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Electrogenic K^+/H^+ exchange in excised wheat roots

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Abstract

Root excision from *Triticum vulgare* L. var. *muticum* seedlings induced a membrane potential drop, homeostasis disturbances, and loss of absorbing capacity in roots. During subsequent 3 - 4 h incubation the initial physiological properties of the roots were restored. At that period K^+ absorption could be blocked by tetraethylammonium (TEA) without changing pH of the incubation medium. After 5 - 6 h of incubation the membrane hyperpolarization and the enhancement of the absorbing capacity were observed. At that period K^+ influx, at presence or absence of valinomycin in incubation medium, was coupled with acidification of the external medium and was not blocked by TEA.

Additional key words: ion uptake, tetraethylammonium, *Triticum vulgare*, valinomycin.

Introduction

Excised roots are widely used in investigations of ion absorption of higher plants. However, excision of roots and segmentation lead to stress reactions in root cells and subsequent restoration of physiological properties (non-specifc adaptive syndrome) (Gronewald and Hanson 1982, Pakhomova and Pakhomov 1992). The aim of the present paper is investigation of dynamic states of the transport systems in cell membrane of excised roots.

Materials and methods

Plant material: The experiments were carried out with excised roots of 4 - 6-d-old seedlings of wheat (*Triticum vulgare* var. *muticum* L.) cv. Moskovskaya-35. Seeds were moistened in tap water during 24 h and then were drilled on glass covered by moist gauze and placed in cuvette with 0.25 mM $CaCl_2$ solution. The plants were grown in 0.25 mM $CaCl_2$ at room temperature and natural illumination. Seedlings

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Abbreviations: f.m. - fresh mass; MP - membrane potential; TEA - tetraethylammonium; VM - valinomycin.

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with roots were easily graged from the gauze without injury. Roots (150 mg) were placed in 0.25 mM solutions (3 cm^3) immediately after excision and then were incubated for 1 - 7 h under 30 °C and moderate shaking. Substances were added immediately after root excision and after preliminary incubation for 2, 3 and 5 h in control solution (0.25 mM $CaCl_2$). Following substances were used: 0.02 mM valinomycin (VM) (*Serva*, Heidelberg, Germany), 10 mM tetraethylammonium (TEA) (*BDH*, Poole, England) and 0.2 mM K_2SO_4 (*Reakhim*, Sankt-Petersburg, Russia). Initial pH of the incubation medium was 6.1 - 6.3.

K^+ efflux and influx: K^+ efflux was determined by changes of its content in the incubation medium for 1 - 7 h. Measurements of K^+ were carried on flame photometer (*PFM*, Zagorsk, Russia). K^+ absorption was registered in the same manner for 2 - 6 h in the incubation medium containing 0.2 mM K_2SO_4 + 0.25 mM $CaCl_2$.

Membrane potential of root cells was measured by standard microelectrodes (resistance 10 - 20 × 10^9 mS^{-1}) filled with 3 M KCl solution. Comparison electrode was teflon tube filled with 3 M KCl solution and agar and connected with electrode Ag-AgCl through 3 M KCl solution.

Results

Excision of roots from wheat seedlings led to a membrane potential decline (Fig. 1). Membrane potential decline was accompanying by leakage of endogenous K^+ out of the cells (Fig. 1, Table 1). Lost potassium was present in the incubation medium during 1 - 2 h. At that period exogenous K^+ was not absorbed by the roots; as before K^+ efflux into salt solution was observed (Fig. 1).

During subsequent 3 - 4 h of incubation the initial physiological properties of root cells were restored. By the 2nd - 3rd h of incubation membrane potential was restored and then its gradual enhancement (hyperpolarization) was registered (Fig. 1).

By the 4th h re-uptake of lost endogenous K^+ was observed (Fig. 1, Table 1). At that time period absorption of potassium from exogenous salt was also observed. K^+ influx was decreased by TEA without changing pH of the incubation medium (Fig. 1, Table 1). Moreover enhancement of K^+ uptake rate (development of absorbing capacity) was observed during incubation (Fig. 1, Table 1).

Addition of exogenous K^+ after 5 h led to its absorption and was accompanied by acidification of the incubation medium. Analogous phenomenon was observed under simultaneous action of K_2SO_4 and VM (Table 1). At that period (5 - 6 h) K^+ absorption was not blocked by TEA with or without VM present (Table 1). 5 h after VM addition acidification of the incubation medium was registered; herewith loss of endogenous K^+ was not observed (Table 1).

Table 1. Effect of TEA and VM on pH change of the incubation medium, leakage and absorption of potassium by the excised wheat roots during incubation. K^+ efflux is designated by minus sign, K^+ uptake by plus sign. Excised roots were incubated for 2, 3 and 5 h in control solution and then for 1 h in corresponding solutions; after that K^+ efflux, K^+ uptake and pH of the incubation medium were measured.

Treatment	K^+ [μeq g^{-1}(f.m.)]			Medium pH			
	2	3	5	0 (initial)	2	3	5
Control	−0.6±0.1	0	0	6.3	6.1±0.0	6.2±0.1	6.2±0.1
0.2 mM K_2SO_4	+2.9±0.6	+4.7±0.3	+6.4±0.4	6.2	5.9±0.1	5.9±0.1	5.6±0.1
10 mM TEA	−2.0±0.2	−0.7±0.3	0	6.1	6.1±0.0	6.2±0.0	6.0±0.0
K_2SO_4 + TEA	+2.0±0.3	+2.9±0.2	+7.3±0.0	6.2	6.1±0.0	6.0±0.1	5.7±0.2
0.02 mM VM	-	-	0	6.3	-	-	5.6±0.1
K_2SO_4 + VM	-	-	+5.5±0.5	6.3	-	-	4.9±0.1
K_2SO_4 + TEA	-	-	+5.3±0.0	6.3	-	-	5.0±0.1

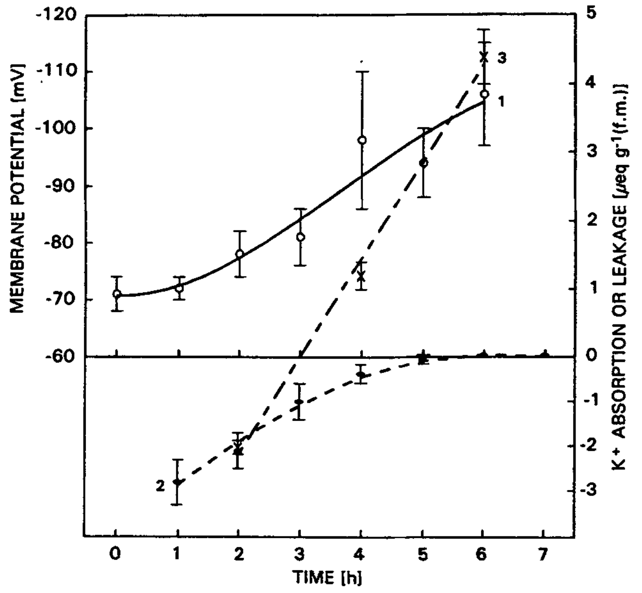

Fig. 1. Change of various physiological indices in wheat root cells during non-specific adaptive syndrome. *1* - membrane potential, MP [-mV]; *2*, *3* - K^+ concentration in the incubation medium of different composition: *2* - 0.25 mM $CaCl_2$, *3* - 0.2 mM K_2SO_4. Minus sign designates leakage of endogenous K^+; plus designates K^+ absorption by the roots. MP of root cells in the intact seedling is -84 ± 4 mV; 0 corresponds to MP of freshly excised roots.

Discussion

Increased permeability in the initial period after root excision is most likely attendant by opening of K^+-channels. Foregoing suggestion is supported by decline of K^+

leakage under blockade of K^+-channels at this time period (Nikolaev 1988). Moreover under MP reduction the open state of K^+ channels is probable. MP decline and loss of the absorbing capacity of excised roots during the first 1 - 2 h of incubation indicate the inhibition of electrogenic H^+-pump in the cell plasmalemma. This is possible in condition of open state of passive channels for transport of cations and anions (Vorobev 1988).

During the 2nd - 3rd h of incubation MP is increased up to the initial level (that indicates active functioning of plasmalemma H^+-pump at this time period) (Fig. 1), and moving force for non-specific transport of cations into the cells (electrogenic antiport) is created (Vorobev 1988). This leads to K^+ absorption (Fig. 1, Table 1). More slow recovery of K^+-influx in comparison with H^+-efflux after root excision was also established (Chastain *et al.* 1981). Supression of K^+ absorption by TEA (Table 1) indicate open state of K^+-channels, inasmuch as substances of this type block only the open K^+-channels (Vorobev 1988). At this period K^+ absorption is not coupled with pH change of the incubation medium (Table 1). Negligible correspondence between the rates of H^+-efflux and K^+-influx during the initial 4 h of incubation was also demonstrated in excised maize roots (Lin and Hanson 1976). This idea was also supported by acidification of the incubation medium under blocking of K^+-permeability at this period (Nikolaev 1988).

The presented facts reflect electric manner of H^+-pump and K^+-channels coupling (Vorobev 1988). By the 5th - 6th h of incubation cell polarization is appeared as a consequence of active functioning of ATPase. After that in accordance with data of Lin and Hanson (1976) MP of maize root cells leveled off. Authors considered that hyperpolarization was stipulated by electrogenic H^+-extrusion; inasmuch as it was blocked by uncouplers (Lin and Hanson 1976). Analysing of presented information have led us to the conclusion that at this period (5 - 6 h) a transition of H^+-ATPase to the state carrying out primary active transport on the manner of K^+/H^+-ATPase mechanism (chemical coupling) might occur (Vorobev 1988). Foregoing suggestion is proved by coupling between K^+ uptake by the cells and acidifications of the incubation medium at this period (Table 1). Analogous phenomenon is observed with VM present (Table 1). Moreover acidification of the incubation medium under VM and K_2SO_4 action was blocked by inhibitors of plasmalemma ATPase (Nikolaev 1988). This indicate the active mechanism of H^+ leakage out of the cells. Moreover root response on excision and subsequent incubation was established to resemble fusicoccin effect: in both cases membrane hyperpolarization, the enhancement of the absorbing capacity and H^+-extrusion were registered (Gronewald *et al.* 1979). Fusicoccin was suggested to induce a transition of plasmalemma H^+-pumps to the hyperpolarized state (Vorobev *et al.* 1987). The transition of H^+-pump to the electrogenic state is coupled with decrease of population of open K^+-channels (with channel close) and with reduced H^+-pumps. Closed K^+-channels with depressed H^+-pump stipulate low membrane conductance (Vorobev 1988). This is confirmed by the fact that at this time period K^+ uptake is not blocked by TEA (Table 1). Foregoing is also supported by others investigations showing H^+-efflux decline (Hanson and Lin 1977, Gronewald *et al.* 1979), MP stabilization and membrane conductance decrease (Lin and Hanson 1976).

It may be concluded that in condition of multihour incubation of excised roots electrogenic K^+/H^+ exchange change from depressed state (1 - 2 h after excision) through transitional state of electric coupling (3 - 4 h) to the state of chemical coupling (5 - 6 h).

References

Chastain, C.J., La Fayette, P.R., Hanson, J.B.: Action of protein synthesis inhibitors in blocking electrogenic H^+-efflux from corn roots. - Plant Physiol. **67**: 832-835, 1981.

Gronewald, J.M., Cheeseman, J.M., Hanson, J.B.: Comparison of the responses of corn root tissue to fusicoccin and washing. - Plant Physiol. **63**: 255-259, 1979.

Gronewald, J.M., Hanson, J.B.: Adenine nucleotide content of corn roots as affected by injury and subsequent washing. - Plant Physiol. **69**: 1252-1256, 1982.

Hanson, J.B., Lin, W.: A model for creating and maintaining electrogenic cell potentials in corn root cells. - In: Harrison, G., Reashar, A. (ed.): Exchanges Ioniques Transmembranaires chez les Végétaux. Pp. 199-204. Rouen-Paris 1977.

Lin, W., Hanson, J.B.: Cell potential, cell resistance and proton fluxes in corn root tissue. Effect of dithioerythritol. - Plant Physiol. **58**: 276-282, 1976.

Nikolaev, B.A.: Dykhatel'nyï gazoobmen korneï pshenitsy pri modifikatsii pronitsaemosti plasmalemmy. [Respiratory gas exchange of wheat roots under modification of plasmalemma permeability.] - Thesis. Institute of Biology, Kazan Research Center of the Academy of Sciences of Russia, Kazan 1988. [In Russ.]

Pakhomova, V.M., Pakhomov, D.V.: [Non-specific adaptive syndrome of excised roots]. - Uspekhi sovremen. Biol. **112**: 398-409, 1992. [In Russ.]

Vorobev, L.N.: Regulirovanie Ionnogo Transporta: Teoreticheskie i Prakticheskie Aspekty Mineral'nogo Pitaniya Rasteniï. [Ion Transport Control: Theoretical Aspects of Plant Mineral Nutrition.] - VINITI, Moscow 1988. [In Russ.]

Vorobev, L.N., Magerramov, M.G., Babakov, A.V., Muromtsev, S.: [Effect of fusicoccin on membrane conductance and potential in *Nitellopsis obtusa* cells.] - Fiziol. Rast. **34**: 342-349, 1987. [In Russ.]