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EFFECT OF ABSCISIC ACID ON EXUDATE OSMOLARITY AND PROTON FLUX IN EXCISED MAIZE ROOTS

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Abstract

The effect of abscisic acid (ABA) on the xylem exudate osmolarity and proton flux in excised roots of maize (*Zea mays* L.) cv Anjou 210 was determined. It was observed that ABA does not alter the exudate osmolarity until 24 h from excision, but beyond this, there was a little drop in the exudate osmolarity. ABA increased potassium flux (Qoi_K) leaving the proton flux (Qio_H) little or unaffected. The noticeable point, however, was the increased chloride flux (Qoi_{Cl}) by ABA, and differential response of root segments to create a proton gradient. These data suggests that ABA has an additive effect on the proton pump in excised maize root cells. This happened to maintain primary-energy requiring process "Qio_H" for increased K transport into the symplasm.

Introduction

In higher plants, abscisic acid (ABA is probably a key messenger in regulation of salt and water transport under stress conditions (Pitman *et al.*, 1974). ABA has many complex effects such as stomatal closure, abscision and dormancy in different crop species (Milborrow, 1974, 1975; Walton, 1980). ABA formed in the leaves (Mizrahi *et al.*, 1970; Most, 1970) or its precurser(s) formed in the shoot (Zeevaart, 1977) can be translocated through the phloem to the root (Hocking *et al.*, 1972), where it acts as an instrument for fine regulation of water and salt transport under stress conditions.

In previous works (Collins & Channa, 1983; Channa & Collins 1986, 1988) it was reported that ABA stimulates K transport and the hydraulic conductivity of the cell membrane in excised maize roots. It is, however, observed that in plant cell, the membrane located K-Na pump is energised by the electron transport and may be linked with proton extrusion pump (Smith, 1970), depending on the pH (Robert, 1979) and salt status of the medium (Pitaman *et al.*, 1974), in which plants were grown.

In the present study experiments were carried out to determine the influence of ABA on net K transport into the xylem exudate and primary energy-requiring process "Qio_H" which is partly balanced by "Qoi_K" (Raschke, 1975; 1977) coupled to an active flux of chloride "Qoi_{Cl}" (Hall & Baker, 1977). Such an approach may provide an understanding of ABA action on energy-mediated salt transport mechanism across the plant roots..

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Materials and Methods

Seed germination and plant growth conditions: Seeds of maize (*Zea mays* L.) cv Anjou 210 were soaked in running tap water for 2 h. After soaking the seeds were blotted and set on moist tissue paper, also covered with it and kept in the dark for germination at 25±1°C. After 48h of incubation the germinated seeds having about 2-5 mm long radicles were transferred to a 20l capacity container containing 0.5mM $CaCl_2$ solution. The culture solution was aerated continuously with a stream of moist air to facilitate root growth and seedling development. After 3-days of growth in $CaCl_2$ solution the plants were removed for further studies.

Exudation experiments: Exudation experiments were carried out in a similar way as described previously (Collins & Channa, 1983; 1985).

Ion analysis and osmolarity: Ion analysis were made on Pye Unicam SP 90A Series 2 Atomic Absorption Spectrophotometer. Chloride concentration in the exudate were determined by chloride meter and the osmolarity of the exudates were determined by Fiske Freezing point Osmometer.

pH determination: In this experiment two lots of roots were used. Each lot contained 20 roots with a minimum of three replications. The first lot consisted of 1 cm apical segments and the second lot contained 9 cm basal segments of the root. Each lot of roots was placed separately in a glass Petri dish (i.d. 6") containing 50 cm^3 of 1/10 Long Ashton culture medium with or without 10^{-6} M ABA. The pH of the medium was measured frequently at required intervals with laboratory pH meter (ELL Model 7020).

Ion fluxes into the exudate: The ion flux through the root from the external medium, to the xylem conduit was calculated by the following equation:

$$Js = Jv \cdot Cs^x$$

where:

JS, is the net ion flux into the xylem exudate in nmol cm^{-2} h^{-1} (=2.8 x 10 m^{-2} s^{-1}).

JV, is the water flow (strictly volume flow) in ul cm^{-2} h^{-1} (= 2.8 x 10^{-9} m^3 m^{-2} s^{-1}).

Cs^x, is the concentration of any solute in the xylem exudate in mM (= mol m^{-3}).

Results and Discussions

Effect of ABA on Osmolarity and Ion flux into the exudate: The data revealed that ABA does not alter exudate osmolarity until 24h from excision, but beyond this period there was a small drop in the exudate osmolarity caused by ABA, which might have resulted from increased water flow (Table 1). ABA increased potassium flux 'Qoi_K' and chloride flux 'Qoi_{Cl}' leaving calcium flux 'Qoi_{Ca}' little or not affected (Table 2).

Effect of ABA on proton flux: ABA had no profound effect on H^+ exchange from cell to external medium (Fig. 1). The noticeable point, however, was the difference in response of different root segments for creating the proton gradient 'ΔH' in the external medium.

Table 1. Effect of abscisic acid (ABA) on osmolarity of xylem exudate of 3-day old maize roots.

	Osmolarity (m Osmoles)			
	Hours from excision			
	1-6	6-12	12-24	24-36
Control	60.50 ± 0.40	62.22 ± 0.63	58.00 ± 0.64	54.71 ± 0.68
ABA 10^{-6}M	58.00 ± 0.40	58.11 ± 0.88	57.30 ± 0.33	45.72 ± 0.54

Plants were grown in 0.5 mM $CaCl_2$ solution in the dark at 25°C. Uptake medium was 1/10 Long Ashton culture solution. Each value mean of 5 samples ± s.e.m.

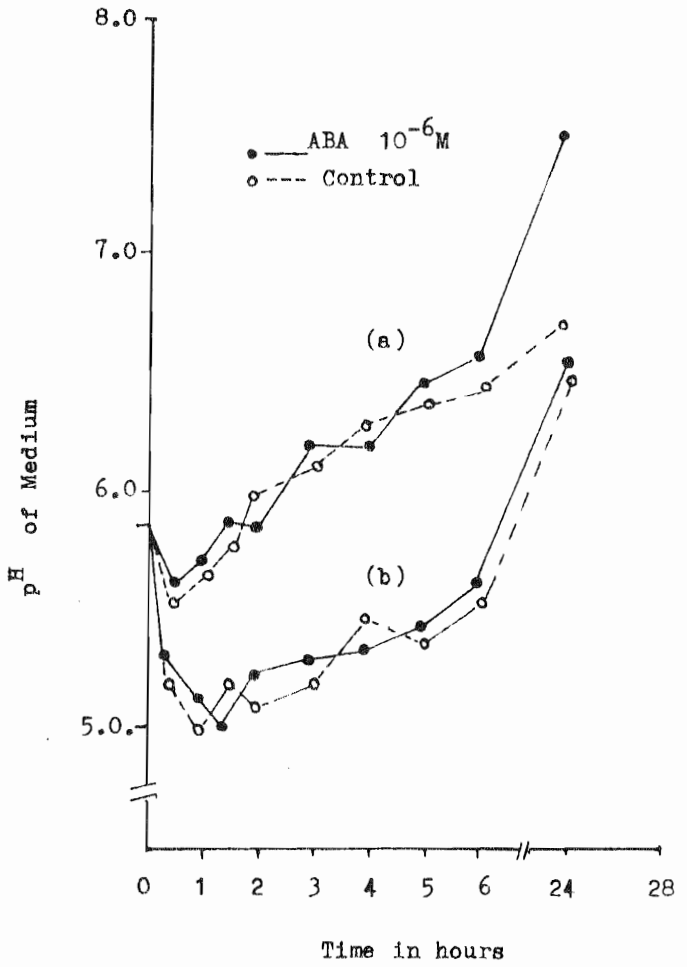

Fig. 1. Change in pH of the medium. (a) 1 cm apical root segments, (b) 9 cm basal root segments.

Table 2. Effect of abscisic acid (ABA) on the volume flux (Jv) and ion flux (Qio_j) in the xylem exudate of 3-day old maize roots.

	Jv ($\mu l\ cm^{-2}h^{-1}$)	Qoi_K	Qoi_{Ca} ($nmol\ cm^{-2}h^{-1}$)	Qoi_{cl}
	Hours from excision			
	1–6h			
Control	1.6 ± 0.31	36.35 ± 0.45	4.15 ± 0.23	28.29 ± 0.50
ABA $10^{-6}M$	3.00 ± 0.20	54.60 ± 0.44	5.04 ± 0.01	36.00 ± 0.34
	6–12h			
Control	2.01 ± 0.02	29.75 ± 0.73	7.29 ± 0.40	23.16 ± 0.75
ABA $10^{-6}M$	3.47 ± 0.01	44.50 ± 0.84	7.26 ± 0.23	27.68 ± 0.54
	12–24h			
Control	1.36 ± 0.30	21.76 ± 0.58	4.40 ± 0.22	28.69 ± 0.88
ABA $10^{-6}M$	4.07 ± 0.30	48.44 ± 0.86	5.40 ± 0.20	36.00 ± 0.66
	24–30h			
Control	0.87 ± 0.03	9.24 ± 0.01	6.80 ± 0.23	5.22 ± 0.20
ABA $10^{-6}M$	1.47 ± 0.32	16.28 ± 0.36	8.23 ± 0.22	6.02 ± 9.36

Plants were grown in 0.5mM $CaCl_2$ in the dark at 25°C. Uptake medium 1/10 Long Ashton culture solution. Each value mean of 5 samples ± s.e.m.

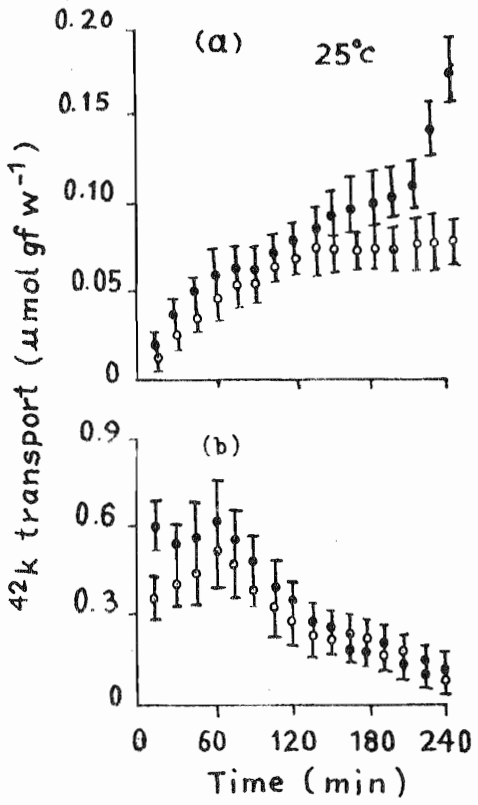

Fig. 2. Effect of ABA on 42_K transport in the exudate. (1) Isotope applied to the bathing medium, (b) Roots pretreated with 42_K for 18 hours. • – ABA 10^{-4}M, o – control.

These data are similar to our previous reports (Channa & Collins, 1985, 1986, 1988) where it was observed that ABA increases both water permeability and salt transport through the maize root. The remarkable point in the present data is the increased chloride flux by ABA (Table 2). Although ΔH^+ is not much pronounced as compared to the stimulation of Cl transport, this, however, implicated that ABA is likely to interact with primary energy-linked H^+ extrusion pump driven by a H^+-ATPase located in the plasmalemma of the excised maize roots (Sze, 1984), which is partly balanced by 'Qoi_K' and remainder of the 'Qio_H' is balanced by 'Qio_{OH}' coupled to an active 'Qoi_{cl}' (Smith, 1970). The major effect of ABA seems to maintain energy-requiring process at cell membrane while keeping on increased K flux into the symplasm. This possibility is supported by the data of Fig. 2a. When the isotope was added to the bathing medium of low-salt roots, there was about 2-fold increase in K transport. There was a differential response of root segments to bring about ΔH^+ of the external medium (Fig. 1). This might be due to the different levels, distribution and compartmentation of ABA and its metabolites in maize roots (Rivier & Pillet, 1981; Behl & Jeschke, 1981), and also due to the presence of Ca^{++} in the medium which has been found to cause a linear H^+ efflux associated with plasmalemma membrane H^+ – ATPase in maize roots (Mengel & Schebert, 1985).

Studying the mode of action of ABA on stomatal aperture it has been reported that ABA inhibits both K uptake and proton release by promoting malate leakage from the guard cells of different plant species (Horton & Moran, 1972; Mansfield & Jones, 1971;

Raschke, 1977). Such a response of ABA does not occur in the root cells. An important element might be the different gradients of accompanying anion to regulate H/K exchange process in stomatal complex and in the root cells (Baker & Hall, 1973; Humble & Raschke, 1971).

In the present study the influence of ABA on H^+ flux (Fig. 1) reveals that ABA does not inhibit H^+ extrusion pump in excised maize roots for creating proton gradient, and it increases K transport into the symplasm (Fig. 2a). This H/K exchange process could possibly be maintained by an additive effect of ABA on H^+ pump, which is presumably linked directly or indirectly to K influx in maize roots under the low-salt conditions (Cheeseman & Manson, 1979).

References

Baker, D.A. and J.L. Hall. 1973. Pinocytosis and ion uptake by plant cells. *New Phytol.*, 72: 1282-1291.

Behl, R. and W.D. Jeschke. 1981. Influence of abscisic acid on unidirectional fluxes and intercellular compartmentation of K and Na in excised barley root segments. *Physiol. Plant.*, 53: 95-100.

Channa, A.N. and J.C. Collins. 1985. Effect of abscisic acid on water and ion uptake in excised roots of maize, millet and sorghum. *Pak. J. Bot.*, 17: 25-31.

Channa, A.N. and J.C. Collins. 1986. Effect of abscisic acid and kinetin on volume flow and potassium selectivity in excised maize roots. *Pak. J. AAEVS*, 1: 1-6.

Channa, A.N. and J.C. Collins. 1988. ABA stimulated intercellular fluxes of potassium in excised roots of maize. *Pak. J. Bot.*, 20: 83-95.

Cheeseman, J.M. and J.B. Hanson. 1979. Energy-linked potassium influx as related to cell potential in corn roots. *Plant. Physiol.*, 64: 842-854.

Clarkson, D.T. 1976. The influence of temperature on the exudation of xylem from detached root systems of rye (*Secale cereale*) and barley (*Hordeum vulgare*). *Planta* (Berlin), 132: 297-304.

Collins J.C. and A.N. Channa. 1983. The effect of abscisic acid on the ion and water relations of excised maize roots. *Proc. Int. Symp. Irding* (Austria). pp. 119. 121.

Hall, J.L. and D.A. Baker. 1977. *Cell membrane and ion trfansport. Integrated Themes in Biology*. Longman, London, New York.

Hoad, G.C.V. 1971. Effect of water stress on abscisic acid levels in white lupin (*Lupinus albus* L.) fruit, leaves and phloem exudate. *Planta*, 142: 287-290.

Hocking, T.C., Hilman, J.K. and M.B. Wilkins. 1972. Movement of abscisic acid in *Phseolus vulgaris*. *Nature New Biol.*, 235: 124-125.

Horton, R.F. and L. Moran. 1972. Abscisic acid inhibition of potassium flux into stomatal guard cells. Z. *Pflazenphysiol.*, 66: 193-197.

Humble, G.D. and K. Raschke. 1971. Stomatal opening quantitatively related to potassium transport. Evidence from elected probe analysis. *Plant Physiol.*, 48: 447-453.

Mansfield, T.A. and R.J. Jones. 1971. The effect of abscisic acid on potassium uptake and starch content of stomatal guard cells. *Planta* (Berlin), 101: 147-158.

Mengel, K. and S. Schubert. 1985. Active extrusion of protons into deionized water by roots of intact maize plants. *Plant. Physiol.*, 79: 344-348.

Milborrow, B.V. 1974. Chemistry and Physiology of abscisic acid. *Ann. Rev. Plant Physiol.*, 25: 259-307.

Mizrahi, V., Blumenfeld, A. and A.E. Richmond. 1970. Abscisic acid and transpiration in leaves in relation to osmotic root stress. *Plant. Physiol.*, 46: 169-171.

Most, R.H. 1971. Abscisic acid in immature apical tissues of sugarcane and in leaves of plants subjected to drought. *Planta*, 101: 67-75.

Pitman, M.G., Luttge, J., Lauchli, A. and E. Ball. 1974. Action of abscisic acid on ion transport as affected by root temperature and nutrient status. *J. Exp. Bot.*, 25: 147-155.

Raschke, K. 1975. Simultaneous requirement of carbon dioxide and abscisic acid for stomatal closing in *Xanthium stramarium*, *Planta*, 125: 243-259.

Raschke, K. 1977. Stomatal turgor mechanism and its response to CO_2 and abscisic acid. Observation and Hypothesis. In: *Regulation of Cell Membrane Activities in Plants.* (Ed.) E. Marre and C. Clif. Elsevier North Holland Biomed. Press. Amsterdam. pp 173-183.

Rivier, L. and P.E. Pillet. 1981. Abscisic acid levels in root tips of seven *Zea mays* L. varieties. *Phytochemistry*, 20: 117-119.

Robert, J. 1979. Les Systems Transport Du phosphate chez chlorella pyrenoidose effect etes fortes concentrations externes en potassium de la valinomycine at d'anters inhibiteurs. *Physiol. Veg.*, 17: 197-205.

Smith, F.A. 1970. The mechanism of chloride transport of Characean cells. *New. Phytol.*, 64: 69-90.

Sze, H. 1984. H^+– translocating ATPases of plasma membrane and tonoplast. *Physiol. Plantarum*, 61: 683-691.

Walton, L.D. C. 1980. Biochemistry and physiology of abscisic acid. *Ann. Rev. Plant. Physiol.*, 31: 453-489.

Zeevaart, J.A.D. 1977. Site of action of abscisic acid synthesis and metabolism in *Ricinus communis* L. *Plant Physiol.*, 59: 788-791.

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