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SALT-STRESS INDUCED CHANGES OF THE ACTIVITY OF H^+-PUMPS IN ROOT CELLS AND CO_2-METABOLISM ENZYMES IN LEAVES OF WHEAT GENOTYPES

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In the present study some local wheat genotypes, including Barakatli 95, Garagylchyg 2 and Gyrmyzy bugda have been divided into 6 biogroups according to the morphological structure of seed embryos and the effects of high NaCl concentrations on the H^+-pums function and the activities of carbon metabolism enzymes have been determined. It was found that under salt stress CA, RBPC, NAD-MDH enzymes in the leaves and H^+-pumps of the root system act in a coordinated manner in various biogroups and thereby play an important role in the formation of salt tolerance mechanisms.

Keywords: *wheat genotypes, H^+-pumps, CA, RBPC, NAD-MDH, salt stress*

Introduction

Plants are exposed to soil and atmospheric water deficiency and in many places to the effect of high salt concentrations. It was established that 6% of the world and 30% of the irrigated area faced salinity problem [14]. Salinity is one of the major severe abiotic factors affecting crop growth and productivity.

The accumulation of salt in leaves increases gradually as water evaporates. Old leaves were found to accumulate more salt compared with young leaves. Finally in old leaves salt concentration can increase to the dangerous levels for cells [9].

Transport ATP-ases of membrane or redox-pumps, performing the proton excretion function, create H^+ electrochemical gradient or proton motive force. Namely this gradient can be spent in cells in various processes of ion and organic compound uptake. Another aspect of the investigation of H^+-pumps-their functional relation to the growth processes and plant productivity-is also of great importance [1].

The proton pumps have been found in cells of all bio organisms. Mitchell supposed the existence of a redox chain, performing active transport of H^+, i.e. the proton pump in plasmatic membrane of plant cells. Later the proton pumps were observed in plasmatic membrane of algae and higher plants. In higher plants it is called ATP synthesizing pump-generator. They are participating in the energy transformation and accumulation in chloroplasts and mitochondria. It was established that the active transport of H^+ ions plays an important role in ion absorption through root [10], functioning of bundle sheath apparatus [11] and ion exchange in reserve tissues. Remove of the H^+ ions from cytoplasm and entry of cations into the cell support cell trophism [3].

CA acting in a coordinated way with H^+-pumps plays an important role in catabolic and anabolic processes [6]. The main enzyme of the Calvin cycle - RBPC takes part in CO_2 metabolism. NAD-MDH catalyzing the reversible conversion of oxaloacetate to malate plays an important role in the metabolism. Under increasing salt concentrations CA, RBPC, NAD-MDH and H^+-pumps are labile. Acting concerted under stress conditions they promote plant tolerance against stress factors.

Thus, further investigations are needed for elucidation of the stress defence reactions in C_3 and C_4 plants [8].

The aim of our study was a comparative study of the effect of salts on the activities of enzymes involved in the metabolism of carbon in leaves and on the function of H^+ pump of the root system of wheat varieties: Barakatli 95, Garagylchyg 2 and Gyrmyzy bugda.

Materials and methods

Plant material and growth conditions. Plants were grown in soil, in a climatic chamber of phytotron at 30-35^{0}C, under fluorescent lamps LB-40, at 40-50 klx, 16-h photoperiod, 60-70 % humidity, which correspond to the energy intensity of about 55-65 W/m^2. On the 25th day the seedlings were transferred to 50 ml weighing bottles. Weighing bottles were filled with the test solution, the volume of which during the experiment was kept constant by adding distilled water. Wheat seeds were grouped according to the morphological structure of embryo [5]. The leaves and roots of 3-10-day-old wheat plants were used for the analysis.

Enzyme activity assay. CA activity was determined by the electrometric method based on the change of initial pH [15]. RBPC activity was measured spectrophotometrically [4]. NAD-MDH activity was also measured spectrophotemetrically at 340 nm [12].

Determination of total protein content. Total protein content was determined according to Sedmak [13].

Determination of H^+-pump activity. The acidity of the nutrient solution was determined by glass pH electrode (pH meter "pH-340) [7]. Wheat seedlings were grown in weak salt solution, then KCl solution was added for the stimulation of H^+-pump activity and kinetics of released H^+ was studied. After the lag period kinetic profile became linear. The slope of the line is used as a measurement unit for the proton pump activity.

Statistical analysis. Calculation of H^+ micro-equivalents, statistical processing of the growth characteristics, the construction of the correlation lines were performed using a microcomputer. The arithmetic mean for every value has been presented in the tables.

Results and discussion

H^+-flows which are the functional parameters of the productivity of wheat seeds were investigated in roots and dynamics of the activity changes of carbon metabolism enzymes carbonic anhydrase (CA, EC 4.2.1.1), RBP-carboxylase, (RBPC, EC 4.1.1.39), NAD-malatedehydrogenase, (NAD-MDH, EC 1.1.1.37) was studied in leaves at increasing NaCl concentrations.

It is known that abiotic factors (drought, salinity, etc.) effect on metabolic process first of all in root and leaf level and then adaptive changes occur in other levels.

Kinetics of the acidification of environment by the root system of wheat genotypes is represented in fig. 1. The strongest acidification (ΔH^+_{max}) for separate biogroups of Barakatli 95 was observed in the II bio group (ΔH^+_{max}=3.7 x 10^{-4}mkeq). For the IV and V biogroups ΔH^+_{max}= 3.2 x 10^{-4}mkeq and the lowest ΔH^+_{max} (ΔH^+_{max}=2.5 x 10^{-4}mkeq) occurs in the I biogroup. Accordingly the II biogroup seedling is highest (12.8 cm). The most development rate is also observed in this group (2.73 cm per day). Barakatli 95 shows a mild degree of salinity tolerance. All four group pumps except the first recovered their work (fig. 1A). It occurred in IV and V bio groups most effectively.

Fig. 1B shows that biogroups IV and V of the variety Garagylchyg-2 contrary to Barakatli 95 reaches its maximum H^+-outflow on the 8th day of its growth, while the groups I, II and III continue to acidify the environment. The initial delay of the H^+-outflow is also characteristic for the III group.

Garagylchyg 2 variety which is also productive was intolerant practically: Root system H^+-pumps could not recover active H^+-outflow during two days. Though we did not observe a plant perish during this period, the inhibition of the work of H^+-pumps may be a symptomatic sign indicating a weak resistance of this variety against salinity.

The variety with the lowest productivity Gyrmyzy bugda appeared to be the most tolerant to salinity. Its H^+-pumps recovered the active outflow of protons at the first day and the most efficient work of the H^+-pumps was observed for the biogroup V as the most salt-tolerant one. The groups of III and II are in the middle positions and the lowest productivity was noted for the first biogroup (fig. 1C).

Since the varieties did not differ in the length of their root system, it allows us to evaluate the rate of the acidification of the experimental medium as the parameter of the H^+-pump power.

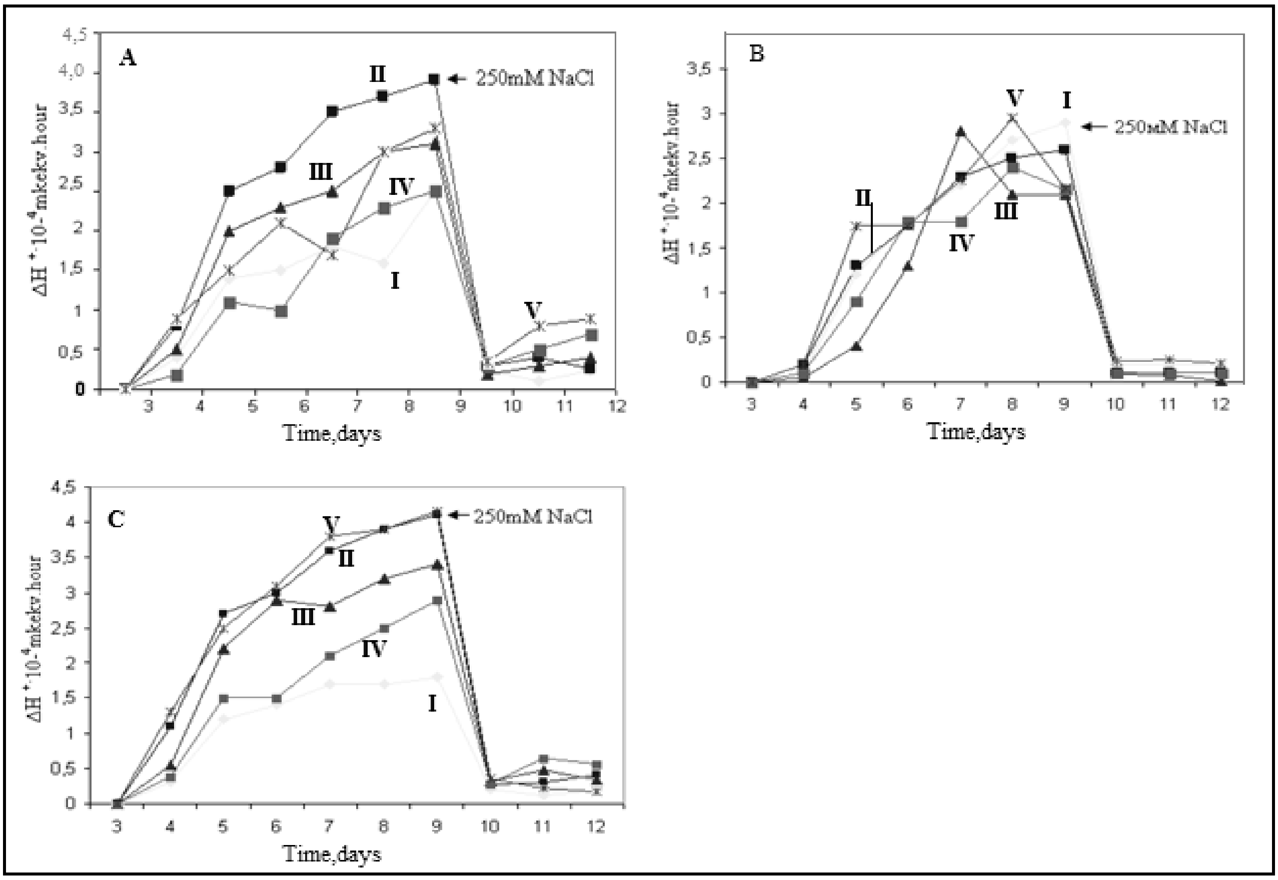

Fig. 1. Kinetics of the H^+-outflow changes in the root system of the 5 biogroup of Barakatli 95 (A), Garagylchyg 2 (B) and Gyrmyzy bugda (C) under high salinity conditions (NaCl). I, II, III, IV, V-number of biogroups

According to the degree of acidification of the H^+-pumps the varieties are ranked as follows: A high-productive Barakatli-95 shows a higher acidification degree than a productive sort Garagylchyg 2 and a low-productive Gyrmyzy bugda is in the last place.

Table shows that the low productive variety biogroups did not differ in seed weight and the rate of the active H^+-outflow, though the seed weight is 32% higher in comparison with productive varieties. On the other hand, the group I of the high-productive variety, which is considered as a non-productive according to the Shevchenko method has the lowest weight. But the biogroup II as the most productive has the highest weight. The biogroup I of the variety Garagylchyg 2 does not concede the biogroup II in the weight of seeds.

Thus on the basis of the obtained data we conclude that the size of the seed endosperm does not necessarily correlate with the power of the H^+-pump work and on the contrary in high productive sorts with the high H^+-outflow, the weight of seeds is lower than in the low-productive Gyrmyzy bugda.

Maximum acidification for the three varieites occurs on the 8th day of the growth for Barakatli 95 – 3.8 x 10^{-4} mkeq H^+, for Garagylchyg 2 2.5 x 10^{-4} mkeq H^+ and for Gyrmyzy bugda 2.05 x 10^{-4} mkeq H^+. The comparison of these parameters of the work power of H^+-pumps with the estimation of the sort productivity performed earlier by Shevchenko (Table) allows determining a strong correlation between them.

As seen in fig. 2 the activities of CA (fig. 2A), RBPC (fig. 2B) and NAD-MDH (fig. 2C) have changed similarly under various salt concentrations in the leaves of the wheat genotypes.

Differential tolerance of different biogroups within the sort population may serve as the selection sign to improve salt-tolerance of plants.

Table

Average weight of seeds (δ-standard error) in each biogroup of three winter wheat varieties with different productivity (Biogroup volume is designated in %)

Varieties		Biogroups				
		I	II	III	IV	V
Barakatli 95	**Bec**	30.2±1,8	47.0±1.1	38.2±1.2	-	39.3±1.3
	%	6.7	40.0	20.0	0.5	33.0
	ΔH^+	22.7	3.8	3.5	3.5	35.0
Garagylchyg 2	**Bec**	22±2.7	43.0±1.0	32.0±1.1	-	44.0±1.3
	%	7.4	41.3	18.1	2.0	31.2
	ΔH^+	2.7	2.45	1.9	2.4	2.7
Gyrmyzy bugda	**Bec**	52.1±1.7	57.1±1.4	54.4±3.0	55.7±4.5	59.0±1.4
	%	32.8	21.2	15.1	13.5	17.4
	ΔH^+	2.1	2.0	2.0	1.9	2.0

Note: *The values of the maximum acidification ΔH^+ (10^{-4}mkeq) created by the seedlings of the each biogroup are given for comparison. Estimated values of the average weight of seeds in the population are 41.42 mg for Barakatli 95, 38.9 mg for Garagylchyg 2 and 55.2 mg for Gyrmyzy bugda*

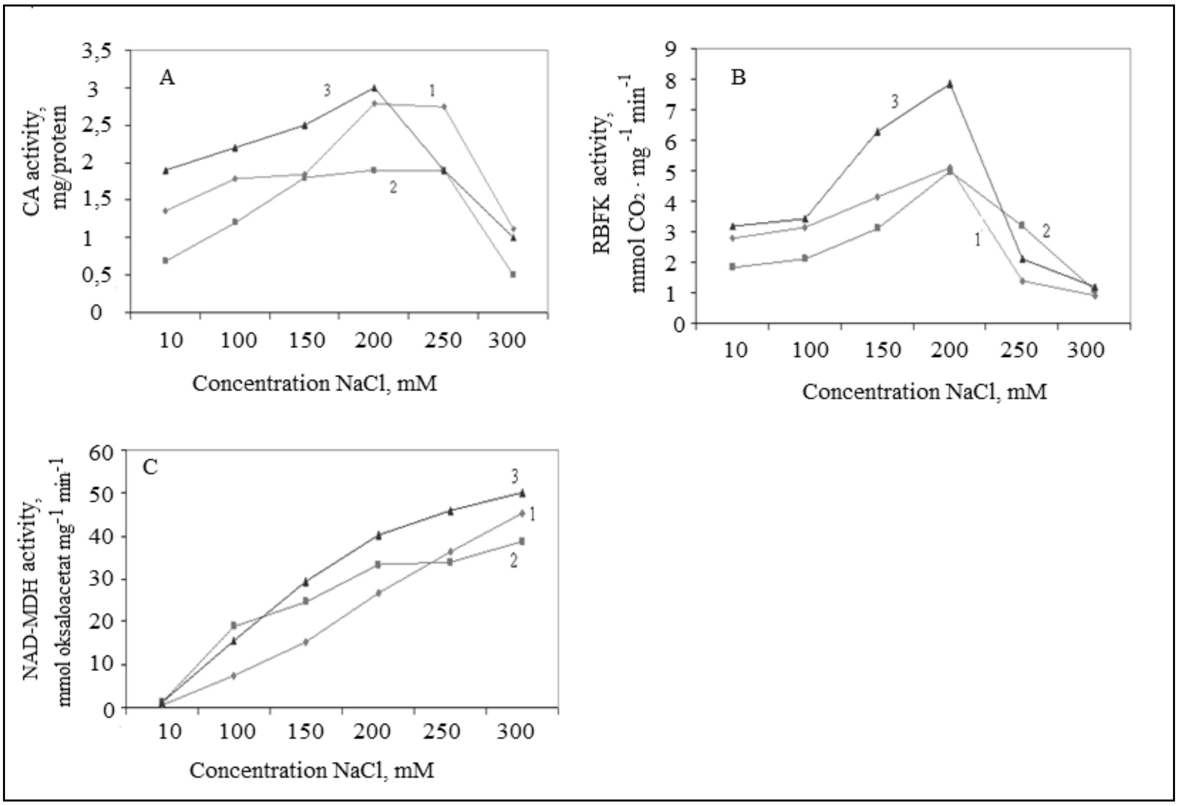

Fig. 2. The dynamics of the changes of carbon metabolism enzyme activities at higher concentrations of NaCl in different wheat genotypes. A - CA, B-RBPC, C-NAD-MDH; *1*- Barakatli 95 (biogroup II); *2* - Garagylchyg 2 (biogroup V); *3* - Gyrmyzy bugda (biogroup II)

Conclusions

1. The evaluation of the potential productivity of the winter wheat on the basis of morpho-physiological types of the seed embryos showed that biogroups of the intensive type-II and IV predominated in more productive varieties while in less productive varieties the biogroup I predominated. 2. Within sort population of seeds energetic estimation of biogroups of different productivity allows determining maximal H^+

gradients and the rate of the active H^+-outflow. 3. Recovery of the work of H^+-pumps was characteristic for the low-productive Gyrmyzy bugda and high productive Barakatli 95 under salinity. Tolerances of the varieties were provided at the expense of biogroups IV and V. 4. It suggests that a part of CO_2 formed during respiration and sent to carboxylation centers is being used in synthesis processes. The rest of CO_2 converts to H^+ and HCO_3^- with CA. Formed H^+ ions penetrate to medium by means of H^+-pumps and cause its acidification, i.e. formation of the specific risosphere. Therefore the medium pH measurement allows estimating H^+-pumps and CA. 5. The increase in NaCl concentration to 200 mM caused an increase in ΔH^+, ΔV_L, chlorophyll and protein amount, activities of CA, RBPC and NAD-MDH. This increase is more pronounced in tolerant and low-productive Gyrmyzy bugda and in tolerant and high-productive Barakatli 95 contrary to Garagylchyg 2, which is intolerant and productive variety. 6. At 200-300 mM NaCl concentrations ΔH^+ and $\Delta V_{L,}$ amounts of protein and chlorophyll were unchanged, CA, RBPC activities decreased and NAD-MDH activity increased 8-9 times. It may be explained as follows: the increase in NAD-MDH activity caused the intensification of synthesis of C_4 -acids (malate, aspartate) and in spite of the closure of bundle sheath cells Calvin cycle reactions were not disturbed. Because malate and aspartate provide Calvin cycle with CO_2 when its intake from atmosphere terminated and gas-exchange weakened.

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BUĞDA GENOTİPLƏRİNİN KÖK HÜCEYRƏLƏRİNDƏ H^+ - NASOSUNUN VƏ YARPAQLARDA CO_2 METABOLİZMİ FERMENTLƏRİNİN AKTİVLİYİNIN DUZ STRESİ İLƏ İNDUKSİYA OLUNAN DƏYİŞİKLİKLƏRİ

H.Q.Babayev

Təqdim olunan işdə Bərəkətli-95, Qaraqılçıq-2 və Qırmızı buğda yerli buğda genotipləri toxum rüşeyminin morfoloji quruluşuna görə 6 bioqrupa ayrılmış, NaCl duzunun yüksək qatılıqlarının hər qrupda H^+-nasosunun və karbon metabolizmi fermentlərinin aktivliklərinə təsiri müqayisəli öyrənilmişdir. Müəyyən olunmuşdur ki, yüksək duz qatılıqlarında KA, RBFK, NAD-MDH fermentləri və kök sistemlərində H^+-nasosları müxtəlif bioqruplarda uzlaşdırılmış fəaliyyət göstərir və duza davamlılığın yaranmasında mühüm rol oynayırlar.

Açar sözlər: *buğda genotipləri, H^+-nasos, KA, RBFK, NAD-MDH, duz stresi*

ИЗМЕНЕНИЯ АКТИВНОСТИ H^+- НАСОСОВ КОРНЕЙ И ФЕРМЕНТОВ CO_2 МЕТАБОЛИЗМА В ЛИСТЬЯХ РАЗЛИЧНЫХ ГЕНОТИПОВ ПШЕНИЦЫ ПРИ ВОЗДЕЙСТВИИ СОЛЕВОГО СТРЕССА

Г.Г.Бабаев

Генотипы пшеницы местной селекции Баракатли-95, Гарагылчыг-2 и Гырмызы бугда по морфологической структуре зародыша семян были разделены на 6 биогрупп и было проведено сравнительное изучение воздействия высоких концентраций NaCl на активность H^+-насосов и ферментов CO_2 углеродного метаболизма. Установлено, что при высоких концентрациях соли ферменты КА, РБФК, НАД-МДГ и H^+-насосы корневой системы действуют согласованно в различных биогруппах, и таким образом, играют важную роль в формировании механизмов толерантности к засолению.

Ключевые слова: *генотипы пшеницы, H^+-насосы, КА, РБФК, НАД-МДГ, солевой стресс*