

HORMONAL MODIFICATION OF PLANT RESPONSE TO WATER STRESS

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Abstract

Addition of either kinetin or abscisic acid (ABA) to the root medium of tobacco plants grown in culture solution greatly modified their response to different types of root stresses that were applied 48 hr after the addition of the hormones.

All the stresses employed impaired the water balance of the plants as evidenced by a decline of shoot turgor. The stresses consisted of cessation in root aeration, reduction of root temperature, and decrease in the osmotic potential of the root media by addition of either NaCl or mannitol.

Response of plants to a stress was estimated by measuring the water saturation deficit (W.S.D.) in the leaves 3 hr after the stress started. Pretreatment with kinetin resulted in an increased W.S.D. in the leaves whereas pretreatment with ABA, with the exception of the 3-hr saline stress, effected decreased W.S.D. in comparison with the appropriate controls.

A possible explanation of this phenomenon and its practical applications are discussed.

I. INTRODUCTION

Many reports implicate hormones in the regulation of water balance in plants. Cytokinins increase transpiration by effecting stomatal opening (Livne and Vaadia 1965; Meidner 1967; Luke and Freeman 1968), and abscisic acid (ABA) effects closure of stomata and consequent reduction of transpiration (Little and Eidt 1968; Mittelheuser and Van Steveninck 1969; Jones and Mansfield 1970; Mizrahi, Blumenfeld, and Richmond 1970), as well as enhancement of root exudation (Tal and Imber 1971). It was also reported recently that ABA increases the permeability of plant tissues to water (Glinka and Reinhold 1971). In addition, work on a wilted tomato mutant has shown that the impairment of the closure of its stomata may be corrected by treatment with ABA (Imber and Tal 1970).

Water and osmotic stresses alter the content of endogenous cytokinin and ABA: cytokinin activity in the root exudate declines drastically after 48 hr of salinity stress (Itai, Richmond, and Vaadia 1968), and ABA content in leaves of such plants increases considerably (Wright and Hiron 1969; Mizrahi *et al.* 1971) the increase starting within 40 min of commencement of salinity stress (Mizrahi and Richmond, unpublished data). This study describes the effects of ABA and kinetin on plant response to various conditions which impair plant water balance. These included decreased osmotic potential in the root medium and reduced root temperature and aeration.

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II. MATERIALS AND METHODS

Nicotiana rustica plants which were 50 or 60 days old were grown in a greenhouse under natural light conditions in a day length of 13 hr approximately and at a diurnal temperature range of 18–30°C. The plants were grown in 2-litre containers in half-strength Hoagland solution which was gently aerated by bubbling compressed air through it. Aeration stress was applied by withholding the air stream and filling the container to capacity with water. Osmotic stress was induced by either NaCl or mannitol (Mallinckrodt), 6 and 31 g/l respectively. Low root temperature was induced by lowering the temperature of the nutrient solution to 4°C and packing ice around the containers to maintain that temperature.

cis-trans-Abscissic acid and *N*-6-furfuryl adenine (kinetin) were added in the nutrient solution to the desired concentration 48 hr before the root stress treatments. Stock solutions were prepared as follows: ABA was dissolved in methanol and made 4×10^{-5} M with distilled water, the final concentration of methanol being 0.2%. Kinetin (Calbiochem) was dissolved by autoclaving, and was made 5×10^{-5} M with distilled water. Methanol was added to match its final concentration in the ABA solution.

Water status of the plants was estimated by measuring the leaf water saturation deficit (W.S.D.), this value being the difference between 100% and the relative water content (R.W.C.), the latter being obtained as follows (Slatyer 1961):

$$\text{R.W.C.} = [(\text{fresh weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight})] \times 100.$$

Leaf disks 23 mm in diameter were punched out from the five largest leaves of each five plants. The disks were weighed immediately (fresh weight) and weighed again at the end of 4 hr flotation on 10 ml distilled water at 4°C under incandescent light of 20 W (saturated weight). The disks were then oven-dried at 85°C for 24 hr and weighed (dry weight). In general, as W.S.D. increases the water potential decreases, i.e. water stress is augmented (Kramer 1969). Every treatment included five plants and each experiment was run at least three times. A given set of experiments yielded similar results, and the data presented here represent a typical experiment of each set.

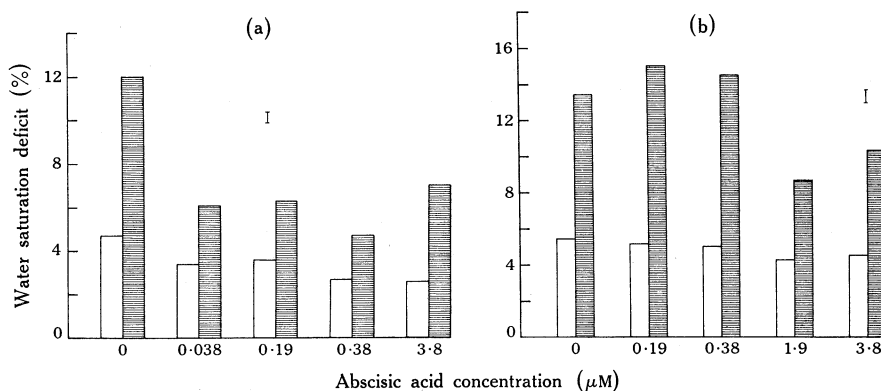


Fig. 1.—(a) Effect of various concentrations of abscisic acid on the water saturation deficit of tobacco plants. Open rectangles, before aeration was withheld; shaded rectangles, after aeration was withheld. Abscisic acid was added at a concentration of 40 μM. (b) Effect of pretreatment with abscisic acid on the water saturation deficit of tobacco leaves before and after a 3-hr exposure to mannitol (at a concn. of 31 g/l) in the root media of tobacco plants. Open rectangles, before mannitol was added; shaded rectangles, 3-hr after mannitol was added. In both parts of the figure results represent averages of five replicate plants, and the vertical lines indicate the respective standard errors.

III. RESULTS

Under our experimental conditions, a 3-hr cessation of aeration resulted in a significant increase in leaf W.S.D. [Fig. 1(a)]. The effects of pretreatments with ABA and kinetin 48 hr before cessation of aeration on plant response to such reduced root aeration are compared in the following tabulation (results are averages of five replicate plants; S.E. = ± 1.06):

Pretreatment	W.S.D. (%)	% of control
None	18.0	100
ABA, 4 μM	10.7	59.4
Kinetin, 5 μM	27.4	152.2

Thus, cessation of aeration for 3 hr resulted in a significant decrease in the W.S.D. of the ABA-treated plants compared with that of the non-treated plants. In contrast, pretreatment with kinetin resulted in a very pronounced increase in W.S.D. (see also Fig. 2).



Fig. 2.—Effect of a 48-hr pretreatment 3.8 μM abscisic acid or 4.0 μM kinetin on leaf turgidity after a 3-hr cessation of root aeration. NS, without hormone.

When the effect of various concentrations of ABA on leaf W.S.D. was tested it was found that before cessation of root aeration ABA at concentrations of 3.8×10^{-9} up to $3.8 \times 10^{-6}\text{M}$ did not significantly affect the W.S.D. [Fig. 1(a)]. However, 3 hr after ceasing aeration, the ABA-treated plants exhibited significantly lower W.S.D. in comparison with the non-treated plants. Although the difference between the effects of the various ABA concentrations was not significant, lowest W.S.D. were obtained at $3.8 \times 10^{-7}\text{M}$ in all the three experiments.

It has been reported that a sudden reduction in root temperature reduces water uptake by the root (Kramer 1940), and we indeed found a sharp rise in leaf W.S.D. 3 hr after the temperature in the root medium was reduced. As shown below, pretreatment with either ABA or kinetin resulted in a significantly decreased and increased leaf W.S.D., respectively, following the temperature stress (results are averages of five replicate plants; S.E. = ± 0.43):

Pretreatment	W.S.D. (%) before lowering temperature	W.S.D. (%) after 3 hr at 4°C
None	6.4	15.7
Kinetin, 5 μ M	7.1	17.6
ABA, 4 μ M	6.0	13.9

The response to a mannitol-induced stress followed a similar pattern—3 hr after mannitol (at a concn of 31 g/l) was added, the kinetin pretreated plants exhibited significantly higher W.S.D., as shown in the following tabulation (results are averages of five replicate plants; S.E. = ± 1.15):

Pretreatment	W.S.D. (%) before mannitol	W.S.D. (%) after mannitol
None	3.9	10.4
Kinetin, 5 μ M	5.0	16.4

On the other hand, those plants pretreated with 1.9 and 3.8 μ M ABA exhibited significantly lower W.S.D. than the non-treated plants [Fig. 1(b)]. Although the difference between the effect of 1.9 and 3.8 μ M ABA was not significant, 1.9 μ M ABA yielded the lowest W.S.D. in all the three experiments.

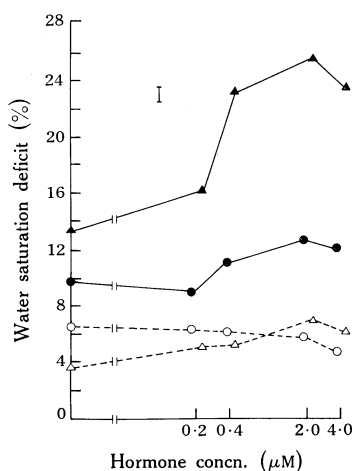


Fig. 3.—Effect of pretreatment with kinetin ($\blacktriangle, \triangle$) or abscisic acid (\bullet, \circ) on the water saturation deficit of leaves before (---) and after (—) a 3-hr exposure to NaCl at a concentration of 6 g/l. The experiments with kinetin and with abscisic acid were conducted on different days, hence the variations in water saturation deficit shown for the non-treated plants. Nevertheless, the standard error (vertical line) was identical for both the experiments with kinetin and with abscisic acid.

When salinity stress was applied (3-hr exposure to NaCl at a concn. of 6 g/l) W.S.D. in the leaves was greatly enhanced (Fig. 3). The response of ABA-pretreated plants to salinity was different from their response to other types of stresses employed.

Three hours after the salinity stress was induced, the ABA-pretreated plants exhibited higher, rather than lower, W.S.D. values. Pretreatment with kinetin, however, caused a similar response throughout and 3 hr in salt resulted in a greatly increased W.S.D.

IV. DISCUSSION

Our results support the thesis that the hormone make-up of plants may have a marked influence on the regulation of their water balance. It is clear that pretreatment with either kinetin or ABA greatly modifies the response of plants to conditions which impair water balance. Pretreatment with kinetin effected magnified W.S.D. upon exposure to water stress, which is predictable in view of reports on the influence of cytokinins on stomatal opening and enhanced transpiration (Livne and Vaadia 1965). Itai and Vaadia (1968) reported a reduction in shoot cytokinins following a decrease in the osmotic potential of the root medium. In view of our results, this phenomenon may be interpreted as reflecting a feature of the regulatory mechanism that facilitates the plant's adaptive response to decreased osmotic potential of the root medium. Therefore, addition of cytokinins to cytokinin-deficient, stressed plants does not alleviate the symptoms associated with water stress, but rather intensifies them. Similarly, the increase in the content of leaf ABA upon exposure to water stress (Wright and Hiron 1969; Mizrahi, Blumenfeld, and Richmond 1970) may be interpreted as reflecting a feature of a mechanism which facilitates lower W.S.D. upon exposure to conditions which impair the plant's water balance.

It should be noted that while pretreatment with ABA reduced the W.S.D. in leaves of plants exposed to the various stresses, it did not have a similar effect on plants exposed to a salinity stress for 3 hr. We cannot yet offer a tested explanation for this phenomenon.

Our essential finding that hormone effects on water balance are greatly intensified after exposure to conditions which effect water stress may be instrumental in investigating hormonal regulation of water balance, and may have practical benefits. Kinetin-mediated enhancement of W.S.D. may be of limited commercial value, but the effect of ABA in imparting resistance to conditions which increase W.S.D. and wilting has obvious economic potential, as already mentioned by Jones and Mansfield (1970). Although many compounds that reduce transpiration are already known, the finding that ABA increases root exudation (Tal and Imber 1971), chloride accumulation (Mizrahi and Richmond, unpublished data), and the permeability of plant tissues to water (Glinka and Reinhold 1971) suggests a more complex mode of action than merely that of an antitranspirant. In addition, commercial utilization of ABA to improve water balance of water-stressed plants seems advantageous in that ABA is an endogenous material with a high turnover (Milborrow 1969; Milborrow and Nodle 1970), thereby diminishing problems of residual toxicity and deleterious metabolic side effects.

V. ACKNOWLEDGMENTS

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VI. REFERENCES

- GLINKA, Z., and REINHOLD, L. (1971).—Absciscic acid raises the permeability of plant cells to water. *Pl. Physiol., Lancaster* **48**, 103–5.
- IMBER, D., and TAL, M. (1970).—Phenotypic reversion of flacca, a wilted mutant of tomato, by absciscic acid. *Science, N.Y.* **169**, 592–3.
- ITAL, C., and VAADIA, Y. (1968).—The role of root cytokinins during water and salinity stress. *Israel J. Bot.* **17**, 187–95.
- JONES, R. J., and MANSFIELD, T. A. (1970).—Suppression of stomatal opening in leaves treated with absciscic acid. *J. exp. Bot.* **21**, 714–19.
- KRAMER, P. J. (1940).—Root resistance as a cause of decreased water absorption by plants at low temperatures. *Pl. Physiol., Lancaster* **15**, 63–79.
- KRAMER, P. J. (1969).—“Plant and Soil Water Relationships: A Modern Synthesis.” (McGraw-Hill Book Company: New York.)
- LITTLE, C. H. A., and EIDT, D. C. (1968).—Effect of absciscic acid on bud-break and transpiration in woody species. *Nature, Lond.* **220**, 498–9.
- LIVNE, A., and VAADIA, Y. (1965).—Stimulation of transpiration rate in barley leaves by kinetin and gibberellic acid. *Physiologia Pl.* **18**, 658–64.
- LUKE, H. H., and FREEMAN, T. G. (1968).—Stimulation of transpiration by cytokinins. *Nature, Lond.* **217**, 873–4.
- MEIDNER, H. (1967).—The effect of kinetin on stomatal opening and the rate of intake of carbon dioxide in mature primary leaves of barley. *J. exp. Bot.* **18**, 556–61.
- MILBORROW, B. V. (1969).—The occurrence and function of absciscic acid in plants. *Sci. Prog.* **57**, 533–58.
- MILBORROW, B. V., and NODLE, R. C. (1970).—Conversion of 5-(1,2-epoxy-2,6,6-trimethylecyclohexyl)-3-methylpentacis-2-trans-4-dienoic acid into absciscic acid in plants. *Biochem. J.* **119**, 727–34.
- MITTELHEUSER, C. J., and VAN STEVENINCK, R. F. M. (1969).—Stomatal closure and inhibition of transpiration induced by (RS)-absciscic acid. *Nature, Lond.* **221**, 281–2.
- MIZRAHI, Y., BLUMENFELD, A., and RICHMOND, A. E. (1970).—Absciscic acid and transpiration in leaves in relation to osmotic root stress. *Pl. Physiol., Lancaster* **46**, 169–71.
- MIZRAHI, Y., BLUMENFELD, A., BITTNER, S., and RICHMOND, A. E. (1971).—Absciscic acid and cytokinin contents of leaves in relation to salinity and relative humidity. *Pl. Physiol., Lancaster* **48**, 752–5.
- SLATYER, R. O. (1961).—Effect of several osmotic substrates on the water relationships of tomato. *Aust. J. biol. Sci.* **14**, 519–40.
- TAL, M., and IMBER, D. (1971).—Abnormal stomatal behavior and hormonal imbalance in flacca, a wilted mutant of tomato. III. Hormonal effects on the water status in the plant. *Pl. Physiol., Lancaster* **47**, 847–50.
- WRIGHT, S. T. C., and HIRON, R. W. D. (1969).—(+)-Absciscic acid, the growth inhibitor induced in detached wheat leaves by a period of wilting. *Nature, Lond.* **224**, 719–20.