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CONTENT

EFFECT OF COMBINED SALT STRESS OF ALKALINE TYPE ON BIOLOGICAL INDICATORS, ANTIOXIDANT AND PROOXIDANT ENZYME SYSTEMS OF SOYBEAN SPROUTS

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Abstract. *The main goal of this research was to investigate the changes in the biological indicators, antioxidant and pro-antioxydant enzyme systems of 10-day-old soybean sprouts under stress conditions created by the equimolar concentration of $NaHCO_3$ + Na_2CO_3 salts. The results of the experiments revealed that the created stress has a serious negative effect on the development of the root and leaf systems of seedlings, including the amount of chlorophyll in the leaves. This effect begins to manifest itself at the weakest applied concentration and becomes stronger as the concentration of salts increases. At 150 mM concentration, the mass of the root system decreases by 3.5 times, and that of the leaves by about 2 times, compared to the control. A similar result is also observed in the dry weight of the root and leaf system of seedlings. The low concentration of salts (50 mM) induced the activity of antioxidant enzymes in the tissues of the root system of seedlings, while in the leaves it had a weak inhibitory effect on the activity of SOD and GR enzymes, and a stimulating effect on the activity of APX and CAT enzymes. Due to salt increase in the habitat, SOD and GP were weakened in both tissue cells, while in APX and CAT enzymes, this situation was observed only at the highest concentration of salt solution.*

Keywords: *alkaline salt stress, soybean sprouts, biological indicators, antioxidant and pro-antioxydant enzyme.*

INTRODUCTION

One of the most important environmental extreme factors for plants is salt stress caused by soil salinity [Munns, 2005]. According to estimates, 7% of the Earth's surface, and more than 20% of cultivated areas to one or another degree are affected by the salinity [Mammadov, 2007]. The main cations involved in salt stress in the soil are Na^+, Ca^{+2}, Mg^{+2} and K^+ ions, and anions are Cl^-, $SO4^{-2}$, HCO_3- and $CO_3^{\,2}$ ions (https://www.fao.org/3/x5871e/x5871e05.htm). Some of them create neutral (for example, NaCl and Na_2SO_4 salts), while others (for example, $NaHCO_3$ and Na_2CO_3) create alkaline-type salinity and corresponding salt stresses [Evgrashkina et all., 2020]. In order to eliminate the consequences of adverse environmental conditions, to adapt to such an environment and to complete their ontogenesis, plants have created a complex and multi-component defense system during the evolutionary process. The study and practical application of these systems can play an important role in the development of new agriculturally important varieties that are tolerant to salinity. Antioxidant [Kaya et all., 2019] and pro-antioxidant enzyme systems [Corpas, & Barroso, 2014] is one of the important components of plant defense system. Some of them by providing NADPH-oxidase with NADPH are involved in the formation of H_2O_2, which is an active metabolite of oxygen and induces a defense reaction in plants [Sagi et all., 2006;

Airaki et all., 2015], while others participate in the neutralization of excess of this substance, which causes damage to cellular components. Such important antioxidant enzymes include superoxide dismutase (SOD), which performs the dismutation (distribution) of electrons of the superoxide anion and creates H^2O^2, catalase (CAT), which serves to neutralize excess H^2O^2, and ascorbate peroxidase (APX) and glutathione reductase (GH), the main component of the glutathione-ascobate cycle, which requires the participation of NADPH, which also are involved in neutralization the excess of H^2O^2.

The main sitotoplazmatic NADPH-generating enzymes include glucose-6-phosphate dehydrogenase (G6PDH), malate dehydrogenase decarboxylating (MDHD) and NADP-dependent isocitrate dehydrogenase (NADP-ISDH) enzymes, which are sometimes considered as pro-antioxidant enzymes and are related to the defense system. They perform a dual function in plant life. The high-energy reducing metabolite, NADPH, synthesized by them is important for the growth and development of the cell, on the one hand, and performs the function of an antioxidant metabolite as a coenzyme in the defense system, on the other hand [Aliyeva et all., 2023].

Considering the fact that not one but several salts are involved in the creation of soil salinity in nature and a number of antioxidant and pro-antioxidant enzymes are involved in the defense against the stress caused by them and the importance of investigating the relationship between their activities, in the presented research the effect of combinative salt stress created by the equimolar concentration of alkaline ($NaHCO^3$ + Na^2CO^3) type salts on the biological parameters, on the activity of antioxidant (SOD, CAT, APX, GR) and pro-antioxidant (G6PDH, MDHD, NADP-ISD) enzymes of soybean seedlings was studied. As is well known, soybean (Glycine max. L), an annual grass plant belonging to the legume family, cultivated in many countries of the world, rich in protein, fat and carbohydrates as an agricultural plant present of strategic importance. At present, the attention to soy plant is increasing in our republic. It is cultivated in many regions of Azerbaijan and its cultivated area is gradually expanding.

MATERIALS AND METHODS

Soybean (Glycine max. L, var., Taleh) seeds supplied by Azerbaijan Agricultural Scientific Research Institute were disinfected with 0.2% sodium hypochlorite solution and washed with distilled water and cultivated in a laboratory fitatron (Plant Growth Chamber) at a temperature of 28/22 °C, 70 % humidity, 700 - 1000 μmol м-2•s-1 intensity illumination and 8/16 hours dark-light regime. 1 kg of soil was added to each pot and 5 soybean seeds were planted. Salt stress was applied to seedlings at the 3-leaf stage. In the control version, the seedlings were watered with 100 ml distillated water, and the experimental versions was supplied with the same volume of equimolar concentration of alkaline salts (NaHCO3 + Na2CO3) solution at 50, 100 and 150 mM a day. Experiments were studied in 5 replicates on 10-day-old seedlings. The dry and wet weight of the plants was measured by standard methods, and the amount of chlorophyll in the leaves was measured spectrophotometrically based on fluorescence. For this, 0.1 g of biological material was crushed in a mortar, dissolved in 10 ml of 95% acetone, centrifuged at 5000 g for 5 min. After spinning for 10 minutes, the amount of pigments in the supernatant was determined based on absorbance at 400, 645 and 663 nm using a SPECORD 250 plus spectrophotometer [Wellburn & Lichtenthaler 1984]. Superoxide dismutase activity was determined based on the methods proposed by Beaucham, Fridovic [Beaucham., Fridovic., 1971, catalase activity by Hadwan [Hadwan, 2018], and ascorbate peroxidase activity by Amako, Chen, Asada [Amako et al., 1994]. To determine the activity of NADPH-reducing enzymes, 1 g of root or stem tissues were washed, disinfected with 0,2% NaOCl solution, dried and crushed in 10 mM tris-HCI solution with pH of 7.5, containing 5 mM DDT, 10 mM MgCl, 1 mM EDTA, and 1% PVP. The homogenate passed through nylon tissue and after spinning for 10 min. at 10,000 x g the supernatant was taken and used as a cytoplasmic enzyme preparation. The activity of all three NADPH-generating enzymes was determined spectrophotometrically as described previously [Aliyeva et al., 2019].

RESULTS AND DISCUSSION

The main goal of the conducted research was to investigate the changes in the biological indicators, antioxidant and pro-antioxidant enzyme systems of 10-day-old soybean sprouts due to the equimolar combinative effect of $NaHCO_3$ + Na_2CO_3 salts.

From the results of the experiments, it was found that the stress created by the presence of NaHCO3 and Na_2CO_3 salts has a serious negative effect on the development of the root and leaf system of seedlings (Table 1). This effect begins to manifest itself at the weakest applied concentration, and as the concentration of salts increases, its inhibitory effect becomes stronger. Thus, at a concentration of 150 mM, the mass of the root system decreases by 3.5 times, and that of the leaves by about 2 times, compared to the control. A similar picture was also reflected in the dry weight of the root and leaf system of seedlings. The created alkaline-type stress conditions also have a sharp negative effect on the amount of chlorophyll in the leaf tissues. In this case, there was a direct correlation between the concentration of salts and their degree of inhibition of the amount of chlorophyll. The negative effect of the relatively high concentration of carbonate-type salt solutions on the biological indicators of plants and the amount of chlorophyll was also observed in cotton [Huijuan et al., 2019] and pumpkin plants [Yong-Dong Sun & Wei-Rong Luo, 2013].

Table 1.

Effects of NaHCO3+Na2CO3 salt solutions on biological indicators and chlorophyll content of soybean seedlings

Concentration $NaHCO_3$+Na_2CO_3	Wet weight (mg/plant)		Dry weight (mg/plant)		Chlorophyll (mq.d-1m-2)
	Root	Leaf	Root	Leaf	Leaf
Control	35±0.5	33±0.3	17±0.5	13±0.4	45±0.4
50 ml	29±0.4	26±0.3	14±0.5	9±0.2	25±0.5
100 ml	18±0.3	21±0.5	9±0.6	6±0.2	18±0.2
150 ml	10±0.3	17±0.4	5±0.2	4±0.11	8±0.5

Table 2 presents the results of the effect of alkaline type combinative stress on the activity of antioxidant enzymes such as SOD, GR, APX and CAT in the root and leaf tissues of soybean seedlings.

Table 2.

Effects of NaHCO3+Na2CO3 salt solutions on the activity of antioxidant enzymes in soybean sprouts

Concentration $NaHCO_3$+Na_2CO_3	SOD (U mg-1 protein)		GR (µg g-1 FW)		APX (µg g-1 FW)	CAT (µg g-1 FW)
	Root	Leaf	Root	Leaf	Root	Leaf
Control	6.2±0.4	7.1±0.5	3.8±0.4	2.05±0.4	28.8±1.2	1.9±2.4
50 ml	8.5±0.4	6.5±0.3	4.±0.3	1.95±0.6	29.9±1.3	2.9±2.3
100 ml	4.1±0.2	5.2±0.4	3.5±0.4	1.25±1.2	33.2±2.1	2.8±2.6
150 ml	3.2±0.3	4.7±0.3	1.7±0.2	1.05±0.8	21.8±2.2	1.5±2.4

The low concentration of salts (50 mM) induced the activity of antioxidant enzymes in the tissues of the root system of seedlings, while in the leaves it had a weak inhibitory effect on the activity of SOD and GR enzymes, and a stimulating effect on the activity of APX and CAT enzymes. Due to the increase in salt concentration, SOD and GR activity were weakened in both tissue cells, while in APX and CAT enzymes this situation was observed only at the highest concentration of salt solution. From the obtained results, it can be concluded that all of the investigated enzymes are involved to one degree or another in eliminating the consequences of mild alkaline (50 mM) type stress created by the equimolar concentration of $NaHCO_3$+Na_2CO_3 salts, and CAT enzyme is involved in the leaf tissues. The stress conditions created by the concentration of 100 mM salts are accompanied by the weakening of the activity of SOD and GR enzymes in both tissues, and the induction of the activity of APX in the root and CAT in the leaf. It seems that the process of neutralization of hydrogen peroxide in the stress conditions created by the presence of alkaline salts in soybean sprouts is mainly carried out by CAT and APX enzymes. Aggravation of stress conditions makes it difficult for all antioxidant enzymes to perform their functions.

The fact of activation of antioxidant enzymes in the leaves of mulberry plant under carbonate-type salt stress was observed by Parvaiz Ahmad et al., [2014], Zepeng Yin et al., [2019] in the leaves of Puccinellia tenuiflora halophyte. It was assumed by Huseynova and colleagues that under drought conditions created by the salinity factor in wheat removal of H_2O_2 is performed mainly by the CAT enzyme [Huseynova et al., 2015].

It is well known that the formation of reactive oxygen species and the processes of neutralizing their excess during activation of a protective reaction in plants under exposure to extreme environmental factors require the participation of the NADPH metabolite [Mammadov, 2012; Mirzoyeva & Mammadov., 2013]. G6PDH, DMDH and NADP-ISDH are among the main enzymes that provide NADPH to the cell [Corpas & Barroso, 2014; Aliyeva et al., 2019]. The following table 3 shows changes in the activity of the cytoplasmic forms of these enzymes in the tissues of the root and leaves of 10-day-old soybean seedlings under the influence of $NaHCO_3$ + Na_2CO_3 salts stress.

Table 3.

Effects of NaHCO3+Na2CO3 salt solutions on the activity of NADPH-producing enzymes in soybean sprouts

Concentration $NaHCO_3$+Na_2CO_3	G6PDH (μM•min^{-1}• g^{-1} FW)		DMDH (μM•min^{-1}• g^{-1} FW)		NADP-İSDH (μM•min^{-1}• g^{-1} FW)	
	Root	Leaf	Root	Leaf	Root	Leaf
Control	4.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	4.6 ± 0.2	2.3 ± 0.1	2.0 ± 0.1
50 mM	5.2 ± 0.2	4.1 ± 0.2	4.3 ± 0.2	6.2 ± 0.2	2.7 ± 0.1	2.7 ± 0.1
100 mM	5.5 ± 0.2	4.4 ± 0.1	4.1 ± 0.2	5.1 ± 0.3	3.2 ± 0.1	3.3 ± 0.1
150 mM	2.2 ± 0.1	2.1 ± 0.1	1.6 ± 0.1	3.2 ± 0.2	2.1 ± 0.1	1.5 ± 0.1

At the stress conditions created by 50-100 mM the activity of all three enzymes increases in both root and leaf tissues, while at a relatively high salt concentration (150 mM) it sharply decreases (Table 3). Such changes in enzyme activity, observed under mild and moderate stress conditions was apparently due to the active participation of all three enzymes in providing the cell with NADPH for the protective process. Acute stress conditions are associated with difficulties in their functioning. This can be explained by profound changes in cellular metabolism (ion toxicity, increased pH, etc.).

CONCLUSION

The obtained results allow us to say that all the studied pro-antioxydant enzymes (G6P-DH, MDHD and NADP-ISDH) are involved to one or another degree in eliminating the consequences of the created alkaline stress. The aggravation of stress conditions seems to lead to the difficulty of the antioxidant and pro-antioxidant enzyme sistems to perform their physiological function.

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