

Abscisic Acid Accumulation Maintains Maize Primary Root Elongation at Low Water Potentials by Restricting Ethylene Production¹

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Previous work showed that primary root elongation in maize (*Zea mays* L.) seedlings at low water potentials (ψ_w) requires the accumulation of abscisic acid (ABA) (R.E. Sharp, Y. Wu, G.S. Voetberg, I.N. Saab, M.E. LeNoble [1994] *J Exp Bot* 45: 1743–1751). The objective of the present study was to determine whether the inhibition of elongation in ABA-deficient roots is attributable to ethylene. At a ψ_w of -1.6 MPa, inhibition of root elongation in dark-grown seedlings treated with fluridone to impose ABA deficiency was largely prevented with two inhibitors of ethylene synthesis (aminooxyacetic acid and aminoethoxyvinylglycine) and one inhibitor of ethylene action (silver thiosulfate). The fluridone treatment caused an increase in the rate of ethylene evolution from intact seedlings. This effect was completely prevented with aminooxyacetic acid and also when ABA was supplied at a concentration that restored the ABA content of the root elongation zone and the root elongation rate. Consistent results were obtained when ABA deficiency was imposed using the *vp5* mutant. Both fluridone-treated and *vp5* roots exhibited additional morphological symptoms of excess ethylene. The results demonstrate that an important role of ABA accumulation in the maintenance of root elongation at low ψ_w is to restrict ethylene production.

Maintenance of root elongation at low water potentials (ψ_w) is considered to be an adaptive feature that promotes survival of the plant under water-limited conditions (Sharp and Davies, 1989; Spollen et al., 1993). For example, the primary root of maize (*Zea mays* L.) maintains substantial elongation at a ψ_w of -1.6 MPa, whereas shoot development is completely inhibited at around -0.8 MPa (Sharp et al., 1988). This differential response is advantageous for seedling establishment under dry conditions.

The mechanisms that allow roots to grow at low ψ_w have received little attention and are only beginning to be understood. Although hormones are likely to play an important regulatory role in the adaptation of root growth to

water stress, the involvement of most of these compounds has not been elucidated. The exception is the accumulation of abscisic acid (ABA), which was shown to be required for maintenance of primary root elongation at low ψ_w in maize seedlings (Saab et al., 1990; Sharp et al., 1994). This was demonstrated by decreasing endogenous ABA levels chemically using fluridone, which inhibits carotenoid (and ABA) biosynthesis, or genetically using the *vp5* mutant, in which carotenoid (and ABA) biosynthesis is deficient. At low ψ_w , root elongation rate of ABA-deficient seedlings was severely inhibited compared with untreated or wild-type seedlings, and fully recovered when the ABA content of the elongation zone was restored to normal levels with exogenous ABA. Since the seedlings were grown at near-saturation humidity in the dark, indirect effects of altered ABA levels on growth due to stomatal control of plant water balance or photosynthesis were avoided.

The role of ABA accumulation in the maintenance of root elongation at low ψ_w is not known. There have been several reports that applied ABA can inhibit ethylene production from various organs in a range of species (e.g. Gertman and Fuchs, 1972; Wright, 1980; Yoshii and Imaseki, 1981; Tan and Thimann, 1989). Furthermore, ABA-deficient mutants have been found to exhibit increased ethylene evolution from shoots (tomato: Tal et al., 1979) and whole plants (Arabidopsis: Rakitina et al., 1994). It was suggested by Wright (1980) that endogenous ABA accumulation may limit ethylene production during water stress, and that this interaction may help to determine many of the effects of water deficit, including the responses of root and leaf growth. These hypotheses have not been tested.

In this study, we examined whether elongation of ABA-deficient (fluridone-treated and *vp5*) maize primary roots at low ψ_w can be restored with inhibitors of ethylene synthesis or action, and whether ABA-deficiency causes an increase in the rate of ethylene production from water-stressed seedlings. The results show that an important role of ABA accumulation in the maintenance of root elongation at low ψ_w is to prevent excess ethylene production.

MATERIALS AND METHODS

Fluridone Experiments

For most experiments with fluridone, seeds of maize (*Zea mays* L. cv FR27 × FRMo17) were germinated for 32 h in

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well-moistened vermiculite (grade 3, Strong-Lite, Pine Bluff, AR) at 29°C and near-saturation humidity in the dark. Seedlings with primary roots about 5 mm in length were transplanted into Plexiglas boxes or glass beakers containing vermiculite at a ψ_w of -1.63 ± 0.18 MPa (mean \pm SD of all experiments), which was obtained by thorough mixing with a small amount of water. The seedlings were then grown under the same conditions for up to 48 h (Sharp et al., 1988). Vermiculite ψ_w was measured for each experiment by isopiestic thermocouple psychrometry (Boyer and Knippling, 1965). When necessary for growth measurements and for harvesting, illumination was provided by a green safelight (Saab et al., 1990).

Fluridone (SePRO, Carmel, IN) was added at a final concentration of 1.5 μ M to the water mixed with the vermiculite in which seeds were germinated and into which seedlings were transplanted. Details of fluridone preparation are described in Ober and Sharp (1994). Ethanol and Tween 20 (final concentrations of 0.006% and 0.002%, v/v, respectively) were added to control treatments. In previous work, 10 μ M fluridone was used to impose ABA deficiency (e.g. Saab et al., 1990). To minimize potential side effects, the relationship of fluridone concentration to root tip ABA level was refined. It was found that inhibition of ABA accumulation at a ψ_w of -1.6 MPa was almost as large with 1.5 μ M as with 10 μ M fluridone (data not shown). Therefore, a fluridone concentration of 1.5 μ M was used for all experiments.

Experiments to determine whether exogenous ABA could overcome the effects of fluridone were conducted at a later date. A different culture protocol was used because the properties of the vermiculite had changed (although the same brand was used) such that seedlings became Ca^{2+} -deficient unless supplied with supplemental Ca^{2+} (M.A. Else and R.E. Sharp, unpublished data). Seeds were imbibed for 23 h in 1 mM CaSO_4 and germinated for 29 h in vermiculite well moistened with 1 mM CaSO_4 (with or without fluridone). Seedlings were then transplanted into vermiculite at a ψ_w of -1.6 MPa, which was obtained by mixing with 1 mM CaSO_4 (with or without fluridone). Preliminary experiments at a range of Ca concentrations showed that seedlings grown using this protocol exhibited maximum root and shoot elongation rates at high and low ψ_w . (\pm)-ABA (Sigma-Aldrich, St. Louis) was added at a final concentration of 0.5 mM together with fluridone to the vermiculite into which the seedlings were transplanted, as described by Sharp et al. (1994). (ABA was not added prior to transplanting because it inhibits germination.) In these experiments, root length at transplanting was approximately 20 mm.

vp5 Experiments

Seeds of the *vp5* mutant and wild-type maize were obtained by selfing plants grown from heterozygous seed (Maize Genetics Stock Center, Urbana, IL). Only those mutant kernels (identified by a lack of carotenoid pigmentation) that survived desiccation on the plant were used (Saab et al., 1990). The limited amount of such seed restricted the number of experiments that could be con-

ducted with the mutant. Mutant and wild-type seedlings were grown using the first of the culture protocols described above, except that germination times and root lengths at transplanting, respectively, were 54 h and 5 to 10 mm for the wild type, and 54 to 72 h and 2 to 24 mm for *vp5*. The limited number of mutant seed required that all were used; analysis of the results showed no relationship between initial root length and root length increase after transplanting.

Inhibitors of Ethylene Synthesis and Action

Aminooxyacetic acid (AOA) and aminoethoxyvinylglycine (AVG) are inhibitors of pyridoxal phosphate-requiring enzymes including 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, a key enzyme of ethylene synthesis, and silver thiosulfate (STS) inhibits ethylene action (Abeles et al., 1992). These inhibitors were used to test for the involvement of ethylene in the effects of fluridone and the *vp5* mutation. In separate experiments, the different inhibitors were added to the water mixed with the vermiculite in which seeds were germinated and into which seedlings were transplanted. Solutions of STS were made as described by Cameron et al. (1985).

Root Growth and ABA Quantification

Primary root length increase at various times after transplanting to low ψ_w was determined either by marking the positions of the root apices on the face against which they were growing or by destructive harvesting. Effects of fluridone and the *vp5* mutation on root tip swelling were quantified by measuring the spatial distribution of root diameter in the apical 15 mm at the end of experiments. Root tips were photographed immediately after harvest, and diameter profiles were measured from enlarged prints.

Root tips were harvested for measurement of ABA content by radioimmunoassay (Quarrie et al., 1988). Depending on the experiment, the apical 6 or 10 mm (excluding the root cap) was measured. This encompassed the elongation zone, which extends approximately 6 mm from the apex in untreated seedlings at a ψ_w of -1.6 MPa and is shortened in fluridone-treated seedlings (Sharp et al., 1988; Saab et al., 1992). Harvesting and extraction procedures and assay validation were described in Saab et al. (1990) and Sharp et al. (1994).

Ethylene Evolution

Ethylene evolution rate was measured from intact seedlings using a continuous flow-through system. After germination, up to 35 seedlings were transplanted into a Plexiglas cylinder (2 L) containing vermiculite at a ψ_w of -1.6 MPa. The cylinder was fitted with a lid containing a rubber O-ring, an air inlet at the bottom, and an air outlet at the top. Ethylene-free air flowed through the chamber at a rate of 40 mL min^{-1} . To maintain the ψ_w of the vermiculite, the relative humidity of the air was increased prior to entering the chamber by bubbling through water at 50°C. Measure-

ments showed that the vermiculite ψ_w decreased by only about 0.1 MPa during the 48-h experiments.

At various times after transplanting, samples (60 or 120 mL) of the exiting air stream were collected with syringes, and the ethylene was trapped by injection into a sample loop containing 100 mg of absorbent (Porapak S, Supelco, Bellefonte, PA) and kept at -95°C with melting acetone (De Greef et al., 1976). The sample loop was then heated with boiling water to release the ethylene into the carrier gas (helium) stream and onto a packed alumina F_1 column of a gas chromatograph (model no. 3400cx, Varian, Palo Alto, CA) equipped with a photo-ionization detector (lamp energy 10.6 eV). In preliminary experiments for all treatments, the putative ethylene peak was confirmed to be an olefin by its ability to be trapped in a HgClO_4 solution and released with the addition of LiCl (Young et al., 1952). In subsequent experiments, the peak was identified by its retention time only. The rate of ethylene evolution from the chamber was divided by the number of seedlings to obtain the average rate per seedling.

Statistical Analysis

Analyses of variance were performed with means compared using Fisher's LSD test at the $P = 0.05$ level.

RESULTS

Root Elongation of Fluridone-Treated Seedlings

Root elongation of seedlings treated with $1.5 \mu\text{M}$ fluridone was inhibited by about 45% compared with untreated seedlings after transplanting to a ψ_w of -1.6 MPa (Figs. 1–3). This was associated with a large decrease in the accumulation of ABA in the apical 6 mm (Fig. 1 inset; the ABA level at high ψ_w is 10 to $20 \text{ ng g}^{-1} \text{ H}_2\text{O}$ in both fluridone-treated and untreated root tips [Saab et al., 1990, 1992]). Preliminary experiments in which a range of ABA concentrations were mixed with the vermiculite into which the seedlings were transplanted determined that an exogenous ABA concentration of 0.5 mM was optimal for restoration of root elongation in seedlings treated with $1.5 \mu\text{M}$ fluridone. Figure 1 shows that the addition of 0.5 mM ABA almost fully prevented the inhibition of root elongation, in association with substantial restoration of the root tip ABA level (Fig. 1, inset). Root elongation was restored by 83% at 49 h after transplanting. These results show that virtually all of the inhibition of root elongation caused by $1.5 \mu\text{M}$ fluridone is attributable to ABA deficiency. The requirement for such a high applied ABA concentration to restore the internal ABA content was due to limited uptake from the dry vermiculite (Sharp et al., 1994).

In previous work in which fluridone was applied at $10 \mu\text{M}$, root elongation was inhibited about 65%, and a higher applied ABA concentration (0.7 mM) and a longer time after transplanting were required to restore the root elongation rate (Sharp et al., 1994). The faster restoration in the present experiments was probably due at least partly to longer root lengths at transplanting, which increased the root surface area for ABA uptake and may have slowed the

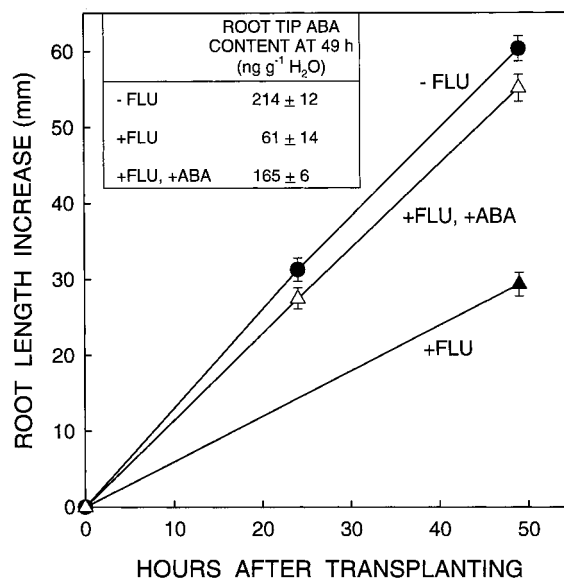


Figure 1. Effect of treatment with fluridone (FLU) with or without the addition of ABA to the vermiculite on root length increase after transplanting to a ψ_w of -1.6 MPa. Length increase at 24 h was determined by marking the position of the root apices (in the +FLU treatment, many roots grew into the vermiculite and were thus not visible), and at 49 h by destructive harvesting. Data are means \pm SE ($n = 11$ –16). The experiment was repeated several times with similar results. Inset, ABA content of the root apical 6 mm at 49 h. Data are means \pm SE of five samples of three root tips each.

rate of stress development (and therefore ABA deficiency) by providing a larger internal source of water.

Inhibitors of Ethylene Synthesis or Action Restore Root Elongation of Fluridone-Treated Seedlings

If the inhibition of root elongation in ABA-deficient seedlings at low ψ_w is caused by ethylene, then elongation should be at least partly restored by inhibitors of ethylene synthesis or action. Therefore, the effects of AOA and AVG, which inhibit ethylene synthesis, and STS, which inhibits ethylene action, were individually examined. The use of three inhibitors was a precaution because of possible side effects of each compound (Abeles et al., 1992). Preliminary experiments determined that the optimum concentrations for restoration of root elongation in fluridone-treated seedlings were $732 \mu\text{M}$ AOA, $43 \mu\text{M}$ AVG, and 2.5 mM STS. A typical experiment using AOA is shown in Figure 2, and the mean results from several such experiments using each of the inhibitors are shown in Figure 3. Representative seedlings are illustrated in Figure 4.

Treatment with AOA almost completely prevented the inhibition of root elongation rate in fluridone-treated seedlings in the 42 h after transplanting to a ψ_w of -1.6 MPa (Fig. 2). The mean restoration of root length increase compared with untreated roots at the end of the experiments was 95% (Figs. 3 and 4). The AOA treatment also caused a small (11%) increase in root elongation of control seedlings. Accordingly, restoration from the effect of fluridone was lessened to 75% compared with the AOA-treated control.

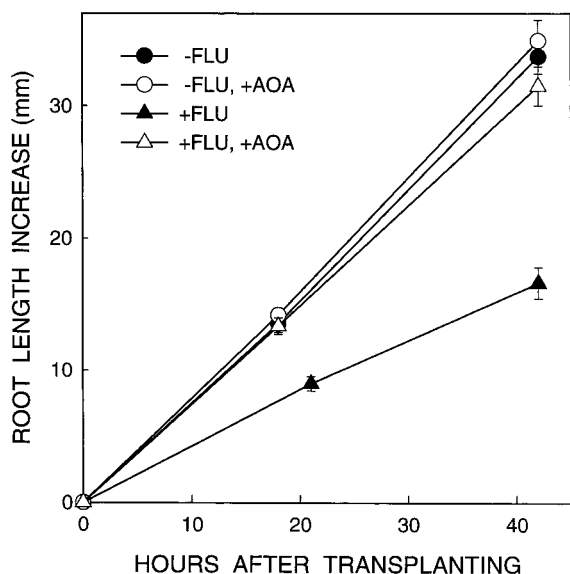
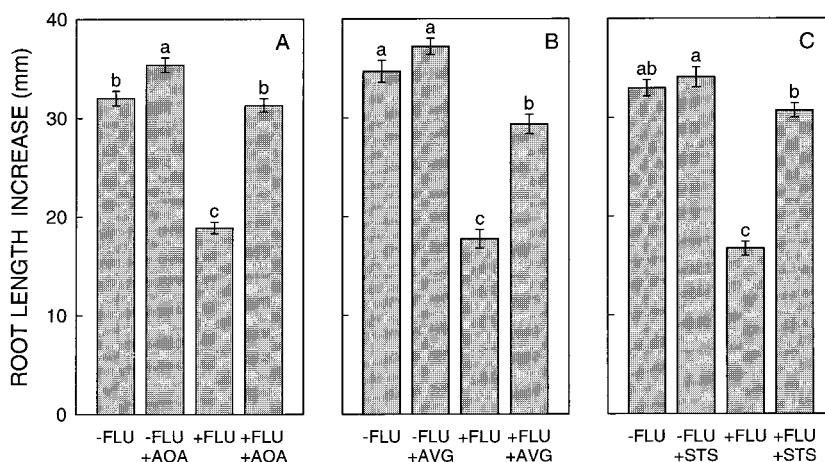


Figure 2. Effect of treatment with AOA on root length increase of untreated and fluridone (FLU)-treated seedlings after transplanting to a ψ_w of -1.6 MPa. Length increases were determined as described in Figure 1, plus an additional harvest of +FLU seedlings was made at 21 h. Data are means \pm SE ($n = 16-18$).

However, the absolute promotion of root length by AOA was much greater for fluridone-treated seedlings (12.4 mm) than for the control (3.4 mm).

Results with AVG and STS were similar. Root elongation of fluridone-treated seedlings was restored by 69% and 86%, respectively, compared with untreated seedlings (Figs. 3 and 4). AVG and STS also caused a slight (not significant) increase in root elongation of control seedlings, so that restoration from the effect of fluridone was reduced to 60% and 81%, respectively, compared with the inhibitor-treated controls. As with AOA, the absolute increases in root length caused by AVG and STS were much greater for fluridone-treated seedlings (11.6 and 14.0 mm, respectively) than for the controls (2.5 and 1.1 mm, respectively). Preliminary experiments using seedlings treated with 10 μ M fluridone showed that root elongation could also be substantially restored with 2,5-norbornadiene, a competitive

Figure 3. Effect of treatment with AOA (A), AVG (B), and STS (C) on root length increase of untreated and fluridone (FLU)-treated seedlings 42 h after transplanting to a ψ_w of -1.6 MPa. Data are means \pm SE ($n = 54-91$) combined from three to five experiments. Within each panel, bars with different letters are significantly different at the 0.05 level.



inhibitor of ethylene binding, and both α -aminoisobutyric acid and CoCl_2 , which inhibit ACC oxidase (data not shown).

To ensure that the inhibitors of ethylene synthesis or action did not restore root elongation of fluridone-treated seedlings by restoring root tip ABA levels, the ABA content of the apical 10 mm was measured 20 h after transplanting in all treatments (Table I). Neither AOA, AVG, nor STS had any effect on the ABA content with or without treatment with fluridone. It should be noted that the ABA contents of untreated and fluridone-treated root tips shown in Table I are lower than those shown in Figure 1, because the apical 10 and 6 mm, respectively, were measured. Previous work showed that the ABA content increases steeply in the apical few millimeters of roots at low ψ_w (Saab et al., 1992).

The results shown in Figures 1 to 4 and Table I indicate that the inhibition of root elongation caused by ABA deficiency in fluridone-treated seedlings at low ψ_w is largely due to ethylene.

Ethylene Evolution Is Increased in Fluridone-Treated Seedlings

Measurements of ethylene evolution rate were made from whole seedlings to assess whether ABA deficiency at low ψ_w causes an increase in ethylene production. Shoot development was minimal at the time of transplanting and was completely inhibited thereafter, and seminal root development during the experiments was limited (Fig. 4). Therefore, the measurements reflect rates of ethylene evolution from the primary root plus an unknown contribution from the kernel.

Figure 5 shows that fluridone-treated seedlings exhibited a 5-fold increase in ethylene evolution rate compared with untreated seedlings at 20 h after transplanting to a ψ_w of -1.6 MPa. This declined to a 3-fold enhancement at 40 h, which is consistent with previous observations that the effects of treatment with fluridone on ABA accumulation and root elongation decrease with time after transplanting (Saab et al., 1990; Sharp et al., 1994). This probably reflects progressive dilution and/or metabolism of the fluridone absorbed during imbibition, combined with limited fluridone uptake from the dry vermiculite.

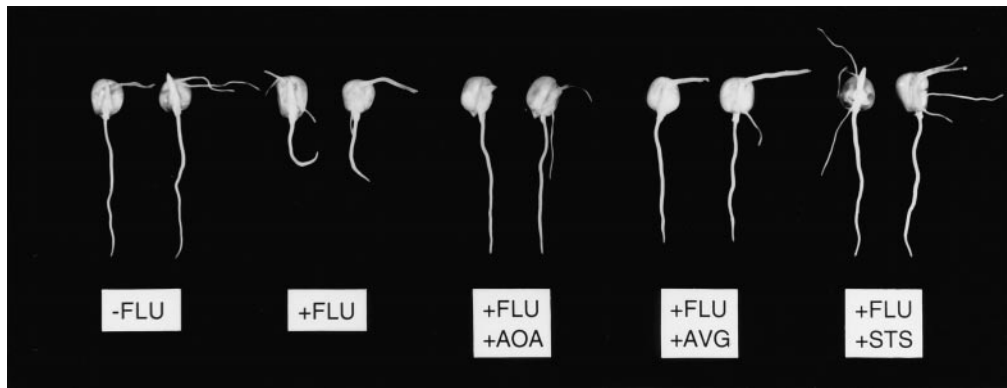


Figure 4. Representative seedlings treated with or without fluridone (FLU), or with fluridone and AOA, AVG, or STS, 42 h after transplanting to a ψ_w of -1.6 MPa. Root lengths of selected seedlings were within $\pm 11\%$ of the treatment means in the particular experiments.

Treatment with AOA completely prevented the fluridone-induced increase in ethylene evolution at both 20 and 40 h after transplanting (Fig. 5). In these experiments, root elongation was inhibited by 47% in fluridone-treated compared with untreated seedlings, and AOA restored elongation of fluridone-treated roots by 78% and 76%, respectively, compared with untreated and AOA-treated controls. These effects are very similar to those described in Figures 2 and 3, showing that the continuous flow of air that was used to collect evolved ethylene did not affect the results. Treatment with AOA also caused a small decrease in the ethylene evolution rate of control seedlings, although this effect was not significant at either time. This may have caused the slight promotion of root elongation in this treatment (Fig. 3). The differences in ethylene evolution rate among treatments were not attributable to differences in seedling fresh weight, which were similar in all cases (Fig. 5, legend).

To confirm that the increase in ethylene evolution rate from fluridone-treated seedlings was due to ABA deficiency and not to other effects of fluridone, exogenous ABA was added at a concentration of 0.5 mM, the same concentration used to restore root elongation (Fig. 1). Figure 6 shows that this treatment completely prevented the fluridone-induced increase in ethylene evolution from 12 to 48 h after transplanting to low ψ_w . The time course of the effect of fluridone on ethylene evolution was similar to that shown in Figure 5, but the increase was less pronounced, perhaps because of the different culture protocol used (see "Materials and Methods").

The ability of both AOA and ABA to prevent the increase in ethylene evolution and the inhibition of root elongation in fluridone-treated seedlings indicates that increased ethylene production is an important cause of root growth inhibition in ABA-deficient seedlings at low ψ_w .

Root Elongation and Ethylene Evolution in *vp5* Seedlings

Similar studies were conducted with the *vp5* mutant to strengthen the validity of the fluridone experiments. Root elongation of the mutant was severely inhibited (by 67%) compared with the wild type after transplanting to a ψ_w of -1.6 MPa (Fig. 7). This was associated with a greatly

decreased level of ABA in the apical 10 mm (Fig. 7, inset). These effects were more pronounced than in fluridone-treated seedlings (Figs. 1–3; Table I) and were associated with a greater increase in ethylene evolution. The ethylene evolution rate per seedling increased by 10-fold to 40 pmol h^{-1} in *vp5* relative to wild type at 20 h after transplanting (Fig. 8), compared with a 5-fold increase to 27 pmol h^{-1} observed in fluridone-treated seedlings grown under the same conditions (Fig. 5). Furthermore, in *vp5* seedlings the increase was sustained at 40 h, in contrast to the decline observed in the fluridone treatment. Since the mutant (and wild-type) seedlings weighed only about 60% as much as the hybrid seedlings (Figs. 5 and 8, legends), the greater ethylene evolution rate of *vp5* compared with fluridone-treated seedlings was even more pronounced on a fresh weight basis.

Treatment with AOA (732 μM , as used in the fluridone experiments) caused a significant restoration of root elongation in *vp5* seedlings (Fig. 7), but less than the almost complete restoration observed in experiments with fluridone (Fig. 3). Elongation was restored by 42% and 47%, respectively, compared with untreated and AOA-treated wild-type seedlings (Fig. 7). The same restoration was obtained using 900 μM AOA, and higher concentrations were less effective. As in the fluridone experiments, the AOA treatment had no effect on the ABA content of the root tips of *vp5* or wild-type seedlings (Fig. 7, inset). Similar results

Table 1. Effect of treatment with AOA, AVG, or STS with or without treatment with fluridone (FLU) on the ABA content of the root apical 10 mm, 20 h after transplanting to a ψ_w of -1.6 MPa

Data are means \pm SE of five samples of three root tips each. The experiment was repeated with similar results.

Inhibitor	Root Tip ABA Content	
	-FLU	+FLU
	$ng\ g^{-1}\ H_2O$	
None	140 \pm 8	44 \pm 4
AOA	149 \pm 6	39 \pm 2
AVG	129 \pm 10	39 \pm 2
STS	146 \pm 9	36 \pm 2

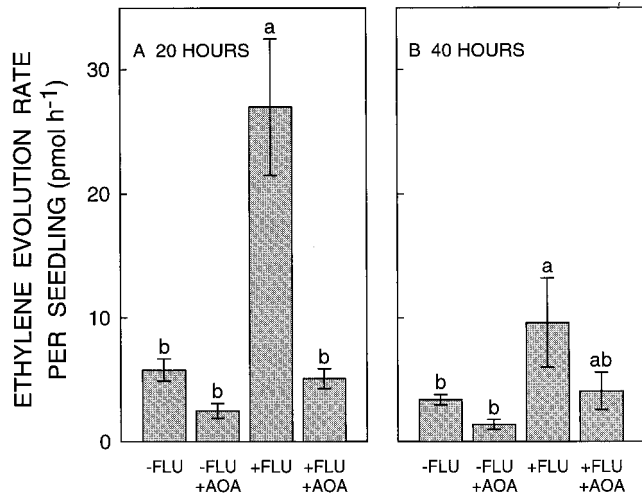


Figure 5. Ethylene evolution rate from untreated and fluridone (FLU)-treated seedlings with or without treatment with AOA at 20 h (A) and 40 h (B) after transplanting to a ψ_w of -1.6 MPa. Data are means \pm SE of four experiments. Within each panel, bars with different letters are significantly different at the 0.05 level. At 40 h, mean seedling fresh weight ranged from 578 to 589 mg in the different treatments.

were obtained in one experiment using STS, in which the maximum restoration of elongation was 28% and 32%, respectively, compared with untreated and STS-treated wild-type seedlings (data not shown).

Measurements of ethylene evolution suggest that the incomplete restoration by AOA of root elongation in *vp5* seedlings was due to incomplete prevention of the increase in ethylene evolution. At both 20 and 40 h after transplanting, AOA prevented only 55% of the increase (Fig. 8), in contrast to the complete prevention observed in fluridone-

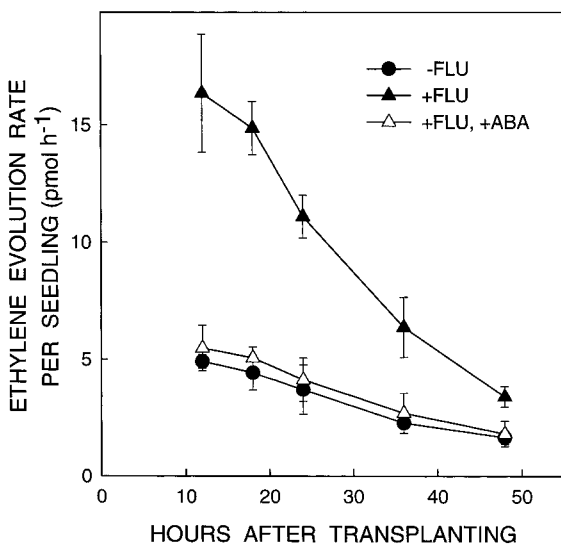


Figure 6. Effect of treatment with fluridone with or without the addition of ABA to the vermiculite on seedling ethylene evolution rate after transplanting to a ψ_w of -1.6 MPa. Data are means \pm SE of three experiments. In these experiments, the ABA treatment restored root elongation by 68% at 48 h.

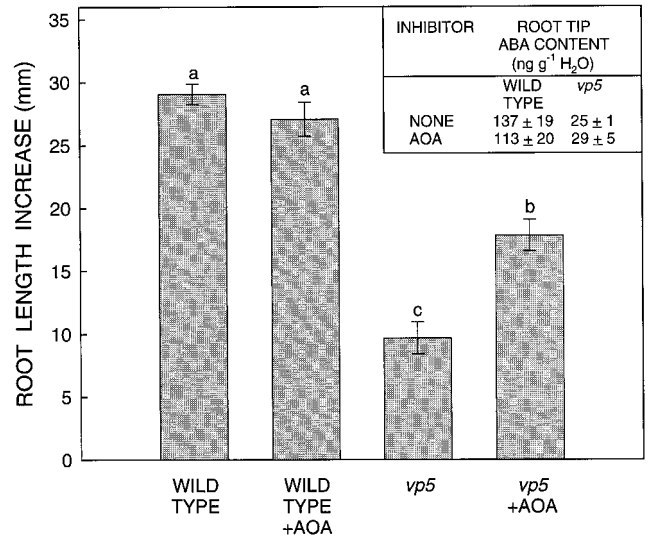


Figure 7. Root length increase of wild-type and *vp5* seedlings with or without treatment with AOA 40 h after transplanting to a ψ_w of -1.6 MPa. Data are means \pm SE ($n = 12-21$). Bars with different letters are significantly different at the 0.05 level. Inset, ABA content of the root apical 10 mm at 40 h. Data are means \pm SE of three samples of three root tips each. The experiment was repeated with similar results.

treated seedlings (Fig. 5). In these experiments, root elongation was restored by 37% and 32%, respectively, compared with untreated and AOA-treated wild-type seedlings. The difference in effectiveness of AOA between the *vp5* and fluridone experiments suggests that the *vp5* mutation causes a more pervasive inhibition of ABA content, such that AOA did not penetrate to all the ABA-deficient cells. Consistent with this explanation, in an ex-

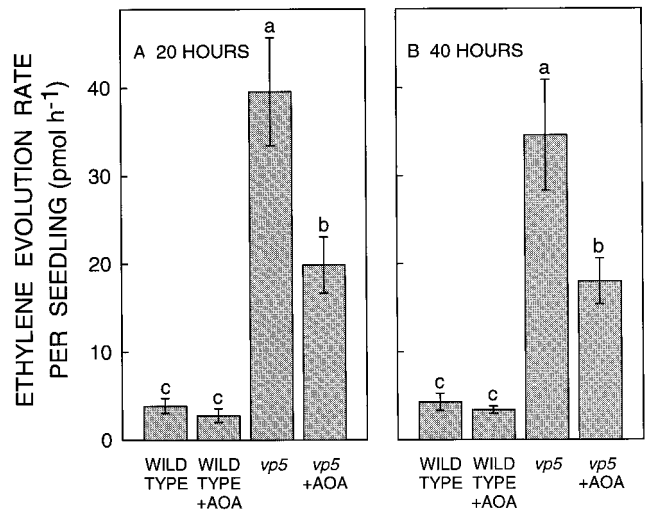


Figure 8. Ethylene evolution rate from wild-type and *vp5* seedlings with or without treatment with AOA at 20 h (A) and 40 h (B) after transplanting to a ψ_w of -1.6 MPa. Data are means \pm SE of four wild-type and six *vp5* experiments. Within each panel, bars with different letters are significantly different at the 0.05 level. At 40 h, mean seedling fresh weight ranged from 323 to 337 mg in the different treatments.

periment using seeds from a single ear, AOA restored root elongation in fluridone-treated wild-type seedlings by 76% but in *vp5* seedlings by only 34% (data not shown).

The large increase in ethylene evolution rate from *vp5* seedlings and the partial recovery of both root elongation and ethylene evolution by treatment with AOA are consistent with the conclusion that increased ethylene production is an important cause of the inhibition of root elongation in ABA-deficient seedlings at low ψ_w .

Morphology of ABA-Deficient Roots at Low ψ_w

In addition to the inhibition of elongation, other morphological characteristics of the ABA-deficient roots at low ψ_w were consistent with an involvement of increased ethylene production. Compared with untreated or wild-type seedlings, root tips of fluridone-treated and *vp5* seedlings grown at a ψ_w of -1.6 MPa were swollen primarily beyond the apical 2 mm (Fig. 9). Exogenous ethylene inhibits elongation and causes a similar pattern of swelling in maize primary roots at high ψ_w (Moss et al., 1988; Whalen and Feldman, 1988). In both fluridone-treated and *vp5* roots, most of the increase in diameter resulted from a greater expansion of the cortex (data not shown). Root tip swelling resulting from treatment with ethylene is also attributable primarily to increase in cortical thickness in roots of maize (Whalen and Feldman, 1988) and barley (Jackson, 1983). In addition, the ABA-deficient roots exhibited a loss of vertical orientation (Fig. 4), which also results when maize roots are treated with ethylene (Curtis, 1968). Both the swelling and the loss of vertical orientation were largely prevented by the addition of 0.5 mM ABA and by treatment with AOA, AVG, and STS (Fig. 4) (determined by visual assessment at the end of the experiments).

In the fluridone-treated roots, swelling became maximal at 10 mm from the apex, where the diameter was 50%

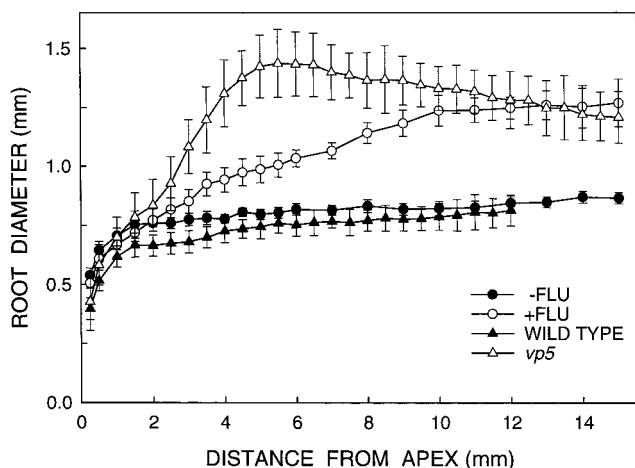


Figure 9. Diameter as a function of distance from the apex for roots of untreated and fluridone-treated hybrid seedlings (40 h after transplanting, $n = 5$) and of wild-type and *vp5* seedlings (48 h after transplanting, $n = 6-8$) growing at a ψ_w of -1.6 MPa. Data are means \pm SE of roots that elongated within $\pm 10\%$ of the mean rate from at least 20 seedlings per treatment.

greater than that of untreated roots (Fig. 9). In *vp5* roots, the increase in diameter was more pronounced and occurred more steeply, reaching a maximum that was 90% greater than that of the wild type at 5.5 mm from the apex. The diameter of the *vp5* roots decreased steadily at greater distances from the apex, but was still larger than the wild type at 15 mm. The difference in pattern between the fluridone-treated and *vp5* roots is consistent with the decreasing effect of fluridone on ethylene evolution with time after transplanting (Figs. 5 and 6). In contrast, the diameter profile of the *vp5* roots indicates that the effect of the mutation increased with time, probably as the tissue water status declined.

DISCUSSION

The combined results of this study provide compelling evidence that an important role of endogenous ABA accumulation in the maintenance of maize primary root elongation at low ψ_w is to prevent excess ethylene production. When ABA deficiency was imposed at a ψ_w of -1.6 MPa by treatment with fluridone, root elongation was largely restored by three inhibitors of ethylene synthesis or action, demonstrating that the inhibition of root elongation was primarily attributable to ethylene. The ethylene evolution rate of fluridone-treated seedlings increased several fold, and this effect was prevented when ABA was supplied at a concentration that restored both the ABA content of the root elongation zone and the root elongation rate. The consistent results obtained when ABA deficiency was imposed using the *vp5* mutant confirm that the increase in ethylene production was not a side effect of the use of fluridone. It should be noted that our findings do not exclude the possibility that the sensitivity of root elongation to ethylene was also increased by ABA deficiency. This question is under investigation.

In addition, since none of the inhibitors of ethylene synthesis or action substantially increased root elongation when ABA deficiency was not imposed, the study also indicates that ethylene is not an important cause of the inhibition of elongation in water-stressed roots that accumulate normal levels of ABA. (At a ψ_w of -1.6 MPa, maize primary root elongation is about one-third of the rate at high ψ_w [Sharp et al., 1988].) The possible involvement of ethylene in the inhibition of growth during water stress is a long-standing question (El-Beltagy and Hall, 1974), but to our knowledge there is no previous information in relation to root growth.

Relationship of the ABA-Ethylene Interaction to Other Processes of Root Elongation

In previous work, ABA accumulation was shown to be required for two other responses thought to contribute to the ability of the maize primary root to elongate at low ψ_w . These are increases in the activity of the putative wall-loosening enzyme xyloglucan endotransglycosylase (XET) (Wu et al., 1994) and in the concentration of Pro (Ober and Sharp, 1994) within the elongation zone. A preliminary study suggested that the inhibitory effects of ABA defi-

ciency on these responses are not caused by the increase in ethylene production. In fluridone-treated seedlings supplied with AOA, neither the activity of XET nor the concentration of Pro was restored (Sharp et al., 1998). This was probably not due to toxic effects of AOA because there was little effect on XET activity or Pro level in roots of control seedlings. Since AOA almost completely restored root elongation (Fig. 3), the increases in XET activity and Pro concentration (at least to their normal extent) are apparently not essential for root elongation at low ψ_w (discussed further in Sharp et al., 1998).

At low ψ_w , the maize primary root becomes thinner, which is believed to be adaptive by increasing the efficiency of resource utilization in the exploration of new soil volume for water (Sharp et al., 1988; Liang et al., 1997). The mechanism of this response is unknown (Baskin et al., 1999). Since ethylene increases lateral expansion of roots (Moss et al., 1988; Whalen and Feldman, 1988), it is tempting to speculate that root thinning at low ψ_w is related to the restriction of ethylene production by ABA accumulation. However, the pattern of swelling in the ABA-deficient roots suggests that this effect was not a reversal of water-stress-induced thinning. First, the diameter of the apical millimeter was minimally responsive to ABA deficiency (Fig. 9; Saab et al., 1992), whereas this region exhibits the largest decrease in lateral expansion rates at low ψ_w (Liang et al., 1997). Second, the *vp5* roots at low ψ_w exhibited a maximum diameter that exceeded that of *vp5* or wild-type roots at high ψ_w (data not shown), despite having a higher ABA content (Fig. 7, inset; Saab et al., 1990). These observations make it unlikely that root thinning at low ψ_w is attributable to the restriction of ethylene production by ABA accumulation.

Generality of the ABA-Ethylene Interaction

Our results confirm that an important role of endogenous ABA accumulation is to limit ethylene production. Moreover, to our knowledge, the study provides the first demonstration that this interaction is involved in the effects of ABA status on plant growth. These ideas were first suggested by Wright (1980) and developed further by Bradford and Hsiao (1982), and were based on the finding that pretreatment with ABA prevented the increase in ethylene production caused by wilting of excised wheat leaves. Results of such experiments have been diverse, however. Although several other studies reported that ABA treatments inhibited ethylene production, there are also many reports of ABA-stimulated ethylene production (Riov et al., 1990 and references therein). Interpretation of these results is further complicated by the uncertainty that effects of applied ABA at high ψ_w are predictive of the role of endogenous ABA accumulation at low ψ_w (Trewavas and Jones, 1991; Sharp et al., 1994), and because in most cases excised tissues were employed. Morgan et al. (1990) and Narayana et al. (1991) have shown that the use of excised plant parts can lead to erroneous conclusions in studies of ethylene production, particularly concerning the effects of water stress. Our approach, using chemical and genetic means to manipulate endogenous ABA levels at

low ψ_w , and our use of intact seedlings for ethylene measurements, avoided these concerns.

Consistent with our results, it was reported that ethylene production is enhanced in ABA-deficient mutants of tomato (Tal et al., 1979) and Arabidopsis (Rakitina et al., 1994) grown under well-watered conditions. In the *flacca* mutant of tomato, it was also shown that ethylene production could be restored to normal levels with exogenous ABA. However, it is uncertain whether the increase in ethylene evolution in those studies was a direct result of ABA deficiency, or if it was an indirect effect of decreased plant water status. Under transpiring conditions, ABA-deficient mutants typically exhibit high stomatal conductance and wilting (Arabidopsis: Koornneef et al., 1982; tomato: Bradford, 1983), and there are many reports that ethylene production can be increased by plant water deficits (but see Morgan et al. [1990]). In fact, Tal et al. (1979) showed that the greater ethylene evolution of *flacca* was partially to fully prevented (depending on plant age) by growing the plants at high humidity. In the present study, the seedlings were grown under conditions of near-zero transpiration (minimal shoot development, darkness, and near-saturation humidity). Accordingly, the increase in ethylene production resulting from ABA deficiency under water stress was not an indirect effect of differences in stomatal control of plant water balance between treatments.

The possibility that an interaction with ethylene production may be involved in the effects of ABA deficiency on growth has not to our knowledge been assessed in previous studies. Leaf and root growth of ABA-deficient mutants are often substantially inhibited compared with the corresponding wild types (Quarrie, 1987). The mutants of tomato also exhibit morphological symptoms characteristic of excess ethylene, such as leaf epinasty and adventitious rooting (Tal, 1966; Nagel et al., 1994). However, several authors have attributed the inhibition of leaf growth in the tomato mutants to their adverse water relations (Bradford, 1983; Neill et al., 1986; Trewavas and Jones, 1991; Nagel et al., 1994). We have recently shown that when *flacca* is grown throughout development at the same leaf ψ_w as well-watered wild-type plants, leaf growth remains greatly inhibited but can be substantially restored by applying ABA or STS (Sharp, et al., 2000). These results indicate that normal ABA levels are required to prevent ethylene-induced inhibition of leaf growth in tomato, at least in well-watered plants, in agreement with the findings of the present study for water-stressed maize roots.

In contrast, our previous work with maize seedlings showed that ABA deficiency at low ψ_w (either in the *vp5* mutant or imposed using fluridone) was associated with increased shoot growth, indicating that ABA accumulation was causally related to shoot growth inhibition (Saab et al., 1990, 1992). This effect of ABA also appears to involve a restriction of ethylene synthesis or sensitivity: preliminary experiments showed that fluridone-induced growth promotion could be prevented by treatment with STS, and that shoot growth could also be increased by applying ACC or ethylene (Feng, 1996). These findings are

consistent with reports that ethylene stimulates mesocotyl growth in some species (Suge, 1971; Cornforth and Stevens, 1973).

The commonality of these observations suggests that restriction of ethylene production may be a widespread function of ABA. Depending on the response to ethylene of the organ in question, ABA accumulation may thereby play a role in growth maintenance or inhibition in response to a range of adverse environmental conditions.

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