The effect of different levels of salinity stress on variations in the protein pattern of barley plants inoculated with *Glomus fasciculatum* and pretreated with salinity

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Abstract

Mycorrhiza fungi play a key role in plants' resistance to environmental stresses. Among environmental stressors, salinity is an acute problem. One way to understand the ability of plants to deal with abiotic stress is to investigate and identify the changes that occur as a result of stress in the levels of certain proteins. The purpose of the present study was to investigate the emergence of special protein bands and their strength in barley plants under increasing salinity concentration and affected by salinity pretreated mycorrhizal fungi. The first factor of the study was pretreatment of mycorrhiza with 0, 25, 50, and 100 mmol of salt, and the second factor included 0, 25, 50, 100, and 200 mmol salinity treatments applied to the plants under study. Barly plants were analyzed by SDS-page gel electrophoresis method and staining to check changes in protein pattern. Results showed that the pattern of protein contents of the plants under stress was significantly different from that of control plants. Considering the enhanced bands or the appearance of new bands on the SDS-page gel, synthesis of some proteins increased in the plants under stress or some new proteins were produced to resist stress conditions compared to control plants. Based on the molecular weight of the proteins that have been determined in different plants and the range of bands obtained on the electrophoresis gel, the proteins of the investigated plant might be identified.

Keywords: barley, *Glomus fasciculatum*, mycorrhiza, protein pattern, salinity

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Introduction

Genetic diversity is a fundamental part of biological diversity and in individuals within a population or different populations of a species, it manifests the evolutionary status of the species. In general, the high genetic diversity in a particular species increases the possibility of carrying out breeding programs for it.

Protein diversity is an essential part of the plant's response to environmental stress and adaptation to environmental conditions (Raza et al., 2020). Salinity stress causes changes in plants' protein pattern and content (Osman et al., 2021). Under stress conditions, plants show complex molecular responses, including the production of stress proteins and compatible osmolytes, which are

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more abundant in salt-resistant cultivars than in salt-sensitive ones, as observed in barley, sunflower, and rice (Abdelhamid et al., 2020; Riaz et al., 2019). One way to get insight into the ability of plants to tolerate environmental stress is to identify changes induced by stress in their protein levels, with the idea that adaptation to stress is caused by changes in gene expression(Sharma et al., 2022; Zhao et al., 2020) . Salinity resistance is the result of cooperation amongst several physiological and biochemical factors (Polash et al., 2019). One of these factors may be the ability to bind ions to proteins. Different proteins are induced in salt-treated plants (Mansour and Hassan, 2022).

Electrophoresis is usually used to study protein changes in tissues affected by stress (Mahmood et al., 2022). In fact, the most extensive technique for the biochemical description of plant populations is protein electrophoresis. The existing electrophoresis methods are very diverse, and their obvious advantage is the low cost and quick results(Alsafran et al., 2022; Enespa and Chandra, 2022). The success of protein electrophoresis in identification and recognition of cultivars is attributed to the fact that the proteins separated through this method are the first product of gene activity (Abril et al., 2022). A report on the effect of salinity on Arachis plant's proteins shows that the new proteins were synthesized in this plant under salt stress (Han et al., 2021).

Metwali et al. (2011) in their studies on the protein pattern of wheat cultivars under salt stress revealed that the synthesis of some polypeptides increased and decreased, and these differences were argued to be probably due to the activity of genes involved in plant adaptation to salt conditions(Hussain et al., 2021a). Therefore, protein pattern and isozyme analysis can be a tool in understanding gene expression in response to stress(Attia et al., 2021) Analysis of soluble proteins extracted from primary leaves of tetraploid, hexaploidy, and wild wheat cultivars under salinity stress, using two-dimensional gel electrophoresis, showed that no new proteins were created under salinity stress compared to normal conditions(Halder et al., 2022), although the protein profiles between species showed differences, and salinity stress causes quantitative changes in proteins(Cao, 2021). The purpose of the present study was to investigate the emergence of special protein bands and their strength in barley plants under increasing salinity concentration and affected by salinity pretreated mycorrhizal fungi.

Materials and Methods

This research was implemented in the Greenhouse of the Faculty of Agriculture of Islamic Azad University, Saveh Branch. The first factor was pretreatment of mycorrhiza with 0, 25, 50, and 100 mmol of salt, and the second factor included 0, 25, 50, 100, and 200 mmol salinity treatment applied to the plants under study.

Barly seeds obtained from Karaj Seeds and Seedlings Institute were disinfected with 5% sodium hypochlorite for 10 minutes before they were washed with distilled water and rinsed in three consecutive steps. *Glomus fasciculatum* mycorrhizal fungus was obtained from Toran Biotechnological Research Institute, Shahrood, and 40 grams of the inoculum was used for each 1 kg pot. Pots with a height of 15 cm, a bottom diameter of 10 cm, and an opening diameter of 16 cm were used for cultivation. The pots were filled with 0.4 mm Lyca up to a height of 10 cm. The prepared seeds were primed by soaking in distilled water for 24 hours. The mycorrhiza was also pretreated with 0, 25, 50, 100 mmol concentrations of sodium chloride for 24 hours and then washed to remove the salt residue and mixed with the primed barley seeds. The inoculated seeds were planted in pots containing lyca at a depth of 2 to 3 cm. Ten seeds were sown in each pot and covered with a thin layer of Lika. The pots were kept in an environment with natural light and a temperature of 28 to 32 ℃.

Seven liters of Hoagland's base solution (pH: 5-6.8) were prepared and poured equally into 7 containers according to the nutritional requirements of the plant. Sodium chloride was used to prepare salinity solutions (0, 25, 50, 100 and 200 mmol/l), which were added to the containers containing Hoagland's to prepare treatment solutions. Then, 40 ml of the treatment solutions were applied to each pot twice a day, for

10 days. After 10 days of treatment, the plants were harvested for subsequent assays.

To compare the changes of leaf proteins in response to salinity, SDS-PAGE electrophoresis system was used, and two gels with different concentrations and pH levels were used. The concentration of the gels was determined as 12.5%. Electrophoresis was performed with a vertical system LKB-2001 device. After the end of electrophoresis, the gels were transferred to the dye solution. Then the dyeing operation was done.

Results

Examining the changes in the protein pattern showed that the protein pattern of stressed plants was different from that of the control plants (Fig. I). In the conditions of no treatment of mycorrhiza with salt, the protein pattern was different in plants treated with different concentrations of salinity. The bands present in the plants under non-stress condition were also present in the treatments with 25, 50, 100, and 200 mmol salinity. These bands included bands with molecular weights of 90-85 kilodaltons, 39-41 kilodaltons, 25-27 kilodaltons, 22-24-24 kilodaltons, 20-22 kilodaltons, 16 kilodaltons, and 15 kilodaltons, which were also present in other treatments. On the other hand, plants treated with 50, 100, and 200 mmol salinity had a number of bands. Additionally, it was observed that these bands were not present in the control treatment and 25 mmol NaCl. These bands included the ones with molecular weights of 105-110 kilodaltons, 94-97 kilodaltons, and 19-21 kilodaltons. Among the treatments without mycorrhizal fungus, the highest number of bands was observed in the treatment of barley plants with 200 mmol NaCl.

Pretreatment of mycorrhiza fungus with 25 mmol salt, resulted in no significant differences in the protein pattern of the plants under different concentrations of salinity. All the bands present in the non-salinity stress condition were also present in all the salinity treatments, although some additional bands were observed in the salinity treatments. Under salinity treatments of 25 and 50 mmol, in addition to the bands in the control treatment, there were bands with molecular weight of 48-52 kDa and 19-21 kDa while in the

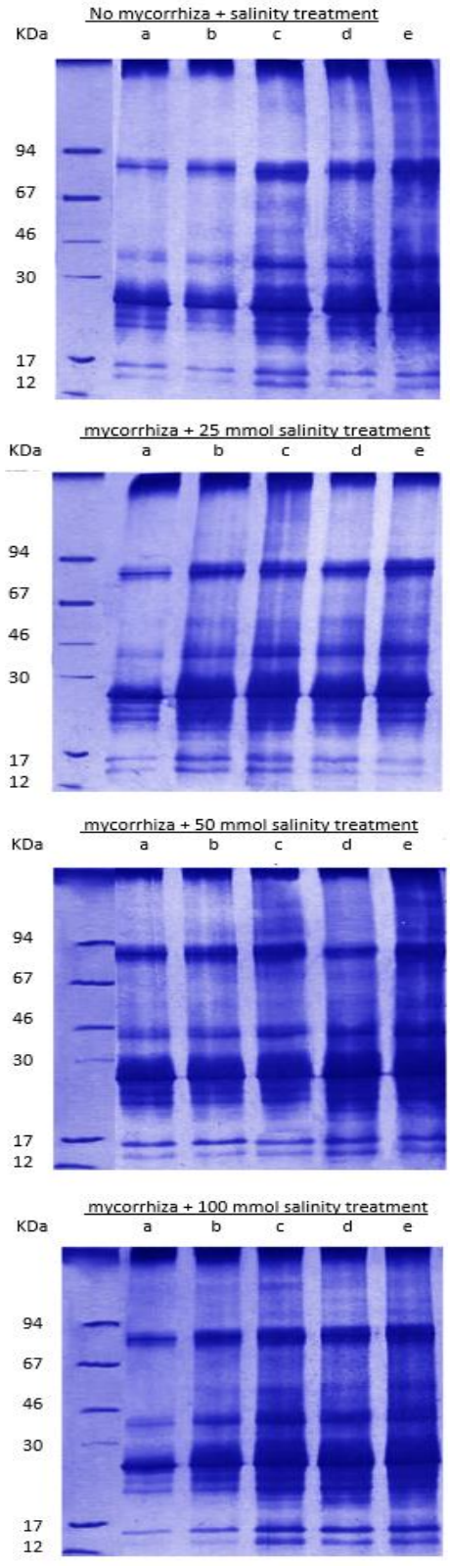

Fig. I. Protein pattern in barley plants under different salinity treatments; a: without salt treatment, b: 25 mmol salinity, c: 50 mmol salinity, d) 100 mmol salinity, d) 200 mmol salinity

treatments with 100 and 200 mmol salinity, the band with molecular weight of 19-21 kDa did not appear. Among the plants receiving mycorrhizal fungus pretreated with 25 mmol NaCl, the highest number of bands was observed in 25 and 50 mmol salinity treatments.

Pretreatment of mycorrhizal fungus with 50 mmol NaCl resulted in significant difference in the protein pattern under various concentrations of salinity treatment. All the bands present in the conditions without salinity stress were also present in all the salinity treatments while some additional bands were also observed in the salinity treatments. At 50 mmol salinity, in addition to the bands in the control group, there were also bands with molecular weights of 105-110 kDa, 94-97 kDa, and 65-70 kDa, whose intensity were different from the 100 and 200 mmol treatments. At 100 mmol salinity, in addition to the bands in the control treatment, there were also bands with molecular weights of 65-70 kDa and 19-21 kDa. The highest number of bands was observed (12 bands) under 200 mmol salinity treatment.

Mycorrhizal fungus pretreated with 100 mmol NaCl resulted in different protein pattern under different concentrations of salinity treatment. At 25 mmol salinity, all the bands that were present in the control were observed, with the difference that their intensity was higher. In the 50 mmol pretreatment, in addition to the bands present in the control, the intensity of these bands was higher than that of the control, and bands with molecular weights of 105-110 kDa, 46-52 kDa and 19-21 kDa also appeared. In the 100 mmol pretreatment, there were bands that appeared in 50 mmol treatment, although in this treatment the band with a molecular weight of 105-110 kilodaltons was absent, and the intensity of the other bands was somewhat similar to that of the 50 mmol NaCL treatment. All the bands present in 100 mmol treatment were also observed in 200 mmol group, although these bands were somewhat more than the 100 mmol treatment, so that in the pretreatment of mycorrhizal fungus with 100 mmol NaCl, the highest number of bands was observed under 50 mmol salinity treatment.

Discussion

In response to environmental stress, the expression of some genes increases while that of some others decreases. This is because due to the lack of immobility, plants must make the necessary metabolites in order to overcome the imposed stress conditions. The change of expression in plants reduces the synthesis of some structural proteins or enzymes involved in certain metabolic activities (Swapnil et al., 2021). Due to the complexity of the plant response mechanism to stress, it is thought that hundreds of genes are involved in the response to biological stress(Sahoo et al., 2020). The products of several genes that respond to salinity, chilling, and drought stresses at the transcript level, either directly protect the plant against abiotic stresses, or regulate gene expressions and message transmission in response to the stress(Hussain et al., 2021b) . Ericson and Karki and coworkers investigated the protein pattern of cultivated tobacco cells under control and salt stress conditions by analyzing the amino acids of these proteins and found that all of them contained hydroxyproline (Karki et al., 2023). Researchers showed the effect of salinity on the protein pattern of barley roots, observed and recorded the changes in the microsomal part and showed that membrane proteins play an important role in the response of plants to salinity(Choudhary et al., 2023). By applying salinity stress to barley seedlings specific tissue proteins were created by the stress(Zeeshan et al., 2020a). Zeeshan and coworkers (2020) pinpointed two main effects as being significant in salinity stress. First, there is a quantitative regulation in the production of certain proteins. This means that the abundance of some proteins with different molecular weight and isoelectric point is increased or decreased. Second, the same group of new proteins are induced. Therefore, he concluded that gene expression changes under salt stress conditions(Zeeshan et al., 2020b).

The stimulation of protein production in barley plants showed that after the application of jasmonic acid, transcripts of polypeptides accumulated in leaves(Ali et al., 2022). Therefore, it can be argued that the gene related to these proteins is expressed after stimulation by jasmonic acid and leads to the production of these proteins. Studies have shown that salinity stress affects the

amino acid methionine in a large number of root polypeptides in resistant barley plants. The most changes take place in three polypeptides related to germin, homopolymeric protein of about 130 kilodaltons consisting of a 26 kilodalton subunit(Chaudhary et al., 2022). Two of these proteins are called pl 3.6 and pl 6.5, which are about 26 kilodaltons, and the third protein is pl 5.6, which decreases during salt stress (Moreno et al., 2020).

Conclusion

According to the molecular weight of proteins which are already known in different plants and the range of bands obtained on gel electrophoresis, it is possible to roughly identify the proteins of the plant under study, such as stress proteins with a molecular weight of 67.32 KDa, named (3-1) β-glucanase GV with a weight of KDa 34.41, smutin with a weight of KDa 26, and germin with a weight of KDa 26 which is within the range of strengthened bands and a protein called catalase 1 with a weight of KDa 56.58 and 6-phosphogluconate dehydrogenase with a weight of KDa 58/ 51 which are in the range of newly appeared bands.

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