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Control of Stomatal Function by Xylem Exudates from Stressed Plants without a Decrease in the Leaf Water Potential : Possible Role for Root Signals ?

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A simple system involving excised leaves which enables maintenance of turgor of the excised leaves was developed to examine the effect of extraneously added substances in the transpirational flow. It was tested with various levels of externally applied abssisic acid which alters stomatal canductance vague.

Addition of xylem exudates from the stressed plants in the transpirational flow of excised maize leaves decreased the stomatal conductance within 5 minutes. After 25 minutes it dropped by more than 80%. This decrease could be observed inspite of the leaf water potential being maintained by steady supply of water. This indicates that the xylem exudates of stressed plants contain certain substances which can close the stomata rapidly. The xylem exudates from the stressed plants also altered the membrane permeability.

Key Words : Stomatal conductance, Water stress, Root signals, Xylem exudates, Leaf water potential, Maize, excised leaves, Abssisic acid

Introduction

Increasing evidence shows that soil water deficits can influence water loss through stomata in ways that are not mediated through leaf water potential and turgor (Bates & Hall 1981, Gollan et al. 1986, Turner 1986). Studies have shown that the stomatal conductance decreased prior to changes in the water potential (Trejo & Davies, 1991). The authors own work also showed no relationship between leaf water potential and stomatal conductance in a number of crops like maize, cowpea and sorghum (Gangadhar Rao & Hebbar, 1996). These findings suggest that plant roots sense the impending soil water deficits much in advance and control

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the water loss through stomata. The mechanism by which the plant roots communicate with the shoots is believed to be mediated through root signals (Gollan et al. 1986, Turner 1986, Munns 1992, Munns et al. 1993). This study was conducted to find out the role of such root signals in the water stressed plants by alternate methodology.

Materials and Methods

Plant growth conditions : Maize seedlings (Zea mays L. cv. Madhuri) were raised in green house in glazed pots of 15 kg capacity in the month of March 1995. The plants were grown in a red soil supplemented with nitrogen as urea equivalent to 25 kg/ha. Two plants were maintained in each pot. 20 pots were divided into two sets of 10 each. Plants in one set of pots were irrigated with tap water, while in the water stress treatment watering was withheld for a period of 5 days starting from 35 days after planting.

Standardization of methodology for maintaining turgor of the excised leaves : A large beaker (2 litres capacity) containing distilled water was placed below the leaf on which a cut was to be made. The leaf was slowly immersed in water and excised under water with the help of a scissors. Care was taken to see that the cut end of the leaf always remained in water and was never allowed to be exposed to air. A small beaker of 50 ml capacity was then dipped inside the large beaker. The cut end of the leaf was transferred into the small beaker. Then the entire assembly of small beaker along with the leaf was taken out. The total volume of water in the small beaker was adjusted to 30 ml. The youngest fully opened leaf was used for all the experiments. Three replications for all the experiments.

Water Potential (Ψ_w) of leaf : It was measured by a pressue chamber using N_2 gas application of abssisic acid (ABA) to the excised leaves. The leaves excised under water were allowed to stabilize for some time before the treatments were imposed. Abscisic acid was dissolved in one drop of 1 N NaOH and stock solutions of ABA were prepared to get the concentrations of 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M in 30 ml of final volume. Different concentrations of ABA, were applied to the transpirational flow of the cut leaves by adding to the small beaker with water containing the excised leaf. Immediately after the application of ABA the stomatal conductance was monitored at regular time intervals.

Application of xylem sap from the stressed plants to the excised leaves : Xylem sap was collected from the leaves of both water stressed and control plants by inserting the leaf into pressure chamber and application of pneumatic pressure. Approximately 30 μl of the sap was collected using a 10 μl micropipette. In well watered plants xylem sap could be collected with a pressure of 0.8 to 1.0 MPa whereas, in stressed plants it required a higher pressure of 1.4 to 1.6 Mpa. Xylem sap from control and stressed plants was applied to the transpirational flow of the cut leaves as described earlier for ABA, by adding to the small beakers with water containing the excised leaf. The stomatal conductance was monitored at regular time intervals.

Measurement of stomatal conductance and electrical conductivity : The stomatal conductance of the excised leaves was measured at the same leaf position at different time intervals using a previously calibrated ΔT porometer (Model AP4, Cambridge, UK). The leaves were handled carefully during the measurements to avoid any mechanical stress or damage. At the end of the experiment the electrical conductivity of the solution was measured with an Electrical conductivity bridge (Model PE-133, Elico, Hyderabad, India).

Results and Discussion

Standardization and Testing of the Simple Leaf System

The stomatal conductance of the maize leaf excised in air declined rapidly as there was no replenishment of water (data not shown). It declined from 0.47 to 0.04 cm s^{-1} within few minutes. The leaf excised under water continued to have almost constant stomatal conductance throughout the experimental period. The stomatal conductance ranged from 0.56 to 0.8 cms^{-1}. This showed that the leaf excised under water was able to maintain the process of absorption and transpiration of water.

To test whether the system works properly, different concentrations of ABA ranging from 10^{-8} to 10^{-5} M were incorporated in the transpirational flow of the excised leaf whose stomatal conductance was monitored. ABA applied to the cut leaves was taken up in the transpiration stream and resulted in the decrease of stomatal conductance (figure 1). ABA at higher concentration of 10^{-5} M resulted in faster decrease in stomatal conductance whereas a low concentration of 10^{-8} M did

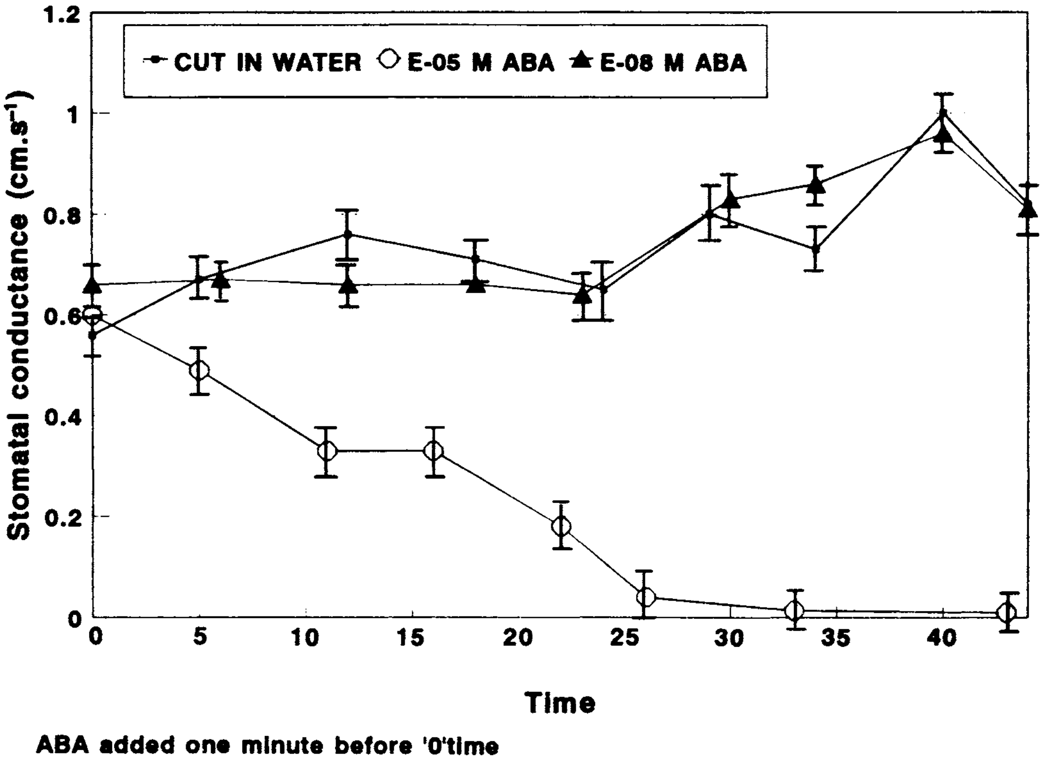

Figure 1 Effect of levels of abscisic acid on stomatal conductance of excised maize leaves

not have any effect on the stomatal function.

This showed that the ABA applied to the transpiration stream of the excised leaf was taken up and after reaching the stomata resulted in the closure of the stomata affecting its conductance. This clearly showed that this system could reliably be used to test the effect of externally incorporated substances on the leaf function.

Effect of Incorporation of Xylem Exudates from the Water Stressed and Control Plants on the Stomatal Conductance

After ascertaining that the excised leaf continues to be stable in terms of stomatal conductance and actually takes up the externally added substances, the xylem exudate from the control and stressed plants was incorporated in the transpirational flow of the excised leaf. The xylem exudates were taken up by the leaves through the transpirational stream. Exudates from the control leaf also reduced the stomatal conductance marginally. However, the reduction in stomatal conductance with stressed leaf xylem exudate was much higher (figure 2). With stressed leaf xylem exudates the stomatal conductance started decreasing within 5 minutes after the xylem sap was added. It declined from a value of 1.2 to 0.04 cms^{-1} within 25 minutes. The leaf water potential of the above leaf measured at the end of the experiment was still around –0.7 MPa, similar to that of a control plant.

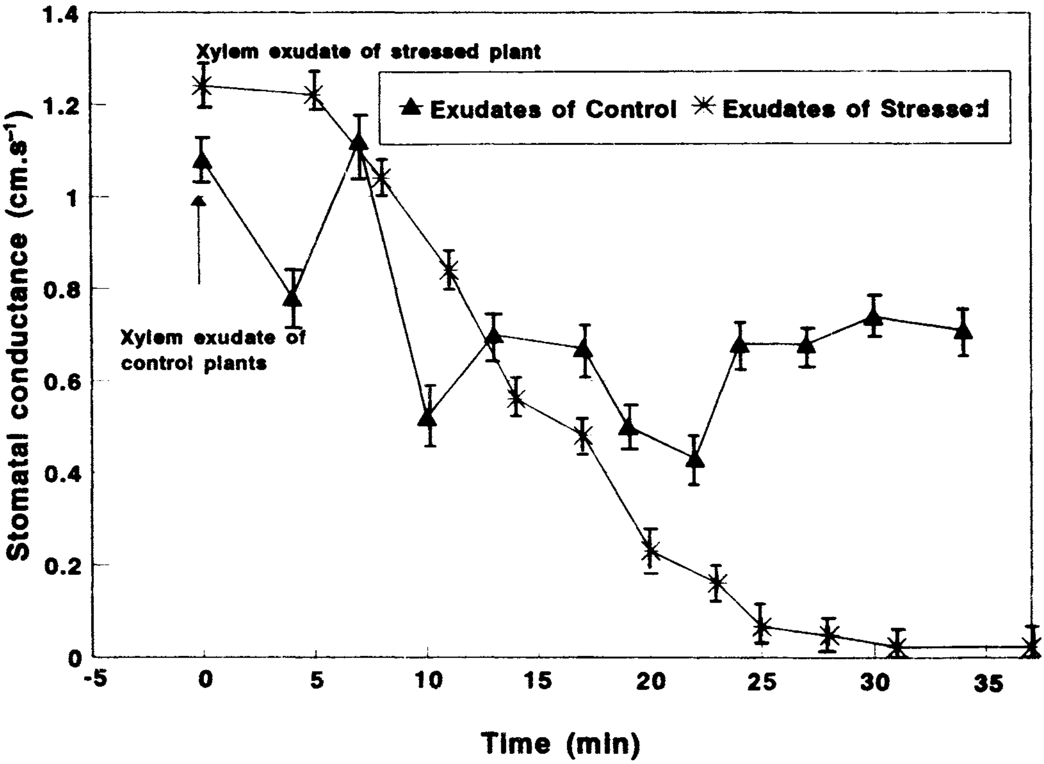

Figure 2 Effect of xylem exudates of control and water stresses plants on the stomatal conductance of excised maize leaves

Even without a reduction in the water potential the sharp decline in the stomatal conductance of the excised leaves, due to the incorporation of xylem exudates from the stressed plants indicates the presence of certain substances in the stressed plants that control stomatal function. These may be acting as root signals produced as a result of water stress and controlling the stomatal function to maintain the plant water status in a depleting soil moisture regime. Such signals may have some advantage in warning the plants of the impending water stress much before the physical forces of water potential gradients ultimately close the stomata. These findings have relevance especially in the light of recent work showing no relationship between leaf water potential and stomatal function (Bates & Hall 1981, Gollan et al. 1986, Turner 1986).

The membrane integrity of the maize excised leaves was altered with the application of xylem exudates from control and stressed plants. However, the loss of membrane integrity was more with the addition of stressed plant xylem exudate than from the control plants (table 1). The application of the leaf xylem exudates from the stressed plants to the excised leaves resulted in the increase of permeability of the cell membranes resulting in the elevated conductivity of water. The EC of the excised leaves with stress xylem exudate was 0.22 m mhos cm^{-1} whereas it was 0.06 m mhos cm^{-1} with control xylem exudate. In excised leaves with distilled water the EC was 0.03 m mhos cm^{-1}. This indicates that the substances present in the xylem exudates of water stressed plants act on the membrane permeability also.

Table 1 *Effect of xylem exudates from the control and the water stressed plants on the membrane permeability of excised maize leaves*

Treatment	EC (m mhos cm^{-1})
leaf in water	0.033 ± 0.01
Control leaf	0.068 ± 0.02
Stressed leaf	0.222 ± 0.04

30 µl of xylem exudates was administered as stated in materials and methods

The next logical question is, what is the nature of these substances that are produced due to water stress and which are controlling the stomatal function?. There is evidence to show that ABA produced by roots in drying soil moves in the transpirational stream and accumulates at or near the guard cells (Cornish & Zeevart 1985, Lachno & Baker 1986, Davies et al. 1987 and Zhang et al. 1987). It has also been reported that ABA produced in the stressed roots may act as chemical signal to the shoots initiating closure of the stomata before any changes in the water status of the leaves are noticed (Davies et al. 1987, Grantz & Meinzer 1990). In the present study also ABA incorporated in the transpirational stream resulted in rapid closure of the stomata. The inclusion of ABA and the stressed leaf xylem exudates altered the membrane permeability suggesting that these substances may be acting at the membrane level which in turn leads to closure of stomata. However, some of the studies have showed that ABA and its derivatives are not the only substances involved in root to shoot communication (Munns & King 1988, Munns, 1990, 1992 and Munns et al. 1993).

However, further work would be needed to characterize the nature of these substances especially due to the reported involvement of substances other than ABA in root to shoot communication (Munns & King 1988).

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