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COMBINED EFFECT OF NaCl AND Na_2SO_4 SALTS ON NADPH - GENERATING ENZYMES IN MAIZE SEEDLINGS AT DIFFERENT RATIOS

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Abstract

Mazı (*Zea mays* L.) seedlings growth and activity dynamics of G6PDH (NADP-glucose-6-phosphate dehydrogenase), DMDH (NADP-malate dehydrogenase) and ISDH (NADP-isocitrate-dehydrogenase) enzymes have been studied under combined stress conditions created by NaCl and Na_2SO_4 salts solution. With regard to the development of seedlings (control), the activity of the G6PDH enzyme gradually decreases and this decrease in 12-day-old seedlings is 35.6% compared to 4-day-old seedlings. On the contrary, during this development phase, the activity of the DMDH and ISDH enzymes has increased by 37% and 13.4%, respectively in comparison with the initial period. This indicates that the formation of cytoplasmic NADPH potential in the early stages of the development of the Zagatala locally improved maize genotype seedlings is mainly done thanks to the activity of G6PDH enzyme, and in the later stages, mainly the activity of the DMDH and ISDH enzymes.

Keywords: *maize seedlings; salt stress; combined stress; NADPH-generating enzymes*

1. Introduction

Most of the plants are subjected to negative impacts of extreme environmental conditions to varying degrees. In order to complete their ontogenesis normally and eliminate the consequences of these negative impacts, they must first generate special protective responses and activate particular adaptation mechanisms to adjust to that environment [5, 9]. Both the initial defense and the adaptation processes are complex in nature and cover many molecular-genetic and biochemical aspects of plant cells. Implementation of both processes often requires the involvement of the NADPH molecule as an universal reduction metabolite [3].

NADPH is one of the most important high-energy cell metabolites specific to all living organisms. As it is involved in the biosynthesis of several biomolecules, including fatty acids, carbohydrates, and others, it is required for the growth, development, and reproduction of cells [4]. Therefore, it is reasonably believed that the antioxidant potential of a plant cell is directly linked to the amount of reduced metabolite supply [7]. There are several enzymes that help keep the NADPH balance of plants at a certain level. Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), decarboxylating-malate dehydrogenase (DMDH, EC 1.1.1.40), and isocitrate dehydrogenase (ICDH, EC 1.1.1.42) are the major enzymes.

The current subject has been conducted for the study of the defense system of maize seedlings grown under extreme conditions created by the individual and combinative effects of NaCl and Na_2SO_4 salt stress. It has been dedicated to the study of the activity of G6PDH, DMDH, and NADP-ISDH enzymes.

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2. Materials and Methods

As an object of the research, the Zagatala locally improved genotype of corn plant (Zea mays L.) belonging to C_4 plants group have been selected. Irrigation through the relevant salt solutions (NaCl and Na_2SO_4) has been carried out to create salt stress.

The activity of the NADPH-generating enzymes has been determined spectrophotometrically, at a wavelength of 340 nm, spectrophotometer in accordance with the rate of reduction of NADP in 1.0 ml spectrophotometric cuvettes ("MRC" (Israel)). The reaction has been done at 23-25 ºC, and the measurements have been repeated 5 times. Tissue: the extraction solution was taken in a ratio of 1g : 5 ml. composition [1, 2].

3. Results and discussion

Has been studied the individual and combined effects of NaCl and Na_2SO_4 salts at different concentrations on maize seedlings.

Taking into account the fact that the plants are more sensitive against the different extreme stress factors during the initial stages of their development [8]. The impact of stress on the development of seedlings has been observed on 4^{th}, 8^{th}, and 12^{th} days of the germination process. Impact on the developmental change of the root and stem system of maize genotypes has been given in Table 1.

Table 1. Impact of combined salt stress containing NaCl and Na_2SO_4 on the maze seedling

Days / Variants	Day 4	Day 8	Day 12
Control	root – 5.1±03	root – 7.4±03	root – 9.9±01
	stem – 3.2±02	stem – 4.8±02	stem – 7.3±01
NaCl 100 mM	root – 3.5±03	root – 5.1±01	root – 6.0±01
	stem – 2.9±01	stem – 3.8±02	stem – 4.5±02
NaCl 75 mM 25 mM Na_2SO_4	root – 3.3±02	root – 4.4±02	root – 5.2±01
	stem – 2.6±01	stem – 3.4 ±01	stem – 4.2±03
NaCl 50 mM 50 mM Na_2SO_4	root – 3.0± 01	root – 4.2±03	root – 5.0±02
	stem – 2.3±02	stem – 3.0±02	stem – 3.8±03
NaCl 25 mM 75 mM Na_2SO_4	root – 2.7 ±02	root – 3.7±01	root – 4.4±02
	stem – 2.0±03	stem – 2.6±01	stem – 3.1 ±01
Na_2SO_4 100 mM	root – 2.2±01	root – 3.1±01	root – 3.8±01
	stem – 1.7±02	stem – 2.3±02	stem – 2.7±02

As can be seen from the table (Table 1) both salts lead to an increase in stress. The gradual replacement of NaCl salt by Na_2SO_4 in the environment led to a further increase in this variation in proportion to the concentration of Na_2SO_4 salt in the environment

Impact of NaCl and Na_2SO_4 salts on NADPH - generating enzymes in maize seedlings. With regard to the development of the Zagatala locally improved maize genotype seedlings, the activity dynamics of NADPH - generating enzymes and results of the individual and combined impact of NaCl and Na_2SO_4 salt solutions on the course of this process have been illustrated in Table 2.

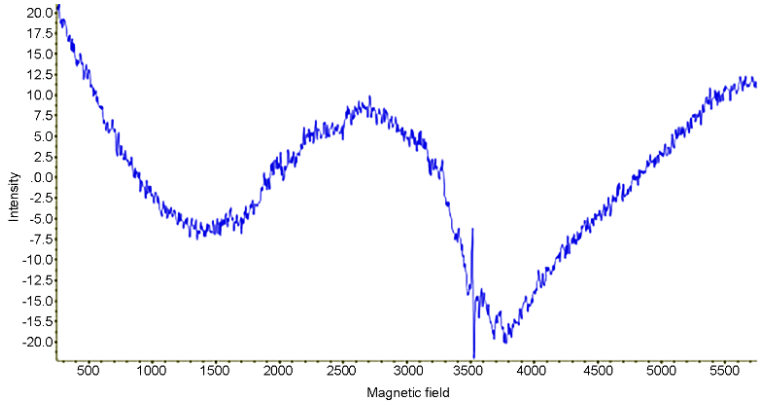
Table 2. Impact of combined NaCl and Na_2SO_4 salts on the activity of NADPH - generating enzymes in the maze seedlings

Variants	G6PD activity			NADP-Malic activity			NADP-ISD activity		
	day 4	day 8	day 12	day 4	day 8	day 12	day 4	day 8	day 12
Control	103.3	80.4	66.5	77.6	95.4	106.3	93.6	108.1	106.6
100 mM NaCl	110.1	99.8	76.2	88.7	117.3	123.4	96.8	105.5	108.3
50 mM NaCl + 50 mM Na_2SO_4	97.8	91.1	75.4	84.4	99.7	110.0	98.3	109.8	113.6
100 mM Na_2SO_4	92.3	77.1	67.8	80.3	96.2	99.6	105.7	121.1	127.9

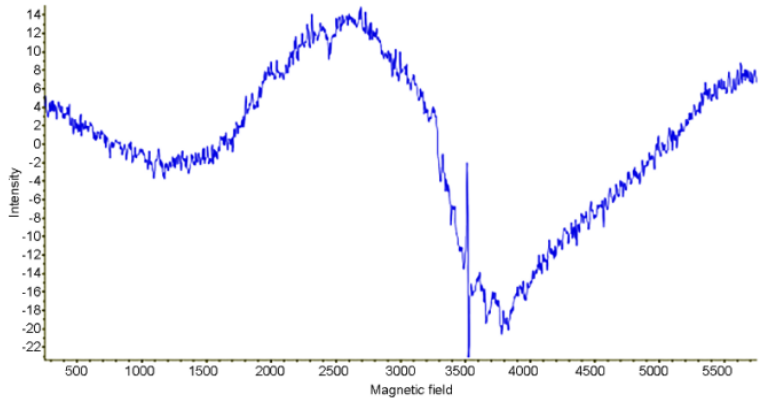
According to the results obtained, the increase in the concentration of Na_2SO_4 salt compared to NaCl salt in the solution has an extremely negative impact on the growth of both root and stem systems of seedlings. Increasing the rate of concentrations of both Na_2SO_4 and NaCl salts and their continuous effect has caused the plant stagnation. Under the salinity stress condition, it's been observed the induction of the activity of G6PDH enzyme in the early stages of the growth of the root system of seedlings, and the activity of DMDH enzyme in the relatively later stages. The activity of the NADP - dependent - ICDH enzyme has not been subjected to significant changes compared to other enzymes. The influence of Na_2SO_4 on the induction of the activity of all three enzymes has been higher than that of NaCl. Similar results have been made with respect to the tissues of the stem system, however, the effect of salts on the course of the process has been relatively low in this regard [1].

Study of Electron Paramagnetic Resonance (EPR) in Zagatala locally improved maize genotype seedlings.

In our research work, Electron Paramagnetic Resonance (EPR) has been analyzed under the control and NaCl conditions in order to study the paramagnetic centers in seedlings of different genotypes of maize plant. Paramagnetic centers in different genotypes of maize seedlings exposed to NaCl (100 mM) solution have been studied through the EPR method and comparatively analyzed with control samples (Fig. 1).



a) control



b) 100 mM NaCl

Fig. 1. EPR spectra in maze seedlings (control a) and 100 mM NaCl b))

As can be seen from the figure 1, the intensity of the free radical signal (g=2.0023) in the control sample is significantly lower than the intensity of the signal generated in the sample exposed to salt stress (a and b). At the same time, when we compare the broad EPR signals generated in both samples, it can be clear that the amplitude of the broad EPR signal in maize seedlings exposed to salt stress is higher than in the control sample (Fig. 1. a and b). The values obtained in this regard are in close agreement with those obtained for other species of C_3 and C_4 plants [6].

4. Conclusions

The research work studies the characteristics of the activity dynamic of enzymes with different dynamics depending on the nature of stress factors. Thus, it has been observed that the activity of NADP-G6PD and NADP - ME enzymes has mainly been used in the process of eliminating the impacts of NaCl salt, and the activity of NADP - ISD enzyme has been used in eliminating the complications of Na_2SO_4 salt. Also, in the initial phase of plant development, G6PD enzyme has scored a higher value, whereas, in the later stage, NADP - ME and NADP - ISD enzymes have gotten higher values. In order to study the paramagnetic centers in Zagatala locally improved genotype of maize seedlings, the Electron Paramagnetic Resonance (EPR) has been studied under the control and impact of NaCl conditions, and the amplitude of the broad EPR signal has been recorded to be higher in the variants exposed to stress compared to the control sample.

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