Plant Physiol. (1984) 76, 392–394
0032-0889/84/76/0392/03/$01.00/0

Stomatal Responses to Water Stress and to Abscisic Acid in Phosphorus-Deficient Cotton Plants

Received for publication March 5, 1984 and in revised form June 11, 1984

JOHN W. RADIN*
United States Department of Agriculture, Agricultural Research Service, Western Cotton Research Laboratory, Phoenix, Arizona 85040

ABSTRACT

Cotton (*Gossypium hirsutum* L.) plants were grown in sand culture on nutrient solution containing adequate or growth-limiting levels of P. When water was withheld from the pots, stomata of the most recently expanded leaf closed at leaf water potentials of approximately −16 and −12 bars in the normal and P-deficient plants, respectively. Pressure-volume curves showed that the stomata of P-deficient plants closed when there was still significant turgor in the leaf mesophyll. Leaves of P-deficient plants accumulated more abscisic acid (ABA) in response to water stress, but the difference was evident only at low water potentials, after initiation of stomatal closure. In leaves excised from unstressed plants, P deficiency greatly increased stomatal response to ABA applied through the transpiration stream. Kinetin blocked most of this increase in apparent sensitivity to ABA. The effect of P nutrition on stomatal behavior may be related to alterations of the balance between ABA and cytokinins.

Suboptimal N nutrition of cotton plants increases stomatal sensitivity to water stress and to exogenous ABA (20, 22). The enhancement of response to ABA could be almost completely blocked by simultaneous application of kinetin in the transpiration stream along with the ABA (22). These data imply that N effects on stomatal response to water stress may be governed by the balance between endogenous ABA and cytokinins, and they are consistent with numerous reports that suboptimal N nutrition decreases cytokinin content of plant tissues (13, 24, 25, 27, 28).

Low P also decreases cytokinin levels of plants (8, 13, 24) and, by analogy with low N, may also increase stomatal sensitivity to water stress and to exogenous ABA. Experiments to test this hypothesis are the subject of this paper.

MATERIALS AND METHODS

Plant Growth. Cotton (*Gossypium hirsutum* L. cv Deltapine 70) plants were grown from seed in a greenhouse in 14-L sand-filled pots; growth conditions were described earlier (22). The plants were watered 3 times weekly with a modified half-strength Hoagland solution which contained either 0.5 mM (control) or 0.05 mM (deficient) P_i as KH_2PO_4. Deionized H_2O was supplied on other occasions as needed to prevent wilting. Leaf area per plant was decreased about 50% by P deficiency under these conditions. Total leaf P concentrations (21) were 220 ± 27 and 74 ± 15 μmol/g dry weight, respectively, for the high P and low P plants.

Some batches of sand contained enough residual P that any additions of P restored growth to the control rates. On those occasions, plants grown with no added P had growth rates, tissue P concentrations, leaf areas, and carbohydrate concentrations typical of the P-deficient plants described above. Plants therefore were selected as suitable low P experimental material based upon growth rates rather than upon amount of P added to the sand. Growth on high P was not similarly affected by variability among batches of sand.

Leaf Water Relations and Stomatal Conductance. After the plants had five fully expanded leaves, watering was discontinued. During drying, abaxial and adaxial conductances to water vapor were followed in the fifth leaf (most recently expanded) with a LiCor LI-1600 steady state porometer (LiCor Instruments, Lincoln, NE).[1] Leaf conductances are reported as the sum of the two parallel conductances. Immediately after measurement of conductances, each leaf was excised and its Ψ_w[2] determined in a pressure chamber. Ψ_π and ψ_p were estimated from pressure-volume curves generated by methods described earlier (22). All measurements were made in early afternoon, at or near the time of minimum daily Ψ_w.

In some instances, pressure-volume curves were constructed using leaf RWC as the volume term. After the last pressure chamber reading, the leaf petiole was excised and the blade weight recorded (final fresh weight). The entire leaf was floated on distilled H_2O at 30°C in dim white light for 24 h to obtain saturated weight, then dried at 70°C for 24 h in a forced draft oven to obtain dry weight. At each step on the pressure-volume curve blade fresh weight was calculated by adding final fresh weight to the weight of water lost subsequent to that step. The RWC was calculated from the relationship:

$$\mathrm{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Saturated weight} - \text{Dry weight}}$$

Tissue Analyses for ABA. Water potentials of leaves were determined in early afternoon, and the leaves were immediately frozen at −80°C and lyophilized for ABA analysis. Sample extraction, purification, and analysis by GC were as described earlier (9, 22) except that the internal standard was pure *trans, trans*-ABA. The *trans, trans* isomer was isolated by HPLC from mixtures of *cis, trans*, and *trans, trans* isomers of ABA (Sigma Chemical Co.)

Stomatal Sensitivity to Applied ABA. The procedures described earlier (22) were followed. Briefly, petioles of excised leaves were placed in water to which ABA or kinetin, or both, were added and the leaves were incubated in light. Stomatal conductances were measured after 3 h of transpiration. Results

---

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

[2] Abbreviations: Ψ_w, water potential; ψ_π, osmotic potential; ψ_p, pressure potential; RWC, relative water content.

are shown as conductances plotted against the concentration of exogenous ABA, which is the dominant factor determining rate of ABA uptake into the leaf (22).

RESULTS AND DISCUSSION

The low P nutrient solution reduced leaf area by about half in these experiments. Leaf area was decreased more than dry matter accumulation per unit leaf area. A more complete discussion of effects of P on growth is presented elsewhere (21). No senescence (yellowing) was visible in plants grown on either level of P, even in the cotyledonary leaves.

Pressure-volume curves revealed only very small effects of P nutrition on internal water relations of leaves. Low P typically decreased turgor by about 0.5 bar, a difference that was essentially constant over a wide range of Ψ_w (Fig. 1). Based upon analysis of nearly 100 pressure-volume curves, P nutrition did not significantly or consistently influence the slope of the relationship between Ψ_p and Ψ_w, nor the slope of the relationship between Ψ_p and RWC. These observations suggest that cell wall elasticity was unaffected.

Stomatal conductance began to decrease after water was withheld and Ψ_w decreased (Fig. 2). In both treatments, conductance was high at a Ψ_w of -11 bars or higher. Assuming a maximum conductance of 3.0 cm/s, 50% closure occurred at a Ψ_w of approximately -16 bars for high P plants and -12 bars for low P plants (Fig. 2). At these values of Ψ_w, Ψ_p was 0.6 and 3.1 bars in high P and low P leaves, respectively (Fig. 1). Thus, P nutrition clearly altered the relationship between mesophyll turgor and stomatal conductance. The high P leaves behaved 'normally' with stomatal closure occurring near zero turgor (19). Low P, however, increased the turgor threshold for stomatal closure during the drying cycle. This increased turgor threshold was also observed with suboptimal N nutrition (20, 22).

Stomatal closure during water stress is believed to be mediated by ABA (14, 23). Low P not only sensitized stomata to water stress, it also increased the accumulation of ABA during drying (Fig. 3). However, differences in leaf ABA concentration became apparent only at low Ψ_w (below about -17 bars), at which point the stomata had already begun to close in both the high P and low P plants (Fig. 2). Bulk levels of ABA in leaves thus seemed unrelated to P effects on stomatal behavior except during the later stages of drying.

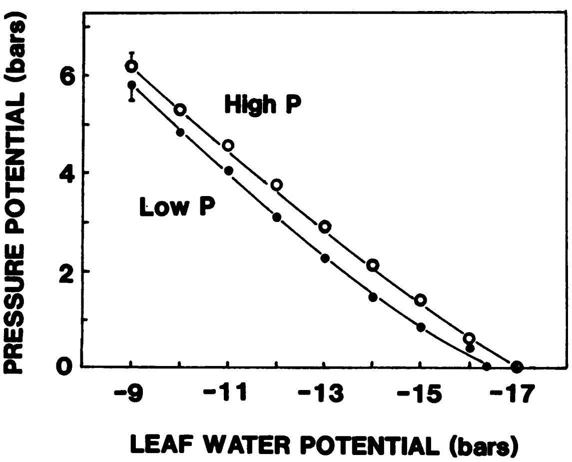

FIG. 1. Relationship between Ψ_w and ψ_p in leaves of low P and high P plants. Turgor was calculated from pressure-volume curves for each 1-bar increment of Ψ_w. Data are the means of three leaves of each type; representative SEs are indicated at $\Psi_w = -9$ bars. Lines were fitted by eye.

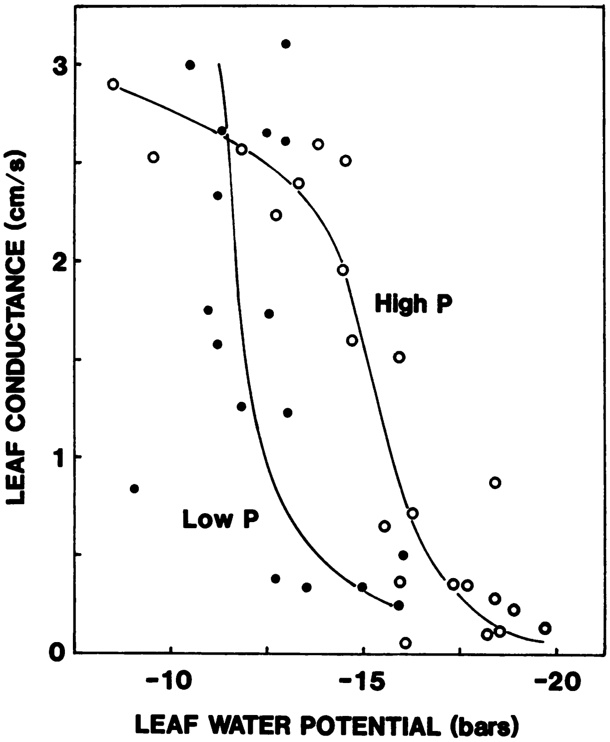

FIG. 2. Stomatal conductances of leaves of low P and high P plants during drying. All measurements were taken on the fifth true leaf in early afternoon. Each point represents a measurement on a single leaf. Lines were fitted by eye.

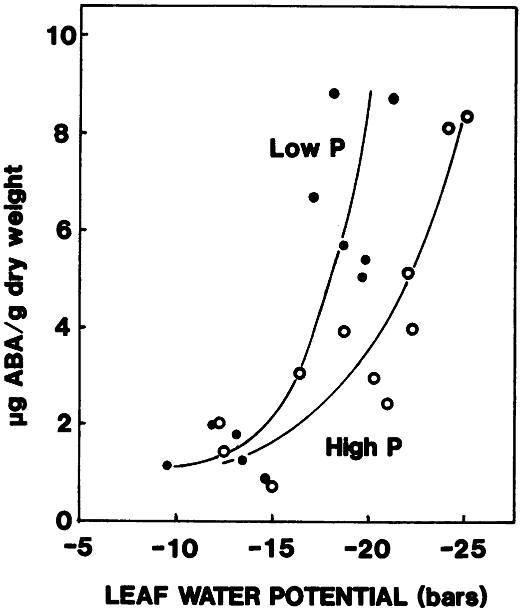

FIG. 3. ABA concentrations in leaves of low P and high P plants during drying. All samples were single leaves at the fifth node, taken in early afternoon. Leaves were frozen and lyophilized immediately after measurement of Ψ_w in the pressure chamber. Lines were fitted by eye.

The active pool of ABA is equated with that ABA released into the transpiration stream and carried to the guard cells (2, 23). Excised leaves were therefore presented ABA in the transpiration stream to test for differences in sensitivity to the substance. Low P greatly increased apparent stomatal sensitivity to exogenous ABA, and this response to P nutrition was largely prevented by simultaneous application of kinetin to the leaves along with the ABA (Table I). In low P leaves, a concentration of ABA as

Table I. *Effects of ABA and Kinetin on Stomatal Conductances of Excised Leaves*

The growth substances were presented to the leaves through the transpiration stream. The kinetin concentration was 10 μM in all cases. Conductances of leaves in water were 1.65 ± 0.09 and 1.50 ± 0.06 cm/s for high and low P, respectively. All reported values are the means ± SE of triplicate leaves.

Solution	Stomatal Conductance	
	Low P	High P
	% of control	
Water	100	100
ABA (0.1 μM)	62 ± 14	95 ± 6
ABA (0.3 μM)	41 ± 3	95 ± 5
ABA (1.0 μM)	47 ± 2	83 ± 7
Kinetin	87 ± 9	112 ± 6
Kinetin + ABA (0.1 μM)	91 ± 13	97 ± 7
Kinetin + ABA (0.3 μM)	74 ± 16	87 ± 9
Kinetin + ABA (1.0 μM)	40 ± 9	94 ± 10

low as 0.3 μM (0.15 μM of the active [+]-isomer) caused greater than 50% closure by itself, but 10 μM kinetin decreased this effect by more than half. The effect of kinetin alone was not significant. In high P leaves, neither kinetin nor ABA at these concentrations had large effects (Table I). In all respects these effects of P deficiency on stomata are similar to the effects of N deficiency (22).

The results presented here show that P levels which decrease growth by about half also cause profound changes in plant responses to water stress. Stomatal responsiveness to water stress and to applied ABA is increased, perhaps from an alteration of the balance between ABA and endogenous cytokinins. This change is independent of the internal water relations of the leaves, which remain almost unchanged. Although I have not performed cytokinin analyses on these plants, other reports show that P deficiency strongly decreases cytokinin content of leaves and xylem exudate (8, 13, 24). Similar dual effects of stress on cytokinin content (either of plant tissue or of exudate) and stomatal sensitivity to ABA occur with N deficiency (13, 22, 24, 25, 27, 28), water stress (1, 3, 7, 15, 18), and possibly flooding (4, 5). The widespread association of these stress-induced characteristics suggests that they are related, and a cause-and-effect relationship can reasonably be inferred from the ability of applied cytokinins to counteract the effects of the stress (Table I; 3, 4, 22).

The data in this paper do not reveal the mechanism(s) of increased stomatal responsiveness to ABA. As one possibility, P nutrition could alter the partitioning of ABA between active and inactive pools. The inactive pool is largely chloroplastic, with the rate of transfer between compartments (pools) determined by pH gradients (11). Such an explanation thus might require only that P nutrition alter pH gradients between chloroplast and cytosol (6). Hartung *et al.* (12) recently demonstrated that osmotic stress alters partitioning of ABA between pools in the mesophyll and thereby increases ABA accumulation in the epidermis.

Stomatal responsiveness to ABA may also be mediated directly at the guard cell plasmalemma, the presumed site of ABA action (10, 17). Following arguments of Trewavas (26), in this case P nutrition might regulate the number of ABA binding sites. Similarly, kinetin can be postulated to act at either the mesophyll or the guard cells in its reduction of P deficiency-induced stomatal responsiveness to ABA (Table I). Jewer and Incoll (16) reported an effect of cytokinins on stomatal aperture in epidermal peels, implying a direct effect on the guard cells. However, such reports are scarce. Clearly, generalizations are not yet possible about mechanisms by which environmental or nutritional stresses affect stomatal behavior.

Acknowledgments—I thank Marie P. Eidenbock and Linda L. Parker for capable technical assistance, Scott Hodge for maintaining the plants, Dr. Donald L. Hendrix for the isolation of *trans, trans*-ABA, and Dr. Robert C. Ackerson and Dr. Kent Bradford for valuable comments.

LITERATURE CITED

1. ACKERSON RC 1980 Stomatal reponse of cotton to water stress and abscisic acid as affected by water stress history. Plant Physiol 65: 455–459
2. ACKERSON RC 1982 Synthesis and movement of abscisic acid in water-stressed cotton leaves. Plant Physiol 69: 609–613
3. AHARONI N, A BLUMENFELD, AE RICHMOND 1977 Hormonal activity in detached lettuce leaves as affected by leaf water content. Plant Physiol 59: 1169–1173
4. BRADFORD KJ 1983 Involvement of plant growth substances in the alteration of leaf gas exchange of flooded tomato plants. Plant Physiol 73: 480–483
5. BURROWS WJ, DJ CARR 1969 Effects of flooding the root system of sunflower plants on the cytokinin content in the xylem sap. Physiol Plant 22: 1105–1112
6. COWAN IR, JA RAVEN, W HARTUNG, GD FARQUHAR 1982 A possible role for abscisic acid in coupling stomatal conductance and photosynthetic carbon metabolism in leaves. Aust J Plant Physiol 9: 489–498
7. DAVIES WJ 1978 Some effects of abscisic acid and water stress on stomata of *Vicia faba* L. J Exp Bot 29: 175–182
8. DHILLON SS 1978 Influence of varied phosphorus supply on growth and xylem sap cytokinin level of sycamore (*Platanus occidentalis* L.) seedlings. Plant Physiol 61: 521–524
9. GUINN G 1982 Fruit age and changes in abscisic acid content, ethylene production, and abscission rate of cotton fruits. Plant Physiol 69: 349–352
10. HARTUNG W 1983 The site of action of abscisic acid at the guard cell plasmalemma of *Valerianella locusta*. Plant Cell Environ 6: 427–428
11. HARTUNG W, B HELLMANN, H GIMMLER 1981 Do chloroplasts play a role in abscisic acid synthesis? Plant Sci Lett 22: 235–242
12. HARTUNG W, WM KAISER, C BURSCHKA 1983 Release of abscisic acid from leaf strips under osmotic stress. Z Pflanzenphysiol 112: 131–138
13. HORGAN JM, PF WAREING 1980 Cytokinins and the growth responses of seedlings of *Betula pendula* Roth. and *Acer pseudoplatanus* L. to nitrogen and phosphorus deficiency. J Exp Bot 31: 525–532
14. HSIAO TC 1973 Plant responses to water stress. Annu Rev Plant Physiol 24: 519–570
15. ITAI C, Y VAADIA 1971 Cytokinin activity in water-stressed shoots. Plant Physiol 47: 87–90
16. JEWER PC, LD INCOLL 1980 Promotion of stomatal opening in the grass *Anthephora pubescens* Nees by a range of natural and synthetic cytokinins. Planta 150: 218–221
17. LURIE S, DL HENDRIX 1979 Differential ion stimulation of plasmalemma adenosine triphosphatase from leaf epidermis and mesophyll of *Nicotiana rustica* L. Plant Physiol 63: 936–939
18. MIZRAHI Y, A BLUMENFELD, AE RICHMOND 1970 Abscisic acid and transpiration in leaves in relation to osmotic root stress. Plant Physiol 46: 169–171
19. PIERCE M, K RASCHKE 1980 Correlation between loss of turgor and accumulation of abscisic acid in detached leaves. Planta 148: 174–182
20. RADIN JW, RC ACKERSON 1981 Water relations of cotton plants under nitrogen deficiency. III. Stomatal conductance, photosynthesis, and abscisic acid accumulation during drought. Plant Physiol 67: 115–119
21. RADIN JW, MP EIDENBOCK 1984 Hydraulic conductance as a factor limiting leaf expansion of phosphorus-deficient cotton plants. Plant Physiol 75: 372–377
22. RADIN JW, LL PARKER, G GUINN 1982 Water relations of cotton plants under nitrogen deficiency. V. Environmental control of abscisic acid accumulation and stomatal sensitivity to abscisic acid. Plant Physiol 70: 1066–1070
23. RASCHKE K 1975 Stomatal action. Annu Rev Plant Physiol 26: 309–340
24. SALAMA AMS, PF WAREING 1979 Effects of mineral nutrition on endogenous cytokinins in plants of sunflower (*Helianthus annuus* L.). J Exp Bot 30: 971–981
25. SATTELMACHER B, H MARSCHNEER 1978 Nitrogen nutrition and cytokinin activity in *Solanum tuberosum*. Physiol Plant 42: 185–189
26. TREWAVAS AJ 1981 How do plant growth substances work? Plant Cell Environ 4: 203–228
27. WAGNER H, G MICHAEL 1971 Der Einfluss unterschiedlicher Stickstoff-Versorgung auf die Cytokininbildung in Wurzeln von Sonnenblumenpflanzen. Biochem Physiol Pflanzen 162: 147–158
28. YOSHIDA R, T ORITANI 1974 Studies on nitrogen metabolism in crop plants. XIII. Effects of nitrogen top-dressing on cytokinin content in the root exudate of rice plant. Proc Crop Sci Soc Jpn 43: 47–51