Plant Physiol. (1980) 65, 537–540
0032-0889/80/65/0537/04/$00.50/0

Abscisic Acid Promotes Both Volume Flow and Ion Release to the Xylem in Sunflower Roots

Received for publication May 23, 1979 and in revised form October 15, 1979

ZVI GLINKA
Department of Botany, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

ABSTRACT

The effect of abscisic acid (ABA) on the exudation process in excised sunflower root was investigated. A promotion of both ion flux and volume exudation rate was observed. Cutting off the external supply of ions to the roots markedly increased the magnitude of the ABA effect. The promotive effect of ABA on exudation rate was extremely rapid (less than 6 minutes) and seemed to be biphasic. Reasons are given for relating the ABA effect to two separate actions, namely: (*a*) on water permeability; and (*b*) on the release of ions both from vacuoles to the cytoplasm and from symplasm to the xylem. Kinetin inhibited movement of ions to the xylem and its effect did not appear to be related to that of the ABA.

ABA is known to affect both volume exudation and ion movement to the xylem in excised roots, phenomena believed to have a bearing, respectively, on water permeability and on ion transport, either from the medium to the symplasm or from the symplasm to the xylem (10).

Promotive (2, 5, 6, 8, 14) as well as inhibitory (3, 4, 12) effects, however, have been reported with the use of ABA, depending on its concentration, the plant species, the growing conditions, and the temperature (11). It was recently shown that in some cases the change in volume flow (increase or decrease) results entirely from corresponding changes in the ion transport (8, 13).

In a previous paper (5), I showed that in sunflower root systems, the hydraulic conductivity was increased markedly by ABA. The present paper investigates the effect of this hormone on the release of ions to the xylem in sunflower roots and its relation to the increased water flow. To distinguish between the effect of ABA on the release of ions to the xylem and its possible effect on transport into the root cells, the external supply of ions to the roots was cut off. Apart from increasing water permeability, ABA also promotes ion movement by acting on ion release both from vacuoles to cytoplasm as well as from symplasm to the xylem.

MATERIALS AND METHODS

Four-week-old sunflower plants (*Helianthus annuus* L.) grown in a growth chamber were used throughout. Seeds were germinated in Vermiculite and the 1-week-old seedlings transferred individually to 1-liter plastic jars containing half-strength Hoagland solution. The nutrient solution was renewed every 4th day and on the day before each experiment. The temperature in the chamber was 25 C day/18 C night. The photoperiod was 14 h (0500–1900) and the light intensity was 280 μE m^{-2}s^{-1}. Because of the circadian fluctuations in the rate of exudation (15), the experiments were started routinely at 7 AM. Throughout the experiments the jars were kept at 25 C and the solutions aerated continuously. The plants were decapitated 1 cm above the transition zone and pieces of tightly fitting rubber tubing were affixed to the cut stumps.

At selected time intervals the exuding sap was collected by means of a syringe and subjected to the appropriate measurements. For rapid determination of volume flow, a 0.2-ml pipette was connected to the rubber tube and the exudation rate was measured by observing the movement of exudate column in the pipette. To ensure uniformity, only roots with a 1st-h exudation rate of 0.5–0.8 ml h^{-1} were used in the experiments. Roots were allocated to the various treatments in such a way that almost identical mean 1st-h exudation rates were obtained. The fresh weight of root systems ranged from 8 to 12 g.

In order to deprive the roots of an external supply of ions, they were rinsed for 2 min in distilled H_2O and then transferred to jars containing 0.2 mM $CaSO_4$ solution. The $CaSO_4$ solution was used throughout in order to retain the integrity of cell membranes.

Abscisic acid was added, from a fresh prepared stock solution containing 40 μg cm^{-3}, to a final concentration of 4 μM. This concentration had been shown previously (15) to produce maximum promotion of volume exudation in sunflower roots. The osmotic concentrations of the exuding sap and of the root's medium were determined by means of an automatic osmometer (Osmette A, Precision Systems, Inc., Sudbury, Mass.). The exudates, after suitable dilution, were further assayed for K^+ by flame photometry and for NO_3^- by UV spectrophotometry (1).

RESULTS

Preliminary experiments were run on excised sunflower roots to test the effect of cutting off the ion supply to the root's medium on the release of ions and on the volume flow to the xylem. Deprivation of the roots from an external supply of ions resulted in a gradually diminishing release of K^+ to the xylem, the rate of release decreasing within 4 h from about 0.5 to 0.2 μmol h^{-1} g^{-1} fresh weight. However, a low rate of net K^+ flux was observable even after 24 h (Fig. 1A). The volume flow also changed when the roots were transferred from the nutrient solution to the $CaSO_4$ solution. After a transient elevation in flow, stemming presumably from the sudden increase in outer water potential, there was a gradual decrease in water flow, the rate diminishing to about half the initial value within 6 h, but persisting at low level also by next morning, 24 h after excision (Fig. 1B).

The effect of 4 μM ABA on K^+ and NO_3^- release to the xylem in roots placed in 0.2 mM $CaSO_4$ solution, is shown in Figure 2. ABA was applied to the roots 2 h after their removal from the nutrient solution, by which time the concentrations of K^+ and NO_3^- in the apoplast became presumably very low.

Increased flux of both K^+ and NO_3^- in the ABA-treated roots was clearly evident within 1 h. The ABA effect reached its maximal value (250-300% as compared to the control) 3 h after application of the hormone and persisted at a high level for at least 2 h.

A comparison of ABA effect in roots kept in their nutrient

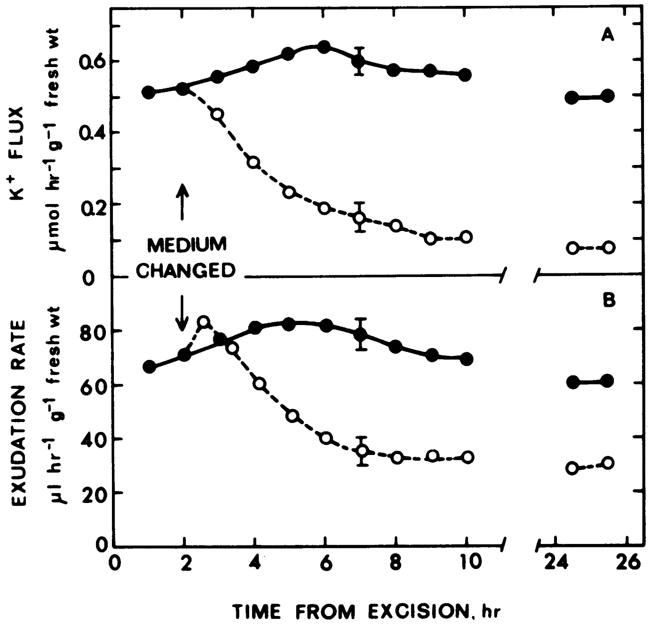

FIG. 1. Effect of excluding ions from root's medium on course of K^+ release to xylem (A) and on exudation rate (B). ●: Nutrient solution; ○: 0.2 mM $CaSO_4$ solution. Each point is mean of six different roots measured. Vertical bars indicate ± SE.

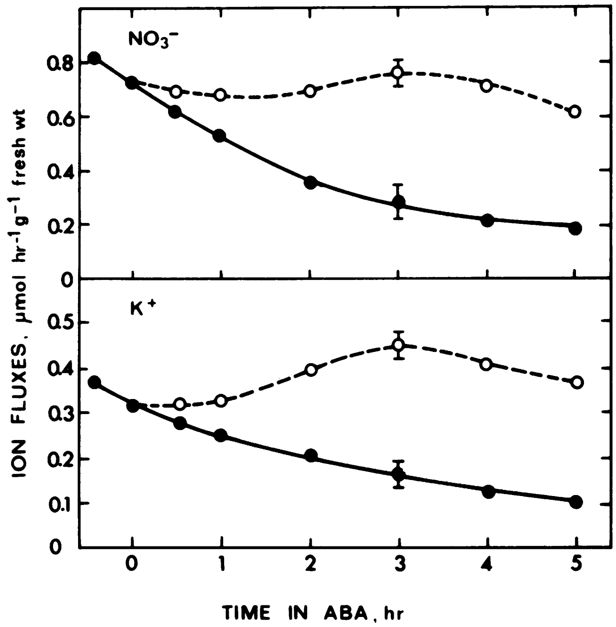

FIG. 2. Effect of ABA on the release of NO_3^- and K^+ to the xylem of sunflower root systems. The roots were excised and placed in 0.2 mM $CaSO_4$ solution 2 h before applying ABA (ABA was added at zero time). ○: Four μM ABA; ●: control. Each point is the mean of eight roots measured. Vertical bars indicate ± SE.

solution and those placed in $CaSO_4$ solution is presented in Table I. Data pertaining to the 4th h of ABA treatment were chosen because they best represented the continuously steady effect of ABA (Fig. 2).

The relative promotive effect of ABA on the flux of ions as well as water was much stronger in roots immersed in $CaSO_4$ solution than in those kept in the nutrient solution (Table I). Both the K^+ and NO_3^- fluxes increased more than 3-fold in the former roots, as compared to only about 160% in the latter.

Table I. *Effect of 4 μM ABA on Exudation Rate (J_v) and Release of Ions to Xylem of Sunflower Root Systems Bathed in either Nutrient or 0.2 mM $CaSO_4$ Solution*

Values given are for 6th h post-root excision and solution change which is 4th h post addition of ABA. Values are means of six roots ± SE.

Medium and Treatment	K^+	NO_3^-	J_v
	μmol $h^{-1}g^{-1}$		*μl $h^{-1}g^{-1}$*
Nutrient solution	58 ± 2	95 ± 6	70 ± 4
Nutrient solution + ABA	87 ± 6	155 ± 8	142 ± 10
ABA/control	1.50	1.63	2.03
$CaSO_4$ solution	10 ± 0.6	18 ± 0.9	31 ± 2
$CaSO_4$ solution + ABA	35 ± 2	60 ± 3	111 ± 7
ABA/control	3.50	3.33	3.58

The effects of ABA on the ions flux and on the volume exudation rate, in the roots placed in $CaSO_4$ solution, were closely correlated. This resulted in an almost equal concentration of ions in the exudate of ABA-treated and control roots.

The effect of ABA on the hydraulic conductivity of the roots was calculated by using a somewhat simplified equation for the exudation process:

$$J_v = L_p(\pi_x - \pi_o) \quad (1)$$

where J_v is the volume flow, L_p is the hydraulic conductivity, and π_x and π_o are the osmotic concentrations of the xylem and medium, respectively. It was assumed that the reflection coefficient for both xylem and medium solutes was equal to 1 and that any possible nonosmotic component of the driving force could be ignored. These assumptions seem justified inasmuch as they affect treated and untreated roots to the same extent.

Table II summarizes the measurements of osmotic concentration of exudate and medium in the different treatments. A substantial decrease in π_x and, consequently, in $(\pi_x - \pi_o)$ was obtained in ABA-treated roots in the two solutions. The calculated values of $J_v/(\pi_x - \pi_o)$ show a 3- and 5-fold ABA-induced increase in L_p for roots immersed in nutrient and $CaSO_4$ solutions, respectively.

To observe the effect of kinetin on release of ions to the xylem, this hormone was applied to the roots in concentrations of 10, 5, and 1 μM. No consistent results were obtained, for in some of the experiments a strong inhibition of both K^+ release and volume flow was observed within 2 h, whereas in others even a 24-h treatment produced no adverse effect.

Noteworthy in this connection was the observed independence of the roots' response to kinetin from that to ABA. Table III, in which two separate experiments are summarized, shows that an almost identical promotive effect of ABA was obtained in both kinetin-sensitive (experiment A) and kinetin-indifferent (experiment B) roots.

In a different set of experiments, the volume flow of exudate was recorded at 2-min intervals and the effect of ABA on the flow evaluated accordingly. Figure 3 summarizes graphically the results of a typical experiment in this series. A significant promotive effect of ABA on volume flow was detected within 6 min from application of the hormone.

The course of the ABA-induced increase in rate of volume flow seemed to be composed of two phases. The initial phase starting almost instantaneously, presented about a 40% increase in flow rate within 20 min. Then, after a plateau of about 30 min, there was resurgence of the volume flow and within 2 h attained 300% or more than that of the control.

DISCUSSION

The results presented in this paper show that 4 μM ABA promotes the net flux of K^+ and NO_3^- to the xylem of excised sunflower roots when these are placed in a solution of 0.2 mM

Table II. *Effect of 4 μM ABA on Exudation Rate (J_v), Osmotic Concentrations of Medium (Π_o) and Exudate (Π_x), and Hydraulic Conductivity (L_p) of Sunflower Root Systems*

Values given are for 6th-h post-root excision and solution change, comprising the 4th h post-ABA addition. Values are means of six roots ± SE.

Medium and Treatment	J_v	Π_o	Π_x	L_p
	$\mu l\ h^{-1}g^{-1}$	*atm × 10^2*		*$\mu l\ h^{-1}g^{-1}$/atm*
Nutrient solution	74 ± 4	26	99 ± 6	1.0
Nutrient solution + ABA	139 ± 10	26	72 ± 5	3.0
$CaSO_4$ solution	32 ± 2	3	69 ± 4	0.5
$CaSO_4$ solution + ABA	122 ± 8	3	51 ± 4	2.5

Table III. *Effects of 5 μM Kinetin and 4 μM ABA on Release of K^+ to Xylem of Excised Sunflower Roots*

Values are from two separate experiments (A and B).

Duration of Treatment	Rate of Release			
	Experiment A		Experiment B	
	Kinetin	ABA	Kinetin	ABA
h	*% of control*			
2	30	320	105	350
24	5	200	95	180

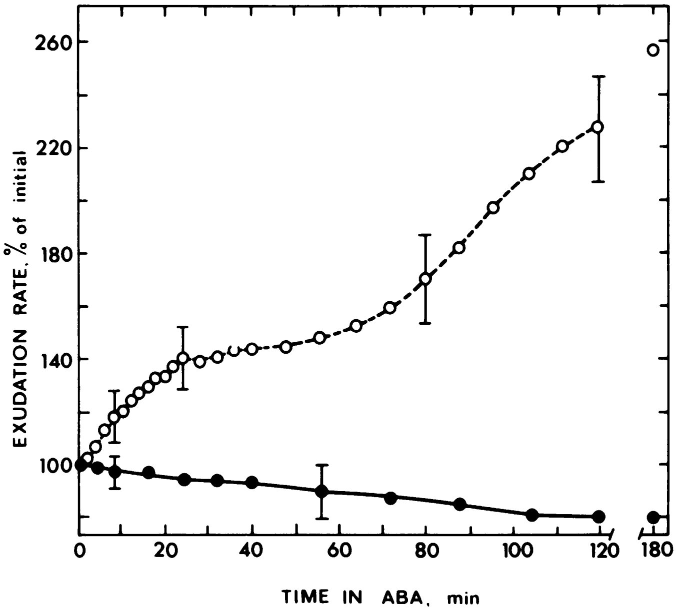

FIG. 3. Short time measurements of course of volume flow as affected by ABA in sunflower root systems. Roots were excised and placed in 0.2 mM $CaSO_4$ solution 4 h before ABA was applied. ○: Four μM ABA; ●: control. Each point is mean of 10 measurements expressed as percentage of initial exudation rate. Vertical bars indicate ± SE. Mean initial exudation rate: 40 μl $h^{-1}g^{-1}$ fresh weight.

$CaSO_4$. Moreover, the magnitude of the relative effect of ABA was much greater than that obtained when the roots were kept in the nutrient medium.

The rate of ion release to the xylem is governed by two processes: (*a*) transfer from the symplasm to the vessels; and (*b*) loading of the symplasm from either the medium or the vacuoles (10). On excluding ions from the medium, the vacuoles probably become the main source of ions for the symplasm. The fact that cutting off the external supply of ions enhanced the relative promotive effect of ABA on ion release to the xylem seems to indicate that ABA enhances the flow of ions from the vacuoles to the cytoplasm, an enhancement which was probably masked partly when a continuous transport of ions from the medium to the symplasm took place.

Concurrent with the ABA-induced enhanced release of ions to the xylem, there was an increase in volume flow. This increase in volume exudation rate may be due to the stronger flow of solutes to the xylem, elevating the osmotic driving force for water movement. My calculations, however, showed a marked increase in $J_v/(\pi_x - \pi_o)$, confirming my previous finding (5) that ABA enhanced the hydraulic conductivity of sunflower roots (see equation 1).

The determination of L_p by presenting the osmotic concentration of the collected exudate as π_x is subject to some criticism (9). The osmotic concentration of the collected exudate may vary from that of the xylem at the region of the osmotic water flux due to an exchange of solutes between the vessels and the surrounding cells in the upper parts of the root. An effect of ABA on that exchange may misleadingly be credited to the sum effect of the hormone on L_p. Enhancement of the hydraulic conductivity by ABA is nevertheless undeniable, else the concentration of solutes at the osmotic region would have to be five times greater than that actually measured to account for the observed exudation rate in the ABA-treated root (Table II).

The higher water flow ensuing from increased L_p may stimulate the movement of ions to the xylem by lowering the concentration of ions on the inner side of the osmotic barrier (= dilution effect [7]) or possibly also by intensifying the apoplastic ions movement.

This notwithstanding, it is hard to believe that an increase of as much as 350% in the rate of ions released to the xylem (Table I) could have resulted exclusively from the faster water flow (*cf.* 10). Inasmuch as it is reasonable to assume that water-flow-dependent movement of ions to the xylem would be more effective upon continuous supply of ions to the root, the results presented in Table I support the view that ABA indeed promotes the release of ions to the xylem. In the experiments summarized in Table I the ratio of ABA effect on release of ions *versus* its effect on volume flow was higher in roots immersed in $CaSO_4$ solution (3.50/3.58) than in roots kept in nutrient solution (1.50/2.03).

It may be justifiably concluded that ABA brings about two discrete effects in sunflower roots: (a) elevated hydraulic conductivity; (b) increased transport of ions to the xylem. And indeed, the typical biphasic nature of the ABA-induced increase in the rate of exudation (Fig. 3) seems to strengthen this conclusion. It is hard to ascertain whether the rapid response of roots to the added ABA is effected primarily via ion transport or hydraulic conductivity. In a root system as highly branched as that used in these experiments, any attempt to follow the changes in the rate of ions released to the xylem during the two observed phases of water flow, would *a priori* be doomed to failure because the sap released from the cut end enters the xylem at an earlier time (depending on the distance of the entering point from the cut end and on the rate of flow in the vessels of the different branches) so that it becomes impossible to ascertain the precise moment of the ions' entry.

Nonetheless, it is tempting to relate the immediate elevation in the volume exudation rate to the increased hydraulic conductivity of the roots and to attribute the second stage of the increase to an ABA effect on the release of ions from vacuoles to cytoplasm and from symplasm to the xylem.

Opposite effects of kinetin and ABA on ion transport in roots were reported previously (2, 14). It was also suggested that the observed effect of ABA resulted from an upsetting of the endogenous balance between kinetin and ABA (8, 11).

In some of the present experiments, kinetin inhibited K^+ release to the xylem whereas in others, no such effect of exogenous kinetin was detected (Table III). Insofar as in both instances a similar promotive effect of ABA was obtained, this seems to suggest an apparent independence of the ABA action.

LITERATURE CITED

1. Bastian R, R Weberling, F Palilla 1957 Ultraviolet spectrophotometric determination of nitrate. Anal Chem 29: 1795–1797
2. Collins JC, AP Kerrigan 1974 The effect of kinetin and abscisic acid on water and ion transport in isolated maize roots. New Phytol 73: 309–314
3. Cram WJ, MG Pitman 1972 The action of abscisic acid on ion uptake and water flow in plant roots. Aust J Biol Sci 25: 1125–1132
4. Erlandsson G, S Petterson, S Svensson 1978 Rapid effects of abscisic acid on ion uptake in sunflower roots. Physiol Plant 43: 380–384
5. Glinka Z 1973 Abscisic acid effect on root exudation related to increased permeability to water. Plant Physiol 51: 217–219
6. Glinka Z 1977 Effects of abscisic acid and of hydrostatic pressure gradient on water movement through excised sunflower roots. Plant Physiol 59: 933–935
7. Jackson JE, PE Weatherley 1962 The effect of hydrostatic pressure gradients on the movement of potassium across the root cortex. J Exp Bot 13: 128–143
8. Karmoker JL, RFM Van Steveninck 1978 Stimulation of volume flow and ion flux by abscisic acid in excised root systems of *Phaseolus vulgaris* L. cv. Redland Pioneer. Planta 141: 37–43
9. Meiri A, WPA Anderson 1970 Observations on the exchange of salt between the xylem and neighbouring cells in *Zea mays* primary roots. J Exp Bot 21: 908–914
10. Pitman MG 1977 Ion transport into the xylem. Annu Rev Plant Physiol 28: 71–88
11. Pitman MG, U Lüttge, A Lauchli, E Ball 1974 Action of abscisic acid on ion transport as affected by root temperature and nutrient status. J Exp Bot 25: 147–155
12. Pitman MG, N Schaefer, RA Wildes 1974 Effect of abscisic acid on fluxes of ions in barley roots. *In* M Zimmermann, J Dainty, eds, Membrane Transport in Plants. Springer-Verlag, Berlin, pp 391–396
13. Pitman MG, D Wellfare 1978 Inhibition of ion transport in excised barley roots by abscisic acid; relation to water permeability of the roots. J Exp Bot 29: 1125–1138
14. Tal M, D Imber 1971 Abnormal stomatal behavior and hormonal imbalance in *Flaca*, a wilty mutant of tomato. III. Hormonal effects on the water status of the plant. Plant Physiol 47: 849–850
15. Vaadia Y 1960 Autonomic diurnal fluctuations in rate of exudation and root pressure of decapitated sunflower plants. Physiol Plant 13: 701–717