to 1 July.

The differences found among the four BDLs for most of the traits investigated were due to significant differences between BDL families (i.e. Os420.1 versus IABO78.2), between (+/+) and (−/−) BDLs, while the families×[(+/+) versus (−/−)] interaction was not significant for any trait. This latter finding indicates that the QTL effects did not change significantly from one family to the other. For this reason, only the mean values of the (+/+) and of the (−/−) BDLs across the two families are presented in the WW and the WS trials (Table 4). Differences between (+/+) and (−/−) BDLs were larger in the WW trial for root lodging and kernel weight and, in the WS trial, for L-ABA, pollen shedding, plant height, grain yield, number of ears per plant, and harvest index. As for grain yield, in particular, the difference between the two mean values was negligible in the WW trial while it was highly significant in the WS trial, consistent with the significant interaction (WW versus WS)×[(+/+) versus (−/−)] reported previously. In the WS trial the (+/+) BDLs showed much lower grain yield than the (−/−) BDLs (4.88 versus 6.27 Mg ha^{-1}, i.e. −22.2%); this finding was attributable to a decrease in both number of ears per plant (−15.6%) and kernel weight (−5.7%).

Table 4. *Test-crosses evaluated in Italy: mean values of the (+/+) and (−/−) BDLs across two families (Os420.1 and IABO78.2) and five inbred line testers in the well-watered (WW) and water-stressed (WS) trials and in the mean*

Trait	BDLs	WW	[a]	WS	[a]	Mean	[a]
Root lodging (%)[b]	(+/+)	23.3		32.7		28.0	
	(−/−)	50.7	*	54.3	ns	52.5	**
L-ABA (ng g^{-1} DW)[c]	(+/+)	327		442		384	
	(−/−)	312	ns	391	**	351	**
RWC (%)	(+/+)	91.6		90.5		91.1	
	(−/−)	91.9	ns	89.9	ns	90.9	ns
Pollen shedding (d)[d]	(+/+)	4.8		5.4		5.1	
	(−/−)	3.7	*	3.8	*	3.7	**
ASI (d)	(+/+)	3.9		5.2		4.6	
	(−/−)	4.4	ns	4.9	ns	4.6	ns
Plant height (cm)	(+/+)	162		162		162	
	(−/−)	168	ns	172	*	170	**
Grain yield (Mg ha^{-1})	(+/+)	9.61		4.88		7.24	
	(−/−)	9.64	ns	6.27	**	7.95	**
Ears per plant (no.)	(+/+)	1.09		0.76		0.93	
	(−/−)	1.06	ns	0.90	*	0.98	ns
Kernels per ear (no.)	(+/+)	557		454		512	
	(−/−)	525	ns	448	ns	490	ns
Kernel weight (mg)	(+/+)	287		265		276	
	(−/−)	308	**	281	*	295	**
Harvest index (%)	(+/+)	46.6		35.3		40.9	
	(−/−)	45.4	ns	39.4	ns	42.4	*

[a] Comparison between (+/+) and (−/−) mean values: * and **, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; ns, not significant.
[b] Mean values refer to untransformed data.
[c] Mean values across two families and four inbred line testers (A632, B99, B103, and K55).
[d] The values given indicate the dates of pollen shedding in July with 1 refers to 1 July.

When data were averaged across WW and WS trials, the (+/+) BDLs showed a much lower mean value than the (−/−) BDLs for root lodging (28.0% versus 52.5%) and such a comparison accounted for 38.1% of the variation detected among the 20 TCs. Moreover, the mean value of (+/+) BDLs was higher for L-ABA (according to expectation) and pollen shedding date, and lower for plant height, grain yield, kernel weight, and harvest index.

Test-crosses evaluated in China

The difference between mean values of WW and WS trials was significant for pollen shedding date and highly significant for all other traits (Table 5). The WS trial showed, in comparison with the WW trial, a higher mean value for pollen shedding date and ASI, and a lower mean value for all other traits. The reduction observed in the WS trial was rather sizeable for grain yield (10.55 versus 3.75 Mg ha^{-1}, corresponding to −64.4%) and its component number of ears per plant (−35.5%), number of kernels per ear (−20.0%), and kernel weight (−31.0%).

The (WW versus WS)×TCs interaction, although significant for all traits, was mainly due to the size of relative

Table 5. *Test-crosses evaluated in China: mean values of the well-watered (WW) and water-stressed (WS) trials across 26 TCs*

Trait	WW	WS	[a]
Pollen shedding (d)[b]	21.8	27.3	*
ASI (d)	3.7	5.6	**
Plant height (cm)	279	203	**
Grain yield (Mg ha^{-1})	10.55	3.75	**
Ears per plant (no.)	1.21	0.78	**
Kernels per ear (no.)	456	365	**
Kernel weight (mg)	335	231	**
Harvest index (%)	64.3	36.3	**

[a] Comparison between WW and WS trials: * and **, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; ns, not significant.
[b] The values given indicate the dates of pollen shedding in July with 1 refers to 1 July.

effects and not to changes in ranking of the TCs. In fact, differences among TCs were larger in the WW trial for grain yield and its components, while differences for ASI were larger in the WS trial. The comparison among TCs across WW and WS trials was significant for all traits and due to gca effects of both testers and BDLs. As for the results provided by the testers (not presented), the highest mean performance for grain yield across BDLs was shown by Moqun17 (8.53 Mg ha^{-1}), while Fu123 showed the lowest value (5.29 Mg ha^{-1}).

Table 6 shows the mean values of the two BDLs in the two water regimes and across the 13 testers. The BDLs differed significantly for grain yield, number of ears per plant and kernel weight at both WW and WS conditions, and for plant height only in WW conditions. The comparison between the mean values of the two BDLs across the water regimes was significant for grain yield and number of ears per plant ($P \leq 0.01$) and for kernel weight ($P \leq 0.05$). In particular, the (+/+) BDL TCs were less productive than the (−/−) BDL TCs (6.83 versus 7.49 Mg ha^{-1}, corresponding to −8.8%) due to a lower number of ears per plant (−8.7%) and kernel weight (−1.5%).

The significance of the differences among TCs was also due to sca effects, i.e. to the testers×[(+/+) versus (−/−)] interaction, for plant height, grain yield and its component number of ears per plant (data not shown). As for grain yield, the performance of TCs obtained with the (+/+) BDL was significantly higher than the performance of the TCs obtained with the (−/−) BDL only in two cases (i.e. in combination with the testers L042 and Zongxi241), while it was significantly lower in six other cases (i.e. with the testers Fu123, Cai11-8, Xin10-513, Moqun17, Nongda178, and Mo17).

Discussion

For the investigations conducted in Italy and in China, the effects due to irrigation levels and to the inbred lines used as testers were significant for most traits. These results

Table 6. *Test-crosses evaluated in China: mean values for the IABO78.2 (+/+) and (−/−) BDLs across 13 inbred line testers in the well-watered (WW) and water-stressed (WS) trials and in the mean*

Trait	BDLs	WW	[a]	WS	[a]	Mean	[a]
Pollen shedding (d)[b]	(+/+)	21.5		26.9		24.2	
	(−/−)	22.0	ns	27.6	ns	24.8	ns
ASI (d)	(+/+)	3.72		6.00		4.9	
	(−/−)	3.59	ns	5.23	ns	4.4	ns
Plant height (cm)	(+/+)	274		206		240	
	(−/−)	284	*	201	ns	242	ns
Grain yield (Mg ha^{-1})	(+/+)	10.16		3.50		6.83	
	(−/−)	10.95	**	4.03	**	7.49	**
Ears per plant (no.)	(+/+)	1.15		0.74		0.95	
	(−/−)	1.26	**	0.82	**	1.04	**
Kernels per ear (no.)	(+/+)	465		362		414	
	(−/−)	452	ns	368	ns	410	ns
Kernel weight (mg)	(+/+)	333		229		281	
	(−/−)	337	*	234	*	285	*
Harvest index (%)	(+/+)	66.2		34.6		50.4	
	(−/−)	62.4	ns	37.9	ns	50.1	ns

[a] Comparison between mean values: * and **, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; ns, not significant.
[b] The values given indicate the dates of pollen shedding in July with 1 refers to 1 July.

thus indicate that the study was adequate for assessing the effects of *root-ABA1* QTL under different water regimes and in different genetic backgrounds. In particular, the drought stress conditions were more pronounced in China, as they lasted from the mid-stem elongation (around V7) until the mid-end of the milk stage of kernel development (R3); in Italy such conditions were shorter (V14–R2). Differences in stress conditions between the two sites were also emphasized by the fact that irrigation volumes for the WS trial corresponded to 40% of the evapotranspiration in Italy and to 30% in China.

The QTL effect, as determined by the comparison between (+/+) and (−/−) BDLs across testers and irrigation levels, was rather sizeable for root lodging, which, however, occurred only in Italy. The lower root lodging of the TCs obtained by the (+/+) BDLs is consistent with the results of previous studies (Giuliani *et al.*, 2005; Landi *et al.*, 2005), which showed that near-isogenic hybrids obtained by crossing Os420 (+/+) and IABO78 (+/+) BDLs were much less susceptible to root lodging than the corresponding near-isogenic hybrids derived from the (−/−) BDLs. Such a consistency is noteworthy not only because it was obtained by testing different genetic backgrounds but also because root lodging occurred at different growth stages, i.e. at R2 in Landi *et al.* (2005), at V10–V11 and at R2 in the two-year experiment of Giuliani *et al.* (2005), and at V9–V10 in this study. Altogether, these results strongly emphasize the consistent effect of *root-ABA1* on root lodging across irrigation treatments, genetic backgrounds,

and growth stages. The control of root lodging exerted by *root-ABA1* should be largely related to a primary effect on root characteristics, as the (+) QTL allele has been shown to determine a higher number of roots per node and roots with wider angle of insertion on the stem, greater diameter, with more branching in the shallow soil layers (i.e. more superficial), and with greater dry weight (Giuliani *et al.*, 2005). Indeed, these root characteristics are known to positively affect the strength of soil anchorage and, hence, tolerance to root lodging (Hebert *et al.*, 1992; Ennos *et al.*, 1993; Guingo and Hebert, 1997; Bruce *et al.*, 2001). Giuliani *et al.* (2005) postulated that the *root-ABA1* QTL first affects root characteristics and, only at a later stage, L-ABA as well as a consequence of the differences in flux of xylem ABA as related to differences in root mass and distribution in the soil. Because the more superficial soil layers dehydrate more quickly even under irrigated conditions, a larger and more horizontal root system would imply a greater flux of xylem ABA towards leaves, thus accounting for the higher L-ABA of the test-crosses obtained with the (+/+) BDLs. It is worth mentioning that a recurrent selection for grain yield in maize under water-limited conditions caused a change in the distribution of roots in the soil profile; in particular, the population obtained after eight selection cycles showed higher grain yield and a lower density of superficial roots (Bolaños *et al.*, 1993).

The *root-ABA1* QTL also exerted a sizeable effect on grain yield, both in Italy and in China. In the WS trial in Italy, as well as in both WW and WS trials in China, the TCs of (+/+) BDLs were out-yielded by the (−/−) BDLs, while similar yield levels were attained by the two groups of TCs in the WW trial in Italy. These findings could be ascribed to differences in growing conditions experienced by the plants in the two sites, differences in genetic backgrounds (as different TCs were evaluated) and/or effects of root lodging, which occurred in Italy but not in China. In fact, root lodging is known to affect grain yield negatively (Carter and Hudelson, 1988; Duvick, 2005), and such an influence might vary depending not only according to the growth stage in which the phenomenon occurs but also on the different stress conditions determined by the irrigation levels. In the studies of Giuliani *et al.* (2005) and Landi *et al.* (2005), both affected by severe root lodging, the effect of *root-ABA1* on grain yield was rather negligible. Giuliani *et al.* (2005) suggested that the hybrids derived from (−/−) BDLs were more penalized in terms of grain yield because they were subjected to more severe root lodging; therefore, in the absence of root lodging, these hybrids are expected to out-yield the hybrids derived from (+/+) BDLs. This hypothesis is corroborated by the yield superiority (in both WW and WS conditions) of the TCs obtained with the (−/−) BDL when tested in China, where root lodging did not occur.

An appreciable effect of the QTL was also observed for plant height (especially in Italy), as the TCs derived from

(+/+) BDLs were of shorter stature; this finding is consistent with the well-known effect of ABA in reducing plant height (Quarrie, 1991). For the other traits investigated, i.e. RWC, pollen shedding date, ASI, and harvest index, the effect of the QTL was either negligible or largely affected by environmental conditions and genetic backgrounds.

Finally, this study confirmed the effect of *root-ABA1* on L-ABA, as the (+/+) BDLs showed higher mean values for L-ABA across several inbred line testers of different origins. Previous studies have shown a very strong and consistent effect of this QTL on L-ABA evaluated across different water regimes (Tuberosa *et al.*, 1998; Giuliani *et al.*, 2005; Landi *et al.*, 2005). Although it has long been recognized that ABA has a positive role in the adaptive response of plants to conditions of water deficit (Larqué-Saavedra and Wain, 1976; Zeevaart and Creelman, 1988; Quarrie, 1991; Landi *et al.*, 2001; Tuberosa *et al.*, 2002), an excessive production of ABA might become detrimental under the prevailing field conditions that characterize modern agriculture. A negative association between L-ABA concentration and grain yield was detected in a field study conducted in maize to investigate the effects of a divergent selection for L-ABA in the Os420×IABO78 background (Landi *et al.*, 2001). Moreover, a recent survey conducted on an historical series of maize hybrids released during the past 70 years has shown a significant and sizeable reduction of the capacity of the hybrids to accumulate ABA when seedlings are exposed to a water-deficit treatment induced in hydroponics by using polyethylene glycol (Sanguineti *et al.*, 2006). Based on these findings, it was proposed that the decline in the capacity to accumulate ABA at a given level of water stress might be related to a negative association between ABA concentration and reproductive fertility in cereals (see also Saini and Westgate, 2000), a trait for which a strong indirect selection was exercised throughout modern maize breeding (Duvick, 2005). Indeed, in the present study the number of ears per plant (at both WW and WS in China and at WS in Italy) was significantly reduced in the presence of the (+) allele of the *root-ABA1* QTL, i.e. the allele determining high ABA concentration. The significant effect of *root-ABA1* on reproductive fertility and kernel weight could tentatively be ascribed to an effect of the QTL on accumulation of photosynthates consequent to the well-known inhibitory action of ABA on stomatal conductance (Davies and Zhang, 1991; Quarrie, 1991; Davies *et al.*, 2002). An involvement of photosynthates in regulating reproductive fertility is well documented in maize (Boyle *et al.*, 1991; Boyer and Westgate, 2004; McLaughlin and Boyer, 2004). Although stomatal conductance and the level of photosynthates were not measured in this study, Giuliani *et al.* (2005) showed that stomatal conductance in the genotypes homozygous for the (+) *root-ABA1* allele, as compared with the geno-

types homozygous for the (−) allele, was significantly reduced. The materials herein evaluated provide the basis for further experiments to ascertain more accurately the mechanisms by which ABA concentration may affect reproductive fertility in maize.

Conclusions

The present results, besides confirming the effect of *root-ABA1* on L-ABA, point out its sizeable effect on root lodging across a wide range of genetic backgrounds and different water regimes. The results also point out the effect of the QTL on grain yield, with the (+) allele reducing its value. However, the effects of *root-ABA1* on grain yield can vary, to a certain extent, depending on the genetic background, the growing conditions and the level of root lodging. Due to the low level of genetic resolution provided by BDLs that differ for an *c*. 30-cM-long chromosome segment (Landi *et al.*, 2005), it is not possible to ascertain whether the effects attributed to *root-ABA1* are due to a single gene with a pleiotropic action or to the presence of closely linked genes, each controlling just one of these traits. In order to gain a better genetic resolution of the chromosome region underlying the peak of *root-ABA1*, a fine mapping study has been undertaken. Eventually, based upon the results of the fine mapping and the utilization of the procedures already used for cloning other plant QTLs (Salvi and Tuberosa, 2005), it may be possible to resolve the genetic basis of *root-ABA1*, opening new and exciting opportunities for the manipulation of root architecture via genetic engineering and/or marker-assisted selection.

Acknowledgements

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