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*Short Communication*

# Abscisic Acid Effect on Root Exudation Related to Increased Permeability to Water

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It has been recently reported (7) that application of abscisic acid to the root medium of decapitated tomato plants increases their exudation rate.

This effect could be due to an increase in driving force or to increased permeability to water. The distinction between these two possibilities cannot easily be made by determining the effect of ABA on the osmotic concentration of the exudate. The common practice of assessing xylem osmotic concentration by measuring the osmolarity of the collected exudate has recently been subjected to serious criticism. Klepper (5) showed that the measured value is an underestimation of the real solute concentration in the water absorption region, whereas Meiri and Anderson (6) proved that under their experimental conditions the value obtained is an overestimation. It is accepted that the discrepancy between the osmolarity of the exudation fluid collected at the cut surface and that of the xylem sap at the region of osmotic water flux is a result of absorption or secretion of ions by cells surrounding the xylem. Any treatment which affects such ion transport may cause an error in determining the parameter affected.

The present paper describes experiments in which bi-directional water fluxes through the root system were measured. Evidence is presented that ABA affects the hydraulic conductivity of the root system.

## MATERIALS AND METHODS

Twenty-five-day-old sunflower plants (*Helianthus annuus* L.), grown in a greenhouse, were used throughout this investigation. Seeds were germinated in vermiculite and seedlings, 1 week old, were transferred individually to 1-liter plastic jars containing one-half strength Hoagland's solution. The nutrient solution was renewed every 4th day. The temperature in the greenhouse ranged from a maximum of 30 C at noon to a minimum of 18 C before sunrise. The photoperiod was 14 hr (0500–1900). Noon light intensity was not less than 30,000 lux.

The plants were transferred to the laboratory the evening before an experiment. The jars were kept in a thremostated bath at 27 C, and the nutrient solution was aerated continuously. These conditions were maintained throughout the experiment. At 8 o'clock in the morning the nutrient solution was renewed, and the plants were decapitated. The cut stumps were connected with a short, tightly fitting rubber tube to a 0.1-cc pipette, graduated in 1-$\mu$l units, and bent horizontally.

The exudation rate was determined by observing the movement of the exudate column in the pipette. A full pipette could be emptied by pressing the connecting rubber tube and absorbing the liquid issuing from the top of the pipette. To minimize variability the exudation rate was measured 2 hr after decapitation and was referred to as the "initial rate." Only those plants whose initial rate was higher than 20 $\mu$l min$^{-1}$ were used in the experiments.

In order to obtain a reversed exudation, the osmotic concentration of the medium surrounding the roots was raised from 8 to 170 milliosmole/kg (=4.2 atm at 27 C) by adding an appropriate amount of 1 M mannitol solution. The addition was made while a measurement was in progress, the continuous bubbling bringing about a rapid mixing of the solution. The osmotic concentrations were routinely checked by means of a Knauer electronic semimicro osmometer. A drop in the osmotic concentration around the roots was achieved by transferring the root system, via a rinsing glass, to a new jar containing pure one-half strength Hoagland solution. This procedure lasted approximately 30 sec. The ABA was dissolved in nutrient solution.

## RESULTS

Figure 1 summarizes experiments performed to determine conditions for a maximal optimal effect of ABA. A concentration of 1 mg/l was most effective. An increase in exudation rate could be detected within 30 min of applying the hormone to the root medium. After 60 min the exudation reached a steady rate which lasted at least 3 hr and was about twice that of untreated roots. Removal of ABA from the root medium after 30 min did not weaken its effect compared to a continuous supply of the hormone.

Measurements made occasionally the next morning, 24 hr after a 30-min treatment with ABA, still showed an increased rate of exudation.

Figure 2A shows the effect of adding mannitol to the root medium on the course of exudation. As expected, an immediate drop in the exudation rate was obtained and exudation subsequently became negative (*i.e.*, water flowed into the cut surface of the stump). It reached its lowest value in the second period of 30 sec. Then the rate increased slowly, approaching zero after 10 min.

On transfer of the root system to pure nutrient solution, the exudation rate returned to its initial value within 20 min. This indicates that the 10-min mannitol treatment did not alter the mechanism of the exudation process.

The effect of ABA treatment on negative exudation rate is shown in Figure 2B. The roots whose response to mannitol treatment was previously recorded (Fig. 2A) were transferred into a nutrient solution with or without 1 mg/l ABA for 30 min. They were then transferred into a pure nutrient solution for another 30 min. The exudation rate of the ABA-treated roots was increased to 45 $\mu$l min$^{-1}$ while that of the untreated remained almost unchanged at 28 $\mu$l min$^{-1}$. Now the same 170

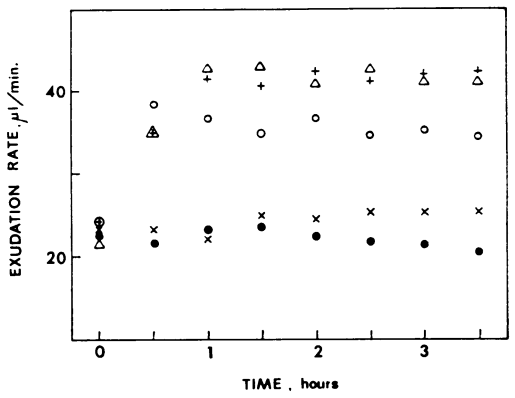


FIG. 1. Effect of ABA on the exudation rate of decapitated sunflower plants. The roots were treated at time zero, 2 hr. after decapitation. ×: 0.2 mg/l; +: 1 mg/l; ○: 5 mg/l—continuous ABA treatment. △: 1 mg/l ABA treatment for the first 30 min; ●: control. Each point is the mean of measurements on five roots.

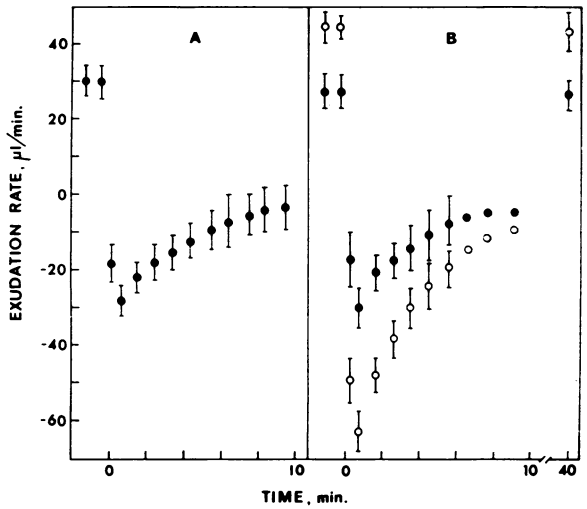


FIG. 2. Effect of adding mannitol to the root medium on the course of root exudation. A: Before ABA treatment, 2 hr after decapitation. At time 0, mannitol (170 milliosmole/kg) was added to the medium. After 10 min the roots were transferred to a pure nutrient solution and half were treated with ABA (see text). B: After ABA treatment, 3.5 hr after decapitation. At time 0 mannitol was again added to the medium. ●: Untreated roots; ○: ABA-treated roots. Each point is the mean of measurements on 16 (A) or 8 (B) roots. Vertical bars indicate ± SE

milliosomole/kg mannitol treatment was again applied. The initial negative exudation was much more pronounced in the ABA-treated roots. It could already be very clearly observed within the first 30 sec and in the second period of 30 sec it reached the value of $-62$ $\mu$l min$^{-1}$ as compared to $-30$ $\mu$l min$^{-1}$ in the case of the control roots. During the 10 min of mannitol treatment, the gap between the treated and untreated roots diminished, both fluxes approaching zero. When the roots were transferred to a pure nutrient solution, their exudation rates returned to the rates observed before the mannitol treatment.

## DISCUSSION

Abscisic acid treatment increases the exudation rate from decapitated sunflower plants anad also increases negative exudation when the osmotic concentration of the root medium is increased by adding mannitol. Water flux through the root is thus increased in both the inward and outward directions, a fact which indicates, as will be shown later, that ABA increases the hydraulic conductivity of the root system.

The exudation process can be expressed by the following equation:

$$J_v = L_p (\sigma_x \pi_x - \sigma_m \pi_m) + \phi_w \quad (1)$$

where $J_v$ is volume flow of water, $L_p$ is the coefficient of hydraulic conductivity, $\pi_x$ and $\pi_m$ are the osmotic concentrations of the xylem and medium respectively, $\sigma_x$ and $\sigma_m$ are the reflection coefficients for the xylem and medium solutes, respectively, and $\phi_w$ is the water flux from medium into the xylem independent of any osmotic gradient.

The interpretation of $\phi_w$ is controversial, but its polar nature, namely from the medium into the xylem, is generally accepted (2).

If some treatment brings about an increase in $J_v$, then according to equation 1 this can result from an increase in $L_p$, $\pi_x$, $\sigma_x$, or $\phi_w$ or a decrease in $\sigma_m$.

When the osmotic concentration of the root medium is increased by the addition of mannitol to such an extent that $J_v$ becomes negative, *i.e.*, water flows from the cut stump surface through the root system into the surrounding medium, then any increase in $\pi_x$, $\sigma_x$, or $\phi_w$ or a decrease in $\sigma_m$ should cause a negative $J_v$ of smaller magnitude.

The observed effects of applying ABA to the roots, *i.e.*, an increase in both positive and negative $J_v$, eliminate the possibility that a change in $\sigma_x \pi_x$, $\sigma_m \pi_m$, or in $\phi_w$ is responsible for the increased exudation rate. (An increase in the reflection coefficient of mannitol seems very unlikely since its normal value is virtually 1.) An increase in the hydraulic conductivity of the root system is, thus, the only reasonable explanation of the abscisic acid effect.

Raising the external osmotic concentration initiates a typical course of events which has been described in detail in the literature (1, 4): a rapidly decreasing rate of exudation followed by a relatively slow rise to a new level. The first stage, where the rate is falling, presumably indicates that time is needed to build up the external concentration at the outer border of the osmotic barrier in the root system. The second stage, the subsequent rising rate results from alteration in $\pi_x$ caused by the change in water flux and continued ion transport. In the present investigation, where a much higher external concentration was used, the same general pattern was observed. The highest rate of negative exudation was attained in the second period of 30 sec, after which the rate became less negative, tending to reach zero.

Ideally, the negative exudation rate should have been determined at the moment of increasing the external concentration, before any change in the internal solution occurs. Unfortunately, this cannot be achieved because of the existence of the first stage.

The ABA treatment did not change the duration of the first stage. Nevertheless, since during the first 30 sec the negative water flow in ABA-treated roots was stronger, their $\pi_x$ possibly became higher than that of the control. Determination of the ABA effect on $L_p$ by comparing the negative exudation rates of treated and untreated roots at the beginning of the second stage only is, if so, an underestimation.

Had it been possible to measure the true negative exudation rate at the moment of increasing external concentration and the real solute concentration in the water absorption region of treated and untreated roots, it could have been checked whether the apparent change in $L_p$ was fully symmetrical—*i.e.*, whether the size of the change in $L_p$ indicated

by negative exudation measurements completely accounted for the observed changes in positive exudation.

As it is, the quantitative information available is not adequate to exclude the possibility that other parameters in equation 1, apart from $L_p$, were affected by the ABA. But even so, the increase in hydraulic conductivity must predominate in causing the increased exudation rate, as explained in the discussion of the equation. The effect of ABA on root exudation therefore, in all probability relates to its effect on permeability of plant cell membranes to water reported earlier (3).

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