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EFFECT OF ABSCISIC ACID ON ION TRANSPORT AND TRANSLOCATION IN *HORDEUM VULGARE* SEEDLINGS

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ABSTRACT

The effect of abscisic acid (ABA) on potassium ion transport into roots and ion translocation into shoots of intact *Hordeum vulgare* seedlings was studied. The presence of 10^{-6}, 10^{-5}, or 10^{-4} molar ABA in the absorption solution caused an increase in potassium transport into roots and prohibited translocation of ions to the shoot. These effects were observed at both 0.1 mM and 20 mM absorption solution potassium ion concentrations. The results are discussed in terms of water stress adaptation by the intact seedling.

INTRODUCTION

The phytohormone abscisic acid (ABA) plays many roles in plant growth and development. Water stress adaptation, root geotropism, seed development and germination, dormancy, correlative inhibition of buds, and ion movement are just a few of the many responses that are mediated in one way or another by ABA (Walton, 1980).

One of the most studied aspects of ABA action on ion transport is the regulation of the movement of potassium ions, protons, and chloride ions which cause turgor changes within guard cells. It is known that ABA inhibits potassium ion uptake by guard cells (Horton and Moran, 1972). Since an active potassium ion -proton exchange is thought to be involved in the regulation of guard cell turgor (Raschke, 1977), it is easy to see that ABA could affect turgor changes within guard cells by altering the movement of potassium ions.

In addition to its apparent effect on potassium ion transport in guard cells, ABA can also affect ion transport in other tissues. ABA has been shown to inhibit potassium ion uptake and proton extrusion in germinating seeds of *Raphanus* *sativus* (Ballarin-Denti and Cocucci, 1979), and to inhibit potassium and chloride ion uptake by *Avena* coleoptiles (Reed and Bonner, 1974).

The effects of ABA on ion transport in roots are still a matter of dispute in the literature. It has been reported that ABA both promotes (Karmoker and Van Steveninck, 1979) and inhibits (Shaner *et al.*, 1975) the uptake of ions into roots, and both promotes (Collins and Kerrigan, 1974) and inhibits (Cram and Pitman, 1972) the movement of ions across roots and into the xylem exudate. Pitman, *et al.* (1974) have shown that, depending on the conditions under which plants were grown, ABA either promotes or inhibits ion transport and translocation across the root. They found that in roots excised from dark-grown, low-salt plants, the presence of ABA in the absorption solution caused a decrease in the amount of potassium ion in the xylem exudate. If dark-grown, high-salt roots were used, ABA in the absorption solution caused the amount of potassium ion in the root exudate to increase. This increase was not seen in roots excised from light-grown, high-salt plants. Therefore, it is obvious that the conditions under which plants are grown play an important role in determining how the roots of those plants will respond to the application of exogenous ABA.

It is also known that the effects of ABA on ion uptake by excised roots are different from those observed in intact plants of the same species. In excised roots ABA stimulated potassium ion uptake, while in intact seedlings ABA inhibited potassium ion uptake (Karmoker and Van Steveninck, 1979). This discrepancy in the effect of ABA was explained by these authors as an indication that a variety of factors, such as sugar levels in the tissue, endogenous phytohormone levels, and possible interactions between endogenous and exogenous phytohormones, could be involved in the action of ABA upon ion transport and translocation.

Reports concerning the effects of ABA on the exudate volume flow rate from roots are also contradictory. ABA in the absorption solution increased the exudation volume flow rate in decapitated plants (Glinka, 1973). This increase in volume flow was attributed to an increased hydraulic conductivity of root systems caused by ABA (Glinka, 1977). Other reports in the literature indicate that ABA decreases the volume flow rate and the hydraulic conductivity of root systems (Markhart *et al.*, 1979; Fiscus, 1981). This discrepancy in the literature concerning the effects of ABA on exudate volume flow and hydraulic conductivity in roots was explained by Markhart *et al.*, (1979) as being a combination of differences in species, cultural practices, and ABA concentrations used by different researchers.

As indicated above, there is a considerable amount of disagreement in the literature concerning the effect of ABA on ion transport, translocation, and exudate volume flow in intact plants and excised roots. In an attempt to resolve this disagreement, an experiment was designed in which the effect of ABA on potassium ion transport and translocation in intact *Hordeum vulgare* L. (barley) seedlings was studied.

These experiments should help clarify the contradictory literature for several reasons. First, it is known that damaging plant tissue causes a phytohormone-induced wound response (Dieffenbach *et al.*, 1980). Wounded tissue may respond differently to exogenous application of phytohormones than non-wounded tissue. In experiments reported here, only intact seedlings were used. By using only intact seedlings, the possibility of obtaining erroneous results caused by wounding tissue has been eliminated.

Second, the volume and potassium ion concentration of guttation fluid, as well as the percent ash weight and potassium ion content of root and shoot tissue was determined in this experiment. These determinations considered together allowed the effect of ABA on ion distribution to be quantified in a precise manner.

Third, two different absorption solution potassium ion concentrations (0.1 and 20 mM) and three different ABA concentrations (10^{-6}, 10^{-5}, and 10^{-4}M) were used in these experiments. Most of the authors, as cited above, used only one absorption solution potassium ion concentration and only one concentration of ABA. By using several different concentrations of both potassium ion and ABA, it would be possible to obtain a more accurate representation of ABA effect on ion transport and translocation.

A study of the effect of ABA on ion transport and translocation, therefore, offered the possibility of giving additional insight into how this phytohormone affects ion movement in intact seedlings. Because of this, the present investigation was undertaken.

MATERIALS AND METHODS

Seeds of barley (cv. Jefferson) were imbibed in Nystatin-treated (0.5mg/100ml), aerated, 0.5 mM calcium sulfate solution. After 24 hours, seeds were placed between moist layers of sterile cheese cloth suspended over 700 ml of aerated 0.5 mM calcium sulfate solution in a 1000 ml beaker. Seedlings were allowed to grow in darkness for 6 days at 25 C.

At the end of 6 days, the calcium sulfate solution was replaced with 0.5 mM calcium sulfate solution containing either 0.1 or 20 mM potassium choride. Abscisic acid (sodium salt) was added to a final concentration of 10^{-6}, 10^{-5}, or 10^{-4} M. A control group of seedlings received equal amounts of sodium with no ABA. All treatments were done in triplicate. The seedlings were covered with plastic bags and left in darkness. After a 24 hour period, guttate was collected, measured with a micro-pipette, and stored in the freezer for later potassium ion analysis. The seedlings were then harvested, weighed, and placed in a 70 C drying oven. After 48 hours, the tissue was weighed again, and placed in a muffle furnace for 6 hours at 500 C. Percent ash weight was determined by dividing the ash weight by the dry weight and multiplying by 100. Percent ash weight indicates that total mineral percentage of the tissue. The ashed tissue was used to determine potassium ion content by method of flame emission spectrophotometry.

RESULTS

In this experiment, the effects of different concentrations of ABA on percent ash weight, potassium ion content, guttate volume flow, and guttate potassium ion concentration were determined.

The presence of ABA in the absorption solution caused the percent ash weight of roots of seedlings grown in 0.1 mM potassium chloride to significantly increase, and had no effect on the percent ash weight of roots of seedlings grown in 20 mM potassium chloride when compared to controls. Roots of seedlings grown in both 0.1 mM and 20 mM potassium chloride showed a significantly higher potassium ion content than did the controls (Table 1).

In shoots of seedlings grown in both 0.1 mM and 20 mM potassium chloride, the presence of ABA caused a significant reduction in the percent ash weight, but no significant differences in the potassium ion concentration, when compared to the controls (Table 1).

Table 1

EFFECT OF ABSCISIC ACID ON
ION TRANSPORT AND TRANSLOCATION

Mean percent ash weight and potassium ion content for six-day-old, dark-grown *Hordeum vulgare* seedlings given 0.1 mM or 20 mM potassium chloride with different concentrations of abscisic acid for twenty four hours. Values represent three replicate samples per treatment.

Tissue	Percent Ash Weight %	Potassium Ion Content μMoles/g D.W.
0.1 mM KCl		
Roots		
Control	4.10 ± 0.58[1] a[2]	358.3 ± 61.4 a
10^{-6} M ABA	4.97 ± 0.17 b	667.8 ± 118.7 b
10^{-5} M ABA	5.95 ± 0.89 b	625.8 ± 95.3 b
10^{-4} M ABA	9.69 ± 0.70 b	628.6 ± 157.7 b
Shoots		
Control	6.38 ± 0.80 a	464.6 ± 37.1 a
10^{-6} M ABA	3.82 ± 0.03 b	607.3 ± 92.3 a
10^{-5} M ABA	3.82 ± 0.82 b	588.9 ± 22.1 a
10^{-4} M ABA	3.89 ± 0.91 b	574.1 ± 239.6 a
20 mM KCl		
Roots		
Control	12.04 ± 2.94 a	1130.6 ± 62.1 a
10^{-6} M ABA	10.33 ± 1.67 a	1621.2 ± 243.8 b
10^{-5} M ABA	12.96 ± 0.81 a	1657.1 ± 152.8 b
10^{-4} M ABA	9.71 ± 1.55 a	1378.9 ± 156.3 ab
Shoots		
Control	9.56 ± 1.51 a	764.1 ± 92.9 a
10^{-6} M ABA	6.29 ± 1.30 b	762.1 ± 174.5 a
10^{-5} M ABA	5.67 ± 0.67 b	634.8 ± 70.3 a
10^{-4} M ABA	4.66 ± 0.41 b	577.4 ± 56.8 a

[1] Values represent means ± standard deviation.

[2] Values followed by the same letter are not significantly different (Duncan's Multiple Range, $P = 0.05$).

Table 2.

EFFECT OF ABSCISIC ACID ON GUTTATE VOLUME FLOW AND POTASSIUM ION CONCENTRATION

Mean guttate volume flow and guttate potassium ion concentration for six-day-old, dark-grown seedlings of *Hordeum vulgare* given 0.1 mM or 20 mM potassium chloride with different concentrations of abscisic acid for twenty four hours. Refer to footnotes for sample sizes.

Treatment	Guttate Volume Flow μliters/hour		Potassium Ion Concentration μMoles/ml
0.1 mM KCl			
Control	2.3 ± 0.6[1]	a[2]	0.04[3]
10^{-6} M ABA	1.1 ± 0.2	b	0.02
10^{-5} M ABA	1.1 ± 0.3	bc	0.02
10^{-4} M ABA	0.8 ± 0.2	c	0.02
20 mM KCl			
Control	1.7 ± 0.4	a	0.16
10^{-6} M ABA	0.8 ± 0.2	b	0.08
10^{-5} M ABA	0.7 ± 0.2	b	0.04
10^{-4} M ABA	0.4 ± 0.1	c	0.04

[1] Values represent mean ± standard deviation (n = 15).

[2] Values followed by the same letter are not significantly different (Duncan's Multiple Range, P = 0.05).

[3] Represents value for one sample.

The guttate volume flow of seedlings grown in 0.1 mM and 20 mM potassium chloride was significantly smaller than the controls, and the potassium ion concentration of the guttate was lower in the ABA treated seedlings than in controls (Table 2).

DISCUSSION

The fact that roots of ABA-treated, intact, dark-grown, low-salt, barley seedlings showed an increase in the potassium ion content suggests that ABA promotes potassium ion transport into the roots. The fact that shoots of ABA-treated seedlings showed a decrease in percentage ash weight and a decrease in the potassium ion concentration of the guttate suggests that ABA inhibits ion translocation across the root and up to the shoot.

These results are in general agreement with other reports in the literature concerning ABA effects on ion transport and translocation in excised roots of barley (Pitman and Wellfare, 1978), and for short-term (3 hour) experiments using intact, light-grown barley seedlings (Dieffenbach *et al.*, 1980). It has been shown that ABA inhibits translocation of ions across the root to the xylem without inhibiting the uptake of ions from the absorption solution by the root (Cram and Pitman, 1972). The data reported here support this view for two reasons. First, the presence of ABA increased the potassium ion content found in the root tissue. If ABA had inhibited ion uptake by roots, it would be expected that the potassium ion content of the roots treated with ABA would be less than the controls. Second, the presence of ABA decreased the potassium ion concentration in the guttate. If ABA had enhanced or had had no effect on potassium ion translocation across the root to the xylem, it would be expected that the potassium ion concentration of the guttate would have been greater or equal to the controls.

The results also indicate that treatment with ABA decreased the volume flow of the guttate. This observed decrease in the guttate volume flow was most likely the result of the ABA-induced decrease in the potassium ion concentration of the xylem sap. This concentration decrease would cause a decrease in the passive movement of water into the xylem of the root, with a corresponding decrease in root pressure, and a subsequent decrease in guttate volume flow.

In shoots, the significant decrease in percent ash weight over that of controls indicates that the total amount of inorganic minerals present in the shoot tissue is reduced by ABA treatment. Subsequent analysis indicated no significant differences in the amount of potassium ion concentration of shoot tissue from ABA treated or control seedlings. One possible explanation for this observation is as follows. It has been reported that, in addition to inhibiting the movement of potassium ions, ABA also inhibits the movement of calcium ions (Pitman and Wellfare, 1978) and chloride ions (Cram and Pitman, 1972) across the root and up to the shoot. Since the absorption solution used contained these ions, it is quite possible that their absence in the shoots of the ABA treated seedlings caused the percent ash weight to be less than that of the shoots of the control seedlings.

SUMMARY

The observed effects of ABA on ion transport and translocation in intact seedlings of barley are as follows:

1) ABA in the absorption solution promotes the transport of potassium ions from the absorption solution into the root.

2) ABA in the absorption solution reduces ion translocation across the root and into the shoot.

It has long been known that ABA accumulates in plants in response to water stress (Wright, 1969). Since exogenous ABA alters the partitioning of ions between the root and shoot, it is quite possible that this altered partitioning plays a role in the development of water stress resistance.

It is quite possible, therefore, that in water stressed seedlings, increased levels of ABA would lead to higher concentrations of ions in roots, causing a reduction in the osmotic potential of the root tissue. Water would move into the root in response to this reduced osmotic potential and water stress would be alleviated.

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