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Short Communication

Abscisic Acid and Transpiration in Leaves in Relation to Osmotic Root Stress[1]

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The adaptive response of plants to a decrease in osmotic potential of the root medium involves adjustments that facilitate the maintenance of their water balance. Thus Bernstein (2, 3) showed that turgor pressure may be maintained under salinization through a decrease in the osmotic potential of the whole plant. However, actual turgor may also be maintained by a decrease in transpirational water loss. Indeed, the turgor of salinized cotton plants was even higher than the controls according to Gale *et al.* (4), who reported that, despite the high turgor, the stomata of the salinized plants were only partially open and transpiration was considerably reduced. They observed lower transpiration rates both in cotton and bean plants which had undergone complete osmotic adjustment and in onion plants which exhibited only partial osmotic adjustment. They concluded that salinity affected stomatal aperture by a mechanism other than reduced turgor.

Itai and Vaadia (6) and Vaadia and Itai (19) found a significant reduction in the amount of cytokinins in the root exudate of stressed plants. They suggested that the modified leaf metabolism and physiology of stressed plants may result from the decreased supply of cytokinins to the shoot. Pertinent to this finding is the discovery by Livne and Vaadia (9) that kinetin affected stomatal opening and enhanced transpiration. Abscisic acid, on the other hand, was recently discovered by Little and Eidt (8) to reduce transpiration of some woody cuttings. Mittelheuser and Van Steveninck (10) reported that ABA[3] affected closure of stomata and reduced transpiration in excised first leaves of wheat and barley. Earlier, Tal (18) had described a wilting tomato mutant in which transpiration was excessive owing to the inability of the stomata to close. Imber and Tal ("Phenotypic reversion of *flacca*, a wilting mutant of tomato, by abscisic acid." Science. In press.) recently discovered that when ABA was sprayed on these mutants, the stomata would close as in normal plants; transpiration was reduced and the wilting mutant resumed a normal appearance. There is thus good evidence for the involvement of both cytokinins and ABA in regulating stomatal aperture and transpiration.

This study is part of a broader project to test the hypothesis that ABA plays a controlling role in the adaptation of plants to decreased osmotic potential in the root medium. This particular investigation queries whether ABA could be involved in the reduction of transpiration that generally follows a decrease in the osmotic potential of the root medium.

Sixty-day-old tobacco plants (*Nicotiana tabacum*) grown in a greenhouse in half-strength Hoagland solution were used for these experiments involving osmotic stresses. Osmotic stresses were induced by the addition of 6 g of NaCl or 31 g of mannitol per liter. The plants were sprayed three successive times with either 5×10^{-5} M kinetin, 4×10^{-5} M *dl*-abscisic acid (R. J. Reynolds Tobacco Company), or water. Tween-80 (final concentration 0.01%, v/v) was added to all sprays. The first spray was given at the initiation of the osmotic stress, the second spray took place 24 hr later, and the third spray yet another 24 hr later, 1 hr before detachment. The first fully expanded leaves below the apex were detached and placed in beakers of distilled water. The beakers with the leaves were placed in $20 \times 20 \times 50$ cm open glass containers which were illuminated by 2300 lux of fluorescent light. The difference in water loss between a beaker containing leaves and one without leaves was used to calculate transpiration. Leaves from stressed plants transpired less than leaves from the nonstressed controls (Fig. 1). Treatment with kinetin and ABA increased and decreased, respectively, the rate of transpiration of leaves from both stressed and nonstressed plants. In leaves of stressed plants, treatment with kinetin increased the rate of transpiration to that of the nonstressed, water-sprayed plants. In leaves of the nonstressed plants, ABA reduced the level of transpiration to that of the stressed, water-sprayed plants. The same patterns were obtained whether the root stress was imposed with NaCl or with mannitol. These patterns proved reproducible in several repetitions of the experiment. The transpiration rate exhibited by leaves sprayed with both ABA and kinetin lies between those rates obtained by application of each of the two hormones alone (Table I). These experiments were run three times, with two replications each, and the same patterns of transpiration were observed each time. Thus in our system, one hormone seemed to negate the effect of the other hormone, as observed for other physiological phenomena (7, 13, 14, 17). This is reminiscent of other cases where the ratio of hormones plays a regulatory role in the control of a physiological process (12, 15).

Possible differences in water content in the various test plants were evaluated by determining the relative water content of the leaf tissue (16), with the use of leaf discs measuring 23 mm in diameter. Although initially, 4 hr after the application of the osmotic stress, relative water content was slightly lower than in the control, 48 hr later the relative water content of stressed leaves was slightly higher (Table II). Thus their turgor must be equal to or greater than in the control, and the decrease in transpiration exhibited by stressed plants could not be due to a reduction in the turgor of the leaves.

Our tentative explanation for the transpiration patterns observed (Fig. 1, Table I) was that leaves from the stressed plants might be relatively low in cytokinins and high in ABA,

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[3] Abbreviation: ABA: abscisic acid.

whereas leaves of nonstressed plants may be relatively high in cytokinins and low in ABA. Itai and Vaadia (6) have already shown that the cytokinin content of root exudate of stressed plants was greatly reduced. We therefore concentrated our efforts on analyzing the ABA-like inhibitor content of stressed leaves.

The endogenous amount of ABA-like inhibitors in leaves was estimated by a method devised after the principles described by Addicott and Lyon for the extraction of ABA (1). All the leaves of the plant except the first leaf and the small leaves in the apical whorl were taken for analysis. The main veins were removed,

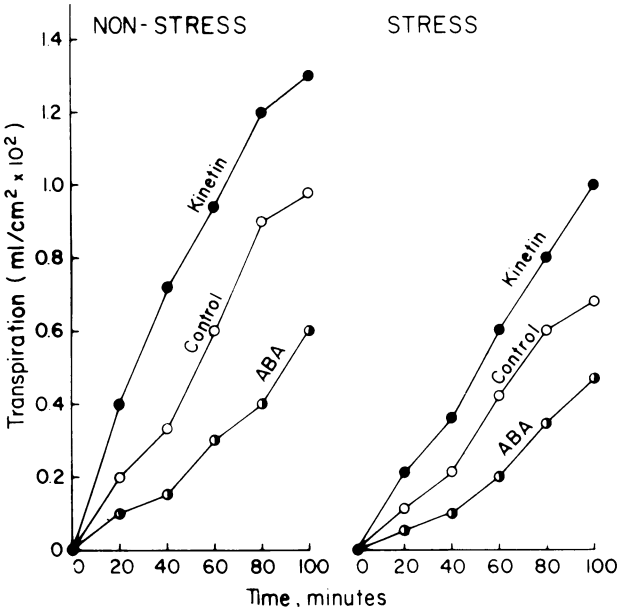

FIG. 1. The effect of NaCl-induced root stress and hormone sprays of the shoot on the transpiration rate of detached tobacco leaves.

Table I. *Effects of Mannitol-induced Root Stress and Hormone Sprays of the Tops on the Rate of Transpiration of Detached Tobacco Leaves*

ABA concentration, 4×10^{-5} M; kinetin concentration, 5×10^{-5} M.

Root medium	Transpiration Rate			
	Water spray	ABA spray	Kinetin spray	ABA and kinetin sprays
	ml/cm²·hr			
Stress (with mannitol)	0.72	0.42	0.84	0.60
No stress (no mannitol)	0.96	0.66	1.26	0.78

Table II. *Effect of NaCl-induced Root Stress on Relative Water Content of Leaves*

Time from Initiation of Stress	Leaf Water Content	
	No root stress (no NaCl)	Root stress (with NaCl)
hr	%	%
4	82.4	74.5
48	82.4	85.8

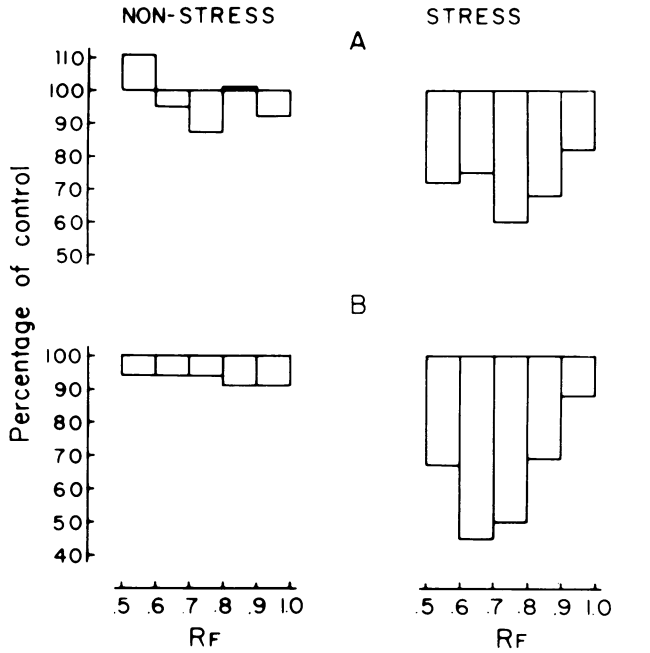

FIG. 2. The effect of osmotic root stress on the ABA-like inhibitor content of tobacco leaves as assayed by coleoptile elongation. A: 4 hr after initiation of root stress, relative water content of stressed plant is below that of nonstressed control (Table II); B: 48 hr after initiation of root stress, relative water content of stressed plant is above that of nonstressed control (Table II).

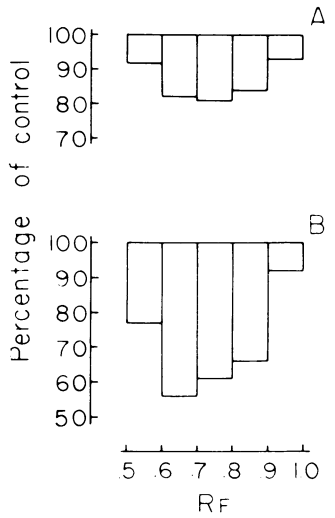

FIG. 3. The effect of osmotic root stress on the ABA-like inhibitor content of tobacco leaves in the α-amylase induction bioassay. Extracts of leaves from stressed plants were bioassayed against 10^{-7} M GA_3. Extracts from the leaves from nonstressed plants showed no inhibitor effects. A and B as for Figure 2 for leaves from stressed plants.

and the leaf sections were frozen in liquid air and lyophilyzed. A sample weighing 2 g was extracted three times in 70% (v/v) methanol and evaporated to 15% of the original volume. The pH was adjusted to 8.5 with $NaHCO_3$, and the solution was extracted once with petrol ether and then with ethyl acetate. The water phase was separated, and its pH was adjusted to 2.7 with HCl. It was then extracted twice with ethyl acetate and evaporated at 40 C to dryness. The dried residue was dissolved in methanol, and four equivalent samples each equaling 100 mg of the original 2 g (dry wt) sample were taken for chromatography.

The samples were run ascendingly on Whatman No. 3 paper for a distance of 20 cm with isopropanol-ammonia-water (10:1:1). In this system, ABA had an R_F of 0.7–0.8.

The biological activity in the sections of R_F 0.5 to 1.0 was bioassayed in two systems. In one bioassay the elongation of wheat coleoptiles was measured according to Nitsch and Nitsch (11) except that the variety used was Florence Aurore, the coleoptiles of which elongate without exogenous auxin. The other bioassay was based on determination of the extent of inhibition of α-amylase activity induced by treating barley seed endosperm (var. Omer) with 10^{-7} M GA_3 (5). As shown in Figures 2 and 3, an inhibitor with biological activity similar to that of ABA and with R_F values that corresponded to ABA (kindly supplied by Hoffman-La Roche, Switzerland) was found in tobacco leaves. Leaves taken from either NaCl-stressed plants (shown) or mannitol-stressed plants (not shown) contained substantially greater amounts of this inhibitor. In addition, more inhibitor was observed in the plants subjected to 48 hr of stress than in those subjected to 4 hr of stress. We suggest that the reduction in transpiration which follows a decrease in the osmotic potential of the root medium may be partly due to increase in the amounts of ABA in the leaves or to a marked increase in the ABA-cytokinin ratio, or to both.

Wright and Hiron (20) recently reported that the ABA content of detached wilting leaves rises markedly 4 hr after the onset of wilting. They theorized that the physiological responses of plants to water stress such as closure of stomata might be regulated by an increase in the ABA content of leaf cells. Our data indicate that the ABA content remains high well after the initiation of osmotic stress and the apparent recovery of turgor. Thus the increase in the ABA content of leaves is not necessarily conditional on reduced leaf turgor. It may indeed relate to a more general aspect of water balance in plants, namely, the regulation of the adaptive response of plants to osmotic stress. Whether the stress-induced rise in ABA and the presumed modification in ABA-cytokinin ratio play a controlling role in the adaptation of plants to osmotic stress or are essentially a result of it awaits further investigation.

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