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Effect of salinity stress on protein changes in different cultivars of wheat (*Triticum aestivum* L.)

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ABSTRACT:Salt stress is one of the most destructive abiotic stresses which restrict crop production worldwide and a great threat to the world's major crop, wheat. Natural tolerance to salinity stress is present in some cultivars of wheat. One way to detection salt tolerant cultivars is polypeptides identification which their levels change under salinity stress. In this study, four wheat cultivars were investigated to evaluate their salt tolerance under different increments of salinity levels (0, 70, 140 and 210 mM NaCl). Leaf sampling was done 2 and 10 days after completion of each salinity treatment. Quantitative and qualitative data showed that, the cultivars reaction in terms of their growth was similar in short term stress and the differences were observed in long term stresses. Falat cultivar had a higher decrease in growth than the others. Thus, for comparing salt tolerance in wheat cultivars, time period is a main factor.

KEYWORDS: Wheat, salt stress, Falat, Time period

I.INTRODUCTION

As sessile nature, plants are forced to endure different abiotic stresses like salinity, drought, high or low temperature and etc. [1]. Salinity is a major abiotic stress which restricts plant growth and crop productivity[2, 3] and a great threat to wheat as one of the world's major crops. A considerable quantity of worldwide lands is salt-affected, which amount of that increase every day [4]. Natural or human process cause salinity occurrence through dissolving salt accumulate in the soil water that inhibits plant growth [5]. Salinity at high level in the soil has negative effects on plant growth and development; mainly due to signature of Na^+ and Cl^- ions in plants. The negative effects are destruction of plant metabolism, inhibition of seed germination, reduction of photosynthetic rates and ultimately lead to decrease of crop yield [6, 7].

There are two main mechanisms for salt tolerance in plants: reduction of salt entrance to the plant and minimize of salt concentration in the cytoplasm. Halophyte plants have both of mechanisms and hence, they can grow for a long time in the salt land. But glycophyte plants have low ability to exclude salt, and accumulation of toxic ions lead to sensitivity of these plants to salinity [8]. In most salt tolerant plants, Na^+ and Cl^- ions absorption are restricted while uptake of macronutrients such as K^+, $NO3^-$ and Ca^{2+} improved [9]. Therefore, one of the main objectives of plant breeding programs is improving crop tolerance to salt stress. Some efforts have been made for breeding in salinity tolerant crops, but little progress obtained and studies still cannot explain the mechanism of plant salt tolerance [10, 11]. In fact, Salt tolerance is a complex trait regulated by hundreds of thousands of genes and dozens of physiological mechanisms. There are several wheat genes Ta-UnP, TaZNF, TaSST, TaDUF1, and TaSP that significantly improve the salt tolerance of transgenicplant [10].One way for detection of salt tolerant cultivars, is identification of polypeptides involved in salt stress response which their levels changed under stress. In some cases, salinity leads to decrease or increase of proteins, while sometimes it causes to completely disappear of some proteins and induces new proteins [12]. Accumulated proteins under salinity may be synthesized de novo in response to salinity [11]. According to the result of different studies, salt stress can change level of soluble proteins. In salt tolerance cultivars of barley, sunflower, finger millet and rice, demonstrated that amount of soluble proteins increased after salinity [13], But according to the result of Agastian et al.(2000) [14], soluble proteins amount in mulberry cultivars increased at low salinity and decreased at high salinity. Therefore, the aim of this study is elucidation of leaf protein changes in different cultivars of wheat in response to salinity.

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II. METHODOLOGY

A)Plant materials and growth condition

this study was made to investigate salinity stress tolerance in four wheat cultivars; containing Kavir and Roshan as tolerant cultivars, Falat as semi-tolerant and Inia as sensitive ones.seeds were provided from Seed and Plant Improvement Research Institute, Karaj, Iran. After sterilization of seeds with 3% sodium hypochlorite solution, Seeds were germinated at 20°C in dark in petri dishes for 48h. Then germinated seeds were transferred to tubes filled with vermiculite. After one week, two-leaf seedlings were grown at Hydroponic culture, including Hogland nutrient solution. Seedlings were grown in a controlled condition (25°c with supplementary lighting provided by 10000 lux phytotron for 16 h daily and 45% relative humidity). Nutrient solution was renewed every one week.At three-leaf stage, salt (NaCl) treatment was applied to the nutrient solution gradually for Falat and Roshan cultivars (at increments of 35 mol m^{-3} every other day) and abruptly for Kavir and Inia cultivars until the final concentration of 0 (control), 70, 140 and 210 mol m^{-3} were reached. Leaf sampling was done in 2 and 10 days after completion of each salinity treat. Samples frozen in liquid N_2 immediately and stored at -80°c for later analysis.

B) Protein extraction

Total soluble proteins were extracted from the leaves of control and NaCl-treated seedlings of wheat genotypes. Leaf tissues were ground in liquid nitrogen using a mortar and pestle. Homogenate extract was prepared by composite of ground samples and extraction buffer containing Tris-HCl 25 mM, pH 7.5, KCl 150 mM, 20% glycerol (v/v) and DTT 1 mM with ratio of 4:1 (tissueextraction buffer). The suspension was centrifuged at 14000 rpm (4°C) for 15 min. supernatant was separate as soluble proteins and stored at –20°C for analysis.

C) Quantification of protein

Protein concentrations of extracts were determined by Bradford method [15]. In this method protein concentration is determined by quantifying the binding of the dye, Coomassie Brilliant Blue G-250, to the unknown protein solution, as compared to known standards. For protein determination, 100 µl of protein extracts were added to tubes. Blank tubes without protein sample and containing 100 µl extraction buffer were also prepared. Then 5 ml of Coomassie Dye Reagent (containing 0.01 gr Coomassie Brilliant Blue G-250, 5 ml ethanol (96%), 10 ml phosphoric acid (85%) which final volume of that reached to 100 ml with distilled water) was added to each tubes and the mixtures vortexed. For each sample absorbance at wavelength of 595 nm using Spectrophotometer was recorded. The proteins concentration of each sample was determined using Bovine Serum Albumin (BSA) standard curve. Each sample was assayed with three replicates.

D) Protein electrophoresis

SDS-polyacrilamide gel electrophoresis (SDS-PAGE) was carried out on 10% acrylamide gels. The leaf protein extracts were mixed with buffer containing 0.3 gr Tris-HCl (pH 6.8), 0.92 gr SDS, 2 ml β-mercaptoethanol, 4 gr glycerol and 2 ml bromophenol blue (0.1%) and then heated at 100°C for 3 min. 30 µg of soluble protein was loaded in each well. Bio-Rad low molecular weight marker used to standard. For staining, the gels were placed in staining solution (containing of 0.25 gr coomassie brilliant blue R-250, 125 ml methanol, 25 ml glacial acetic acid, and 100 ml distilled water) and shaked for 24-48h. Distaining of gels was done with solution containing 100 ml methanol, 100 ml glacial acetic acid and 800 ml distilled water for 48h at room temperature. The gels were photographed and quantitative amounts of the bands were determined by densitometry.

E) Data analysis

Electrophoresis pattern of each genotypes were scored based on presence (1) or absence (0) of each band for qualitative data. Dendrogram of cluster analysis drew based on Euclidean distances and by UPGMA (Unweighed Pair Group Method Using Arithmetic Average) method with SPSS software.

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III. EXPERIMENTAL RESULTS

A) Comparison of protein changes on salinity

In this study effect of salinity on protein changes surveyed in two forms, gradually for Falat and Roshan cultivars and abruptlyfor Kavir and Inia ones. Two days after gradual stress, clear differences for seedlings growth were not observed between Falat and Roshan cultivars in control and salt treatments, while 10 days after salinity, decrease in seedling growth was observed at high salinity level. This decrease in growth was a little higher in Falat cultivar than the others.Electrophoretic pattern study of leaf soluble proteins showed fundamental similarity between Falat and Roshan cultivars in control and salt treatments. 18 to 20 polypeptide bands were observed, but no polypeptide bands belonging to the specific cultivar or treatment was observed. Two days after exposing to salinity stress sampling showed no considerable difference among the polypeptide bands, and color intensities of polypeptide bands were low, which is according to same seedling growth at 2 days after stress in different control and salt treatments (Figure 1). While 10 days after salinity stress, in two 54 and 56 kDa bands, high quality bands were observed, and in some other bands, different color intensities were seen (Figure 2).

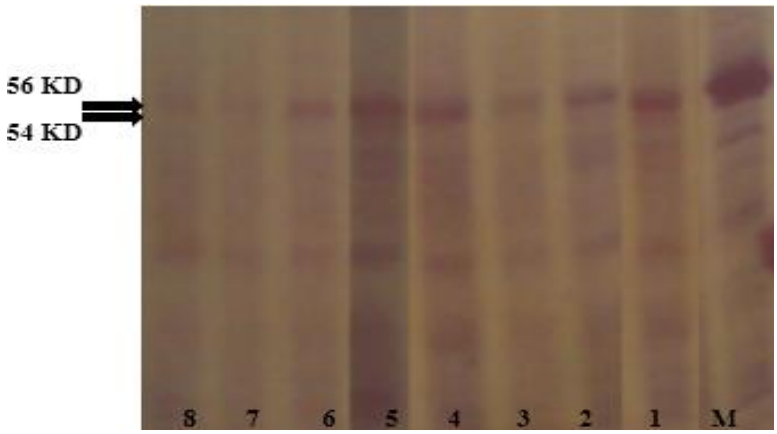

Fig 1: SDS-PAGE pattern of leaf soluble proteins 2 days after gradual salinity. M: molecular marker, Falat at 1:control, 2: 70 Mm, 3: 140 Mm, 4: 210 Mm, Roshan at 5:control, 6: 70 Mm, 7: 140 Mm, 8: 210 Mm.

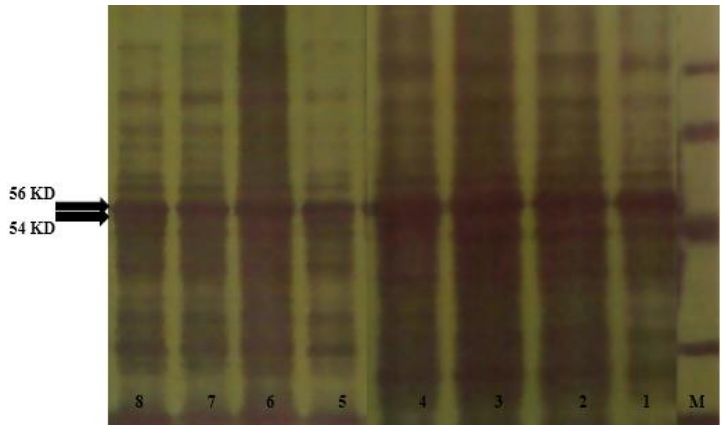

Fig 2: SDS-PAGE pattern of leaf soluble proteins 10 days after gradual salinity. M: molecular marker, Falat at 1 control, 2: 70 Mm, 3: 140 Mm, 4: 210 Mm, Roshan at 5:control, 6: 70 Mm, 7: 140 Mm, 8: 210 Mm.

Densitometry analysis showed 10 days after salinity in Falat cultivar, 54 kDa band was removed in all salinity treatment. In Roshan cultivar this band was detected in control and 70 mol m-3 salinity, but it also removed in 140 and

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210 mol m-3 salinity.Also, in present study, Kavir (as tolerant) and Inia (as sensitive) cultivars were compared in abrupt form of salinity stress. In this way, all treatments were applied simultaneously and completely. Qualitative pattern of polypeptide bands was basic similar between cultivars, and 16-20 polypeptide bands were detected. Salinity treatment did not induced synthesis of any specific polypeptide bands belonging to the specific cultivars or one of the salinity treatments (Figure 3). Only in Inia cultivars intensity of 54 and 56 kDa bands decreased at 210 mol m-3 at 10 days after stress (image not shown).

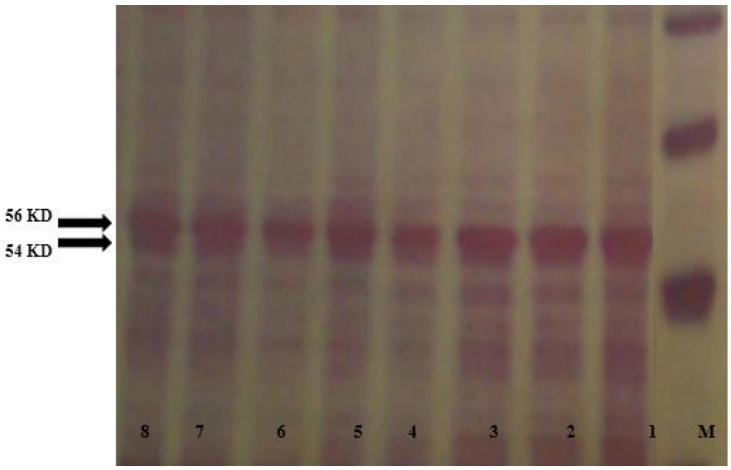

Fig 3: SDS-PAGE pattern of leaf soluble proteins after abrupt stress. M: molecular marker, Kavir at 2 days after stress, 1: control, 2: 70 Mm, 3: 140 Mm, 4: 210 Mm, Kavir at 10 days after stress, 5:control, 6: 70 Mm, 7: 140 Mm, 8: 210 Mm

Quantitative changes were observed between genotypes in two 54 and 56 kDa polypeptide bands. According to the densitometry analysis, in Kavir cultivar (2 days after salinity) 54 kDa band was detected in 70 and 210 mol m-3 salinity, while it was not observed in control and 140 mol m-3 salinity levels. Ten days after salinity, this band was observed in all treatment, however intensity of that was low in control and its accumulation increased at 70 mol m-3 salinity.
In Inia cultivar, two days after stress, accumulation of 54 and 56 kDa bands decreased in 210 mol m-3 salinity, and ten days after salinity, accumulation of 54 kDa band decreased in 140 and 210 mol m-3 salinity.

B) Cluster analysis

Cluster analysis of genotypes in different treatment was done by UPGMA method according to the result of densitometry analysis and qualitative data. Dendrogram of cluster analysis for Falat and Roshan cultivars from quantitative data was shown in Figure 4.

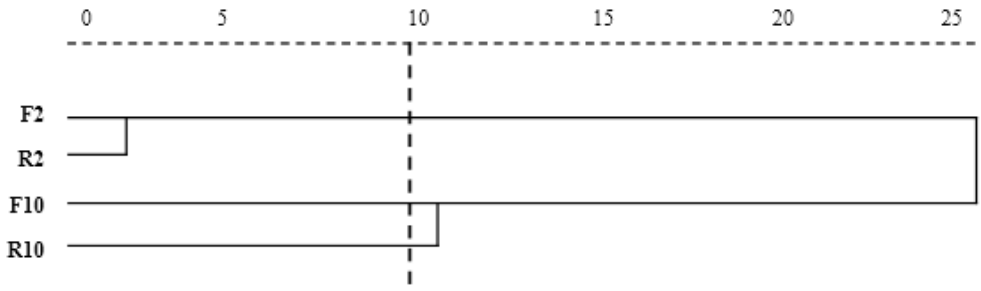

Fig 4: Dendrogram of cluster analysis from quantitative data at all treatment. F2: Falat cultivar at 2 days after salinity, R2: Roshan cultivar at 2 days after salinity, F10: Falat cultivar at 10 days after salinity, R10: Roshan cultivar at 10 days after salinity

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Dendrogram revealed three groups: first group contained Falat cultivar at 10 days after salinity, second group belonged to Roshan cultivar at 10 days after stress. Falat and Roshan cultivars at 2 days after salinity were categorized in third group. Also, cluster analysis for qualitative data showed same grouping with quantitative data.
For Inia and Kavir cultivars, cluster analysis was performed based on quantitative data and dendrogram of that was generated (Figure 5). According to this diagram, 3 groups were formed. Kavir cultivar (2 days after salinity) in one group, Inia cultivar (10 days after salinity) in another group and third group was belonged to Kavir (10 days after salinity) and Inia (2 days after salinity) cultivars.

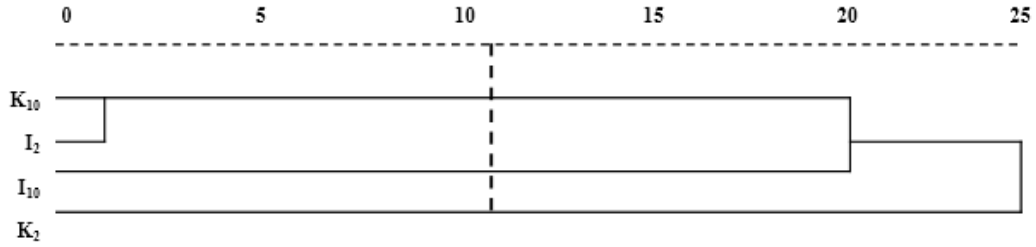

Fig 5: Dendrogram of cluster analysis from quantitative data at all treatment. K10: Kavir cultivar at 10 days after salinity, I2: Inia cultivar at 2 days after salinity, I10: Inia cultivar at 10 days after salinity, k2: Kavir cultivar at 2 days after salinity.

IV.DISCUSSION

Electrophoresis study of leaf soluble proteins in Falat and Roshan cultivars did not show fundamental difference among polypeptide bands belong to different treatment in 2 days after salinity. In sampling 2 days after salinity, two 54 and 56 kDa polypeptide bands detected in all control and salinity treatment, which only color intensity of those decreased in salinity treatments. While 10 days after salinity, quantitative changes were revealed in control and salinity treatment. The 54 kDa band was removed in high salinity levels (140 and 210 mol m^{-3} salinity) in Roshan cultivar and in all salinity treatment in Falat cultivar. These results are according to the result of Yeo et al. (1991) [16], which showed response of different cultivars of rice to the salinity stress is different in short and long term.
Also, cluster analysis confirmed these results. According to this analysis, Falat and Roshan cultivars were located in 2 separate groups in 10 days after salinity while they grouped in one cluster in 2 days after salinity. It seems that in short term after salinity, plants yet are adapting with new condition and therefore difference between cultivars is not revealed aspect of response to salinity.
In Kavir (as tolerant cultivar) and Inia (as sensitive cultivar) in 2 and 10 days after salinity, fundamental difference between polypeptide bands were not revealed, and mainly decrease in color intensity was seen. The most different is belong to 54 and 56 kDa bands. Also, Ashraf and Fatima (1995) [17] reported that in salt tolerance and salt sensitive cultivars of safflower there were not significantly difference in leaf soluble proteins. Comparing the protein bands in short and long term, shows that short period effects of salinity stress are studied under non-steady state. This duration is the time that the plant is adapting itself with the salinity condition. The long period effects are probably examined under the type of steady state condition. This condition indicates a real picture of the plants adaptation and its response to the salinity stress.
Quantitative and qualitative study showed that in short term stress, the genotype reaction was similar and the difference between genotypes were determined in long term stresses. Investigation show that durum wheat is more salt-sensitive than bread wheat [18] but Munns et al. (1995) [19] find that in short term salinity there are not differences between durum and bread wheat cultivars. Thus for compared of response cultivars to the salinity, time period is a main factor.

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