Amelioration of Chilling-Induced Water Stress by Abscisic Acid-Induced Changes in Root Hydraulic Conductance[1]

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ABSTRACT

Pretreatment of soybean (*Glycine max* L. var Ransom) root systems with abscisic acid (ABA) ameliorates the deleterious effect of low temperatures on root hydraulic conductance. ABA treatment of root systems subsequently chilled to 10°C with shoots at 25°C resulted in higher leaf water potentials and lower stomatal resistances. If the root systems are left at 25°C, ABA causes stomatal closure. Membrane alterations are suggested as a mechanism for the ABA action in plant response to chilling stress.

Abscisic acid has been implicated in plant response to environmental stress. ABA accumulates in plants during water stress (8, 21) and closes stomata when applied to the transpiration stream (15, 16, 19). ABA also plays a role in plant response to low temperature stress (2, 17, 18). Treatment of cucumber cotyledons with ABA reduced injury during subsequent exposure to 4°C (18). Treatment of potato stem cultures with ABA increased the freezing tolerance of the tissue by 3 to 5°C (2). Despite increased interest in ABA, the mechanism by which ABA acts is unknown.

Several investigations have shown significant effects of ABA on water and ion transport in tissue. Treatments of carrot root tissue or whole sunflower root systems with ABA have been reported to increase the hydraulic conductivity of the tissues (6, 7). More recent experiments with *Glycine max* L. Merr. and *Phaseolus vulgaris* L. have documented a decrease in the hydraulic conductivity of root systems at 25°C at high flow rates (4, 12). Of considerable interest is the apparent interaction of temperature and ABA on water flux through soybean root systems (12).

The hydraulic conductance of non-ABA-treated root systems subjected to a 5 bar pressure gradient decreased slightly with decreased temperature to 15°C. Below 15°C, however, L^2 decreased precipitously. ABA treatment (10 μM) decreased L at 25°C and eliminated the dramatic change in L at the lower temperatures. When presented as an Arrhenius plot, the natural log of the flux from the ABA-treated root system decreased linearly with reciprocal temperature with a slope in between the two portions of the non-ABA-treated root system. These results suggest that, at low temperatures, L of the ABA-treated root system was greater than the non-ABA-treated root system.

The experiments presented here further test the hypothesis that treatment of root systems with ABA reduces the decrease in the hydraulic conductance of root systems at low temperatures.

MATERIALS AND METHODS

Soybean plants (*Glycine max* L. var Ransom) were grown in environmental growth chambers under a 12-h photoperiod at 25°C. The photosynthetic photon flux density at plant height was 600 μE m^{-2} s^{-1}. Seeds were sown in plastic pots (7.5 × 30 cm) containing a 1:1 mixture of sand and Turface. The bottom of the pot was cut off and replaced with a single hole stopper. Plants were watered daily with alternate applications of half-strength Hoagland solution and distilled H$_2$O. Plants were 21 to 25 d old with one fully expanded trifoliolate leaf at the time of the experiment.

A stock solution containing 0.1 M ABA (Sigma ± *cis-trans*) in 95% ethanol was prepared and stored in the dark at 4°C. Treatment solutions were prepared with distilled H$_2$O the day before the experiment and kept in the dark at 22°C. One and one-half hours prior to the beginning of the photoperiod, the holes in the pots were sealed and 100 ml of 50 μM ABA or 100 ml of distilled H$_2$O (with equivalent amounts of ethanol) were added. After 1 h of treatment, the drainage holes were uncovered and the pots allowed to drain for 5 min. The holes of eight treated and eight nontreated plants were resealed and the pots randomly placed into a water bath cooled to 10°C. Thermocouples placed in the center of selected pots indicated that the roots reached 10°C in 15 min and that there was less than 0.5°C variation in temperature among individual pots. One-half hour after cooling, the lights were turned on. The remaining ABA-treated and non-treated plants were left with both roots and shoots at 25°C.

Stomatal resistances of the upper and lower leaf surface were measured every 2 h with a diffusion porometer (Delta Instruments). Because of a limitation in the number of plants which would fit into the water bath, plant wiltedness, a nondestructive technique, was used to measure plant water status. Plant wiltedness was measured every 2 h by placing a protractor behind the stem of the plant. The angle of the apical internode relative to the rest of the stem was recorded. Zero degress meant a completely turgid plant, whereas 180° meant a completely wilted plant. Four hours after the beginning of the photoperiod, the water potential of representative plants was measured with a pressure bomb.

The entire experiment was repeated twice with the chilled root and warm root treatments each done separately two additional times. The data are presented as the means and standard error of four measurements for a typical experiment. Student's *t* test was used to establish the significance of the differences between means.

[2] Abbreviation: L, hydraulic conductance.

RESULTS

Non-ABA-treated chilled plants showed signs of severe water stress. The plants rapidly wilted, had water potentials 4 bars lower 4 h after the light came on than the non-ABA-treated nonchilled plants (Table I), and had stomatal resistances significantly higher than the non-ABA-treated nonchilled controls (Figs. 1 and 2).

Pretreatment with ABA, however, significantly reduced the water stress in the shoot of plants with chilled roots. The ABA-treated plants wilted less (Fig. 3), had a higher leaf water potential (Table I), and had a lower stomatal resistance for both upper and lower leaf surfaces (Fig. 2; Table I). Figure 4 is a picture of a 10 μM ABA-treated and a nontreated plant with root systems chilled to 10°C. The picture clearly shows the effect of ABA treatment

Table I. *Leaf Water Potential and Stomatal Resistance 4 Hours after the Beginning of the Photoperiod*

Shoots of all plants were kept at 25°C. Roots of the chilled plants were cooled to 10°C 0.5 h prior to the beginning of the photoperiod. Roots of nonchilled plants remained at 25°C. ABA-treated plants were treated with 10 μM ABA 1.5 h prior to cooling. Means ± SE of the means for four replicates are presented.

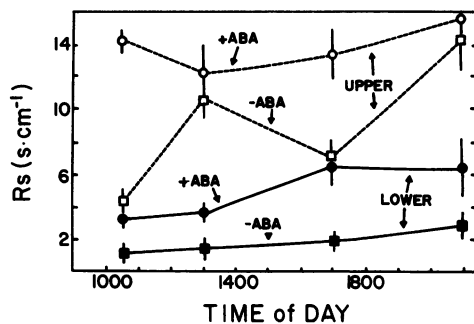| Treatment	Leaf Water Potential	Stomatal Resistance		
		Lower	Upper	Total
	bars	*s cm^{-1}*		
Chilled				
−ABA	−9.5 ± 0.25	24.2 ± 2.1	36.1 ± 3.5	14.5
+ABA	−8.0 ± 0.30	6.2 ± 1.0	28.2 ± 1.5	5.1
Nonchilled				
−ABA	−5.5 ± 0.2	1.4 ± 0.5	8.1 ± 1.0	1.2
+ABA	−3.5 ± 0.2	3.6 ± 0.4	12.2 ± 1.8	2.8

FIG. 1. Stomatal resistance (R_s) for upper and lower leaf surfaces of ABA-treated and untreated plants with roots and shoots at 25°C. Data points are means ± SE for four plants.
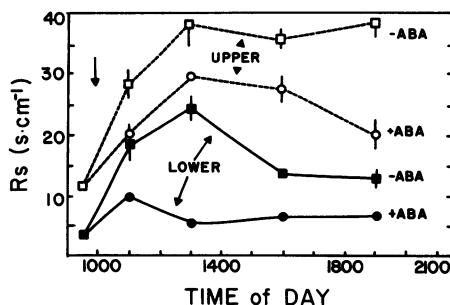

FIG. 2. Stomatal resistance (R_s) for upper and lower leaf surfaces of ABA-treated and untreated plants with roots chilled to 10°C and shoots at 25°C. (↓), Beginning of photoperiod. Data points are means ± SE for four plants.

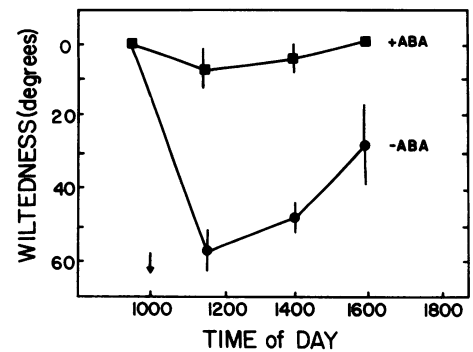**FIG. 3.** Angle of wilt *versus* time for ABA-treated and nontreated plants with roots chilled to 10°C and shoots at 25°C. (↓), Beginning of photoperiod. Data points are means ± SE for five plants.
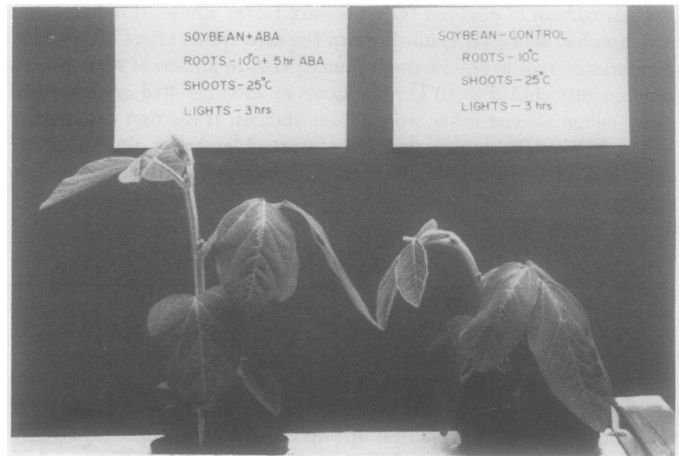

FIG. 4. Soybean seedlings treated (left) or nontreated (right) with ABA. Picture taken 3 h after the beginning of the photoperiod and 5 h after root systems were irrigated with 10 μM ABA.

on chilling-induced water stress.

Pretreatment of roots with ABA left at 25°C resulted in stomatal closure (Fig. 2) and higher leaf water potentials and stomatal resistances than the non-ABA-treated nonchilled control plants. (Table I; Fig. 1). Chilling the root systems of the ABA-treated plant resulted in a 4.5 bar lower water potential than the ABA-treated nonchilled plants (Table I).

DISCUSSION

In plants with roots and shoots at 25°C, ABA added to the root system caused stomatal closure. Soybean root systems are permeable to ABA with significant amounts of the chemical appearing in the xylem water in less than 1 h (5, 11). This suggests that at 25°C ABA is transported to the shoot causing stomatal closure. Similar results have been reported for wheat (3). The increase in stomatal resistance prevents (Fig. 1) a decrease in leaf water potential as compared to the non-ABA-treated nonchilled plants (Table I).

When roots were at 10°C and shoots at 25°C, ABA treatment had a different effect. The leaf water potential of the ABA-treated plants was higher than the nontreated. This was not, however, due to stomatal closure. Stomatal resistances were lower in the ABA-treated plants than the non-treated plants (Table I; Fig. 2). Although not as pronounced as at 4 h after chilling, at 9 h the stomatal resistances were still lower in the ABA-treated plants (Fig. 3). Total resistances after 9 h were 5.2 and 9.9 s cm^{-1} for the ABA-treated and nontreated plants, respectively. These results support the hypothesis that ABA resulted in a greater root

hydraulic conductivity in the treated plants with chilled root systems than in the non-ABA-treated chilled root systems. This conclusion is consistent with the observations from detopped root systems reported earlier (12).

Davies and co-workers observed in intact wheat plants that ABA treatment of chilled roots resulted in a rapid increase in water uptake (3). They imply that this could be due to a stimulation of ion transport increasing the osmotic component of the driving force moving water into the plant. Further experiments are needed to determine if the change in flux is due to ABA-induced changes in driving force or the resistance to water movement.

A membrane has been suggested as the rate limiting barrier for water flow through root systems (9, 10, 13), and the decrease in hydraulic conductivity at low temperatures is due to a change in the fluid properties of this membrane (10, 13). The results presented here suggest that ABA alters the properties of this membrane ameliorating the deleterious changes which result in the large decrease in L. One might further speculate that other membranes are also affected and protected from temperature-induced changes.

Although the leaf water potential in the treated plants was only 1 to 2 bars greater than the untreated plants, this was sufficient to prevent significant wilting of the leaf tissue (Fig. 3). In other words, a 2 bar difference resulted in positive turgor in the leaf, an important factor in maintaining photosynthesis and leaf expansion (8).

Conclusions on the role of endogenously produced plant growth regulators based on exogenously applied compounds must be aproached with caution. Of specific concern is whether the concentration generated in the tissue from exogenous application is within normal physiological levels. Although values of tissue ABA content vary, concentrations in stressed leaf tissue of 100 to 300 μg kg^{-1} fresh weight are typical (14). Walton et al. (20) measured levels in osmotically stressed bean roots of 100 μg kg^{-1} fresh weight. At first glance, the concentration used in this experiment, 10 μM or 2640 μg kg^{-1} solution, is at least an order of magnitude larger than endogenous levels. Recent evidence, however, indicates that ABA is not sequestered in the vacuole of the plant cell (1). Since the vacuole occupies 80 to 90% of the cell volume, the concentration of ABA in the protoplasm is at least 10-fold higher than when expressed on a total fresh weight basis.

Therefore, the concentration of ABA used in this experiment is to a first approximation not an excessively large challenge to the tissue.

Because of its hydrophobicity and carboxyl group the ABA molecule may partition into membranes and be sequestered in organelles of high pH. It is, therefore, impossible to estimate the effective ABA concentration at any site in the cell based on bulk tissue analysis. Further studies with refined techniques will clarify this important issue.

The results presented in this paper suggest membrane alterations as a mechanism for the action of ABA in plant response to low temperatures. Future work will determine if endogenous ABA acts similarly to exogenously applied ABA and if there are long term benefits to the plant of ABA treatment.

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