The Role of Enzymes in Wheat Plant Adaptation to Soil Salinity

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Geliş Tarihi / Received: 01.09.2022
Kabul Tarihi / Accepted: 24.09.2022
Araştırma Makalesi/Research Article
DOI: 10.5281/zenodo.7130745

ABSTRACT

The global climate changes occurring in the modern world have led to the aggravation of the ecological situation on earth, the development of stress factors such as drought and salinity, and the destruction of a number of valuable plant species, which may lead to serious difficulties in meeting people's demand for food products in the future. Therefore, the creation of new plant varieties and forms that are more productive and resistant to stress factors by using plant genotypes that are resistant to various stresses, including drought and salinity, that can be cultivated in unfavorable soil and climate conditions, is one of the urgent problems ahead.

Keywords: wheat, stress, adaptation, abiotic factor, climate.

INTRODUCTION

Stress factors negatively affect the life activity of living organisms, especially plants, limiting their development and reducing their productivity. In this regard, the study of the effect of stress factors on plants and the discovery of adaptation mechanisms play an important role in the regulation of stress in the cell. From a biological point of view, stress is considered as any change in external environmental conditions that weakens the normal development of the plant or changes it in a negative direction. Biotic (pathogenic, competition with other organisms, etc.) and abiotic (drought, salinity, radiation, high and low temperature, etc.) stresses cause changes in the physiological activity of plants, weaken the process of biosynthesis in the cell, disrupt normal life activities, and ultimately can cause the death of plants. [3 In the classification of currently used land areas according to stress factors, drought, which is a natural stress factor, covers more than 26% of the area. This is followed by salinity stress at 20% and cold or frost stress at 15%. Other stresses make up 29%. Only 10% of the area is not affected by any stress. [1,2]

Drought stress, being one of the most common environmental factors affecting growth and productivity, induces many physiological, biochemical and molecular responses in plants, and plants develop tolerance mechanisms to adapt to adverse environmental conditions. The study of these mechanisms is of great theoretical and experimental importance in the creation of plant varieties and forms resistant to adverse environmental factors. The defense response of plants against salinity is complex and related to many biological processes. The negative effect of salt stress manifests itself in all stages of plant development, including germination, seedling, vegetative and generative development stages. On the other hand, salt tolerance varies in different plants during the developmental stages of plants. For example, it was determined that depending on the stages of development of rice, barley, and wheat [6], their degree of salt tolerance differs.

Slowing down of root growth during salt stress can also be caused by the toxic effect of salts on metabolism. Neves and colleagues found that salt stress thickens the cell wall of root cells, leading to its lignification, which inhibits root growth [5]. During salt stress, the diameter of the roots decreased, while in some plants it increased. Experiments conducted with a number of plants, including desert halophytes, Cnyodon dactylos, Oryza sativa, Triticum spp., Prosopis tamarugo tree sprouts, showed that the diameter of the roots decreases with increasing salinity [10, 7]. However, in Hordeum spp., Gossypium hirsutum, Citrus volkameriana, and Tessaria absinthioides plants, an increase in root diameter was observed as salinity increased [9]. From the experiments conducted with the leaves of

the plants, it was clear that the amount of dry matter in the radish (Raphanus sativus) exposed to salt stress decreases, and 80% of the decrease is due to the decrease in the surface area of the leaves, and the remaining 20% is due to the decrease in the number of stomata, disruption of gas exchange [8] Other It was clear from one of the experiments that the density of the nozzles on the leaves of the tomato plant (Lycopersicon esculentum) decreases as a result of the effect of salt. At the same time, the number of leaves, height, length of the root and its coverage decrease [11]. İn plants growing in highly saline soils, even the leaves change color, becoming grayish and bluish (most likely due to pigment changes). During salt stress, the intercellular apoplast space in the leaves of plants is gradually narrowed and the epidermal and mesophyll layers are thickened and the cells are elongated [11]. The division and growth of cells in the leaves, roots and stems of plants planted in saline soils slows down, therefore the development of plants is weakened, the growth rate of the leaf surface decreases and, as a result, the intensity of photosynthesis decreases. Continued stress can completely stop the development and growth of plants. In addition to reducing plant growth, salt stress causes chlorosis, necrotic spots, yield and quality reduction [14].

As already mentioned, the adaptation of plants to the environment, including unfavorable environmental conditions, is accompanied by changes in metabolism, and NADPH is required for the implementation of these changes. There are four known enzymes (Q6PDH, 6PQDH, ISDH, and DMDH) that generate NADPH money in the cell, the main ones being Q6PDH and DMDH [12, 15]. Re garding their involvement in salt stress, Q6PDH is relatively studied, and DMDH is poorly studied. The analysis of literature data shows that the Q6PDH enzyme plays an important role in the adaptation of various plants to unfavorable environmental conditions, in the elimination of the effects of biotic (in the fight against various pathogens) and abiotic (in response to drought, extreme temperature conditions, salinity, etc.) stress [13, 16, 19]. Due to the influence of biotic and abiotic stress factors, the amount of active metabolites of oxygen in the cell increases sharply. Q6PDH is involved both in the formation of such metabolites and in eliminating their harmful effects. The localization of the DMDH enzyme in plant tissues almost coincides with the localization of the Q6PDH enzyme. Considering that the main product of both enzymes is NADPH, it can be assumed that it also participates in the functions specific to the Q6PDH enzyme related to this metabolite [17].

The involvement of the DMDH enzyme in the defense response of plants against extreme environmental factors and its role in adaptation to such unfavorable conditions is relatively poorly studied compared to the Q6PDH enzyme. However, some information about this problem can be found in the literature. Perhaps it would be worthwhile to consider some of them.

MATERIAL AND METHODS

The wheat plant, a representative of the monocotyledon class, was chosen as the research object. As is well known, wheat and barley plants themselves are relatively salt resistant, while beans and peas are salt sensitive. Experiments were carried out on sprouts of wheat seeds. For the first time, the activity dynamics of Q6PDH and DMDH enzymes were comparatively studied under salinity stress conditions created by using different concentrations of NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 salt solutions, information was obtained about the participation of both enzymes in eliminating the effect of salinity stress, and the enzymes are involved in this process. it was shown that the degree of formation depends on the anion content of salts, their concentration, the type of plants and their development stage. One of the main points in determining the activity of enzymes is the correct selection of the extraction medium. For this purpose, our experiments used 0.1 M tris-HCI buffer solution containing 0.01 M β-mercaptoethanol as a reducing agent and 1% polyvinylpyrrolidone (mol mass 24 kDa) to neutralize phenolic compounds as an extraction solution.

Based on literature data, buffer solutions with a pH value of 8.0 were taken for the extraction of the Q6PDH enzyme, and buffer solutions with a pH value of 7.0 were taken for the extraction of the DMDH enzyme. In preparing the homogenate, 2 ml of extraction solution was taken for 1 g of biological object and crushed in a mortar using an ice bath in a cold environment. In both cases, the

obtained homogenate was filtered through a double kapron tissue and the obtained filtrate was spun in a centrifuge at a speed of 5,000 revolutions/min for 10 minutes, the supernatant part was taken and used to determine the activity. Enzyme preparations prepared by this method had stable activity in a cold environment for several hours and did not cause difficulties in carrying out measurements.

The activity of both enzymes was determined by the spectrophotometric method, at a wavelength of 340 nm, based on the rate of reduction of NADP. ΔE103340/min/g /min/g wet weight was taken as enzyme unit.

Before addition to the incubation medium, malate was neutralized with K_2CO_3 salt. Instead of malate solution, distilled water was added to the control tub. In this case, the NADP solution was used to start the reaction, and the activity of the enzyme was determined at 25 o C. measurements were repeated 3-5 times.

The obtained results were processed statistically the accuracy indicator was less than 5%.

As a result of the conducted research, it was determined that different concentrations of NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 salts in wheat sprouts have different effects on the activity dynamics of the cytoplasmic DMDH enzyme of the root system. At relatively low concentrations of salts, the activity of the enzyme is significantly induced, and at high concentrations, on the contrary, it is inhibited. Underlying the implementation of these two different effects are apparently different mechanisms. Referring to literature data, it can be concluded that the induction of DMDH enzyme activity during salinity stress is related to the defense reaction of roots and is carried out at the level of enzyme expression (18). The effect at high concentrations of salts is probably non-specific and is related to the direct effect of salts on the catalytic activity of the enzyme.

The activity of the DMDH enzyme of the root system of the wheat sprouts of the control variant does not actually change significantly during the 24-hour incubation period. Apparently, such a noticeable stability in the activity dynamics of the enzyme is related to the nature of the demand for its catalytic activity during this period of wheat germ development.

Despite the fact that the stress created by the tested salts led to the stimulation of DMDH enzyme activity in all cases, the observed changes in its dynamics had a different character depending on the type of salt used to create the stress state, its concentration, and the exposure time. At relatively low concentrations of NaCl salt (25 and 50 mM), there is a direct relationship between the degree of stimulation of enzyme activity and the concentration of salt and the duration of exposure. For this reason, maximum stimulation of DMDH enzyme activity is observed 24 hours after the beginning of incubation at 50 mM concentration of NaCl salt. During this period, the activity of the enzyme is 37.9% higher than the activity during the same period in the control variant.

Increasing the concentration of NaCl salt leads to shortening of the time required for maximal stimulation of DMDH enzyme activity on the one hand, and on the other hand to weakening of the activity stimulation effect. At a high concentration of salt, the activity of the enzyme is inhibited. For example, at concentrations of NaCl salt of 75 and 100 mM, the activity of the enzyme reaches its maximum limit not after 24 hours, but only after 12 hours. In the following periods, the stimulation effect weakens and after 24 hours, the activity in the variant of 75 mM concentration is only 10.6% higher than the similar activity in the control, and the activity in the stress created at 100 mM concentration is 12.1% lower than the activity in the control. It seems that eliminating the stress situation caused by low concentrations of NaCl salt requires the intensification of the DMDH enzyme of the root system of wheat sprouts, while at high concentrations the normal functioning of the enzyme itself becomes a problem.

If certain minor nuances are not taken into account, it can be said that the nature of the dynamics of DMDH enzyme activity changes during the stress created by Na_2CO_3 salt is similar to the effect in the NaCl salt variant. Among the tested salts, $NaHCO_3$ salt had the strongest inducing effect on enzyme activity. In the range of 25-50 mM concentration, its stimulating effect changed directly proportionally depending on the time and concentration factor, and the maximum effect was observed

after 24 hours of incubation at 50 mM concentration. As in all variants, in this case, high concentrations of salt had an inhibitory effect on DMDH enzyme activity.

RESULT AND DISCUSSION

Thus, NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 salt solutions have a negative effect on the germination of wheat plant seeds. Salts that have a negative impact on the process can be placed in the order $NaCl<Na_2SO_4<NaHCO_3\leq Na_2CO_3$.

The effect of NaCl, Na2SO4, NaHCO3 and Na2CO3 salt solutions on the growth dynamics of wheat sprouts was studied and it was shown that there is a positive relationship between the concentration and exposure time of the salt solutions and their negative effect on this process. Due to the negative effect on the growth dynamics of sprouts, salts can be arranged in the order $NaCl<Na_2SO_4<NaHCO_3\leq Na_2CO_3$. Mild stress conditions created by salt solutions mainly induce Q6PDH, and relatively severe stress conditions lead to the induction of DMDH enzyme.

CONCLUSION

As a result of the research conducted in this way, it was determined that wheat sprouts of different concentrations of NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 salts have different effects on the activity dynamics of the cytoplasmic DMDH enzyme of the root system. At relatively low concentrations of salts, the activity of the enzyme is significantly induced, and at high concentrations, on the contrary, it is inhibited. Underlying the implementation of these two different effects are apparently different mechanisms. Referring to literature data, it can be concluded that the induction of DMDH enzyme activity during salinity stress is related to the defense reaction of roots and is carried out at the level of enzyme expression (18). The effect at high concentrations of salts is probably non-specific and is related to the direct effect of salts on the catalytic activity of the enzyme.

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