Stimulation of Volume Flow and Ion Flux by Abscisic Acid in Excised Root Systems of Phaseolus vulgaris L. cv. Redland Pioneer

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Abstract. Abscisic acid (ABA) caused a 7-8-fold increase in volume flow in excised bean root systems and this was coupled with an increase in $^{42}\text{K}$, $^{36}\text{Cl}^-$, and $^{24}\text{Na}$ flux into the xylem. The transport of $^{42}\text{K}$ and $^{36}\text{Cl}^-$ increased by a factor larger than the stimulation of volume flow, resulting in an increase in the concentration of those ions in the xylem exudate. Carbonylcyanide-m-chlorophenyl hydrazine, on the other hand, eliminated ABA-stimulated $^{42}\text{K}$ transport and caused a further inhibition of $^{42}\text{K}$ flux, thus providing additional support for the proposition that ABA stimulation may involve an energised process of ion transport. ABA also increased the accumulation of $^{24}\text{Na}$ and $^{36}\text{Cl}^-$ in bean root tissue, but not that of $^{42}\text{K}$.

Key words: Abscisic acid – Ion flux – Phaseolus – Root (ion transport) – Transport (ions) – Xylem (ion transport).

Introduction

Abscisic acid (ABA) is known to play a key role in the regulation of physiological phenomena which govern water balance in plants. It causes rapid closure of stomata (Mittelheuser and Van Steveninck, 1969, 1971; Cummins et al., 1971) and therefore reduces the rate of transpiration (Little and Eidt, 1968; Mizrahi et al., 1970). It was also found to increase the exudation rate of decapitated tomato plants (Tal and Imber, 1971) and excised maize roots (Collins and Kerrigan, 1974) and this increase in volume flow ($J_v$) was reported to be based on a direct effect on the hydraulic conductivity of the roots (Glinka and Reinhold, 1971; Glinka, 1973). Subsequently Cram and Pitman (1972) found that ABA had no effect on the permeability of maize roots to water while it inhibited both $J_v$ and potassium transport into the xylem exudate of single maize roots.

Exudation from excised root systems is believed to arise from a difference in osmotic potentials of external solution and xylem sap (Anderson et al., 1970). ABA-mediated control of exudation and stomatal regulation is likely to be based upon its effect on ion transport (see discussions in Raschke, 1975; Vaadia, 1976). The fact that ABA may regulate the distribution of $K^+$ at the cellular level (Mansfield and Jones, 1971; Horton, 1971; Horton and Moran, 1972) gives rise to some expectation that it may also be involved in the regulation of the long-distance transport of ions. Since ions are moving from the cortex into the xylem mainly through the endodermis and the xylem parenchyma (see reviews by Anderson, 1972, and Higinbotham et al., 1973), any change in their movement through the xylem parenchyma and the endodermal layer will affect the long-distance transport of ions and their distribution.

In this paper we show that ABA increased both $J_v$ and the transport of $^{42}\text{K}$, $^{24}\text{Na}$ and $^{36}\text{Cl}^-$ into the xylem of excised root systems of the bean (Phaseolus vulgaris L. cv. Redland Pioneer). It will also be shown that in this bean cultivar stimulation of $J_v$ follows the stimulation of ion transport.

Material and Methods

Culture of Plants

Seeds of Phaseolus vulgaris L., cv. Redland Pioneer (obtained from New World Seeds, Galston, N.S.W., Australia) were germinated in vermiculite in darkness at 25°C. Before secondary roots were developed the germinated seeds were transferred to a plastic container (8.5 x 8.5 x 8 cm$^3$) containing modified half-strength culture solution (Hoagland and Arnon, 1950). The concentration of $K^+$ in the culture solution was adjusted to 0.5 mM and the solution renewed every 48 h. The culture solution was always replaced with 0.5 mM KCl (or 0.5 mM NaCl)+0.1 mM CaSO$_4$ solution 15 h...
before decapitating the plants. The plants were grown under the following conditions: 14-h day at 26° C with light intensity (measured at the foliage level) of ca. 300 µmE m⁻² s⁻¹, 18° C night, 60–65% relative humidity. The chemical composition of full-strength solution was as follows: KNO₃ 0.5 mM, Ca(NO₃)₂ 5 mM, MgSO₄ 2 mM, K₂HPO₄ 0.5 mM, FeEDTA 100 µM, H₂BO₃ 46 µM, MnCl₂ 9 µM, ZnCl₂ 0.8 µM, CuCl₂ 0.3 µM, Na₂MoO₄ 0.1 µM.

Exudation Experiments with Excised Root Systems

Uniform 9- to 12-day-old plants were decapitated ca. 1.5 cm above the root-stem junction. A piece of vinyl tubing was carefully fitted to the short stump and sealed to the stem with low-melting-point wax (Paraffin, M.P. 49° C, mixed with paraffin oil to lower M.P. to 42° C). The detached root systems were kept under observation for 1 h, and specimens having uniform exudation rates were then selected and transferred to plastic containers containing solutions radioactive K⁺, Na⁺ or Cl⁻, with or without added ABA. Thevinyl tubes were covered with discs of modelling clay to prevent evaporation of the collected exudate. The "radioactive" media contained 0.5 mM KCl + 0.1 mM CaSO₄ or 0.5 mM NaCl + 0.1 mM CaSO₄. The specific activities of ⁴²K, ²⁴Na and ³⁶Cl in the solutions were ca. 5.71 gCi/mg K⁺, 9.67 gCi/mg Na⁺ and 1.13 µCi/mg Cl⁻. ABA stock solution, 4 × 10⁻⁶ M, was prepared by dissolving ABA in distilled water by shaking overnight. ABA was used at concentrations of 5 × 10⁻⁷, 10⁻⁶ and 10⁻⁵ M. The solutions were aerated continuously at a rate of 12 l h⁻¹ using No. 20G, 3-inch hypodermic needles. The exudation experiments were carried out at a constant temperature of 25° C in diffuse light/darkness 12/12 h. The exudate was collected at 3-h intervals for 12 h and then every 12 h (except where otherwise stated), using 100 µl microsyringes which facilitated measurement of the exudate volume.

Radiochemical Analysis of Exudates and Tissues

After an experimental period of 48 h each root system labelled either with ⁴²K or ²⁴Na was briefly rinsed in two 5-min changes of 150 ml 0.2 mM CaSO₄, and packed in a polyethylene tube for counting. The radioactivities of ⁴²K and ²⁴Na in the exudates were recorded directly to a minimum of 10,000 and 2,000 counts, respectively, using a Packard (Packard Instrument, Downers Grove, Ill., USA) Autogamma Spectrometer (Model 5022/5023, tandem console). After rinsing, the roots labelled with ³⁶Cl were briefly rinsed in two 5-min changes of 150 ml boiling deionised water. One ml of the extract was transferred to a scintillation vial containing 8 ml of TRIPOP scintillation liquid (2,5-diphenyloxazole (PPO) 0.4 g, Triton X114 500 ml, xylene 1500 ml). ³⁶Cl was recorded to at least 10,000 counts using a Packard Tricarb Liquid Scintillation Spectrometer (Model 3320).

The amount of radioactivity was expressed as an absolute quantity (Q) rather than counts per minute. Q was calculated by dividing the activity of the radioactive element in the samples by the specific activity of the labelling solution (Greenway and Pitman, 1965; Cram and Pitman, 1972). "K⁺", "Na⁺" and "Cl⁻" represent the amounts of ⁴²K, ²⁴Na and ³⁶Cl equivivalent to the observed counting rates and are expressed in µ-equivalents or n-equivalents. Concentrations of K⁺, Na⁺ and Cl⁻ are based on the amounts of K⁺, Na⁺, Cl⁻ per volume of exudate. By definition, these are measured relative to the specific activity of the labelling solution. ⁴²K and ²⁴Na were obtained from the Australian Atomic Energy Commission, Lucas Heights, Sutherland, N.S.W., Australia, and ³⁶Cl from Radiochemical Centre, Amersham, Bucks, U.K. ±-ABA was supplied by Polysciences, Warrington, Pa., USA.

Atomic Absorption Spectroscopy

Potassium in the exudate was measured using an atomic absorption spectrophotometer (Varian Techtron, Melbourne, Vic., Australia; model AA5).

Results

Increase of Jv in Bean Root Systems by ABA

Within 9–12 h of treatment ABA caused a 6- to 8-fold increase in the rate of Jv in excised bean root systems bathed in 0.5 mM KCl (Fig. 1). This stimulatory effect of ABA on the rate of exudation was observed even when root systems were exposed to KCl solutions with concentrations ranging from 0.5 to 100 mM KCl (Table 1).

Although Jv decreased from 9 to 48 h in both ABA-treated and untreated roots, the stimulatory effect of ABA on Jv was maintained after 48 h of treatment (Fig. 1).

Enhancement of ³⁶Cl, ⁴²K and ²⁴Na Transport by ABA into the Xylem

ABA stimulated ³⁶Cl transport (Fig. 2) and ⁴²K transport (Fig. 3) to the xylem. This effect reached

![Fig. 1](image-url)

The effect of different concentrations of ABA on volume flow in excised root systems of Phaseolus vulgaris cv. Redland Pioneer bathed in 0.5 mM KCl+0.1 mM CaSO₄. Control; ○ 5 × 10⁻⁷ M ABA; △ 10⁻⁶ M ABA; ● 10⁻⁵ M ABA. Each point represents the mean of six replicates; the bars represent ± standard error.