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ACTIVITY OF ANTIOXIDANT SYSTEM IN DROUGHT AND SALINITY CONDITIONS

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ABSTRACT

The effect of antioxidant system on the effect of drought and salt stress on the Shirvan lucerne variety and Chokhyarpag accessions (*Medicago sativa L.*) has been studied. The antioxidant defense system consists of antioxidant enzymes and small molecular organic compounds. The activity of guaiacol peroxidase and the amount of proline were determined in the affected plants. The conception about antioxidant enzyme system activity in Shirvan variety and small-molecular antioxidant system activity in Çokhyarpag accesion have been discussed.

KEYWORDS: Lucerne, stress, drought, proline, peroxidase.

INTRODUCTION

The majority of all flesh on Earth cannot live without oxygen. Oxygen oxidize the nutrients and plays a key role in the energy of the cell. Molecular oxygen is non-toxic to the cell, but its incomplete oxidized derivatives - active forms of oxygen (AFO): superoxide radical, hydrogen peroxide, singlet oxygen, hydroxyl radical are dangerous. In normal conditions, AFO constantly is formed in the cell, and this is a normal metabolic process. They are involved in defensive reactions, such as pathogens, as well βas second mediators in transmission of signals. However, in unfavorable conditions occurs extreme collection of AFO (drought, salinity, high and low temperatures, heavy metals, etc.). This can lead to serious functional impairments. Thus, the destruction of biological membranes, photosynthetic apparatus activity, ingestion of the cell division, modification of nucleic acids, and so on. may occur.

Defense from AFO harmful effects is carried out due to the antioxidant system's work by enzymatic and non-enzymatic methods. Antioxidant protection system consists of antioxidant

enzymes (superoxidismutase, catalase, peroxidase), adaptive small-molecular organic compounds called osmoliths (proline, polyamines, carotenoids, etc.) [Y.G.Sherudilo et al., 2013]. The accumulation of adaptive osmolites in the cell is associated with the activation of hydrolytic enzymes and the slowing down of the stream out of the cell. Adaptive osmolytes reduce water potential, reduce water potential, restore water supply, protects enzymes from inactivation, ensures integrity of structural proteins, and maintains functional activity of cell membrane [V.V.Polevoy, 1989; O.S. Sakariyavo et al., 2001].

Photodynamic, β-oxidation of bytiric acids, H_2O_2 produced during oxidation of polyamines are essential for many metabolic reactions. However, the excess amount of H2O2 produced in stress conditions creates irreversible changes in the structure of biomolecules. H2O2-detoxifying enzymes in plants are catalase and peroxidase family: guaiacol peroxidase, ascorbate-peroxidase, glutathione peroxidase. Guaiacol peroxidase catalyzes the oxidation of a number of aromatic compounds by using hydrogen peroxide and participates in protecting the cell from oxidative stress [T.N.Soshinkova et al., 2013]. The purpose of this study was to study the effectiveness of antioxidant system in lucerne plants by the effects of stress factors.

MATERIAL AND METHODS

In the study, Shirvan (*Medicago sativa* L.) variety and Chokhyarpag accession were used. After the plants, taken from the experimental area were subjected to drought and salt stress (11% sucrose and 11% NaCl solutions) within 24 hours in laboratory conditions, the amount of proline was determined by Bates et al. al (1973) method [L.S.Bates et al., 1973].

The spectrophotometric determination of the peroxidase activity is based on the measurement of the optical density of products produced during the oxidation reaction of guaiacol. 200-500 mg leaves are pressed in small quantities (5-10 ml) with phosphate buffer (pH 5,4) in porcelain mortar and centrifuged at a speed of 4000-5000 rpm for 10 minutes. The optical density of the reaction mixture consisting of 0.5 ml substrate (guaiacol), 1.5 ml of phosphate buffer, 0.5 ml supematant (fermentative plant material), 0,5 ml H2O2 1 min. was measured at 470 nm wavelength in spectrophotometer (UV-3100 PC) [A.I.Yermakov et al.,1987].

RESULTS AND DISCUSSION

Determination of the proline content and the activity of guinea-peroxidase in plants stressful revealed a difference between control and experimental plants (Figure 1). In the Shirvan variety accessions were also significantly increased (for 1,2 times) guaiacol-peroxidase

activity during drought and salinity. Proline aminic acid content increased twicely in drought, 1.7 times in salinity conditions.

If we take into consideration that the indicators for the control are more than 100 percent, the idea about activation of defense systems at the level of both fermentative and small molecule antioxidant compounds can be discussed.

Proline has been found to be involved in antioxidant defence against oxidative stress [N.L.Radyukina, 2015]. According to Sochinkova and colleagues, in stress conditions, in leaves of *Th.salsiginea*, exogenous proline is involved in the protection of complete plant and *in vitro* cells (eg UB-B rays, oxidative stress caused by H_2O_2) and *in vitro* cell culture. Thus, with the addition of exogenous proline, the activity of antioxidant enzymes such as superoxide dismutase and guaiacol-peroxidase has decreased.

This was considered as the role of "trap" for the proliferation of the AFO. [T.N.Soshinkova et al., 2013].

In our experiments, in Chokhyarpag lucerne accession, the activity of guaiacol peroxidase was decreased by 60.6% (by 1.4 times) and in salinity conditions by 93% (min.diminution). According to control, the amount of proline increased about 3 times in drought and 2 times in salinity condition. Indicators show that small-molecular defense system (proline) has become more active in Chokhyarpag accession. Although the activity of the fermentative antioxidant system has diminished in drought, it has been very close to control in salinity conditions.

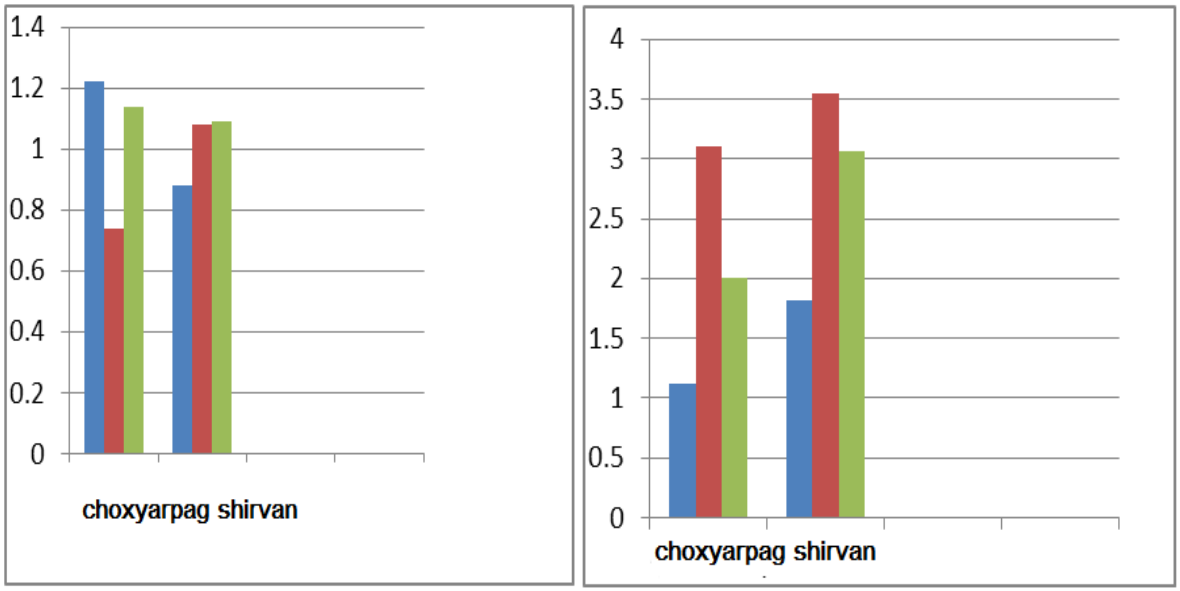
According to literature data, the observed decrease in the activity of peroxidase in the drought because of the enzyme is used and inactivated quickly by H_20_2 in the cell. The synthesis of new enzymes requires some time for gene expression. It is believed that in some cases, small-molecular antioxidants present in the cell, are more effective than the fermentative protection system [N.L.Radyukina, 2015].

During the salinity stress, it is indicated that peroxidase activity is more in resistant varieties, but it is persistent or unchanged in sensitive accessions [Y.F.Khu, et al., 2008; Yaşar et al., 2008]. According to many experimental data, the change in the activity of antioxidant enzymes depends on the dose, duration of the stress factor, the type of plant used in the study, and the initial activity level of the enzymes. However, the effectiveness of antioxidant enzymes is not always effective for the detoxification of large quantity of active forms of

oxygen, which was accumulated during stress management. During stress, active radicals can inactivate enzymes rapidly, and induction of their transcriptional genes takes short time [O.Blokhina et al., 2003].

In our study, the activity of guaiacol-peroxidase was decreased during the drought and salinity stresses in the Chokhyarpag accessions. Particularly, this indicator has dropped significantly in drought condition. Apparently, small-molecular antioxidant compounds have been more active in this lucerne accession and have been closely involved in the extinguishing of hydrogen peroxide and AFOs.

Thus, based on the numerous literature data and the results we have obtained the idea about the antioxidant enzyme system in the Shirvan variety, investigated under the stress factors and the activation of the small molecular antioxidant system in the Chokhyarpag accession can be discussed.



(a) **(b)**

Figure 1: (a) - activity of guaiacol peroxidase, (b)- amount of the proline (µM/q) in drought and salinity condition;. 1 control, 2 drought, 3 salinity. choxyarpag shirvan

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