THE ACTION OF ABSCISIC ACID ON ION UPTAKE AND WATER FLOW IN PLANT ROOTS

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Abstract

Abscisic acid was shown to inhibit transport of potassium and chloride from the cut ends of excised barley and maize roots. Transport to the shoot of intact barley seedlings was also inhibited. Total uptake into the excised root of barley did not appear to be affected by abscisic acid. There was an increase in tracer accumulated in the root accompanying the reduction in transport from the cut end. Concentrations of abscisic acid above 10^{-6}M produced the maximum effect.

Measurements of exudation from excised maize roots showed that abscisic acid inhibited both water flow and net potassium transport into the exudate. The reduction in water flow was completely accounted for as an osmotic consequence of inhibition of potassium secretion, so that there was no evidence for an effect of abscisic acid on the permeability of the root to water.

The results are discussed in relation to the regulation of ion and water distribution within the whole plant.

I. Introduction

Abscisic acid (ABA) causes rapid closure of stomata (Mittelheuser and van Steveninck 1969) probably by inhibiting potassium transport into the guard cells (Horton and Moran 1972). Abscisic acid also accumulates in leaves of plants exposed to drought stress (Wright and Hiron 1969; Most 1971). It was of interest to know whether ABA affects transport of ions in other plant cells, and in particular whether it affects transport of ions from root to shoot.

A part of the ions entering a root is accumulated in the root cells, and the rest secreted into the xylem (Pitman 1971). In whole plants the ions secreted into the xylem are carried upwards to the shoot in the transpiration stream. In excised roots the ions secreted into the xylem are transported out of the root in the exudate from the cut end. This exudation is an osmotic consequence of the higher concentration of solutes in the xylem than in the external solution (Anderson, Aikman, and Meiri 1970). One might expect ABA to have an effect on this exudation since a decrease in the rate of shoot growth associated with stomatal closure would seem to need a correlative decrease in the supply of salts to the shoot, similar to the relationship between relative growth rate and rate of salt transport found under other circumstances (Pitman 1972).

We have measured accumulation and exudation in the excised root system to find whether ABA has any effects on cellular accumulation, secretion into the xylem, and the hydraulic conductivity of plant roots.

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II. Materials and Methods

(a) *Plant Material*

Barley (*Hordeum vulgare* L. cv. Cape) seedlings were grown on aerated 0·5 mM $CaSO_4$ in the dark at 25°C for 5 days, and then transferred to 10 mM KCl + 0·5 mM $CaSO_4$ for 24 hr, after which steady levels of potassium and chloride had been reached in the roots (Pitman 1971). Maize (*Zea mays* L.) seedlings were grown for about 6 days in moist vermiculite, from which they could be removed without damage to the roots.

(b) *Measurement of Tracer Uptake and Transport from the Cut End*

The procedure used was the same as described in previous papers (Pitman 1971, 1972). Roots of barley seedlings grown in aerated 10 mM KCl + 0·5 mM $CaSO_4$ were excised into the same solution and 2 hr later set up in boxes to measure accumulation in the roots and transport from the cut ends. About 70% of the excised root was in a chamber containing aerated 10 mM KCl + 0·5 mM $CaSO_4$ labelled with ^{36}Cl or ^{86}Rb. The cut end was in a collecting chamber containing unlabelled solution. An intermediate chamber containing unlabelled solution acted as a "guard chamber" between the collecting chamber and the labelled solution. Measurements were made of ^{36}Cl or ^{86}Rb exuded from the cut end into the collecting chamber. At the end of the experiment the roots were washed for 2 min in ice water, which would remove 70–80% of the tracer from extracellular spaces, and the amount of tracer accumulated in the roots was then measured. Amounts transported out of the cut end and accumulated are expressed relative to the weight of root in labelled solution and converted to μ-equivalents per gram fresh weight of root using the specific activity of the solution. "Accumulation" and "transport" are used as defined earlier (Pitman 1971).

(c) *Measurement of Water Flow and Potassium Concentration*

Maize roots were excised 10 cm from the tip and set up with most of the root in an aerated solution and the cut end sealed into a capillary tube (Anderson, Aikman, and Meiri 1970). Experiments were performed on roots which had been in the solution overnight and were exuding at a fairly steady rate. The volume of exudate was measured every 40–80 min, and the rates of volume flow were calculated. The capillary was replaced at intervals so that the exudate could be collected for analysis of potassium concentration.

(d) *ABA Concentrations*

Concentrations quoted are of total ABA. The active component was not determined and could be 25–50% of the total. Solutions were prepared by dissolving ABA in a minimal volume of 100 mM KOH, neutralizing with HCl, and making up to a known volume. Aliquots of the stock solution were added to solutions as required. The amount of KCl added at the same time was negligible.

III. Results

Figure 1 shows the effect of ABA on transport of ^{36}Cl out of excised barley roots. Two hours were allowed for the tracer transport to rise to a steady level (2·0 μ-equiv. g^{-1} hr^{-1}) and then ABA was added ($t = 0$ in Fig. 1). Two sets of roots were used as controls without added ABA; the others were treated with concentrations of ABA ranging from 0·4 to $1·9 \times 10^{-5}$M. Measurements were made over successive 40-min periods. Transport is expressed relative to the controls as 100%. No difference in effect was found for these different ABA concentrations, so the results are pooled in Figure 1.

For the first 40 min there was little effect of ABA but then over the next 2 hr the rate of transport fell to about 20% of the control value. A lag of about 40–60 min before ABA had any effect was generally observed (see also Table 1 and Fig. 3).

The inhibition of transport was found for ^{86}Rb (used as a tracer for potassium) as well as for ^{36}Cl. Table 1 gives rates of ^{86}Rb and ^{36}Cl exudation (μ-equiv. g^{-1} hr^{-1}) over successive 40-min periods after adding $0 \cdot 8 \times 10^{-5}$M ABA. The extent of inhibition was about the same as in Figure 1, but the lag before inhibition began was somewhat longer.

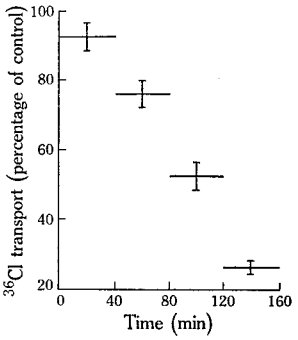


Fig. 1.—Chloride transport from barley roots. Means of six samples $\pm$S.E.M. Abscisic acid added at $t = 0$. There was no difference in response over the abscisic acid concentration range $0 \cdot 4$–$1 \cdot 9 \times 10^{-5}$M. Uninhibited level was $2 \cdot 0 \pm 0 \cdot 2$ μ-equiv. g^{-1} hr^{-1}.

Roots treated with ABA were found to have accumulated *more* ^{36}Cl and ^{86}Rb than the controls. In the roots used in the experiments shown in both Figure 1 and Table 1 this increased accumulation was about 2 μ-equiv. g^{-1}. This was nearly the same as the reduction in ^{36}Cl or ^{86}Rb exudation; about 2 μ-equiv. g^{-1} over 160 min in Figure 1 and $2 \cdot 7$ and $2 \cdot 4$ μ-equiv. g^{-1} over 200 min in Table 1.

TABLE 1

EFFECT OF ABSCISIC ACID ON TRANSPORT OF ^{86}Rb AND ^{36}Cl FROM LABELLED SOLUTIONS OF 10 mM KCl + 0·5 mM $CaSO_4$

Time after adding ABA (min)	Rates of tracer transport (μ-equiv. g^{-1} hr^{-1})*			
	Control		ABA added	
	^{86}Rb	^{36}Cl	^{86}Rb	^{36}Cl
0– 40	1·9	1·6	1·9	1·9
40– 80	2·2	2·2	1·9	1·9
80–120	2·2	2·2	1·5	1·7
120–160	1·8	2·5	0·9	1·0
160–200	2·2	2·5	0·4	0·4

* Mean of two replicates.

A series of measurements on excised roots in labelled culture solution confirmed that ABA increased the amount of ^{36}Cl and ^{86}Rb accumulated in the root. Over a period of $4 \cdot 5$ hr, treatment with 10^{-5}M ABA increased the accumulation of ^{36}Cl and ^{86}Rb by $6 \cdot 5$ and $5 \cdot 2$ μ-equiv. g^{-1} respectively.

This increase was in reasonable agreement with the reduction in tracer transport. Figure 1 and Table 1 do not cover a period as long as $4 \cdot 5$ hr, but from 3 hr onwards transport was at the most only 20% of the control level. On this basis we

can estimate that over 4·5 hr transport of ^{36}Cl and ^{86}Rb should have been reduced by about 5 μ-equiv. g^{-1}.

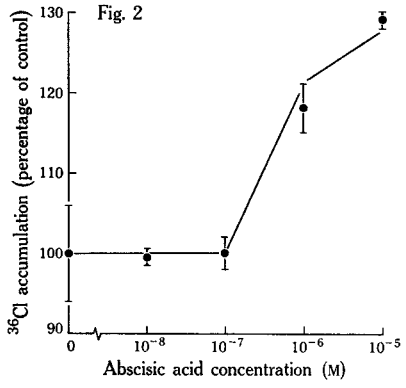
It seems, therefore, that in excised roots ABA does not alter the total amount of potassium (rubidium) or chloride moving into the root from the solution but does inhibit secretion into the xylem.

Inhibition of transport was also found using whole plants, but the equivalence between increased accumulation and decreased transport was not found in experiments with intact seedlings. In this case transport to the shoot was reduced much more than the increase in accumulation by the root. For example, after 5 hr in solution labelled with ^{36}Cl, seedlings were found to have taken up the following amounts of chloride (in μ-equiv. per gram fresh weight of the root; mean$\pm$S.E.M.):

	Control	With 10^{-5}M ABA
^{36}Cl in roots	7·0$\pm$0·2	8·3$\pm$0·3
^{36}Cl in shoots	12·0$\pm$1·0	6·1$\pm$1·0

The reduction in transport from the cut end (5·9 μ-equiv. g^{-1}) was significantly ($P < 0·05$) greater than the increase in accumulation (1·3 μ-equiv. g^{-1}).

The results combined in Figure 1 showed that the degree of inhibition by ABA had reached a maximum at $0·4 \times 10^{-5}$M. The increased accumulation by roots was used to determine the effectiveness of different concentrations of ABA. Figure 2 shows that accumulation was increased at concentrations above 10^{-7}M. ABA can alter the rate of transport therefore only between about 10^{-7} and 10^{-6}M. Within this range transport would be sensitive to changes in ABA concentration.



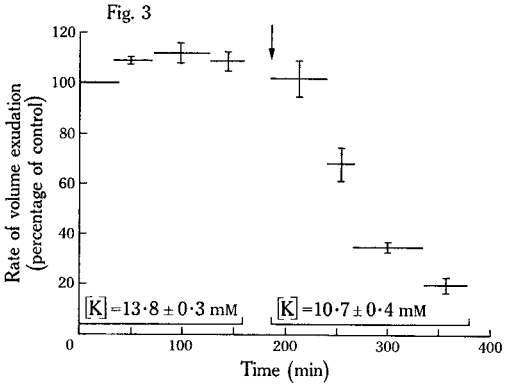


Fig. 2.—Effect of abscisic acid concentration on accumulation of ^{36}Cl by roots. Control level = 9·7 μ-equiv. g^{-1} hr^{-1}. Means of three samples$\pm$S.E.M.

Fig. 3.—Exudation from maize roots in 0·6 mM KCl+0·5 mM $CaSO_4$. Means of five roots $\pm$S.E.M. as percentage of control mean. Concentration of potassium in the exudate measured before and after the addition of $2·7 \times 10^{-5}$ mM abscisic acid (arrow). 100% = 1·2 μl cm^{-2} hr^{-1} at 25°C.

Figure 3 shows the effect of ABA on volume flow and potassium concentration in the exudate from roots immersed in 0·6 mM KCl+0·05mM $CaSO_4$. Under these conditions only potassium and chloride are found in the exudate from maize roots

(Anderson, Aikman, and Meiri 1970). We assume that ABA does not initiate the secretion of other substances.

There was no significant effect of ABA on the rate of volume flow in the first 53-min period, but over the next 2 hr the volume flow fell to 20% of the control value. The time course of the effect was similar to that found for inhibition of tracer exudation from the cut end of barley roots (Fig. 1).

The average concentrations of potassium in the exudate during the period before and after adding ABA are also shown in Figure 3, and were $13 \cdot 8 \pm 0 \cdot 3$ and $10 \cdot 7 \pm 0 \cdot 4$mM respectively.

IV. Discussion

(a) *Effect of ABA on the Hydraulic Conductivity of Maize Roots*

At any point in the root the volume flow into the stele is assumed to be proportional to the osmotic pressure difference between the external solution and the xylem (Anderson, Aikman, and Meiri 1970). This relationship may be written

$$J_v = L_p \cdot \boldsymbol{R}T(c_i - c_o), \tag{1}$$

where J_v is the volume flow (μl cm^{-2} hr^{-1}), $\boldsymbol{R}$ and T have their usual significance, c_i and c_o are the internal and external osmolarities (mosmoles l^{-1}) (and $c_i - c_o = \Delta c$), and L_p is the constant of proportionality or hydraulic conductivity (μl cm^{-2} hr^{-1} bar^{-1}).

Although L_p and Δc may vary along the root (Anderson, Aikman, and Meiri 1970), there is a fairly good linear relation between J_v and Δc when summed over the whole of the root (House and Findlay 1966), so it seems not unreasonable to interpret changes in J_v for the whole root in terms of general changes in L_p, Δc, or both.

The main osmoticum in the exudate is assumed to be KCl. The rate of potassium secretion (J_s) is given by

$$J_s = [\mathrm{K}] \cdot J_v . \tag{2}$$

After the addition of ABA, J_v was reduced by 80%. Since [K] was reduced it is clear that J_s was also reduced by at least 80%. The problem is to determine whether there was also any effect of ABA on L_p. If L_p is not affected by ABA, then equations (1) and (2) can be used to calculate the amount of potassium that would have been secreted in the successive periods during which J_v was measured after the addition of ABA (Fig. 3). These amounts when summed and divided by the total volume exuded show what the average potassium concentration in the exudate would have been over the 3-hr period after adding ABA if L_p had remained constant. The average potassium concentration calculated for these conditions was 9·5 mM, which is only slightly less than the observed value of $10 \cdot 7 \pm 0 \cdot 4$ ($P < 0 \cdot 05$).

If ABA had increased L_p as well as reducing J_s the concentration in the exudate would have been smaller than 9·5 mM. For instance, if L_p were increased by a factor of 2 over the last two measuring periods the average concentration in the exudate would have been 8·9 mM, which is significantly lower than the observed value. Any faster or larger effect of ABA would have led to an even greater reduction in the average potassium concentration.

It can be concluded, therefore, that ABA reduces salt transport into the xylem, but has little effect on the permeability of the maize root to water. Tal and Imber (1971) claim that ABA increases the permeability of tomato roots to water, but it is possible that the response to ABA they observed was due to a change in growth rather than a change in permeability to water, since L_p and J_s vary along the root (Anderson, Aikman, and Meiri 1970).

(*b*) *Action of ABA on Ion Transport in Barley Roots*

The predominant effect of ABA in barley roots was again an inhibition of salt secretion into the xylem. The reduction in tracer transported out of the root was found both for excised roots and for whole plants. The reduced transport to the shoot therefore seems to have been due to an effect on root secretion and not to a reduction in transpiration brought about by an effect of ABA on stomata.

Though secretion of ions into the xylem in excised roots was reduced, total uptake was unaffected by ABA, the decreased secretion being balanced by increased accumulation in the root cortex. Similarly, in whole plants total uptake was reduced by 26%, which is less than the reduction in transport to the shoot (50%), so that there was a small increase in accumulation in the root. The equivalence of increased accumulation and reduced transport was possibly a fortuitous result of the particular experimental conditions; the main point we wish to emphasize is that entry to the cytoplasm and vacuoles of cortical cells is much less affected by ABA than is secretion into the xylem.

The effects of ABA on excised roots therefore support the "two pump" rather than the "one pump" hypothesis of secretion into the xylem, as discussed previously (Pitman 1972). The influx to the cytoplasm of cortical cells appears to be an active process, but is not altered by ABA. The secretion into the xylem also appears to be an active process, but is inhibited by ABA, and is therefore different in nature from the influx to the cortical cell cytoplasm.

The action of ABA in inhibiting only the secretion into the xylem is unlike the effect of the metabolic inhibitor carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), which suppresses both the entry to the root and the secretion into the xylem. There was a difference, too, in the rate at which the inhibition became established. The effect of ABA on exudation took about 3 hr to reach completion whereas the effect of CCCP was completed in 30 min (Pitman 1972).

The slowness of the effect of ABA also does not seem to be due to a diffusion restriction on entry to the roots, since the speed of response to ABA was not increased when its concentration was increased from $3{\cdot}9\times10^{-6}$ to $1{\cdot}9\times10^{-5}$M.

The effect of ABA on ion transport in roots is therefore much slower than its effect on stomata (Kriedemann *et al.* 1972), which suggests that the mechanisms of the two effects may differ.

ABA does not affect respiration or phosphorylation in barley aleurone layers (Chrispeels and Varner 1966), and does not affect respiration in *Phaseolus vulgaris* cotyledons (Yomo 1971). ABA therefore probably does not act on salt transport in roots by inhibiting its energy supply. Instead, the primary effect of ABA may be several steps removed from the transport process, analogous to the effect of ABA on α-amylase secretion by barley aleurone layers (Chrispeels and Varner 1967).

(c) *ABA Regulation of Ion Transport in the Whole Plant*

In recent years it has become apparent that ABA may be an important natural regulator of the water balance of a plant. The present results show that ABA could also be part of a larger regulating system which includes transport of ions.

With regard to the regulation of water balance, Mittelheuser and Van Steveninck (1969) showed that ABA inhibited stomatal opening and reduced transpiration. Wright and Hiron (1969), Mizrahi, Blumenfeld, and Richmond (1970), and Most (1971) have shown that the ABA concentration increased in leaves of plants subjected to drought or saline conditions. Hocking, Hillman, and Wilkins (1972) have shown that ABA can be translocated from the leaves to the roots. There is also evidence that cytokinins may have a complementary role to ABA in this overall regulatory system in plants (Mizrahi, Blumenfeld, and Richmond 1970).

The results reported in this paper show that ABA has no effect on the permeability of maize roots to water over a period of several hours. Therefore it appears that ABA does not control the water potential in the plant by altering the resistance to entry of water.

With regard to the regulation of ions in the plant, it appears that the plant can control net transport of the major ions to the shoot (potassium, sodium, and accompanying anions). Various experiments with plants growing on culture solutions show that there is a correlation between transport to the shoot and relative growth rate, or that uptake is more closely related to growth than to the concentration in the solution. Under conditions of low light this regulation may simply be due to availability of respiratory substrates, but there are other conditions where metabolic reserves increase without a corresponding increase in growth or uptake, and some other form of regulation is implied (Pitman 1972).

The demonstration that ABA can inhibit secretion into the xylem in roots provides the basis for a regulatory mechanism. Basically, the suggestion is that levels of ABA change in the leaf in response to changing ion levels or their osmotic effects. Translocation of ABA takes place to the root (Hocking, Hillman, and Wilkins 1972) transmitting a change in ABA levels, which then controls the rate of secretion of ions to the xylem.

Consider such a system in relation to what is known of the effects of salinity or drought. Both conditions lead to increases in ABA levels in the leaf, as already discussed. Transport of this ABA to the root would reduce further salt secretion into the root xylem. Qualitatively one can see that in the absence of this control of secretion the concentration of salt would build up in the xylem, and ultimately in the leaf. Unless the leaf were able to accumulate these ions in the cell vacuoles, or export an equivalent amount, the osmotic pressure in the extracellular spaces would rise, increasing the water deficit of the leaf cells. Since photosynthesis can be expected to be reduced by the ABA-induced closure of stomata, growth of the plant would not provide an adequate means of coping with salt transport to the leaf, if this continued at the same rate as in the unstressed plant.

The same system could operate in the normally growing plant, providing a fine negative feedback to allow regular adjustment of ion transport to growth. In this way ABA, and also possibly cytokinins, could be part of a system regulating ion levels in the plant as well as water potentials.

V. Acknowledgments

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VI. References

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