

Effects of Abscisic Acid and of Hydrostatic Pressure Gradient on Water Movement through Excised Sunflower Roots

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ABSTRACT

The effect of abscisic acid on the exudation rate from decapitated roots of sunflower plants (*Helianthus annuus* L.) was investigated in the presence and absence of an imposed hydrostatic pressure gradient. The magnitude of the abscisic acid effect was constant even when suction up to 60 cm Hg were applied to the cut stumps.

When roots were bathed in a THO-labeled nutrient solution, the course of the appearance of radioactivity in the exudate, expressed as a function of exudate volume, was not affected by abscisic acid treatment but was strongly speeded up by applying suction.

The implications of those findings with regard to the water pathway through the root and the location of the abscisic acid effect are discussed.

It is well established that ABA causes a considerable increase in exudation rate from decapitated roots (4, 5, 10), a phenomenon which was attributed to an increase in hydraulic conductivity of the roots. However, these findings do not indicate unequivocally that ABA has an identical effect, if any, in an intact root of a transpiring plant. While exudation experiments are carried out ordinarily when the driving force for water flow is solely an osmotic potential gradient, in the intact plant, on the other hand, an important fraction of the driving force for moving water through the root is a hydrostatic pressure gradient formed by the transpiration pull.

The nonequivalence of these two components on water flux was clearly demonstrated (7). It was suggested that hydrostatic pressure gradient causes either changes in water permeability or changes in the root's absorbing area and in the water pathway inside it (1, 2, 7).

It was of interest, if so, to test the ABA effect on water flux through the root when a gradient in hydrostatic pressure was imposed, a state which may be comparable to that of a transpiring plant.

In addition, the ABA effect was used to obtain further information on the relationship between the nature of the driving force for water flux and its pathway through the root.

MATERIALS AND METHODS

Twenty-five-day-old sunflower plants (*Helianthus annuus* L.) grown in a greenhouse were used throughout these experiments. Seeds were germinated in vermiculite and seedlings, 1 week old, were transferred individually to 1-liter plastic jars containing 0.5 strength Hoagland 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 thermostated bath at 27 C and the nutrient solution was aerated continuously. These conditions were maintained throughout the experiment. At 8 AM, the nutrient solution was renewed, and the plants were decapitated. The cut stumps were connected with a short, tightly fitting rubber tube to a graduated pipette. The exudation rate was determined by observing the movement of the exudate column in the pipette.

To minimize variability, the exudation rate was measured 1 hr after decapitation and was referred to as the "initial rate." Only those roots whose initial rate was 8 to 16 $\mu\text{l min}^{-1}$ were used in the experiments. Roots were allocated to the various treatments in such a way that identical initial rates were obtained.

The air pressure above the exudate column was reduced by connecting the pipette to a suction system which was composed of a pressure-leveling vessel connected to a vacuum pump. The pressure was monitored with a mercury manometer and was adjusted by means of a needle valve on the pressure-leveling vessel.

In order to observe THO transfer from the medium to the exudation fluid, the root system was transferred to a nutrient solution containing tritiated water. When a desired volume of exudate had been collected in the pipette, its radioactivity was determined using a Packard liquid scintillation spectrometer.

Abscisic acid (Sigma, \pm *cis-trans* isomer) was applied at a concentration of 4 μM in the nutrient solution. Measurements of ABA-treated roots began 1 hr after the roots were transferred to the ABA solution. It had been shown (5) that 1 hr is sufficient to obtain a full effect of ABA on exudation rate.

Osmotic potentials were measured by means of a Knauer electronic semimicroosmometer. Values of 9 and 48 milliosmol/kg for the medium and the exudate, respectively, were obtained, giving a $\Delta\Psi$ of 0.96 atm at 27 C.

RESULTS

A typical experiment showing the effect of ABA on exudation rate as a function of hydrostatic pressure difference between the root surface and the exudate column is summarized in Figure 1.

It can be seen that the effect of suction on ABA-treated roots was similar to that on untreated ones. The ratio in exudation rate of ABA-treated *versus* untreated roots ranged between 1.8 and 1.9 at all of the suction applied from 0 to 60 cm Hg.

The fact that the effect of ABA on exudation rate was constant no matter whether the driving force for water movement was of a purely osmotic nature (when no suction was applied), or included a significant hydrostatic pressure component, led us to test the possible influence of pressure gradient on water pathway in our root system.

The experiment summarized in Table I was conducted for this purpose. After determining the initial exudation rate, the roots were transferred to THO-labeled nutrient solution and their cut stumps were subjected simultaneously to either 50 cm Hg suction or to atmospheric pressure. When a volume of 2.8 ml of sap

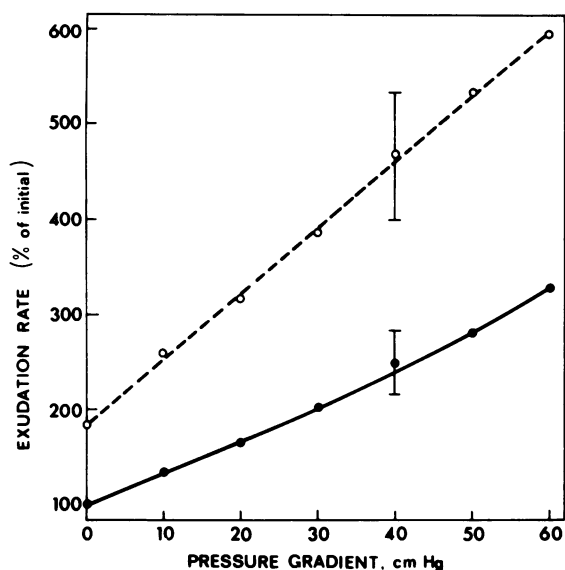


FIG. 1. Effect of hydrostatic pressure gradient between root surface and exudate on the exudation rate of ABA-treated sunflower roots. Each root was subjected to increasing suction from 0 to 60 cm Hg. ○: ABA; ●: control. Mean initial exudation rate: $13.2 \mu\text{l min}^{-1}$. Each point is the mean of measurements of 12 roots, expressed as per cent of their initial rate. Vertical bars indicate \pm SE.

Table I

Effect of Hydrostatic Pressure Gradient on THO Appearance in the Exudate of Sunflower Roots
External THO concentration was 105,000 cpm/ml. The data are the means \pm standard errors of 12 roots.

Treatment	Relative exudation rate ¹	Length of treatment ² min	Radioactivity of exudate cpm $\times 10^{-2}$
No suction applied	1.15	240	784 ± 82
Suction (50 cm Hg.) applied	3.45	84	1232 ± 98

¹Relative to the mean initial rate ($9.9 \mu\text{l min}^{-1}$).

²Time required to obtain 2.8 ml of exudate.

had been secreted from a given root, its pipette was disconnected, and the radioactivity of the secreted solution was determined. The amount of THO secreted into the xylem of roots under 50 mm Hg hydrostatic pressure gradient was approximately 160% as compared with roots lacking pressure gradient (Table I).

Since the THO was present exclusively in the external medium at zero time, the increased amount of tritiated water in an identical volume of sap indicates that by applying hydrostatic gradient, a "shortened" pathway between the medium and the root xylem was operating.

The effect of ABA on the course of THO appearance in the exudate is shown in Figure 2. In this case, short glass tubes were connected to the cut stumps and at selected time intervals the exudate was collected by means of a syringe and its volume and radioactivity were determined. The exudate radioactivity did in fact rise much more steeply with time in ABA-treated roots as compared to control roots (Fig. 2), but when the data were expressed as a function of exudate volume (Fig. 3), it appears that in contrast to the case of the effect of pressure gradient, no speeding up by ABA on the arrival of THO in the exudate was observed.

In Table II, an experiment of combined ABA and pressure gradient treatments is summarized. After the ABA effect on exudation rate reached its steady value (first phase), the roots were transferred to THO-labeled nutrient solution and were subjected to either 40 cm Hg suction or atmospheric pressure until a volume of 2.5 ml of sap, individually for each root, was

collected in the pipette (second phase). The second phase lasted, according to treatment, from the mean of 27 up to 155 min.

The effect of pressure gradient on THO appearance in the exudate of ABA-treated roots was clear (treatments 3 and 4)

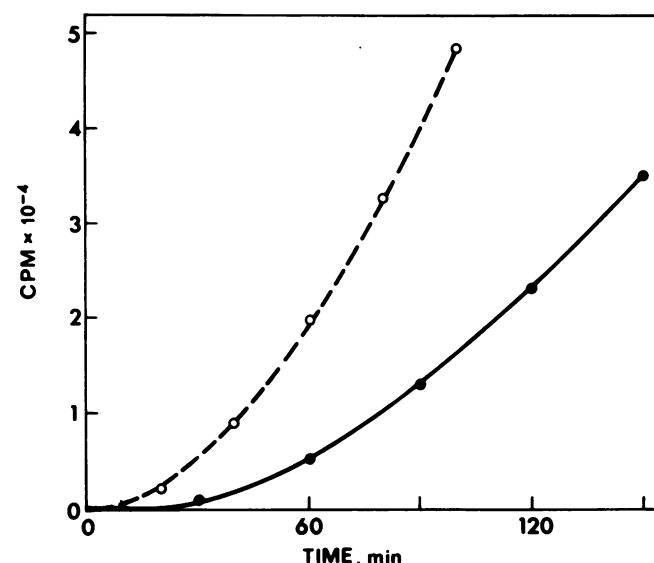


FIG. 2. Course of THO appearance in exudate as a function of time in ABA-treated and control sunflower roots. ○: ABA; ●: control. Exudation rate: $20.8 \mu\text{l min}^{-1}$ in ABA-treated roots; $12.2 \mu\text{l min}^{-1}$ in control roots. Each point is the mean of measurements of nine roots.

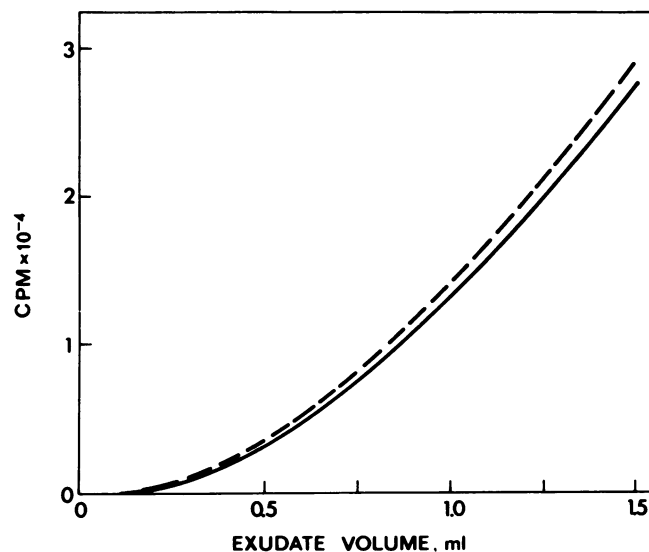


FIG. 3. Course of THO appearance in exudate as a function of exudate volume. Calculated from data of Figure 2. ---: ABA; —: control.

Table II

Effects of ABA and of Hydrostatic Pressure Gradient on the Exudation Rate and on the Appearance of THO in the Exudate of Sunflower Roots

First phase: 1-2 hr after applying ABA.
Second phase: The period of applied 40 cm Hg suction to roots while bathed in THO labelled solutions.
External THO concentration 96,000 cpm/ml.
The data are the means \pm standard errors of 7 roots.

No.	Treatment ABA Suction	Relative exudation rate ¹ 1st phase 2nd phase	Length of 2nd phase (min) ²	Radioactivity of exudate cpm $\times 10^{-2}$
1	- -	1.1 ± 0.1 1.0 ± 0.1	155	312 ± 41
2	- +	2.6 ± 0.4	59	504 ± 49
3	+ -	1.9 ± 0.3 2.1 ± 0.2	74	360 ± 37
4	+ +	5.7 ± 0.7	27	480 ± 42

¹Relative to the mean initial exudation rate ($12.2 \mu\text{l min}^{-1}$).

²Time required to obtain 2.5 ml exudate.

although it was somewhat lower than in untreated roots (treatments 1 and 2). No effect of ABA on the course of THO appearance was obtained in roots subjected to pressure gradient (treatments 2 and 4) when equal volumes of exudate were compared.

DISCUSSION

A hydrostatic pressure gradient across the root has been shown to be more effective in moving water through it than an equal gradient in osmotic potential (2, 7). Two main explanations were suggested for this phenomenon: (a) the actual permeability of the cell membrane to water is increased; (b) the effective absorbing area of the root is increased, and additional pathways come into force, e.g. walls of passage cells or other unsuberized regions of the endodermis (cf. 2).

THO can be considered as a tracer for the water of the medium of the roots. The findings summarized in Table I show that exerting a hydrostatic gradient across the root not only caused an increased water flow which is greater than can be accounted for by the increased $\Delta\Psi$, an increase in $\Delta\Psi$ from 0.96 to 1.61 atm increased flow from 11.4 $\mu\text{l min}^{-1}$ to 34.1 $\mu\text{l min}^{-1}$, but also "shortened" the water pathway from root surface to xylem as indicated by the higher concentration of THO in the exudation fluid after equal volumes of exudate had been collected in the two cases.

The radial pathway of water through the root from surface to xylem is still controversial. Opinions as to its route in the cortex range from the view that the apoplast is the major pathway (1) to regarding the symplasm as the predominant route (8). Assuming that hydrostatic pull is more dependent on pore size than osmosis because of its mass flow nature, and that the larger "pores" in the root system are in the cell walls (cf. 9), the observed difference in pathway when a pressure gradient is applied is probably a result of a shift in the relative importance of the symplasmic and apoplastic water flows in the cortex, toward the latter pathway. The fact that pressure-induced water flow is less sensitive to inhibitors than osmotically induced water flow (7) is compatible with this view.

That ABA increases the exudation rate from excised roots has been clearly demonstrated (4, 5, 10). The present results show that the percentage increase in water flow induced by ABA was constant when the cut stumps were exposed to up to 60 cm Hg suction (Fig. 1). Since it is unreasonable to propose that ABA

affects apoplastic water flow, it may be assumed that even when suction is applied, water must still traverse through a symplasmic barrier, probably the endodermal cells.

It has been shown that ABA increases the water permeability of carrot root cells (6) and of cortical maize root cells (3). Such an effect should express itself in increasing the ratio of symplasmic flow *versus* apoplastic flow through the cortex as compared with the control, and since the volume of the symplasm is large compared to the apoplast, the concentration of THO in the exudate of ABA-treated roots should, consequently, be lower. However, the results presented in Figure 3 and Table II show that ABA did not alter the concentration of THO in the exudate when equal volumes of exudate were compared.

Looking upon the symplasmic and the apoplastic pathways in the cortex as two parallel resistances which are arranged in series with the resistance of the endodermal layer and assuming that the resistance to water flow through the symplasm is higher than through the apoplast, the explanation suggested for the results discussed above is that the water permeability of the endodermal cells was increased by ABA to a higher extent than that of the cortical cells and consequently the ratio of apoplastic *versus* symplasmic flows through the cortex virtually did not change.

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