Plant Physiol. (1996) 112: 379–384

NaCl Reduces Indole-3-Acetic Acid Levels in the Roots of Tomato Plants Independent of Stress-Induced Abscisic Acid[1]

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Indole-3-acetic acid (IAA) was measured in leaves and roots of tomato (*Lycopersicon esculentum*) genotypes subjected to salt stress. An abscisic acid (ABA)-deficient mutant of tomato (*sitiens*), the genetic parent (Rheinlands Ruhm, RR), and a commercial variety (Large Cherry Red, LCR) of tomato were treated with 50 to 300 mM NaCl in nutrient culture. Both LCR and RR had significantly higher levels of IAA in the roots compared with that in *sitiens* prior to treatment. The initial levels of IAA in the roots of LCR and RR declined by nearly 75% after exposure to NaCl, whereas those in roots from the *sitiens* mutant remained unchanged. IAA levels in the leaves of all genotypes remained unchanged or increased slightly in response to NaCl. ABA was highest in leaves from the normal genotypes after exposure to NaCl. ABA levels in the roots of *sitiens* were similar to the levels in the normal genotypes, whereas levels in the leaves were only 10% of the levels found in normal genotypes regardless of the salt treatment. Treatment of LCR and *sitiens* with exogenous ABA increased the ABA levels in leaves and roots, but there were no measurable changes in endogenous IAA. Therefore, the reduction in IAA appears to result from an ABA-independent effect of NaCl on IAA metabolism in the roots of stressed plants.

Endogenous levels of ABA increase in response to a variety of stresses, including drought, salinity, and low temperature; and ABA is thought to modulate the response of plants to these stresses (Zeevaart, 1988). The effects of temperature and water stress on tomato (*Lycopersicon esculentum*) appear to be mediated by ABA (Daie and Campbell, 1981; Bray, 1990). The response to salt stress in plants often mimics the symptoms associated with temperature stress and water deficit. Drought, PEG-mediated water deficit, NaCl, and cold stress induced a 2- to 4-fold increase in the ABA content of tomato leaves (Plant et al., 1991). In this same study, the *le16* gene of tomato induced by drought, low temperature, and ABA also was induced by NaCl. The similarity of responses suggests that ABA may be a common signal for mediating the response to all three environmental stresses in tomato (Daie and Campbell, 1981).

Auxins apparently function in the same way in tomato as they do in many other plants: they promote root growth, maintain apical dominance, and regulate phototropic and gravitropic behavior (Moore, 1989). The role of auxins in tomato development has been investigated using the *dgt* mutant (Daniel et al., 1989). The *dgt* mutant exhibits horizontal growth habit, thin stems, hyponastic leaves, lack of lateral roots, reduced ethylene production, and insensitivity to auxin (Ursin and Bradford, 1989). The inability of auxin to induce growth in the *dgt* mutant apparently results from a defect in auxin-induced cell-wall loosening (Daniel et al., 1989), since the endogenous levels of IAA are the same in the mutant and wild-type parent (Fujino et al., 1988).

Although well documented as a regulator of plant growth, IAA has not received the attention devoted to ABA in the search for endogenous chemical mediators of salinity or water stress responses in plants (Mansfield and McAinsh, 1995). In an early study of RR and the ABA-deficient mutant *flacca*, Tal and Imber (1971) reported that IAA may play a role similar to ABA in protecting tomato plants against water deficit and declining plant turgor. IAA apparently increases stomatal resistance and decreases the resistance to water movement in the roots. Based on the results of a series of studies, Tal and Imber (1970, 1979) have suggested that IAA may function as part of the water-deficit signaling mechanism in tomato plants. Blatt and Thiel (1994) recently reported that the mechanism for auxin-induced stomatal opening exhibited the same pattern of stimulus-response coupling found in ABA-induced stomatal closing.

ABA and IAA may interact to signal changes in plant functions initiated by stress conditions that alter endogenous turgor (Dunlap and Robacker, 1990; Guinn et al., 1990). Therefore, we conducted experiments to examine the possible dependence of auxin metabolism on stress-induced increases in ABA levels. Using an ABA-deficient *sitiens* mutant and exogenous applications of ABA, we measured changes in the concentration of IAA and ABA in the roots and leaves from plants stressed by exposure to NaCl. In making these comparisons, we find that NaCl-induced changes in IAA levels or its distribution within the plant occur independent of increases in ABA.

[1] This paper reports research conducted by the Texas Agricultural Experiment Station, the Texas A&M University system. This research was supported in part by the National Research Initiative Competitive Grants Program, U.S. Department of Agriculture award no. 93-37100-8951.

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Abbreviations: dH_2O, distilled water; *dgt*, *diageotropica*; LCR, Large Cherry Red; RR, Rheinlands Ruhm.

MATERIALS AND METHODS

An ABA-deficient mutant of tomato (*Lycopersicon esculentum sitiens*), the parental origin of *sitiens*, RR, and a normal commercial variety, LCR, were used in treatments that included 50 to 300 mM NaCl in nutrient culture. Plants were grown in a hydroponic system with one-half-strength Hoagland solution (Epstein, 1972). Water was replenished daily and the nutrient solution was exchanged on a weekly basis. When plants had five to seven expanded leaves, saline treatments were initiated by increasing the NaCl concentration in increments of 50 mM d^{-1}. The ABA treatments were applied by amending the nutrient solution with 10 μM ABA (mixed isomers, Sigma) 48 h prior to commencing salinization. For experiments involving ABA, the nutrient solutions were replaced every 48 h.

IAA and ABA Analysis

Tissue samples were harvested, frozen in liquid N_2, and lyophilized. The dried tissues were ground and approximately 300 mg was placed in 20 mm × 150 mm glass tubes. These ground samples were mixed with 10 mL of freshly prepared 90% aqueous acetone (v/v) containing 0.01% ascorbic acid (w/v). The internal standards $^{13}C_6$-labeled IAA (Cohen et al., 1986) and $^2H_5$-labeled ABA (Roberts et al., 1968) were added to each sample at 250 and 750 ng, respectively. The samples were stirred overnight at 4°C. The extract was filtered through one layer of Whatman no. 2 paper in a Swinney attachment (Gelman Sciences, Ann Arbor, MI). The acetone was removed under a stream of N_2 at 40°C and the sample was reduced to the aqueous phase (approximately 200 μL). The sample was immediately cooled on ice for 2 to 3 min. After the addition of 1 mL of dH_2O, the sample was transferred to a 1.5-mL microfuge tube and centrifuged (model 5414, Eppendorf) at 12,000 rpm for 10 min at 4°C. The resulting supernatant was transferred into a 20 mm × 150 mm glass tube and diluted with the addition of 5 mL of dH_2O. The pH was adjusted to 7.0 by the dropwise addition of 0.1 N Na hydroxide while the mixture was continuously stirred.

Each sample was transferred to a 125-mL separatory funnel and partitioned twice against 10-mL volumes of dichloromethane and hexane. The aqueous residue was adjusted to pH 2.5 with 0.1 N hydrochloric acid and partitioned twice against 10-mL volumes of dichloromethane. The aqueous lower phase was discarded and the dichloromethane was collected in a 25 mm × 150 mm tube. A 3-mm glass bead and 200 μL of dH_2O were added to each sample. The dichloromethane was removed and the sample was reduced to the 200-μL aqueous phase by rotary evaporation at 40°C. The resulting aqueous phase was occasionally contaminated by an oily residue from selected tissue extracts. If this occurred, the residual 200 μL was partitioned against 2 mL of hexane and the hexane was discarded prior to any additional purification. In the absence of any immiscible substances, the aqueous residue was acidified with 4 mL of 1 mM hydrochloric acid and filtered through a 0.22-μm nylon syringe filter onto a C_{18} Sep-Pak cartridge (Waters). The microfilter was removed and the Sep-Pak cartridge was rinsed with 3 mL of 1 mM hydrochloric acid and 3 mL of dH_2O. The residual water was removed from the washed Sep-Pak cartridge by centrifugation at low speed followed by elution with 5 mL of methanol into a 7-mL glass vial. The sample was evaporated to dryness at 40°C under a stream of N_2. The dried sample was redissolved in 200 μL of methanol followed by the addition of 200 μL of diazomethane (Cohen, 1984). The solution was reacted for 15 min at room temperature and dried with a stream of N_2 at 40°C. The resulting methylated sample was solubilized in 100 μL of hexane/ethyl acetate (9:1, v/v).

A 2-μL sample was auto-injected in the splitless mode into a gas chromatograph (5890-Series II, Hewlett-Packard) equipped with a mass spectrometer (5971A, Hewlett-Packard). The 2-μL sample was chromatographed on a 10-m capillary column (J&W Scientific, Folsom, CA; DB-1701, 0.18-mm i.d. with a film thickness of 0.4 μm). Ion masses (m/z) associated with the $^{13}C_6$-labeled IAA internal standard (136, 195 m/z) and the endogenous IAA (130, 189 m/z) were selectively monitored and quantified by isotope dilution techniques (Cohen et al., 1986). Natural ABA with ion masses selectively monitored at 162 and 190 m/z was quantified by isotope dilution using $^2H_5$-labeled ABA (165, 193 m/z) as an internal standard (Roberts et al., 1968). Carrier flow was set at approximately 1 mL min^{-1} (column pressure = 10 psi) using He as a carrier. The oven temperature was programmed to start at 65°C for 0.5 min, then increased by 15°C min^{-1} up to 225°C, held for 3 min, increased to 250°C, and held for 5 min. The total run time was 19.67 min, and retention times for IAA and ABA were 11.2 and 13.4 min, respectively. The lower limit of detection for IAA and ABA was less than 3 ng g^{-1} of dried tissue.

Data Analysis

Each treatment was designed with at least three replicate tissue samples bulked from at least two plants. All treatments were repeated on a minimum of three different occasions. Each data point represents the mean ± SE for a treatment.

RESULTS

Hormone Distribution between Roots and Shoots

The levels of IAA in the commercial genotype LCR were higher in the roots (approximately 500 ng g^{-1} dry weight) than in the leaves but declined to approximately 100 ng after exposure to 300 mM NaCl (Fig. 1, bottom). The endogenous levels of IAA in the leaves remained near 75 ng g^{-1} dry weight regardless of the treatment. In contrast to IAA, ABA levels were higher in leaves than in roots and increased to 7000 ng g^{-1} dry weight after exposure to 300 mM NaCl (Fig. 1, top). ABA in the root system was approximately 100 ng g^{-1} dry weight in the nonstressed plants but increased to nearly 500 ng after exposure to 150 or 300 mM NaCl.

The initial levels of IAA in leaves and roots from RR were similar to those of LCR and declined to nearly the same levels at 300 mM NaCl (Fig. 2, bottom). ABA levels in

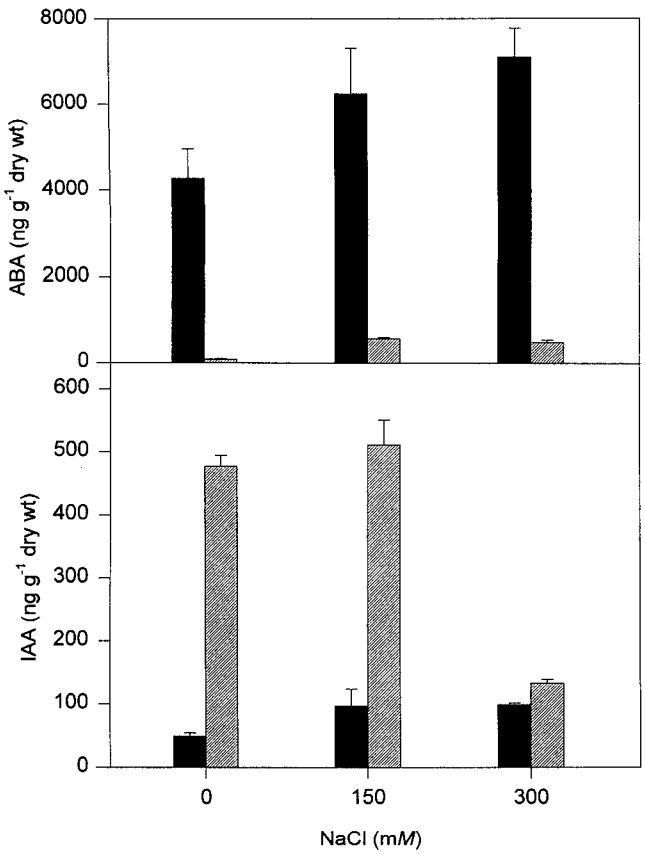

Figure 1. The levels of ABA (top) and IAA (bottom) in leaves (black bars) and roots (hatched bars) from the commercial genotype LCR exposed to 0, 150, or 300 mM NaCl. Each bar represents the treatment mean ± SE.

the roots and leaves of RR plants exposed to 0, 150, or 300 mM NaCl (Fig. 2, top) were nearly the same as the levels measured in LCR (see Fig. 1, top).

LCR and RR reflected similar changes in IAA and ABA and imply a similar if not equivalent hormonal response of the genotypes to salt stress. However, IAA levels in roots from nonstressed plants of the ABA-deficient mutant of RR, *sitiens*, were lower than the levels measured in the two normal genotypes (200 versus 500 ng g^{-1} dry weight, respectively). At concentrations above 150 mM, the *sitiens* plants exhibited severe damage and died within 48 h. The appearance and growth of *sitiens* plants cultured in 150 mM NaCl were comparable with the 300-mM response in the two normal genotypes. For these reasons, 150 mM NaCl was selected as the high-stress treatment for *sitiens*. IAA levels in the roots declined to approximately 100 ng during treatment with NaCl at the maximum concentration of 50 or 150 mM (Fig. 3, bottom). The levels of IAA in leaves increased from approximately 50 ng g^{-1} dry weight in nonstressed plants to 200 ng at 150 mM NaCl, which was a greater increase than that recorded for RR. Although less than the change in *sitiens*, there was a small increase in the IAA of LCR leaves from stressed plants that approached 100 ng g^{-1} dry weight. The levels of ABA in roots from nonstressed *sitiens* plants were equal to or even initially higher at approximately 300 ng g^{-1} dry weight (Fig. 3, top) than the levels found in the RR parent (Fig. 2, top) and LCR (Fig. 1, top). However, the ABA levels in *sitiens* roots remained constant despite increasing salt stress imposed by treatment with 50 and 150 mM NaCl. ABA levels in leaves from the *sitiens* mutant (Fig. 3, top) were approximately 10% of the levels measured in the parental genotype, RR, and the commercial genotype, LCR. ABA levels in the leaves of nonstressed *sitiens* plants were near 300 ng g^{-1} dry weight and did not increase in response to 150 mM NaCl (Fig. 3, top).

ABA Treatment of *sitiens* Plants

It was shown previously that even in the absence of salt stress the roots from the *sitiens* mutant were low in IAA relative to levels in the nonstressed normal genotypes (Fig. 3, bottom). Since the mutant is deficient in ABA, we considered the possibility that unimpaired biosynthesis of ABA present in normal genotypes might be responsible for the higher levels of IAA in the roots. The low endogenous levels of ABA in the mutant were elevated in the absence of salt stress by treating the plants with 10 μM ABA in nutrient culture. The levels of ABA were increased by more than 10-fold in the leaves and roots of *sitiens* plants receiving the ABA supplement (Fig. 4, top). However, IAA levels in the

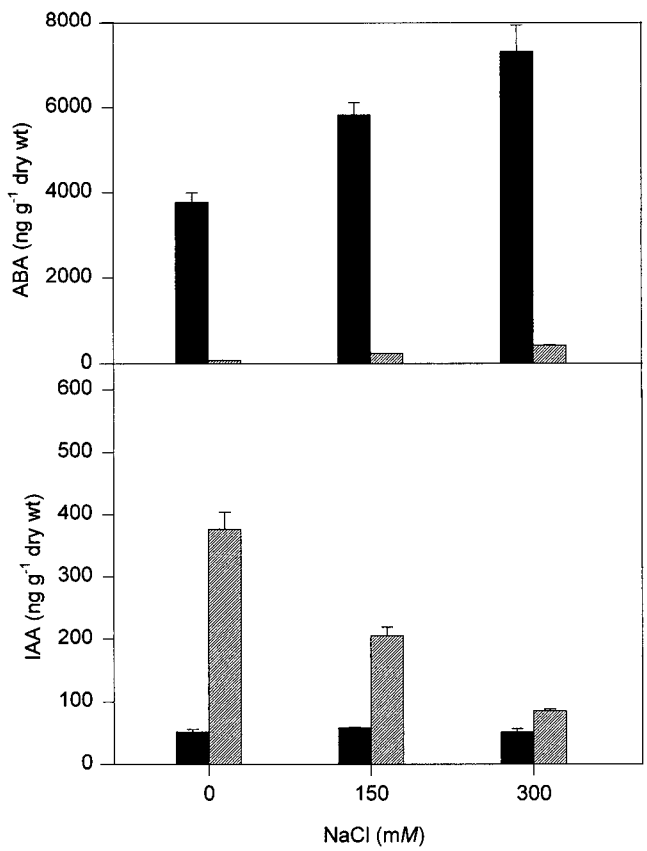

Figure 2. The levels of ABA (top) and IAA (bottom) in leaves (black bars) and roots (hatched bars) from the normal genotype RR exposed to 0, 150, or 300 mM NaCl.

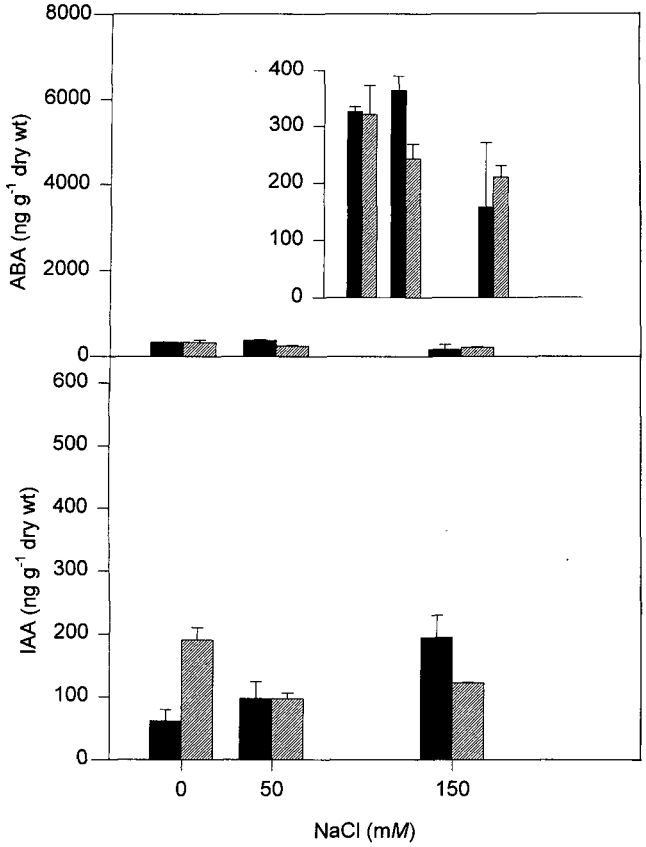

Figure 3. The levels of ABA (top) and IAA (bottom) in leaves (black bars) and roots (hatched bars) from the mutant genotype *sitiens* exposed to 0, 50, or 150 mM NaCl. Inset, The same data on an expanded scale.

roots and leaves of treated plants remained unchanged despite the large increase in endogenous ABA (Fig. 4, bottom).

ABA Treatment of LCR Plants

IAA levels in *sitiens* were not altered by manipulating the endogenous levels of ABA (Fig. 4, bottom). The alternative possibility is that excessive endogenous ABA may be effective only in normal genotypes in which the ABA biosynthetic apparatus remains intact. To examine the possible effect of endogenous ABA on IAA levels in roots and leaves of a normal genotype, LCR plants were treated with exogenous ABA.

The ABA levels in leaves from LCR plants increased more than 300% over the levels in nonstressed plants after treatment with a nutrient solution containing 10 μM ABA (Fig. 5, top). Similar results were obtained when plants were treated with a combination of 10 μM ABA and 300 mM NaCl. Treatment of the plants with only 300 mM NaCl nearly doubled the endogenous levels of ABA in the leaves. However, the treatment was not additive when applied in conjunction with 10 μM ABA, as indicated by the equivalent levels of ABA in leaves from plants treated with ABA and ABA + NaCl. Increasing the endogenous levels of ABA to near 10,000 ng g^{-1} dry weight by treating the plants with 10 μM ABA did not change the accumulated levels of IAA in leaves with respect to the untreated control. IAA levels in leaves treated with 300 mM NaCl increased to approximately twice the levels in the control plants (Fig. 5, bottom). The NaCl-induced increase in leaf IAA was unaffected by the addition of 10 μM ABA to the treatment.

The large increase in endogenous leaf ABA in LCR after treatment with exogenous ABA paralleled increases in root samples from the same plants. The endogenous ABA levels in the roots from ABA-treated plants were only slightly less than the levels measured in the leaves (Fig. 5, top). The elevated endogenous levels of ABA in roots from plants treated with ABA were reduced by the addition of 300 mM NaCl, whereas the NaCl treatment increased the endogenous levels of ABA in the roots of RR and LCR from 85 to nearly 600 ng g^{-1} dry weight (Figs. 1 and 2). IAA levels in the roots of plants treated with 10 μM ABA were not statistically different from the untreated control (Fig. 5, bottom). However, IAA levels in the roots were reduced by more than 60% of the control levels when NaCl was included in the 10 μM ABA treatment. The same reduction in root IAA levels occurred when ABA was excluded from the treatment, leaving only the 300 mM NaCl.

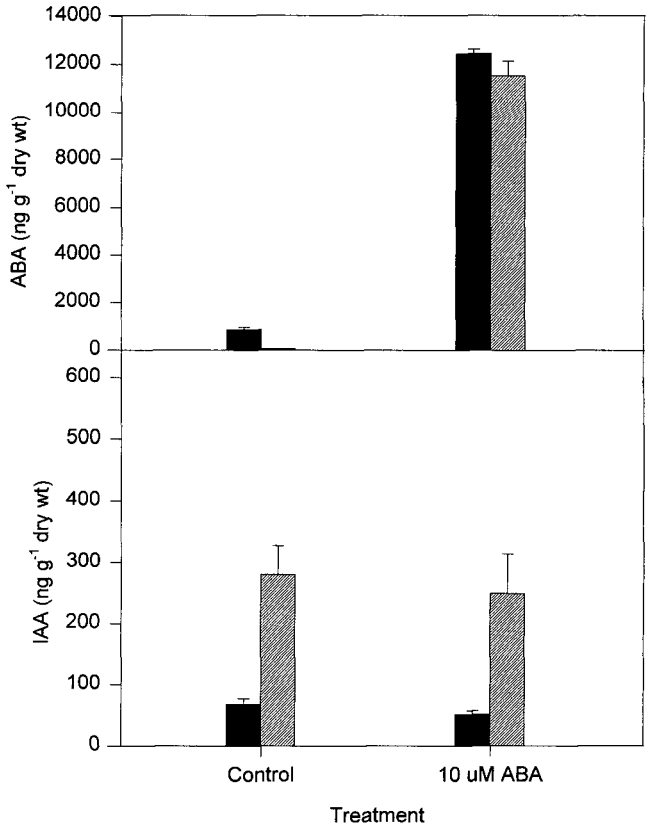

Figure 4. The levels of ABA (top) and IAA (bottom) in leaves (black bars) and roots (hatched bars) from the mutant genotype *sitiens* treated with 10 μM ABA applied in the nutrient solution.

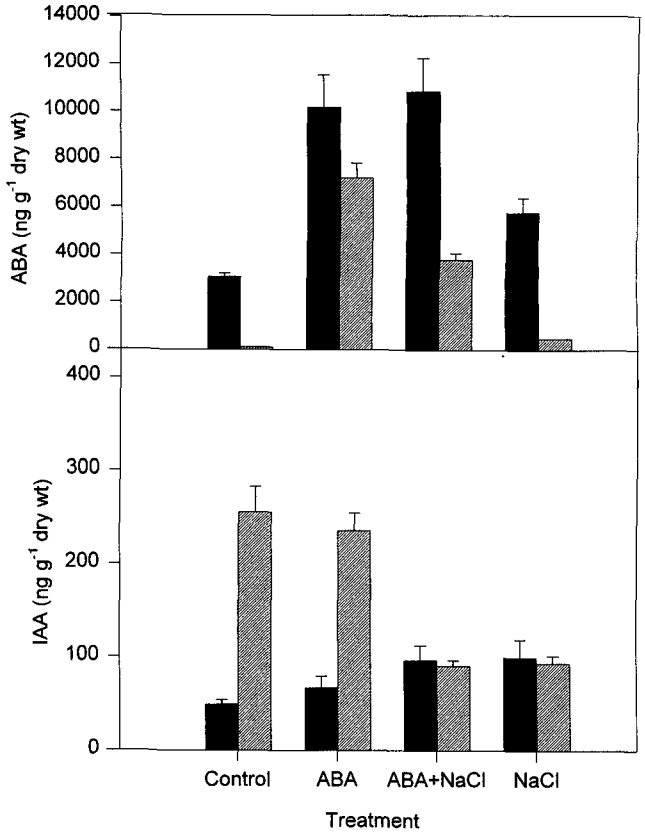

Figure 5. The levels of ABA (top) and IAA (bottom) in leaves (black bars) and roots (hatched bars) from the commercial genotype LCR treated with 10 μM ABA, 10 μM ABA + 300 mM NaCl, or 300 mM NaCl in nutrient culture.

DISCUSSION

The largest quantitative impact of salt stress on IAA levels occurred not in the leaves but in the root systems of both normal genotypes, LCR and RR. The endogenous IAA in roots was reduced by 75% or more of levels in the nonstressed normal genotypes by exposure to 300 mM NaCl (see Figs. 1 and 2, bottom). The endogenous levels of IAA in mature leaves remained unchanged or increased slightly. Thus, the results indicate that salt stress alters the metabolism of IAA and that the change occurs predominantly in the roots.

Are stress-induced changes in IAA metabolism mediated by ABA? Our data suggest that ABA is not playing a direct role in the regulation of auxin metabolism of roots from salt-stressed plants. The application of exogenous ABA to nonstressed plants (0 mM NaCl) failed to alter the endogenous levels of IAA in the roots or leaves. The stable endogenous levels of IAA were maintained despite large increases in root and leaf ABA. The reduction in root IAA levels after exposure to NaCl was not reversed by the presence of ABA in the treatment.

The quantitative data suggest that ABA is not regulating the observed changes in root IAA. The intent of our experimental design was to use data from the ABA-deficient mutant *sitiens* to support our initial proposal that endogenous ABA levels in the roots were not responsible for salt-induced changes in endogenous IAA. However, the ABA-deficient genotype expressed below-normal levels of IAA in the roots of nonstressed plants that typically occurred in salt-stressed normal genotypes. This observation is particularly interesting since *sitiens* is a single gene mutation of RR regulating ABA metabolism (Rick, 1980). Although the mutant was unable to accumulate normal levels of ABA in the leaves, normal or even above-normal levels of ABA were detected in the roots. It appears that the mutation responsible for the ABA deficiency functions primarily in the leaves of *sitiens*.

Despite the similarity in root ABA content, IAA levels in the root systems of nonstressed *sitiens* plants are much lower than levels in the normal genotypes. This observation provides additional evidence that IAA metabolism is not modified by the ABA content of the roots or leaves. It does not preclude the possibility that changes in leaf ABA influence IAA levels in the roots. However, ABA is probably not a factor, since IAA levels were unaffected by elevated ABA levels in the leaves and roots of ABA-treated plants. With respect to IAA levels in the roots, the mutant appears to behave as if it were constantly sensing salt stress.

We have very little information about auxin metabolism in the roots of plants. Most of the studies in tomato have focused on two mutants, *dgt* and *epinastic* (Ursin and Bradford, 1989; Muday et al., 1995). However, the phenotypic characteristics of each mutant are not a consequence of differences in the endogenous levels of IAA (Fujino et al., 1988). Tal and Imber (1970) proposed that another ABA-deficient mutant of RR, *flacca*, was higher in auxins as measured by the wheat-coleoptile bioassay. The *flacca* mutant exhibited "shoot swelling, branch and leaf epinasty, strong rooting along the stem, and increased apical dominance" typical of excess auxin (Tal and Imber, 1970). Using GC-MS analytical procedures, we were unable to confirm an excessive level of IAA in the *sitiens* mutant that was inferred by Tal and Imber (1970, 1979). To the contrary, our results show that the *sitiens* mutant accumulates normal levels of IAA in the leaves but less than normal levels (nonstressed control) of IAA in the roots. If we can assume that the *sitiens* mutant is experiencing water stress even under normal conditions, the reduced level of IAA in the roots may reflect the physiological response of normal tomato genotypes to salt stress. Foliar treatment of the mutant plants with ABA reverses the mutant (wilty) phenotype and increases hydraulic conductance of the plants (Bradford, 1983). These experiments did not consider IAA metabolism or its possible relationship to changes in the hydraulic conductivity of these same plants.

The wilty phenotype expressed by ABA-deficient mutants was considered to be at least partially the consequence of increased resistance to water movement in the roots (Tal and Nevo, 1973; Nagel et al., 1994). Treatment of the roots with ABA resulted in a reversion of the mutant phenotype back to the wild type in conjunction with an increase in plant hydraulic conductance. Unfortunately, the authors did not measure water flow through the roots of the ABA-treated *sitiens* mutant. All of these observations

fail to address the possible role of IAA in root hydraulic conductivity. Foliar treatment of RR with the auxin 2,4-D increased the amount of root exudate produced by the treated plants (Tal and Imber, 1971). Auxins are known to promote stomatal opening (Marten et al., 1991) and act in a similar but opposite manner to the ABA-induced closure of stomates via potassium membrane channels (Blatt and Thiel, 1994). Therefore, it is possible that IAA may be critical to water movement through the root systems of plants, and that the function of this hormone is impaired in both the ABA-deficient mutant and salt-stressed plants. The alteration of IAA metabolism in the roots of these genotypes also may account for the reduction in growth potential via decreasing availability of water and increasing tissue water deficits.

Increasing concentrations of NaCl place osmotic limits on the growth of tomato plants. Our results show that hypersalinity developed with increasing concentrations of NaCl can reduce the endogenous levels of IAA in roots. However, the reduction in root IAA is not mediated by a concomitant increase in endogenous ABA. Auxins including IAA may be critical to water movement through the plant and may represent part of the chemical signal that regulates plant water potential. If we assume that the restriction of water loss through the ABA-regulated stomatal function is critical to maintaining plant turgor, then IAA may perform a complementary but independent function by increasing the hydraulic conductivity of the plant, especially in the root system. Part of the phenotypic response to salt stress expressed by the ABA-deficient *sitiens* mutant and salt-stressed normal genotypes may be the consequence of a common physiological event, the reduction of IAA in roots. The nature of the relationship between IAA metabolism in the roots and salt stress, whether correlative or causal, remains to be clarified by future research. However, here we have shown that the decline in root IAA is a response to NaCl that occurs independent of increases in the endogenous levels of ABA induced by salt stress.

ACKNOWLEDGMENTS

The authors extend thanks to Joe Molina and Bruce Sorenson for invaluable technical assistance.

Received February 20, 1996; accepted June 11, 1996.

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