Short Communication

Abscisic Acid Accumulation in Cotton Leaves in Response to Dehydration at High Pressure

Received for publication November 13, 1981 and in revised form November 2, 1982

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ABSTRACT

Pressure-volume techniques were utilized to examine the control of abscisic acid (ABA) accumulation in dehydrated cotton (Gossypium hirsutum L. cv Tamcot SP 37) leaves. Leaves were rapidly dehydrated in a pressure chamber to a balance pressure coincident with the loss of cellular turgor, and then the pressure was either maintained at that level or released. Rapid accumulation of ABA began within two hours after the balance pressure was achieved, whether or not the high pressure potential of the cells was maintained by the externally imposed pressure. The results show that loss of pressure per se does not trigger ABA accumulation in dehydrated leaves. Rather, the stimulus may be related to cellular shrinkage and relaxation of the elastic wall.

Water stress induces the synthesis and accumulation of ABA in most higher plants (10, 18). Since exogenous application of ABA is known to close stomata (5, 9, 11), the increased endogenous ABA level that occurs during dehydration may represent an important regulatory mechanism for closing stomata, thereby restricting transpirational water loss (14). Despite this important role for ABA in regulating stomatal aperture, there is little evidence to suggest how its synthesis is triggered or controlled. Pierce and Raschke (12) proposed that cellular turgor must approach zero before ABA accumulation could begin, but their evidence did not suggest a control mechanism. Some other work suggests that the threshold turgor need not be zero under all conditions (7).

Our approach to studying the control of ABA accumulation relies heavily on the pressure chamber (15). The theoretical aspects of leaf water relations in a pressure chamber have been well defined (16, 17). For clarity, we briefly review here some of the concepts. Normally, the water in the xylem of a leaf is under tension generated by evaporation from the cell walls of the mesophyll. This tension can be relieved by applying pressure to the blade of the excised leaf. When the applied pressure just equals the tension in the xylem (balance pressure), then the water potential in the xylem xylem is zero (neglecting the small osmotic potential of the xylem sap). Further increases in pressure above the balance pressure will raise the water potential above zero and will cause water to flow to the unpressurized cut surface of the petiole. The leaf will thus be dehydrated until its water potential again reaches zero. At any balance pressure, all compartments of the leaf are in equilibrium, as evidenced by lack of water flow, and thus both the apoplast and symplast must have water potentials of zero. It follows that at this point the pressure potential in the symplast must be numerically equal, but opposite in sign, to the osmotic potential.

Cellular turgor arises from the elastic properties of the cell wall (4, 17). Normally the volume of water in a cell exerts a pressure on the plasmalemma-wall complex while the wall itself exerts a backpressure on the protoplast. When such a leaf is subjected to a small balance pressure, the pressure potential in the symplast includes both internally generated and external components. If the leaf is further dehydrated by pressure to the extent that the internal component of pressure potential is lost (zero turgor), then the external pressure is numerically equal to the osmotic potential (17). The linear portion of a pressure-volume curve indicates such a condition. At such a balance pressure, there is no pressure generated by the elastic cell wall but the pressure potential remains high nonetheless because of the externally applied pressure.

From this analysis, we conclude that the pressure chamber can be used to test the role of pressure potential in triggering ABA accumulation, because it allows leaves to be dehydrated without the pressure potential approaching zero. Here we report results from this type of experiment.

MATERIALS AND METHODS

Experimental work was performed in Wilmington and some in Phoenix. In Wilmington, cotton plants (Gossypium hirsutum L. cv Tamcot SP 37) were grown as described previously (2). Leaves from well-watered plants were placed in a pressure chamber and dehydrated by raising the applied pressure. A balance pressure of 15 to 17 bars was chosen because it represented the leaf water potential coincident with zero turgor when water potential components were determined psychrometrically (2, 3). About 45 to 60 min were required to achieve equilibrium at this pressure. Gas mixtures, conditions of incubation, etc., were as described earlier (2). Leaves were maintained at the balance pressure for 0 to 4 h, removed from the chamber, frozen in liquid N₂, and subsequently analyzed for ABA (1). In a parallel set of experiments, leaves were dehydrated to the same balance pressure, but then the pressure was released and the leaves were incubated at atmospheric pressure for 0 to 4 h before ABA analysis. In both sets of experiments, leaves were weighed before placement in the pressure chamber and ABA levels are reported on an original fresh weight basis (prior to dehydration).

1 Contribution No. 2945 from the Central Research and Development Department, Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.

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RESULTS AND DISCUSSION

Earlier work (see Fig. 3 and Table I of Ref. 2) suggested that pressure dehydration could cause ABA accumulation. This result is confirmed and extended here. In cotton leaves maintained in the pressure chamber at a balance pressure of 15 to 17 bars, ABA levels increased significantly 1 to 2 h after achieving the balance pressure (Fig. 1). Accumulation continued until the ABA levels reached about 1.5 µg/g fresh weight at 4 h (Fig. 1). The time course of accumulation was little changed when leaves were dehydrated to the same extent but incubated without external pressure (Fig. 2). The time lag of 1 to 2 h is similar to that noted earlier by Ackerson (2) and by Pierce and Raschke (12).

Despite the similar behavior of leaves incubated under pressure and in open air, we were concerned about possible artifacts resulting from incubation in the pressure chamber. We therefore modified the experiments by incubating leaves totally within the pressure chamber (petiole not protruding). We reasoned that no turgor or volume should be lost during pressurization, because the cut surface would be in equilibrium with the chamber pressure instead of atmospheric pressure. Table I shows data from this type of experiment. Leaves fully enclosed in the chamber and pressurized to 20 bars accumulated only about 10% as much ABA as did leaves pressure-dehydrated in the standard manner with petioles protruding from the chamber. This difference strongly indicates that neither the pressure applied to the leaf, nor some other unspecified condition of the incubation, could have been responsible for the accumulation of ABA.

It is apparent from these experiments that the high external pressure did not prevent a response to dehydration. Responses to pressure dehydration were similar in two laboratories. This evidence suggests that the transduction system for ABA accumulation must be independent of pressure potential per se.

Acknowledgments—We thank N. Rogers and L. Parker for technical assistance, and Drs. J. Hendrix and W. Hitz for valuable suggestions. Special thanks are extended to Dr. J. Boyer for his helpful comments throughout the development of this paper. We thank E. Sparre for assistance in preparing this manuscript.

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