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Characterization of Maize Lines Differing in Leaf Abscisic Acid Content in the Field. 1 Abscisic Acid Physiology

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The inbred maize lines Polj17 and F-2 have previously been shown to differ by up to three-fold in leaf abscisic acid (ABA) concentration in the field. Lines from the cross Polj17 × F-2 differing in leaf ABA concentrations, and the parents, were studied in the field to characterize the differences amongst the lines in ABA concentrations during the season, during the day and in different parts of the plants. The water status of the plants was measured and leaves were heat girdled to get information on possible causes for the genetic variation amongst the lines in ABA concentration.

Leaf ABA concentrations of the high-ABA lines increased markedly and consistently from flowering time onwards, whereas leaf ABA concentrations of the low-ABA lines gradually fell after flowering. Leaf water potentials of high-ABA and low-ABA lines were similar during this time. Leaf ABA concentrations varied little during the day, and heat girdling caused a rise in ABA concentrations, which was similar in both high-ABA and low-ABA lines, only after girdling for at least 4 h. ABA concentrations were highest in the leaves and it was only in the leaves and developing kernels that substantial differences in ABA concentrations were found between the high-ABA and low-ABA classes. Although aerial brace roots also had high ABA concentrations, other roots and stem internodes had ABA concentrations which were consistently low and the same for both ABA classes.

Differences between the ABA classes were unlikely to be due to differences in leaf water status or in ABA export from the leaves. Other possible explanations for the genotypic differences in leaf ABA concentrations are discussed.

Key words: Maize, *Zea mays* L., abscisic acid (ABA), seasonal and diurnal variation, tissue distribution, genetic variation.

INTRODUCTION

Plants respond to many environmental stresses by producing the plant hormone abscisic acid (ABA). Although drought is the best studied stress known to stimulate ABA accumulation, waterlogging (Jackson and Hall, 1987), low temperature (Smith and Dale, 1988), high temperature (Makins and Shannon, 1979), salt stress (Mizrahi, Blumenfeld and Richmond, 1972) and nutrient stress (Chapin, Walter and Clarkson, 1988) have also been found to increase ABA content in the leaves. Increasing ABA content leads to many changes in physiology, growth and development which, in general, make the plant better adapted to environmental stress.

Much of our present knowledge of ABA physiology has been derived from the study of the physiology of ABA-deficient mutants in various species and the effects of treatments with racemic ABA, reviewed by Quarrie (1987, 1991). Whilst ABA-deficient mutants are very valuable in identifying specific processes that are regulated by ABA, they tell us little about the value to a normal plant of the additional ABA that is accumulated under environmental stress conditions. Likewise, there are difficulties in interpreting the consequences of non-physiological ABA concentration gradients and localization of ABA in tissues treated with ABA. Thus, investigations of the effects of applied ABA may not give a reliable picture of the changes that are likely to result specifically from increases in endogenous ABA concentrations stimulated by stress. Even if the relative importance of a particular ABA concentration in regulating individual processes is known, the net effect of stress-induced ABA accumulation in different tissues on crop yield under stressed conditions would be difficult to predict. Endogenous ABA concentrations necessary to optimize crop yield in a particular stress environment can best be determined by identifying genetic variation in stress-induced ABA accumulation in crops and studying the consequences of this variation.

An initial selection programme to produce closely-related genotypes varying in leaf ABA content was done with spring wheat. Field trials showed that high-ABA genotypes significantly outyielded low-ABA genotypes in some water-limited environments (Innes, Blackwell and Quarrie, 1984). A similar selection programme has now been carried out in maize from the cross Polj17 × F-2 to produce recombinant inbred lines having significantly different leaf ABA contents under field conditions (Pekić and Quarrie, 1988; Quarrie and Davies, 1990).

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When the F_4 generation of the cross between Polj17 and F-2 was screened in the field at around anthesis a range in leaf ABA contents amongst individual plants of about five-fold was present (see Table 1). The aim of the work described here was to characterize some of the lines from this selection programme. In this paper, we examine the distribution of ABA, both spatially and temporally, within the parents and lines from the cross and attempt to determine the physiological basis for the genetic variation in leaf ABA content amongst the genotypes.

MATERIALS AND METHODS

Genotypes

The genotypes of maize (*Zea mays* L.) used for this work were the inbred lines Polj17 (high-ABA) and F-2 (low-ABA) and high-ABA and low-ABA progeny of F_4 plants from the cross between Polj-17 and F-2. The two inbred parental lines had previously been shown to differ by up to four-fold in leaf ABA content under field conditions (Pekić and Quarrie, 1988). The lines were classified into high-ABA (H) and low-ABA (L) lines according to leaf ABA contents of individual plants in the F_4 generation in 1990. The lines tested at the F_5 and F_7 generations in these experiments are listed in Table 1, together with their ABA contents at F_4.

Cultural conditions and seasonal sampling

Plants were grown at the Maize Research Institute (Belgrade-Zemun, Serbia) in a Chernozem soil, four seeds per hill, reduced to two per hill at the seedling stage, in rows 0·8 m apart with three hills per metre. This gave a plant density of 7·5 plants m^{-2}. Plants were grown in two blocks; one block under rainfed conditions and one block irrigated at regular intervals to maintain the soil near field capacity. Seasonal variations in ABA content amongst the lines were assessed in 1991 and 1993 in the mid-third of a leaf. In 1991, leaves were sampled only from the rainfed plot on 21 Jun. (top fully-developed leaf), and 7 and 11 Jul. and 8 and 12 Aug. (third leaf from the top of the stem). In 1993, third leaves from the top were sampled from the rainfed plot on 30 Jun., 10, 20, 22 and 31 Jul. and from the irrigated plot on 21 and 30 Jul. Usually ten plants per genotype were sampled in 1991 and five plants per genotype in 1993. In both years, samples were collected between 0900 and 1100 h, except on 22 Jul. 1993 when they were collected between 0500 and 0700 h. In 1993, the water potential of each sampled leaf was measured with a pressure chamber prior to sampling for ABA content. In 1991, tassel emergence in the parents and in the F_5 lines occurred around 5 Jul. and mid pollen shed was 12–14 Jul., and in 1993 the corresponding dates were about 10–12 and 20–22 Jul., respectively.

Diurnal sampling

Diurnal fluctuations in leaf ABA content and the rate of export of ABA from leaves were examined in 1991 using third leaves from the top of one high-ABA line (132B6) and one low-ABA line (65B6). To examine ABA export, leaves from several plants were phloem girdled by heating a narrow strip for about 25 s half-way along the lamina with a battery-operated hot resistive wire attached to the inside edges of a pair of parallel-action pliers. Leaves were girdled on 13 Jul. at around 0930 h and samples of each leaf were collected approx. 5–10 cm above and below the girdled zone for ABA analysis approximately 2, 4 and 6 h after girdling. Non-girdled leaves were also sampled at about 1500 h. Four replicate leaves of each genotype were sampled on each occasion. By the end of the sampling period the 3 mm strip of leaf treated with the hot wire had turned black.

The third leaf down of four or five plants of Polj17 and F-2 were sampled on 14 Jul. 1991 every 2 h from 0900 until 1900 h. A few leaves from these plants were also sampled up to 4 h after girdling at 0900 h. Plants for these experiments and the seasonal distribution experiment were grown under rainfed conditions.

To test the efficacy of the girdling process, stomatal conductances of both surfaces of the leaves above and below the girdled zone were measured with a purpose-built steady-state diffusion porometer based on the design of Day (1977). Osmotic potentials of leaf segments above and below the girdled zone were also measured, after freezing the leaf segments and thawing them for approx. 20 min. Sap was expressed with a screw press and the solute content of the sap measured with a freezing-point micro-osmometer (Roebling-Camlab, Cambridge, UK).

ABA distribution

The distribution of ABA in various tissues in the parents Polj17 and F-2 and one high-ABA line (132B6) and one low-ABA line (65B6) was studied in plants that were grown under either rainfed or irrigated conditions in 1991. Two plants per genotype were sampled on 15 Jul. from both plots. Plants were dug up and separated into a leaf at the top, middle and bottom of the stem, three stem internodes in positions subtending the corresponding leaves and three root fractions, those in the top 3–10 cm of soil, those from 10 to 20 cm and aerial brace roots developing at the base of the stem. F-2 plants were already flowering and the other lines were about 2–3 d from flowering.

The distribution of ABA in aerial parts of plants of the parents Polj17 and F-2 was also studied in 1990. Plants were grown as above and at about 20 d after flowering (approx. 17 Jul.) six plants of each genotype were sampled. Each leaf on the stem was collected and separated into the lamina either side of the mid-rib and the thicker portion of midrib in the basal half of the leaf. The tassel, top internode, and parts of the ear (silks, kernels, cob, husks and ear stalk) were also collected. Reproductive parts of F-2 had only three or four replicates.

ABA analysis

Prior to ABA analysis samples were stored at −20 °C before freeze-drying. A 50 mg dry weight subsample of the powdered tissue was extracted overnight in 1 ml water at 4 °C and assayed for ABA by radioimmunoassay (Quarrie *et al.*, 1988; Pekić and Quarrie, 1987). ABA sample data were reduced by 10%, as recommended for maize by Quarrie *et al.* (1988), to allow for interference by impurities

TABLE 1. *Leaf ABA content (ng g^{-1} d. wt) at F_4 (1990) of the maize lines used in 1991 and 1993. The first number of the line code indicates the F_2 plant from which the line was derived*

1991				1993			
High-ABA lines		Low-ABA lines		High-ABA lines		Low-ABA lines	
Line No	F4 ABA	Line No	F4 ABA	Line No	F4 ABA	Line No	F4 ABA
13A2	639	25A5	225	122B6	523	65B1	167
66B4	525	65B1	167	154B1	629	65B6	147
122B6	523	65B6	147	159A4	762	157A1	194
127A6	613	71B6	209	167B1	795	176A4	161
132B4	716	83A4	183	Mean	677	Mean	167
132B6	637	83A5	200				
154B1	629	109A6	180				
158B7	537	133A1	279				
159A4	762	157A3	161				
167B1	795	176A2	177				
		176A4	161				
Mean	638	Mean	190				

The third leaf from the top of F_4 plants was sampled shortly after flowering.

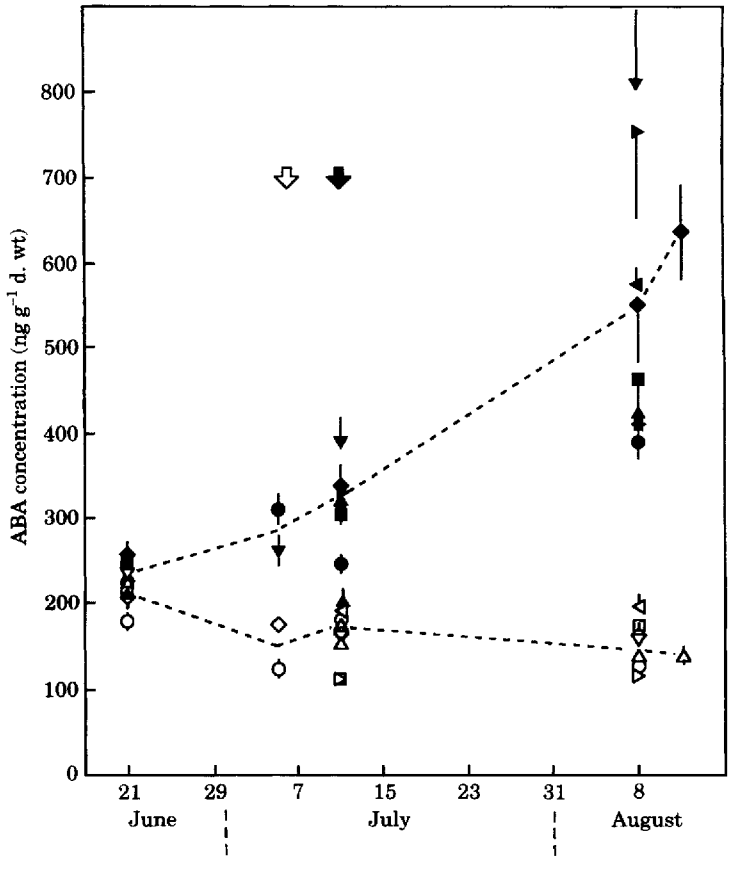


FIG. 1. Changes in leaf ABA concentration in high-ABA (filled symbols) and low-ABA (open symbols) lines during 1991. Different symbols indicate different genotypes. Bars indicate ±1 s.e. Dashed lines join the mean ABA concentrations for high-ABA and low-ABA genotypes on each sampling date. Open arrow indicates mean tassel emergence date. Closed arrow indicates mean flowering date.

in the aqueous extracts. This extraction procedure and immunoassay using crude aqueous extracts has been validated by comparison with GC-MS analyses for maize leaves (Quarrie *et al.*, 1988) and maize roots (Saab *et al.*, 1990).

RESULTS

Seasonal and diurnal fluctuations

As had previously been shown with the parents Polj17 and F-2 (Quarrie, 1991), the differences in leaf ABA content

between the high-ABA and low-ABA lines developed only as the plants reached the flowering phase, shown for 1991 in Fig. 1. Whereas mean leaf ABA content of the high-ABA lines gradually increased from 234 to 549 ng g^{-1} d. wt during this time, in the low-ABA lines the mean ABA content showed a small, but significant ($P < 0{\cdot}01$), reduction with time from 212 to 154 ng g^{-1} d. wt. By the beginning of August high-ABA lines had about 3·6 times as much ABA in the leaves as the low-ABA lines (based on means of eight and seven lines, respectively). This difference in leaf ABA content between the high-ABA and low-ABA lines was shown in 1993 to be independent of the leaf water status, as measured by leaf water potential. Despite similar large differences between the two ABA classes in leaf ABA content that developed as the season progressed (Fig. 2A), ANOVA showed that leaf water potential was, overall, not significantly different between the high-ABA and low-ABA lines (Fig. 2B), though on 10 Jul. water potentials of high-ABA lines were significantly lower than those of low-ABA lines and on 31 Jul. water potentials were significantly lower in low-ABA lines. Because of fluctuations in environmental conditions amongst sampling occasions, the irrigation treatment significantly increased leaf water potentials only of samples collected on 20 Jul. (Fig. 2B).

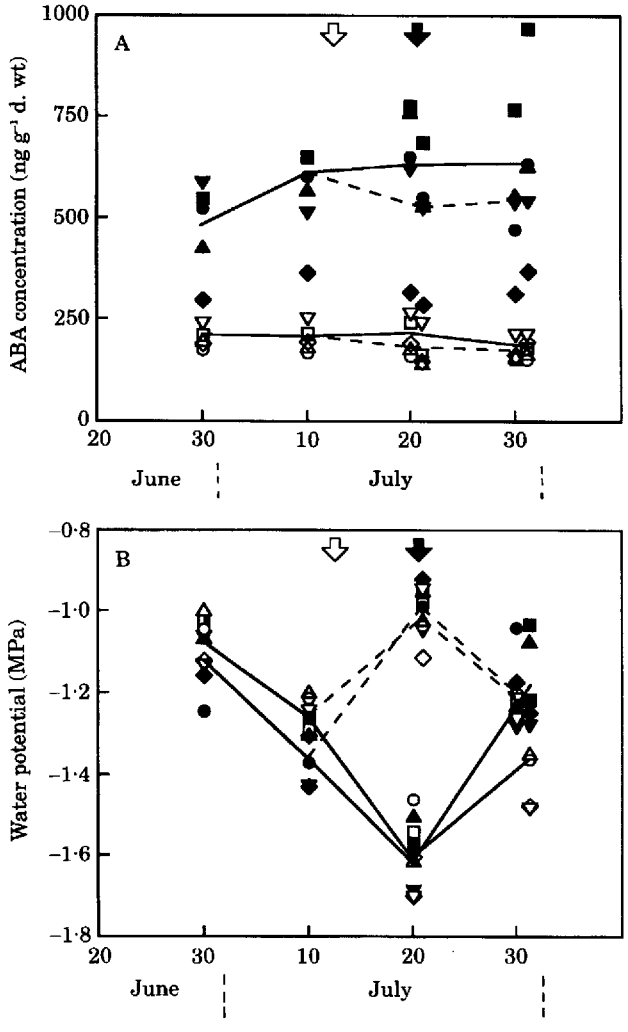


FIG. 2. Changes in leaf ABA concentration (A) and leaf water potential (B) in high-ABA (filled symbols) and low-ABA (open symbols) lines sampled from 0900 h to 1100 h during 1993. Solid lines and dashed lines join mean data for rainfed and irrigated plants respectively. Other details as for Fig. 1.

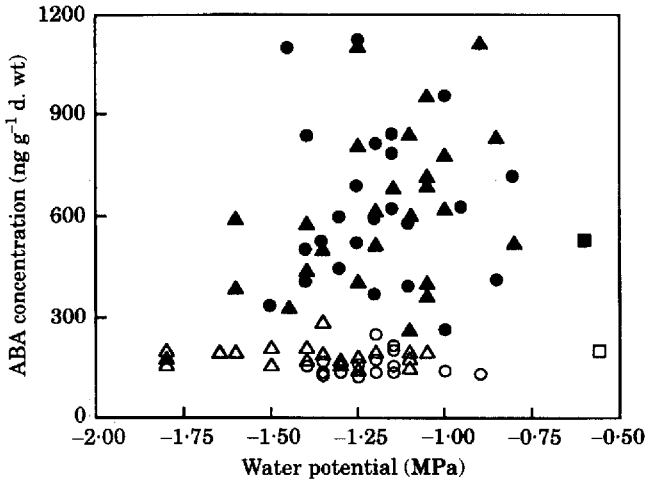


FIG. 3. The relationship between leaf water potential and leaf ABA concentration. Data are for irrigated and rainfed plants on 30 (○, ●) and 31 Jul. (△, ▲), sampled from 0900 h to 1100 h, and ABA class mean data shown for plants sampled on 22 Jul. (□, ■) from 0500 h to 0700 h. Filled symbols—high-ABA lines, open symbols—low-ABA lines.

In neither ABA class was leaf ABA content related to leaf water status (Fig. 3), despite a range, amongst sampling dates, of about 0·6 MPa for means of both ABA classes. Regressions of leaf ABA content on water potential for the two ABA classes on 30 and 31 Jul. were all non-significant. At the end of the sampling periods in both 1991 and 1993 the mean ABA concentrations of the F_5 and F_7 lines were similar to those of individual plants measured at F_4, from which the lines were derived (Table 1): 549 and 630 ng g^{-1} d. wt for high-ABA lines, and 154 and 186 ng g^{-1} d. wt for low-ABA lines in 1991 and 1993 respectively.

As found previously for the parents Polj17 and F-2 (Pekić and Quarrie, 1988), the leaf ABA content of the high-ABA and low-ABA F_5 lines also varied little during the day (Fig. 4), showing a slight but significant ($P < 0{\cdot}001$) increase of 34% between 0930 h and 1530 h. The high-ABA line had nearly twice as much ABA as the low-ABA line throughout the sampling period. The parents in 1991 also showed a slight increase in ABA content towards the middle of the day, reaching 279 and 365 ng g^{-1} d. wt for F-2 and Polj17, respectively at 1300 h. By 1900 h ABA contents in both had declined to 138 and 260 ng g^{-1} d. wt, respectively.

Girdling the leaves with a hot wire had no significant effect on ABA contents above the girdled zone until about 4 h after girdling (Fig. 4), and the two F_5 lines responded similarly, in absolute terms, to the girdling treatment, with an increase in ABA contents caused by girdling of about 200 ng g^{-1} d. wt in both lines after 6 h. In the absence of girdling, ABA contents of two leaf segments sampled in relative positions similar to those sampled above and below the girdle on girdled leaves, were not significantly different

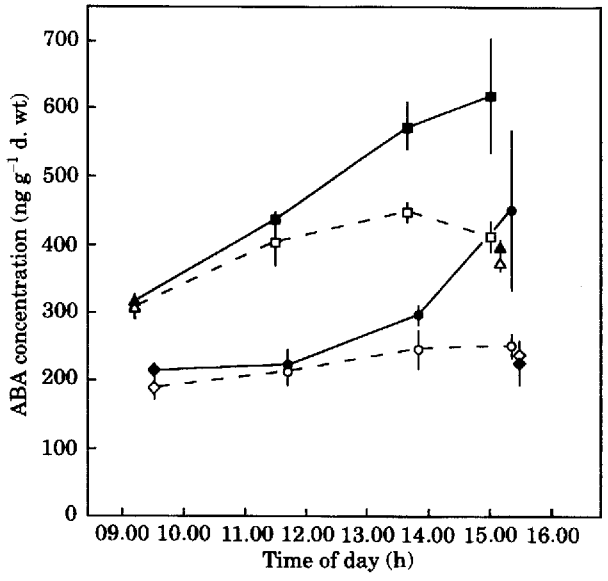
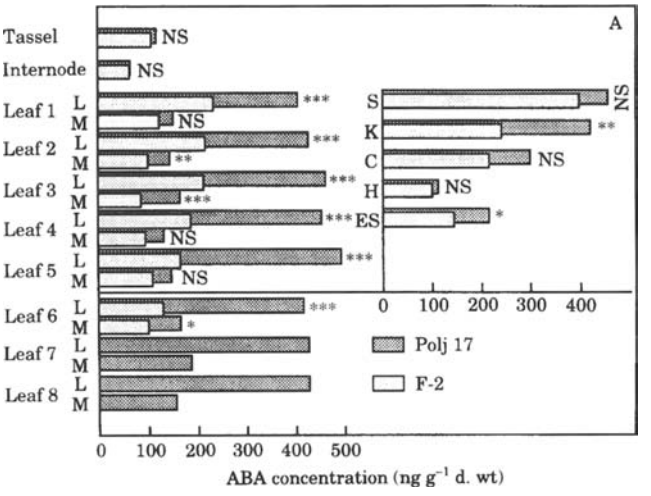


FIG. 4. Changes in leaf ABA concentration in a high-ABA line (132B6) (□, ■, △, ▲) and a low-ABA line (65B6) (○, ●, ◇, ◆) during the day. Filled symbols indicate leaves sampled above a heat girdle, open symbols indicate leaves sampled below a heat girdle. Triangles and diamonds indicate non-girdled leaves, where open and filled symbols indicate proximal and distal positions, respectively. Bars indicate ±1 s.e.



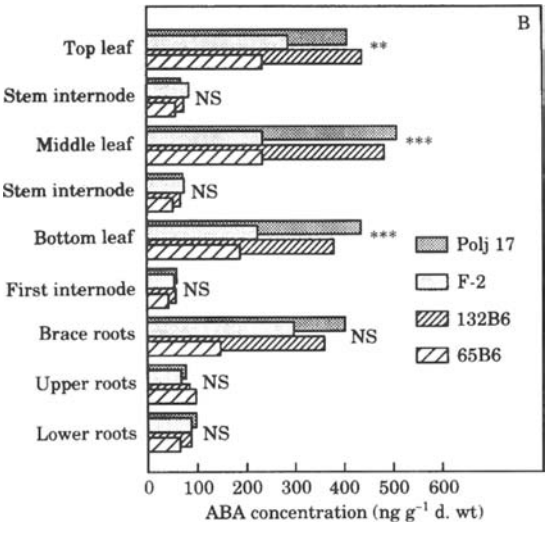


FIG. 5. The distribution of ABA in leaves, tassel internode and reproductive parts of Polj17 (high-ABA) and F-2 (low-ABA) (A), and leaves, stem internodes and roots of 132B6 (high-ABA), 65B6 (low-ABA), Polj17 and F-2 (B). L, Leaf lamina; M, leaf midrib; S, silks; K, kernels; C, cob; H, husks; ES, ear stalk. NS, *, ** and *** indicate differences between high-ABA and low-ABA lines which are non-significant and significant at $P < 0.05$, 0.01 and 0.001, respectively.

(Fig. 4). Girdling leaves of Polj17 (high-ABA) and F-2 (low-ABA) for 4 h had no significant effect (8% probability by paired *t*-test) on leaf ABA contents above the girdled zone (287 and 270 ng g^{-1} d. wt for F-2, and 385 and 346 ng g^{-1} d. wt for Polj17 above and below the girdled zone, respectively).

Girdling had no significant effect on the conductances of leaves until 4 h after the heat treatment, at which time they were 0·16 and 0·29 cm s^{-1} for 65B6 (low-ABA), and 0·15 and 0·49 cm s^{-1} for 132B6 (high-ABA) above and below the girdled zone, respectively. After girdling for 6 h, conductances above the girdle were only 36% and 11% of those below the girdle of leaves of 65B6 (0·15 and 0·49 cm s^{-1}) and 132B6 (0·06 and 0·56 cm s^{-1}), respectively. Osmotic potentials, however, were not affected by the girdling treatment until 6 h after girdling: −2·81 and −1·75 MPa for 65B6, and −2·88 and −2·50 MPa for 132B6 above and below the girdle, respectively. These differences were significant ($P < 0.05$) only for 65B6.

ABA distribution

The distribution of ABA in Polj17 and F-2 in the aerial parts of the plants (Fig. 5A) showed that the much higher ABA content characteristic of Polj17 was confined largely to the leaf laminae. ABA concentrations of the laminae differed between the parental lines on average by over two-fold (444 and 193 ng g^{-1} d. wt for Polj17 and F-2, respectively). ABA concentrations in the midrib portions of the leaves, which had much less chlorophyll, were much lower and more similar, though ANOVA showed that, over all leaves, differences between the two lines in midrib ABA content were still highly significant (152 and 103 ng g^{-1} d. wt for Polj17 and F-2, respectively). However, ABA concentrations in the tassel and tassel internode were the same in the two lines and much lower than those in the leaves. In the ear, ABA concentrations were lowest in the husks (leaves protecting the ear) and highest in the silks, where they reached concentrations similar to those in the leaf laminae. Only in the developing kernels and ear stalk was there evidence that ABA contents were higher in Polj17 than in F-2.

Consistent differences in leaf ABA content between Polj17 and F-2 were also evident in the distribution samples of 1991 (Fig. 5B). The high-ABA and low-ABA F_5 lines also showed similar large differences in the ABA content of each sampled leaf (mean leaf ABA concentrations 438 and 220 ng g^{-1} d. wt for 132B6 and 65B6, respectively). None of the other tissues sampled showed any significant genetic variation in ABA content, though there was a trend for

brace root ABA concentrations to be higher in the high-ABA genotypes. Apart from the aerial brace roots, ABA concentrations in roots and stem internodes were considerably lower than those in the leaves. ANOVA showed that the water regime had a significant effect ($P < 0{\cdot}01$) on the ABA contents of only the middle and upper leaves (435 and 277 ng g^{-1} d. wt for mean leaf ABA contents of rainfed and irrigated treatments, respectively) and the lower roots. However, the ABA contents of the lower roots were significantly *higher* ($P < 0{\cdot}01$) in the irrigated treatment (67 and 102 ng g^{-1} d. wt for ABA contents of rainfed and irrigated lower roots, respectively).

DISCUSSION

We have previously shown (Pekić and Quarrie, 1988; Quarrie, 1991) that Polj17 and F-2 differ consistently in leaf ABA concentrations during the latter part of the season, during the day and from leaf to leaf down the stem. The results shown here confirm these findings for the parents and show that the same seasonal trend is present for all of the high-ABA and low-ABA F_5 and F_7 lines tested (Figs 1 and 2), with leaf ABA concentrations after flowering going up in the high-ABA lines and down in the low-ABA lines, reaching concentrations at the end of sampling periods which were similar to those found in the F_4 generation (Table 1). Diurnal variations in ABA concentrations in one high-ABA and one low-ABA F_5 line (Fig. 4) followed the same pattern of changes as in the parents, and leaf ABA contents were always considerably higher in the high-ABA F_5 line, and high-ABA parent Polj17, as found previously (Pekić and Quarrie, 1988). Comparison with the leaf ABA contents of other maize lines sampled from field-grown plants around the time of flowering (Pekić and Quarrie, 1988; Tuberosa, Sanguineti and Landi, 1994) shows that low leaf ABA contents, similar to those of the low-ABA parent F-2 are more typical of inbred maize lines.

Examination of the distribution of ABA throughout the plants showed that the leaf laminae had, in general, much more ABA than any other organ, except the silks and aerial brace roots and it was mainly in the leaves that lines from the two ABA classes differed significantly in ABA content. Only in the developing kernels did differences in ABA content between the high-ABA and low-ABA genotypes also reach about two-fold.

We examined the water status of leaves of the parents and F_7 lines to determine whether the genetic variation in leaf ABA concentrations could be explained by differences between the ABA classes in leaf water status. It is clear from Fig. 3 that leaf water status is very unlikely to be the cause of the ABA differences. Although we did not measure osmotic potentials and hence deduce leaf turgor pressures in all of these plants, measurements of osmotic potentials during the diurnal sampling with 132B6 and 65B6 showed no significant difference between the two lines during the day in the ungirdled leaves ($-2{\cdot}3$ and $-2{\cdot}4$ MPa in 132B6 and 65B6, respectively). Therefore, turgor pressures in the high-ABA and low-ABA lines were probably similar. Leaf ABA contents in lines of both ABA classes seemed to be completely unaffected by leaf water potentials. Even in the early morning (0500–0700 h) (Fig. 3) when leaves had largely rehydrated (mean leaf water potentials $-0{\cdot}55$ and $-0{\cdot}59$ MPa for low-ABA and high-ABA classes, respectively) leaf ABA concentrations averaged over five low-ABA and five high-ABA lines (203 and 529 ng g^{-1} d. wt, respectively) were still essentially the same as they were later in the day, cf. Figs 2 and 4. Lack of correlation between leaf water potential and ABA content has also been found for maize in the field by others (Tardieu, Zhang and Davies, 1992; Tuberosa *et al.*, 1994).

Differences in leaf ABA concentrations between the ABA classes were not due to differential rates of ABA export from the leaves. Girdling the leaves with a hot wire, which appeared to have been effective in blocking phloem export according to the expected reductions in stomatal conductance and increases in solute concentrations (Henson, 1984; Setter, Brun and Brenner, 1980), had the same absolute effect on increasing leaf ABA concentrations above the girdled zone in both the high-ABA and low-ABA lines. Girdle-induced increases in leaf ABA content were considerably slower and smaller in maize than those found in two other field-grown cereals: millet (Henson *et al.*, 1986) and wheat (Quarrie, unpubl. res.). This suggests that not much ABA is exported in the phloem of maize in comparison with ABA export rates in the other cereals.

It is possible that ABA contents are higher in the leaves of the high-ABA lines because of a greater delivery of ABA to the leaves in the xylem. Preliminary data (Quarrie, 1991) indicated that ABA concentrations in the xylem of mildly stressed plants of Polj17 were about three times those of F-2 (15 and 5 ng ml^{-1}, respectively). We have recently confirmed these differences in xylem ABA content between the parents (Lebreton, Lazić-Jančić, Pekić, Steed and Quarrie, unpubl. res.) and a difference in xylem ABA contents of up to 50% between these two lines was also found by Tuberosa *et al.* (1994). However, it seems unlikely that ABA reaching the leaves in the xylem could account for the large differences in leaf ABA concentration amongst these lines as analysis both of ABA contents of leaves and xylem sap of individual plants and of quantitative trait loci (QTL) regulating those traits indicate that different genes regulate the two traits (Quarrie *et al.*, 1994*a*, *b*, and unpubl. res.).

It is also unlikely that differential compartmentation of ABA away from the sites of ABA catabolism could account for the genotypic variation in leaf ABA content. ABA is distributed amongst subcellular compartments according to pH gradients, with the majority of the ABA accumulating in the relatively alkaline chloroplasts (Cowan *et al.*, 1982; Loveys, 1977), away from the principal site of ABA catabolism in the cytosol (Hartung *et al.*, 1980). For there to be less ABA metabolised in the cytosol in the high-ABA genotypes, the pH gradient between the chloroplast and cytosol would need to be increased considerably. Even a change in chloroplast pH of 0·5 from light to dark (Heilmann, Hartung and Gimmler, 1980) caused only a 60–70% change in chloroplast ABA content (Kaiser, Weiler and Hartung, 1985; Cowan *et al.*, 1982). Genotypic differences in chloroplast/cytosol pH gradients are, therefore, unlikely to be sufficient to allow for differences of up

to three-fold in leaf ABA content between the high-ABA and low-ABA lines.

The most likely explanation for the genotypic variation in leaf ABA content between the two ABA classes would seem to be differences between the ABA classes in their ABA biochemistry (rates of synthesis and/or catabolism), with the high-ABA genotypes having a greater rate of synthesis and/or reduced rate of catabolism.

As the lines have similar percentage dry weight contents (data not presented), cytosolic ABA concentrations are likely to differ amongst the lines by up to four-fold after flowering. However, the physiological consequences of these genotypic differences in ABA concentration are not yet known. Work on the effects of the ABA differences on rooting behaviour and gas exchange properties is in progress. Because of the marked differences between Polj17 and F-2 in their responses to drought stress (Pekić and Quarrie, 1987, 1988), these two inbred lines have been used to create a mapping population of F_2 plants to study the distribution of QTL for responses of maize to drought stress, including ABA contents (Quarrie *et al.*, 1994*a*; Lebreton, Lazić-Jančić, Pekić, Steed and Quarrie, unpubl. res.).

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LITERATURE CITED

Chapin FS, Walter CHS, Clarkson DT. 1988. Growth response of barley and tomato to nitrogen stress and its control by abscisic acid, water relations and photosynthesis. *Planta* **173**: 352–366.

Cowan IR, Raven JA, Hartung W, Farquhar GD. 1982. A possible role for abscisic acid in coupling stomatal conductance and photosynthetic carbon metabolism in leaves. *Australian Journal of Plant Physiology* **9**: 489–498.

Day W. 1977. A direct reading continuous flow porometer. *Agricultural Meteorology* **18**: 81–89.

Hartung W, Gimmler H, Heilmann B, Kaiser G. 1980. The site of abscisic acid metabolism in mesophyll cells. *Plant Science Letters* **18**: 359–364.

Heilmann B, Hartung W, Gimmler H. 1980. The distribution of abscisic acid between chloroplasts and cytoplasm of leaf cells and the permeability of the chloroplast envelope for abscisic acid. *Zeitshrift für Pflanzenphysiologie* **97**: 67–78.

Henson IE. 1984. Evidence of a role for abscisic acid in mediating stomatal closure induced by obstructing translocation from leaves of pearl millet (*Pennisetum americanum* [L.] Leeke). *Journal of Experimental Botany* **35**: 1419–1432.

Henson IE, Mahalakshmi V, Alagarswamy G, Bidinger FR. 1986. Regulation of abscisic acid concentration in leaves of field-grown pearl millet (*Pennisetum americanum* [L.] Leeke): the role of abscisic acid export. *New Phytologist* **102**: 249–259.

Innes P, Blackwell RD, Quarrie SA. 1984. Some effects of genetic variation in abscisic acid accumulation on the yield and water use of spring wheat. *Journal of Agricultural Sciences (Cambridge)* **102**: 341–351.

Jackson MB, Hall KC. 1987. Early stomatal closure in waterlogged pea plants mediated by abscisic acid in the absence of foliar water deficits. *Plant, Cell and Environment* **10**: 121–130.

Kaiser G, Weiler EW, Hartung W. 1985. The intracellular distribution of abscisic acid in mesophyll cells—the role of the vacuole. *Journal of Plant Physiology* **119**: 237–245.

Loveys BR. 1977. Intracellular location of abscisic acid in stressed and non-stressed leaf tissue. *Physiologia Plantarum* **40**: 6–10.

Makins JJC, Shannon C. 1979. Temperature and photoperiod effects on abscisic acid content in "Earliwax" snapbean seed. *Horticultural Science* **14**: 732–733.

Mizrahi Y, Blumenfeld A, Richmond AE. 1972. The role of abscisic acid and salination in the adaptive response of plants to reduced root aeration. *Plant and Cell Physiology* **13**: 15–21.

Pekić S, Quarrie SA. 1987. Abscisic acid accumulation in lines of maize differing in drought resistance; a comparison of intact and detached leaves. *Journal of Plant Physiology* **127**: 203–217.

Pekić S, Quarrie SA. 1988. Abscisic acid in leaves of inbred lines and F1 hybrids of maize growing in the field and its relationship to diurnal and seasonal trends in leaf water potential. *Annals of Botany* **61**: 669–678.

Quarrie SA. 1987. Evaluation of the influence of a metabolic character on drought resistance exemplified by studies on abscisic acid in wheat and maize. In: Monti L, Porceddu E, eds. *Drought resistance in plants: Physiological and genetic aspects.* Luxembourg: Commission of the European Communities, 111–129.

Quarrie SA. 1991. Implications of genetic differences in ABA accumulation for crop production. In: Davies WJ, Jones HG, eds. *Abscisic acid physiology and biochemistry.* Oxford, UK: Bios Scientific Publishers, 227–243.

Quarrie SA, Davies WJ. 1990. Abscisic acid in maize during drought and its control of leaf growth in drying soil. In: *Maize breeding, production, processing marketing in mediterranean countries. Maize '90.* Belgrade: Maize Research Institute, 269–286.

Quarrie SA, Whitford PN, Appleford NEJ, Wang TL, Cook SK, Henson IE, Loveys BR. 1988. A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. *Planta* **173**: 330–339.

Quarrie SA, Gulli M, Calestani C, Steed A, Marmiroli N. 1994*a*. Location of a gene regulating drought-induced abscisic acid production on the long arm of chromosome 5A of wheat. *Theoretical and Applied Genetics* (in press).

Quarrie SA, Lebreton C, Lazić-Jančić V, Steed A. 1994*b*. QTL for drought responses in an F2 population. *Maize Genetics Cooperation Newsletter* **68**: 73–74.

Saab IN, Sharp RE, Pritchard J, Voetberg GS. 1990. Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. *Plant Physiology* **93**: 1329–1336.

Setter TL, Brun WA, Brenner ML. 1980. Effect of obstructed translocation on leaf abscisic acid, and associated stomatal closure and photosynthesis decline. *Plant Physiology* **65**: 1111–1115.

Smith PG, Dale JE. 1988. The effect of root cooling and excision treatments on the growth of primary leaves of *Phaseolus vulgaris* L. Rapid and reversible increases in abscisic acid content. *New Phytologist* **110**: 293–300.

Tardieu F, Zhang J, Davies WJ. 1992. What information is conveyed by an ABA signal from maize roots in drying field soil? *Plant, Cell and Environment* **15**: 185–191.

Tuberosa R, Sanguineti MC, Landi P. 1994. Abscisic acid concentration in leaf and xylem sap, leaf water potential, and stomatal conductance in maize. *Crop Science* **34**: 1557–1563.