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EFFECT OF PREPARATIONS METHYURE AND IVINE ON Ca^{2+}-ATPases ACTIVITY IN PLASMA AND VACUOLAR MEMBRANE OF CORN SEEDLING ROOTS UNDER SALT STRESS CONDITIONS

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Ca^{2+}-ATPases regulate the functioning of Ca^{2+}-dependent signaling pathway SOS which provides removal of Na^+ from the cytoplasm of cells via Na^+/H^+-antiporters in saline conditions. The influence of synthetic preparations Methyure and Ivine on the Ca^{2+}-ATPase activity was investigated. It was shown that exposition of corn seedlings in the presence of 0.1 M NaCl rather enhanced hydrolytic than transport activity of Ca^{2+}-ATPases in plasma and vacuolar membrane of root cells. It was found that seed treatment with such preparations, especially Methyure, caused intensification of the both activities of Ca^{2+}-ATPases, mainly in vacuolar membrane. The results indicate than salt protective activity of preparations, especially Methyure, is associated with increased Ca^{2+}-ATPase activity, which regulates the functioning of Na^+/H^+-antiporters.

K e y w o r d s: Zea mays L., plasma membrane, vacuolar membrane, salt stress, hydrolytic and transport activities of Ca^{2+}-ATPase, Methyure, Ivine.

Salted medium is one of the most negative abiotic factors of permanent action for plants; they create a serious problem for farming which is intensified because of the global warming [1]. Its negative effect may be reduced using the methods of chemical melioration of soils with introduction of calcium [2]. Calcium plays a regulatory role as a secondary messenger in activation mechanisms of numerous signal paths in plant organisms, including the salt stress conditions, activating the processes of sodium removal from cell cytoplasm [3, 7].

The salt stress in plant organisms is multicomponent; it causes the disturbance of osmotic and ionic homeostasis and is accompanied by appearance of secondary oxidizing stress [1]. Plant organisms radically differ from animal ones in their attitude to Na^+ that is redundant and even toxic element for them; it gets into cell cytoplasm through potential-dependent potassium channels of the plasma membrane [4]. Plants adaptation to salinity conditions consists in non-admission of high concentration of Na^+ in cytoplasm by its removal from cells to the outer and vacuolar space with the help of Na^+/H^+-antiporters, which work in the plasma and vacuolar membrane. Their function is regulated with the help of salt overly sensitive system (SOS-system) that consists of the chain SOS3–SOS2–SOS1, where SOS1 is Na^+/H^+-antiporter, and SOS3 i SOS2 – regulatory proteinases. The increase of Ca^{2+} concentration in cytoplasm activates signals transduction through SOS-system that results in removal of Na^+ [5].

Calcium homeostasis in cytoplasm under salinity conditions is maintained by Ca^{2+}-ATPases and Ca^{2+}/H^+-antiporters, which function in various cell membrane structures, the latter being included at high concentrations [6]. Ca^{2+}-ATPases of the plasma and vacuolar membrane play the important role in formation of cell response to different stimuli, including salting conditions characterized by continuous action. They belong to P-type of ATPases, which form intermediate phosphorylated product and are characterized by high affinity, transferring Ca^{2+} in nanomolar concentrations. Ca^{2+}-ATPase of plant membrane is represented by two subtypes: Ca^{2+}-ATPase IIA and Ca^{2+}-ATPase IIB, the latter displaying sensitivity to calmodulin. They are simi-

lar to Ca^{2+}-ATPase of animal cells as to their structure and membrane localization [6-12].

The presence of Na^+ causes temporary accumulation of the both subtypes of Ca^{2+}-ATPases that indicates their role in adaptation to salinity conditions [6, 8-10].

Our previous investigations have revealed salt-protective effect of synthetic preparations Methyure (6-methyl-2-mercapto-4-hydroxypyrimidine) and to a less extent Ivine (N-oxide-2,6-dimethyl pyridine) that determines the fitness of the former for agropractice on salted soils [13]. The work is aimed at finding out the mechanism of these preparations effect on the function of Ca^{2+}-ATPases of the plasma and vacuolar membrane in the presence of Na^+.

Materials and Methods

Seeds were wetted during a day in 10^{-7} M water solutions of synthetic preparations (Methyure and Ivine). Corn seedlings (hybrid Ostrech SV) were grown in water culture on Hogland medium under the conditions of 16-hour light day at 24 °C and illumination 50 W/m^2. They were exposed at a week age in 0.1 M NaCl during 1 and 10 days. Membrane preparations were isolated from roots using a centrifuge Optima TM L-90K Beckman Coulter. The fraction of plasma membrane was obtained by the phase separation method [14], while the fraction of vacuolar membrane – in the stepped gradient of saccharose [15]. Purity of the obtained fractions and availability of membrane additives were determined by the activity of marker enzymes [16], a role of vesicles oriented outwards – by the effect of Triton X-100 on ATP hydrolysis [14], integrity of vesicles – with the help of electron microscopy [17]. Protein content in membrane fractions was determined by the standard method [18].

Hydrolytic activity of Ca^{2+}-ATPase was determined spectrophotometrically (SF-2000, $\lambda = 600$ nm) by the amount of released P_i [19], using special substrate inositoltriphosphate (ITP), activity was expressed in nmol P_i/mg protein/min. Transport activity in the plasma and vacuolar membrane was measured by fluorescent method [20] (sound Fluo 4 AM) [21], using spectrofluorometer Quanta Master 40 PTI (Canada) under excitation of 495 nm, emission 522 nm with the software FelixGX 4.1.0.3096 presenting in Δ%F/mg protein/min.

All the experiments were performed in five biological and three analytical repeats, and reliability of the data obtained was determined following Student's criterion.

Results and Discussion

The research was performed on preparations of plasma and vacuolar membrane represented by closed vesicles equal in size. Some role of plasma membrane (PM) with outward orientation was 72%, and vacuolar – 15%. Composition of PM preparations was as follows: plasma membrane – 83%, vacuolar membrane – 8%, mitochondrial and Golgi apparatus 4-5% each. Composition of vacuolar membrane preparations was: vacuolar membrane – 85%, plasma membrane – 6.4%, mitochondrial and Golgi apparatus – 4.3% each.

Under control conditions the hydrolytic and transport activity of Ca^{2+}-ATPase of PM had to change during 10 days, while a 1-day salt exposure caused their intensification to 40%, which continues during 10 days (Table 1).

Hydrolytic and transport activity of Ca^{2+}-ATPase almost did not change, the 1-day exposure in the presence of 0.1 M NaCl resulted in the 22%

Table 1. Effect of the term of salt exposure on activity of Ca^{2+}-ATPase of plasma membrane from root cells of 8- and 17-days corn seedlings ($M \pm m$; $n = 5$)

Variant	Term of seedlings exposure			
	1 day	% in respect to the control	10 day	% in respect to the control
	Hydrolytic activity (nmol P_i/mg protein/min)			
Control	26.7 ± 1.1	100	27.9 ± 0.9	100
NaCl	36.3 ± 1.7$^{\#}$	136	37.9 ± 1.9$^{\#}$	136
	Transport activity (Δ%F/mg protein/min)			
Control	52.4 ± 2.5	100	53.6 ± 1.5	100
NaCl	72.6 ± 2.6$^{\#}$	139	75.2 ± 3.3$^{\#}$	140

Here and in Tabl. 2-3 $^{\#}P < 0.05$ is probable in respect of control without salt exposure

Table 2. Effect of the term of salt exposure on activity of Ca^{2+}-ATPase of vacuolar membrane from root cells of 8- and 17-days corn seedlings (M ± m; n = 5)

Variant	Term of seedlings exposure			
	1 day	% in respect of the control	10 day	% in respect of the control
	Hydrolytic activity (nmol P_i/mg protein/min)			
Control	32.4 ± 1.2	100	33.5 ± 1.9	100
NaCl	31.8 ± 1.5#	98	49.7 ± 1.3#	148
	Transport activity (Δ%F/mg protein/min)			
Control	48.4 ± 2.1	100	49.5 ± 2.6	100
NaCl	37.7 ± 1.1#	78	54.9 ± 1.9#	111

decrease of transport activity, and only 2% decrease of hydrolytic activity, while prolongation of salt exposure to 10 days intensified hydrolytic activity by 48%, and transport activity only by 11% (Table 2).

The obtained results demonstrate a higher activity of Ca^{2+}-ATpase in plasma membrane compared with vacuolar one on condition of salt exposure that evidences for its important functional role. Such an effect of salt exposure on activity of Ca^{2+}-ATPases in these membrane was also shown on other cultures. The given Ca^{2+}-ATPases play great role in ecologic plasticity of plants that was demonstrated at the early varieties of rice, tomatoes, radish and corn [6-9].

A comparison of Methyure and Ivine effect on activity of Ca^{2+}-ATPase of plasma membrane has shown that under control conditions the preparations something increased the both activities, which grew during the exposure (Table 3). Salt exposure caused further increase in, especially, hydrolytic activity, the effect of Ivine was lower than that of Methyure that explains a stronger salt-protective effect of the latter preparation (Table 4).

Investigations of the effect of these preparations on Ca^{2+}-ATPase of vacuolar membrane have shown that in control conditions the both preparations, especially Methyure and, to a lesser extent, Ivine intensified essentially hydrolytic and to a lesser extent transport activity of Ca^{2+}-ATPase, which almost did not change with time (Table 5).

The preparation, especially Methyure, intensified to a higher extent the both activities, especially hydrolytic one, in the presence of NaCl (Table 6). Thus, the salt protective effect of the above preparations may be connected with intensification of Ca^{2+}-ATPase activity, especially in vacuolar membrane. This results in stabilization of functioning of Ca-dependent SOS-systems by accumulation of Ca^{2+} in vacuolar space.

Table 3. Effect of Methyure and Ivine on activity of Ca^{2+}-ATPase of plasma membrane from the root cells of 8- and 17-days corn seedlings under the absence of salt exposure (M ± m; n = 5)

Preparation	Age of seedlings			
	8 day	% in respect of the control	17 day	% in respect of the control
	Hydrolytic activity (nmol P_i/mg protein/min)			
Control	26.7 ± 1.1	100	27.9 ± 0.9	100
Methyure	29.1 ± 2.5#	109	31.8 ± 2.1#	114
Ivine	28.1 ± 1.4#	105	30.1 ± 1.3#	108
	Transport activity (Δ%F/mg protein/min)			
Control	52.4 ± 2.5	100	53.6 ±1.5	100
Methyure	56.0 ± 4.8#	107	59.5 ±3.5#	111
Ivine	54.5 ± 2.7#	104	56.8 ±1.8#	106

Table 4. Effect of Methyur and Ivine on activity of Ca^{2+}-ATPase of plasma membrane from the root cells of 8- and 17-days corn seedlings exposed in the presence of 0.1 M NaCl (M ± m; n = 5)

Preparation	Term of seedlings exposure			
	1 day	% in respect of the control	10 days	% in respect of the control
	Hydrolytic activity (nmol P_i/mg protein/min)			
Control	36.3 ± 1.7#	100	37.9 ± 1.9#	100
Methyure	47.9 ± 2.1*	132	53.9 ± 2.6*	142
Ivine	42.8 ± 1.6*	118	46.2 ± 1.1*	122
	Transport activity (Δ%F/mg protein/min)			
Control	72.6 ± 2.6#	100	75.2 ± 3.3#	100
Methyure	83.5 ± 3.1*	115	88.1 ± 4.3*	117
Ivine	78.4 ± 2.2*	108	79.7 ± 3.9	106

Here and in Table 6 #$P < 0.05$ is probable in respect of control without salt exposure; * $P < 0.05$ is probable in respect of control with salt exposure

Table 5. Effect of Methyure and Ivine on Ca^{2+}-ATPase activity of vacuolar membrane from root cells of 8- and 17-day corn seedlings without salt exposure (M ± m; n = 5)

Preparation	Age of seedlings			
	8 day	% in respect of the control	17 day	% in respect of the control
	Hydrolytic activity (nmol P_i/mg protein/min)			
Control	32.4 ± 1.2	100	33.5 ± 1.9	100
Methyure	57.3 ± 2.7#	177	62.8 ± 3.1#	187
Ivine	48.6 ± 1.5#	150	51.1 ± 1.7#	153
	Transport activity (Δ%F/mg protein/min)			
Control	48.4 ± 2.1	100	49.5 ± 2.6	100
Methyure	54.1 ± 1.1#	112	58.2 ± 2.3#	118
Ivine	49.9 ± 1.2#	103	52.9 ± 1.9#	107

#$P < 0.05$ is probable in respect of control without salt exposure

Table 6. Effect of Methyure and Ivine on Ca^{2+}-ATPase activity of vacuolar membrane from root cells of 8- and 17-day corn seedlings exposed in the presence of 0.1 M NaCl (M ± m; n = 5)

Preparation	Age of seedlings			
	1 day	% in respect of the control	10 days	% in respect of the control
	Hydrolytic activity (nmol P_i/mg protein/min)			
Control	31.8 ± 1.5#	100	49.7 ± 1.3#	100
Methyure	78.2 ± 2.4*	245	82.1 ± 2.6*	165
Ivine	71.7 ± 2.2*	225	71.9 ± 2.9*	145
	Transport activity (Δ%F/mg protein/min)			
Control	37.7 ± 1.1#	100	54.9 ± 1.9#	100
Methyure	77.6 ± 1.2*	205	79.5 ± 1.6*	144
Ivine	70.8 ± 2.4*	187	71.3 ± 2.9*	129

Ca^{2+}/H^+-antiporters also function in plasma and vacuolar membrane; they take part in the maintenance of cell Ca homeostasis under the effect of salt stress, which causes a considerable increase in calcium level in cytoplasm, the Ca^{2+}/H^+-antiporter of vacuolar membrane being the more mighty [5, 6]. Joint work of Ca^{2+}-ATPases and Ca^{2+}/H^+-antiporters in these membrane maintains functioning of Ca^{2+}/H^+-antiporters which remove Na^+ from cell cytoplasm.

We have shown the capacity of synthetic bioactive preparations to intensify activity of Ca^{2+}-ATPase in the plasma and vacuolar membrane that favors Na^+/H^+-antiporters functioning in them. Under these conditions a higher activity of Ca^{2+}-ATPase in vacuolar membrane provides long-term functioning of its Na^+/H^+-antiporter, which accumulates sodium in vacuolar space, supporting osmotic pressure in cells. Thus, the use of the above preparations, especially Methyure, favors intensification of functioning of Ca^{2+}-dependent paths of Na^+ level normalization in cell cytoplasm, opposing the salt stress formation in them.

ВПЛИВ ПРЕПАРАТІВ МЕТІУР ТА ІВІН НА АКТИВНІСТЬ Ca^{2+}-АТРаз У ПЛАЗМАТИЧНИХ І ВАКУОЛЯРНИХ МЕМБРАНАХ КЛІТИН КОРЕНІВ ПРОРОСТКІВ КУКУРУДЗИ В УМОВАХ СОЛЬОВОГО СТРЕСУ

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Досліджено вплив синтетичних препаратів Метіур та Івін на активність Ca^{2+}-АТРаз, що регулюють функціонування Ca^{2+}-залежного сигнального шляху SOS, який в умовах засолення забезпечує видалення Na^+ із цитоплазми клітин Na^+/H^+-антипортерами. Показано, що експозиція проростків кукурудзи в присутності 0,1 М NaCl більшою мірою посилювала гідролітичну, ніж транспортну активність Ca^{2+}-АТРаз у плазматичних і вакуолярних мембранах в клітинах коренів. Знайдено, що обробка насіння цими препаратами, особливо Метіуром, спричинювала посилення як гідролітичної, так і транспортної активності Ca^{2+}-АТРази, переважно у вакуолярній мембрані. Одержані результати вказують, що солепротекторна дія препаратів, особливо Метіуру, пов'язана з посиленням активності Ca^{2+}-АТРаз, які регулюють функціонування Na^+/H^+-антипортерів.

Ключові слова: *Zea mays* L., плазматична мембрана, вакуолярна мембрана, сольовий стрес, гідролітична і транспортна активність Ca^{2+}-АТРаз, Метіур, Івін.

ВЛИЯНИЕ ПРЕПАРАТОВ МЕТИУР И ИВИН НА АКТИВНОСТЬ Ca^{2+}-АТРаз В ПЛАЗМАТИЧЕСКИХ И ВАКУОЛЯРНЫХ МЕМБРАНАХ КЛЕТОК КОРНЕЙ ПРОРОСТКОВ КУКУРУЗЫ В УСЛОВИЯХ СОЛЕВОГО СТРЕССА

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Исследовано влияние синтетических препаратов Метиур и Ивин на активность Ca^{2+}-АТРаз, регулирующих функционирование Ca^{2+}-зависимого сигнального пути SOS, который в условиях засоления удаляет Na^+ из цитоплазмы клеток. Показано, что экспозиция проростков кукурузы в присутствии 0,1 М NaCl в большей степени повышала гидролитическую, чем транспортную активность Ca^{2+}-АТРаз в плазматических и вакуолярных мембранах клеток корней. Найдено, что обработка семян препаратами, особенно Метиуром, вызывала повышение как гидролитической, так и транспортной активности Ca^{2+}-АТРазы, преимущественно в вакуолярной мембране. Полученные результаты свидетельствуют, что солепротекторное действие препаратов, особенно Метиура, связано с усилением активности Ca^{2+}-АТРаз, регулирующих функционирование Na^+/H^+-антипортеров.

Ключевые слова: *Zea mays* L., плазматическая мембрана, вакуолярная мембрана, солевой стресс, гидролитическая и транспортная активность Ca^{2+}-АТРаз, Метиур, Ивин.

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