Corrigendum

Abscisic acid is correlated with the leaf growth inhibition of four genotypes of maize differing in their response to salinity

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Due to an error in file conversion, lines in Figure 2 on page 113 were extended beyond the data. These portions of the original figure should be ignored. A more accurate figure is reproduced below. Figure 2 as published on the FPB website is correct.

![Figure 2](image)

**Fig. 2.** The association between leaf elongation rate (LER) and the (+)-ABA concentration of the growing zone of the third leaf of four maize genotypes (P3906, L155, Polj 17 and F–2). (+)-ABA concentrations were increased and LER decreased after additions of (+)-ABA to control and salt-stressed plants for 23 h. Solid figures represent the values for 80 mM NaCl-treated plants. Arrows point to values of salt-stressed plants without (+)-ABA added. All values are means. n = 6–8. The crosshairs represent the average s.e. for all means. Each line was fitted by second degree polynomial regression, except for F–2 which was fitted by eye.
Abscisic acid is correlated with the leaf growth inhibition of four genotypes of maize differing in their response to salinity

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Abstract. In this paper we tested the hypothesis that the leaf growth reduction of salt-stressed maize is regulated by the abscisic acid (ABA) concentrations in the growing zone of the leaf. Leaf elongation rate (LER) of maize (Zea mays L.) was rapidly inhibited by salinity (80 mM NaCl), and the (+)-ABA concentration increased significantly in the growing zone of the leaf. Upon removal of salinity, ABA concentrations decreased rapidly in the growing zone and LER increased to control levels. Four maize genotypes differing in their responses to salinity were compared over a range of leaf ABA concentrations. (+)-ABA concentrations in the growing zone of the leaf were highly correlated with LER inhibition for all four genotypes. However, the sensitivity of LER to leaf ABA concentrations differed amongst the genotypes. Thus, for each genotype, ABA concentrations in the growing zone of the leaf were a good predictor of maize LER response to salinity.

Keywords: abscisic acid (ABA), leaf growth, maize (Zea mays L.), salinity.

Introduction

Early reports identified abscisic acid (ABA) as a growth inhibitor having a physiological role in plant responses to environmental stress (reviewed in Milborrow 1974). The concentration of (+)-ABA in a plant increases when it suffers osmotic or dehydration stress (Munns and Sharp 1993). However, different organs may respond differently to the increase of (+)-ABA concentrations. Root growth is much less sensitive to changes in (+)-ABA concentration, such that elongation of water-stressed roots is often maintained or even stimulated by increases in (+)-ABA concentration (Munns and Sharp 1993). On the other hand, leaf elongation is often inhibited by osmotic stress and by increases of (+)-ABA concentrations in the leaf (Cramer 1994; Dodd and Davies 1996; He and Cramer 1996; Montero et al. 1997; Cramer et al. 1998; Montero et al. 1998). This frequently results in an increase in the root to shoot ratio. It was postulated that (+)-ABA affects this ratio via independent effects on root and leaf elongation (Munns and Cramer 1996).

Very little is known about how (+)-ABA inhibits leaf elongation. (±)-ABA can affect the physical properties of the cell wall by decreasing cell wall extensibility (Van Volkenburgh and Davies 1983; Kutschera and Schopfer 1986), or (+)-ABA can increase the apparent yield threshold of the cell wall depending on the (+)-ABA concentration (or sensitivity) of the tissue (Cramer et al. 1998). Both changes in cell wall properties could reduce the ability of cells to expand. A possible mechanism for this action has been proposed (Munns and Cramer 1996). It was suggested that (+)-ABA causes a reduction of cytosolic Ca$^{2+}$ in leaf cells, which results in altered rates of secretion or synthesis of cell wall components, thus altering the properties of the cell wall and its growth rate.

There is indirect evidence to support this model of leaf inhibition by (+)-ABA. (±)-ABA can reduce cytosolic Ca$^{2+}$ concentrations, and reduction of cytosolic Ca$^{2+}$ is correlated with growth reduction caused by (±)-ABA (Cramer and Jones 1996). It is also known that ABA can inhibit hemicellulose deposition in growing tissues (Wakabayashi et al. 1989; Wakabayashi et al. 1991).

Exposure to increased concentrations of salinity induces a proportional increase of (+)-ABA concentration in plants. The increase in (+)-ABA concentration is correlated with the inhibition of leaf expansion by salinity in bean (Montero et al. 1997, 1998), in Brassica species (He and Cramer 1996) and in maize (Cramer 1994). In a detailed study of the response of maize to treatments with the natural (+)-form of ABA (Cramer et al. 1998), leaf elongation rate (LER) was shown to be tightly linked to ABA concentrations in the expanding zone of the leaf. In this paper, we test the hypothesis that reduction of LER in salt-treated maize seedlings is due to the accumulation of ABA in the leaf growing zones, by using maize genotypes known to differ in
their growth responses to salinity. Specifically, we examine whether these responses are consistent across genotypes.

Materials and methods

Experimental conditions were similar to those previously published (Cramer and Bowman 1991). One to four maize genotypes [Zea mays L. Pioneer hybrid 3906 (P3906), and inbred lines L155, Polj 17 and F–2] were used. Some of these genotypes had previously been shown to differ in (+)-ABA production in response to osmotic or dehydration stress (Pekic and Quarrie 1987). All maize caryopses were germinated in moist vermiculite and irrigated with quarter strength modified Hoagland's solution the day before the experiment. Plants were anchored with a foam plug inserted into plastic egg crate-style grids, which were cut to size from larger pieces normally used for light diffusion. The grids sat on top of the glass cylinders. On the day the treatments began, plants were salted from 4–24 h with 80 mM NaCl (depending on the experiment). Salt was added as a concentrate (2 mL of 4 mM NaCl) to the 100 mL of Hoagland's solution, and plants were grown in continuous light (300 µmol m–2 s–1). LER of the third leaf was measured with a displacement transducer. In a preliminary trial, the time course of the response of LER and growing zone ABA concentration to salinization, and recovery from salinization, was measured over a 28-h period. In other experiments, non-salinized and salinized plants were treated with (+)-ABA (0.5, 1, and 2 µM) for 23 h. Salinized plants had salt and (+)-ABA added simultaneously. (+)-ABA was a generous gift from Dr S. Abrams (Plant Biotechnology Institute, Saskatchewan, Canada). At the end of the experiments, the growing zone of the third leaf was removed, frozen in liquid N2 and assayed for (+)-ABA by radioimmunoassay (Cramer et al. 1988; Quarrie et al. 1988).

Results

To determine if (+)-ABA concentrations might be responsible for decreased growth rates in salt-stressed plants, one must first determine when is the appropriate time to assay for (+)-ABA. When plants were suddenly salt-stressed, they went through a series of changes that stabilized after about 4 h of salinization (Fig. 1A), as described previously (Cramer and Bowman 1991; Cramer 1992a, b). Leaf elongation rates declined rapidly upon salinization. This reduction was primarily caused by a reduction in turgor pressure (Cramer and Bowman 1991). After about 30 min, turgor and leaf elongation recovered. However, the recovery rate was dependent upon the salinity concentration. After approximately 4 h, turgor had returned to control values and leaf elongation had reached a steady-state rate that was lower than the control rate. Growth rates remained steady for at least 24 h after salinization, and recovered quickly to control rates (within 2 h) when salinized plants were returned to control solutions (Fig. 1A and Cramer 1992a).

When maize plants were treated with 80 mM NaCl, (+)-ABA concentrations fluctuated in a similar but opposite manner to the response of leaf elongation (Fig. 1B). (+)-ABA concentrations increased when leaf elongation rates decreased, and decreased when leaf elongation increased. First, there was a sharp rise in (+)-ABA concentration that peaked about 2 h after salinization (Fig. 1B). (+)-ABA concentrations decreased from the maximum between 2–5 h, and reached a steady-state concentration that was elevated above control concentrations after about 5 h. (+)-ABA concentrations were still steady after 24 h of salinization. When salinity was removed from the root medium, (+)-ABA concentrations of the growing zone returned quickly to control concentrations (Fig. 1B). Therefore, for the subsequent studies, we chose to sample plants after the effects of salt on LER and ABA concentration had stabilized (23 h of salinization).

The steady-state (+)-ABA concentration of plants treated with 80 mM NaCl was very similar to that of plants of the same genotype treated with 1 µM (±)-ABA (Cramer et al. 1998). Both treatments increased steady-state (+)-ABA concentrations of the growing zone and reduced leaf elongation rates to about the same extent. To test the hypothesis that changes in (+)-ABA concentration in the.

Fig. 1. The effect of salinity on (A) leaf elongation rates (LER) and (B) (+)-ABA concentrations of the growing zone of the third leaf of 8-d old maize leaves (P3906). Salinity was added at 0 h and removed at 24 h. Values are means ± s.e. n = 8 for LER and n = 4 for ABA concentrations.
leaf elongating zone of maize cause the decline of LER in salt-stressed plants, the following experiments were conducted to generate different (+)-ABA concentrations within the leaves of salt-stressed plants. Maize plants differing in their ability to produce (+)-ABA during osmotic or dehydration stress have already been identified (Pekic and Quarrie 1987), but their responses to salinity in terms of growth or (+)-ABA synthesis were unknown. Therefore, (+)-ABA concentrations of the growing zone, and LER of the third leaf of several of these genotypes, were determined 23 h after salinization. Initial results indicated that (+)-ABA concentrations differed between genotypes, but overall, these were poorly correlated with salt tolerance (LER as a % of control and absolute LERs, data not shown). For example, when salinized, genotype F–2 showed very little increase in (+)-ABA concentration relative to the response of the other maize genotypes, yet its LER was more strongly inhibited by salt. However, experiments were repeated with supplemental treatments of 0.5, 1.0, and 2.0 mM (+)-ABA to control and salinized plants to determine the (+)-ABA sensitivity of the tissues. These treatments showed that the LER of F–2 was, in general, much more sensitive to internal (+)-ABA concentrations than the other genotypes (Fig. 2).

For each genotype, (+)-ABA concentration of the growing zone of the leaf was highly correlated with LER (Fig. 2). Sensitivity to ABA for each genotype was assessed by testing for significant differences of the slopes of the log transformed growth data by ANCOVA. There were significant differences in sensitivities to ABA amongst the genotypes ($P=0.0015$ for ABA × genotype interaction). P3906 and Polj 17 had similar sensitivities to internal (+)-ABA concentrations (similar slopes of the lines in Fig. 2) and were the least sensitive of the four genotypes. The sensitivities of L155 and F–2 were significantly different from the other two genotypes, and from each other. L155 was moderately sensitive, and F–2 was the most sensitive, to internal (+)-ABA concentrations. Salinity itself had no additional effect on (+)-ABA sensitivity, with salinized plants responding to increasing internal (+)-ABA concentrations in a manner similar to that of their non-salinized counterparts (also, see Cramer et al. 1998 for the response of P3906 to a broader range of internal (+)-ABA concentrations in non-salinized plants). Plants treated with salinity without supplemental (+)-ABA fell reasonably well within each curve (arrows pointing to the symbols in Fig. 2, and note that those for P3906 and Polj 17 almost coincide). These results are consistent with the responses of Brassica species (He and Cramer 1996) and bush bean (Montero et al. 1997, 1998) to salinity and (+)-ABA.

Different genotypes produced different amounts of (+)-ABA in response to osmotic stress caused by the salt treatment (Fig. 2). F–2 produced the lowest amount ($177.0 \pm 38.1$ ng g DW$^{-1}$), followed by Polj 17 ($243.0 \pm 21.2$ ng g DW$^{-1}$), P3906 ($254.0 \pm 18.2$ ng g DW$^{-1}$) and L155 ($288.0 \pm 19.6$ ng g DW$^{-1}$). On the basis of these four values, the correlation between LER and (+)-ABA concentration appeared very poor. However, once genotype sensitivity to growing zone (+)-ABA concentration was taken into account, the correlation between LER and (+)-ABA concentration was very good across all genotypes.

**Discussion**

Osmotic stress causes an increase in (+)-ABA concentrations in plant tissues (Munns and Sharp 1993). This was confirmed in our work when maize leaf growing zones were sampled from plants stressed osmotically by salinity. An earlier study showed a similar peak of (+)-ABA concentration in expanding leaves, roots and tissue culture, several hours after salt stress (He and Cramer 1996). The subsequent reduction to a lower steady-state concentration of (+)-ABA may be the result of stimulated catabolism of (+)-ABA, although it is possible that the rate of synthesis of (+)-ABA was reduced, or that (+)-ABA transport to or from the growing zone was altered.

The steady-state (+)-ABA concentrations of salt-stressed plants correlated well with the reduction of leaf expansion (Cramer 1994; He and Cramer 1996; Montero et al. 1997; Cramer et al. 1998; Montero et al. 1998). In bean (Montero et al. 1998), salinity increased ABA concentrations and growth inhibition to a greater extent than iso-osmotic macronutrient concentrations. In Brassica species (He and Cramer 1996), salt tolerance was associated with the species

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that had a lower concentration of ABA in the growing tissue. Similarly, in barley, cotton, saltbush (Kefu et al. 1991) and *Suaceda maritima* (Clipson et al. 1988), an increase in ABA concentration correlated with the level of strain (reduced growth), and not the level of stress (NaCl). That is, when growth was optimal (dependent upon species), ABA concentrations were at their lowest. When growth was low from excess salt (or in the case of halophytes, a lack of salt), ABA concentrations were higher than at optimal growth conditions. For example, the ABA concentration of the halophyte, *Suaeda*, was almost twice as high at 0 mM than at 20 or 200 mM NaCl (Clipson et al. 1988).

Our results presented here with four genotypes of maize known to differ in their responses to stress (Pekic and Quarrie 1987, and unpublished results) show that the changes in leaf growth rates following salt stress could be explained in terms of changes in ABA concentration taking place in the leaf growing zones.

Nevertheless, there has been a lack of correlation of internal ABA concentrations with LER in some studies of drought-stressed plants (Dodd and Davies 1996; Salah and Tardieu 1997). In drought-stressed barley, wheat and maize leaves, there appeared to be an interaction between drought stress and the internal ABA concentrations of the growing zone of the leaf (Dodd and Davies 1996). Drought-stressed plants may become more sensitive to internal (+)-ABA concentrations of the growing zone of the leaf, causing a greater reduction of LER per amount of internal (+)-ABA. However, this was clearly not the case in the salt-stressed plants of this study (Fig. 2), or a previous study of salt-stressed *Brassica* species (He and Cramer 1996).

Can ABA concentrations in the growing tissue completely explain the growth reduction in osmotically-stressed plants? Salah and Tardieu (1997) argue that there is a transpirational component, in addition to an ABA component, that is responsible for the reduction of LER in drought-stressed maize leaves. However, their analysis was based upon xylem concentrations of ABA that fluctuate, whereas internal (+)-ABA concentrations of the growing zone may not, it is unclear to what extent this additional component may play a role in growth reduction. Nevertheless, the argument for an additional hydraulic signal may be valid, especially in light of recent convincing evidence supporting the hydraulic signal hypothesis (Nonami et al. 1997; Munns et al. 2000a, b).

In addition to its likely effect on leaf growth rates in response to salt stress, ABA has been shown to induce changes in gene expression (Hurkman 1992; Zhu et al. 1997). In osmotically-stressed plants, there are ABA-dependent and ABA-independent pathways which affect gene expression (Zhu et al. 1997; Hasegawa et al. 2000), although the functions of only a few of the osmotic-stress regulated genes have been established. It is likely that some of these genes play a protective role during dehydration.

Although ABA is generally regarded as a growth inhibitor, it can also stimulate growth in certain salt-stressed plants (Amzallag et al. 1990; Gadallah 1996). Contrary to the inhibition of shoot growth by (+)-ABA in salt-stressed maize, foliar applications of (+)-ABA enhanced shoot growth and accelerated adaptation in salt-stressed sorghum (Amzallag et al. 1990). The beneficial effects of (+)-ABA were apparent only up to 10 d after salinization. Thereafter, exogenously applied (+)-ABA was detrimental to plant growth. The authors noted that if (+)-ABA was applied to the root medium, exogenously applied (+)-ABA inhibited shoot growth instead of stimulating it.

This adaptive response to (+)-ABA appears to be lacking in the short-term in salt-stressed maize. Foliar applications of (+)-ABA (40 µM mixed isomers from Sigma and 0.005% Triton X–100) on salt-stressed maize, similar to those reportedly used on sorghum (Amzallag et al. 1990), had no effect on the response of maize LER to salinity over the first 5 h of salinization (G. R. Cramer, unpublished results).

As well as correlation analysis, another approach to address the function of ABA in salt-stressed plants is the use of ABA synthesis inhibitors or ABA-deficient mutants, which have proven very useful in clearly identifying ABA as an important factor in regulating the responses to drought (Saab et al. 1990; Sharp et al. 1994, 2000). Recent evidence indicates that part of the effect of ABA involves interaction with ethylene. Ethylene does not appear to play a role in the short-term response of maize to salinity (Cramer 1992b). We were not able to obtain ABA-deficient maize mutants to test their response to salinity, so we will investigate the interaction of salinity with ABA mutants of *Arabidopsis* in a following paper.

In conclusion, there is clear evidence that (+)-ABA concentrations increase in salt-stressed maize plants and that, for a given maize genotype, increases in ABA concentration in the leaf expanding zones are correlated with growth reduction. However, further work needs to be done with different ABA mutants to test this hypothesis more completely.

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References


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