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Infuence of short-period incubation of barley sprouts at Na-isocationied soil solutions to the activity of DMDH ferment

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Abstract
Recently, various approaches have been used to study salinity stress and its effects on plant metabolism, including proteomics, genomics, micromixes, transcriptomics, ionomics, metallomics, and others. methods such as. In fact, these methods have become a powerful tool in understanding the mechanism of plants' reactions to salinity stress. The study of genes and proteins involved in the regulation of various environmental stresses may be important in the development of high-yielding plant varieties in saline conditions. With the help of a metabolic approach, changes in the metabolism of plants under stress can be clarified. Plants receive various external and internal signals and use them to create the response necessary for their development.

Keywords: salt solutions, barley sprouts, DMDH, activity, stress factor

1. Introduction

Plants use two main adaptive mechanisms to create tolerance to high salinity. The first is the use of various physical and physiological barriers to prevent the entry of salts into the cell, and the second is the strengthening of the internal adaptive mechanisms that enable survival. Due to the first mechanism, the passage and accumulation of salts inside the cell is limited, especially in the photosynthetic apparatus and cytoplasm [1, 18].

For example, the regulation of Na ion absorption and its transport through the plasma membrane and tonoplast is one of the main mechanisms of the protective reaction of plants against salinity [2, 6, 7].

Prevention of salt absorption can be achieved due to the antagonism between the absorption of metal ions. This mechanism is an effective and complex way of restricting (weakening) the passage of large amounts of some ions, especially Na ions, into the plant tissue through the root. Due to it, the absorption of salts is weakened and their accumulation in the upper (upper) organs of plants, especially in the transpiration organs, is prevented. Many glycophytes skillfully use this strategy to isolate the absorption of Na ions in the root region (level) by maintaining relatively high levels of K ions [3, 11, 19].

As already mentioned, the adaptation of plants to the environment, including adverse environmental conditions, is accompanied by changes in metabolism, and the implementation of these changes requires NADPH. There are four known enzymes (Q6PDH, 6PQDH, ISDH and DMDH) that produce NADPH money in the cell, the main of which are Q6PDH and DMDH enzymes [15, 23, 22]. In terms of their involvement in salt stress, the most studied of these two enzymes is Q6PDH, and the least studied is DMDH.

The enzyme DMDH is the main enzyme in the metabolism of malate (malic acid), which is the second most common metabolite in the living world [8, 17]. This acid is mainly synthesized from glucose, and its main localization in plants is the vacuole. Malate can easily pass from the tonoplast to the cytoplasm and, if necessary, is converted to pyruvate by decarboxylating oxidation due to the catalytic action of the enzyme DMDH. During the reaction, NADPH is formed and CO2 is released. All the resulting products are considered important compounds for the plant cell. Pyruvate can be used for various purposes as a central metabolite, increasing the intensity of CO2 photosynthesis in C-4 plants [25, 20].

The main compound synthesized by the enzyme DMDH is NADPH. It is a high-energy metabolite that acts as a universal electron donor in the cell. For a long time, the scientific view of it was due to its important role in the implementation of biosynthetic reactions [24]. However, views on DMDH and the role of NADPH, which it synthesizes, have recently expanded. It is believed that cell detoxification and antioxidants are one of the important components in the functioning of the immune system.

The localization of the DMDH enzyme in plant tissues almost coincides with the localization of the Q6PDH enzyme. Given that the main product of both enzymes is NADPH, it can be assumed that it also participates in the functions specific to this metabolite, the enzyme Q6PDH [21].

The role of the DMDH enzyme in the protective response of plants to extreme environmental factors and its role in adapting to such adverse conditions has been relatively poorly studied compared to the Q6PDH enzyme. However, there is some information in the literature on this problem. It would probably be appropriate to consider some of them.

Fu and colleagues studied the activity of the enzyme DMDH under abiotic stress in cereals (Triticum aestivum L.) and showed that it played an important role in this process. They play an important role in neutralizing stress caused by NaCl, NaHCO3, Na2CO3 salts, polyethylene glycol (20% PEG 6000, after 72 hours of exposure), water deficit (osmotic stress), changes in pH, as well as the negative effects of stress phytonutrients. They observed an increase in the activity of the enzyme DMDH in cereals by up to 50% as a

result of the action of ABT and salicylic acid, and, consequently, the induction of its protein synthesis and gene transcription [12] Similar experiments rice plant (Oryza sativa) It was found that salt stress increases the activity of its DMDH enzyme and gene expression [6]. (Hordeum vulgare) had higher levels of DMDH activity than the drought-susceptible genotype, and that this phenomenon was based on the resistance of the resistant variety to adverse environmental factors [13]. Yu-Xin Yao, and others show that the enzyme plays an important role in the resistance of the apple plant (Pyrus domestica L.) to cold and salt.

Although the problem of the participation of the enzyme DMDH in the protective reaction against extreme environmental factors has not been studied extensively and comprehensively, the above-mentioned research suggests that it is involved in this process in plants. The analysis of the literature also shows that research on this problem is limited, both in terms of the biological object and the amount of research. The comparative study of DMDH and Q6PDH enzymes in the protective response to extreme environmental factors, including salt stress, has been largely overlooked. I think that conducting research in this form can be important in creating a correct idea of the role of each enzyme in different types of stress factors.

2. Materials and Methods

Barley (Karabakh-2), a representative of the monocotyledonous class, was selected as the object of research. As it is known, barley is characterized by its relative salt resistance. The experiments were performed on the seeds of these plants.

Barley (Hordeum), like the grain plant, is a monoecious grain, a grass plant belonging to the genus of lizards, which has been domesticated and cultivated by humans since ancient times. There are 30 species in nature, of which 3 are cultural and 27 are wild. The most widespread are varieties of common barley (Hordeum vulgaris). Currently, there are many varieties of this species used for various purposes. Among cereals, barley is the second most widely cultivated crop in the world, after corn (Zea mays), rice (Oryza sativa) and grain (Triticum).

The barley plant reaches a height of 1.25 meters and grows quickly after a short growing season. The leaves are ribbon 0.5-2 cm wide. The ears of the leaf are very large and overlap. At each step of the spike axis, three spikes are formed. The flower group is a spike. The spike is a flower. The flower has 3 male stamens. Spike scales are small, narrow-lanceolate. Flower scales stick together. At the end of the outer flower scales, a fissure emerges. It is self-pollinating. The vegetation period of autumn barley is 280-300 days, and that of spring barley is 55-110 days.

Barley seeds begin to germinate at a temperature of 1-2 ^{0} S. The optimum temperature for germination is 20-22 ^{0}S. Barley seedlings tolerate -8 ^{0} S frost. Seeds absorb more than 48-50% of their weight for germination. Plants tolerate -14-16 ^{0} S frost. It is recommended to reach 34-36 ^{0} S during the flowering period. Autumn barley is a long-lived plant. Holds the stage of yarovization in a relatively short time. It starts to sprout 15-18 days after germination. When the seed germinates, it forms 5-8 embryos.

One of the key points in determining the activity of enzymes is the correct selection of the extraction medium. For this purpose, in our experiments, a 0.1 M tris-HCl buffer solution containing 1% polyvinylpyrrolidone (molecular weight 24 kDa) dissolved to neutralize 0.01 M β-mercaptoethanol and phenolic compounds as a reducing agent was used as the extraction solution. Based on the literature, buffer solutions with a pH value of 7.0 were taken for the extraction of the DMDH enzyme. In the preparation of the homogenate, 2 ml of extraction solution was taken for 1 g of biological object and crushed in a cold medium using an ice bath in a mortar. In both cases, after the homogenate was filtered through double kapron tissue, the filtrate was centrifuged at 5,000 rpm for 10 min, the supernatant fraction was removed and used to determine the activity. Enzyme preparations prepared by this method had a stable activity in a cold environment for several hours and did not cause difficulties in making measurements.

The activity of the enzyme was determined spectrophotometrically at a wavelength of 340 nm, based on the rate of reduction of NADP. E103340 / min / g / min / g wet weight was taken as the enzyme unit.

The following incubation medium was used to determine the activity of the DMDH enzyme:

Tris-HCl buffer - 7.0- 0.1 M,
MnSO4 - 10 mM,
Malat - 3 mM,
NADP - 0.1 mM,
Enzyme preparation - 0.25 ml.

Before being added to the incubation medium, the malate was neutralized with K2CO3 salt. Distilled water was added to the control tub instead of malate solution. In this case, NADP solution was used to initiate the reaction, and the enzyme activity was determined at 25 ° S. measurements were repeated 3-5 times.

The obtained results were statistically processed, the accuracy was less than 5%. Exceptions are not included in the results in order not to overload the tables.

3. Results and Discussion

Table 1 below shows the effect of different concentrations of NaCl, Na2SO4, NaHCO3 and Na2CO3 salts on the dynamics of cytoplasmic DMDH enzyme activity of barley seed roots over 24 hours.

As can be seen from the figures in the table, there is virtually no significant change in the DMDH enzyme activity of the control variant during the 24-hour incubation period. Apparently, this stability is due to the nature of the demand for its catalytic activity in the early stages of development of barley seedlings.

Although salinity stress in all cases stimulates the activity of the enzyme DMDH, the changes observed in its dynamics are different depending on the type, concentration and duration of exposure to the salt used to create the stress state. At relatively low concentrations of NaCl salt (25 and 50 mM) there is a direct correlation between the degree of stimulation of the enzyme activity and the salt concentration and duration of action. The maximum stimulation of the enzyme activity manifests itself 24 hours after incubation at a concentration of 50 mM of NaCl salt. During this period, its activity is 44.7% higher than the similar activity in the control variant.

An increase in the concentration of NaCl salt On the one hand, an increase in the concentration of NaCl salt leads to a shortening of the time required for maximum stimulation of the enzyme activity, on the one hand, and a weakening of the stimulating effect of the activity on the other.

Table 1: Infuence of short-period incubation of barley sprouts at Na-isocationied soil solutions to the activity of DMDH ferment

Variant	Incubation period (hours)			
	6	12	18	24
Control	75±1.5	77±1.6	76±1.8	76±1.2
NaCl				
25 mM	78±1.3	90±1.9	96±1.3	98±1.7
50 mM	84±1.5	97±1.7	107±1.5	110±1.1
75 mM	88±1.6	108±1.5	99±1.7	87±1.4
100 mM	91±1.4	105±1.8	85±1.5	70±1.6
Na_2SO_4				
25 mM	77±1.3	96±1.5	102±1.5	86±1.8
50 mM	82±1.5	88±1.4	90±1.3	73±1.3
75 mM	86±1.7	90±1.2	85±1.5	64±1.7
100 mM	84±0.9	86±1.5	60±1.8	30±1.4
$NaHCO_3$				
25 mM	85±1.6	105±1.5	122±1.5	116±1.5
50 mM	89±1.4	114±1.6	139±1.6	151±1.7
75 mM	97±1.15	116±1.4	125±1.4	123±1.5
100 mM	104±1.6	96±1.6	73±1.6	59±1.7
Na_2CO_3				
25 mM	80±1.3	92±1.2	96±1.3	100±1.8
50 mM	85±1.5	104±1.3	113±1.5	126±1.3
75 mM	91±1.4	108±1.4	97±1.6	91±1.5
100 mM	96±1.9	88±1.1	76±1.4	68±1.5

At high concentrations of salt, the activity of the enzyme is inhibited. It seems that overcoming the stress situation at low concentrations of NaCl salt requires the intensification of the DMDH enzyme in the root system of barley seedlings, while at high concentrations the normal functioning of the enzyme itself is disrupted.

The nature of the stress caused by the short-term effects of the Na2SO4 salt on the dynamics of the DMDH enzyme activity is significantly different from that of the NaCl salt. The time required for maximum stimulation of the enzyme's activity under the influence of Na2SO4 salt is shorter than that of NaCl salt, but its stimulating effect is weaker than that of NaCl salt. This emphasizes that the inhibitory effect of Na2SO4 salt is stronger than that of NaCl salt.

It is not known what these differences are due to. In fact, these differences are due to the absorption of salts by the cells of barley sprouts, the expression and translation of the gene encoding the enzyme protein, the direct effect of salts on the enzyme molecule, and so on. can occur at the level of. The effect of stress generated by the Na2CO3 salt on the activity dynamics of the DMDH enzyme is similar to that of the NaCl salt variant.

Among salts, NaHCO3 salt has the strongest effect on the activity of the enzyme. At a concentration of 25-50 mM, its stimulating effect varied in direct proportion to time and viscosity, and the maximum effect was observed after a 24-hour incubation period at 50 mM. As in all variants, in this case, the high concentrations of salt had an inhibitory effect on the activity of the enzyme DMDH.

4. Conclusion

Thus, different concentrations of NaCl, Na2SO4, NaHCO3 and Na2CO3 salts have different effects on the dynamics of cytoplasmic DMDH enzyme activity of barley seedlings root system even during short-term incubation. At relatively low concentrations of salts, the activity of the enzyme increases significantly, while at higher concentrations, on the contrary, it is inhibited. It seems that the induction of the activity of the enzyme DMDH during salinity stress is associated with the protective reaction of seedlings and is carried out at the level of enzyme expression. The effect of high salinity concentrations is probably non-specific and is due to the direct effect of salts on the catalytic activity of the enzyme.

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