International Journal of Nuts and Related Sciences 2(4): 67-74, 2011
ISSN 2008-9937

A Change in Leaves Protein Pattern of Some Pistachio Cultivars under Salinity Condition

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Abstract: Plants are often subjected to several environmental stresses throughout their life cycle. Adaptation of plants to saline conditions may be due to some salt-related changes in the pattern of gene(s) expression. The physiological role of stress induced proteins is not yet clearly understood. However, it is believed that these proteins allow plants to produce biochemical and structural adjustments which enable them to adapt with the stress conditions. In this research we compared protein profile of 4 female cultivars and 2 male genotypes of Rafsanjan Pistachio in salt stress situation. The treatments were irrigated with saline water (EC = 5.5 dSm^{-1}), without drainer, until appearing leaf phytotoxicity. Before and after saline stress leave proteins were extracted by using Damerval method, with some modification. The amount of total proteins was determined according the method of Bradford assay by used of BSA as standard solution. After uniformity of protein concentration, they were loaded on SDS-PAGE, in 12.5% separating gel and 5% stacking gel according to the method of Hames and Rickwood,. The results revealed that salinity induced changes in protein pattern and decreasing of total proteins in leaves of Pistachio. Some bands were decreased, increased, appeared or disappeared after salinity. Additionally a 40 KDa (kilo Dalton) band was increased in all samples. Moreover, this investigation reported the molecular weights of some salt responsive proteins.

Keywords: Protein profile; Pistachio; Salt stress; Protein extraction

INTRODUCTION

Plants are often subjected to several environmental stresses throughout their life cycle. One approach to understanding the ability of plants to tolerate environmental stresses is to identify stress-induced changes in the level of individual proteins, with the assumption that adaptation to stress is the result of altered gene expression [11]. Soil salinity adversely affects the growth and productivity of crop plants mainly because of osmotic stress, ion imbalances and ion toxicity [6, 12]. Adaptation of plants to saline conditions may be due to some salt-related changes in the pattern of gene(s) expression. The physiological role of stress induced proteins is not yet clearly understood. However, It is believed that these proteins allow plants to produce biochemical and structural adjustments which enable them to adapt with the stress conditions. Salinity-induced changes in protein have been reported in several plant species [11, 12, 18, and 19]. Jain [12] in *Brassica juncea L.II* demonstrated that salt-stress induced changes in polypeptide pattern of in vitro selected NaCl-tolerant plants. In this experiment some bands disappeared and some bands appeared after salinity. Zorb [20] showed the short-term effects of salt exposure on maize chloroplast protein pattern. Hurkman and Tanaka [11] showed that salt stress caused changes in protein pattern synthesis in

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barley roots. Garcia [5] investigated differential protein expression in Olive tissues after exposure to salt stress. They observed that 2 bands of approximately 24KD and 40KD were expressed after exposition to salinity. Kong-ngern [13] in leaf sheaths of rice seedlings showed that in SDS-PAGE, 2 protein bonds (22 and 31 KD) increased after salinity stress. Mahmoodzadeh [14] showed that the few polypeptide in tolerant cultivars is higher than sensitive one in seeds of *Brassica napus*. Many efforts have been done to find salt tolerant pistachio cultivars. Few works has been done on the effect of environmental stress on protein synthesis in pistachio. The study of protein profile of inflorescence in dormant and growing season in 5 Pistachio cultivars showed that protein bands in a size of 32 and 20 KDa accumulate in inflorescence during the toleration phase [10]. We have initiated studies to analyze the influence of salt stress on the pattern of protein synthesis in Pistachio. In this research we compared protein profile of 4 female cultivars and 2 male genotypes of Rafsanjan Pistachio in salt stress situation.

MATERIALS AND METHODS

Plant materials

The artificial cross was done between 4 female cultivars (*Shahpasand, Khandani, Badamo-zarand and Ebrahimi)* with Rafsanjan male genotype and 2 male genotypes (m_4 *and* m_{21}) with Ohadi cultivar. In September pollinated seeds were harvested separately. After drying, the nuts were sown in November in glasshouse. The pots were irrigated with city water (EC = 0.6 dSm^{-1}) during the first month. After that pots were irrigated with a saline water characterized in table1, without drainer, until appearing leaf phytotoxicity.

Table 1. The chemical characteristics of water used in the experiments

Water sample	EC dSm^{-1}	pH	$Co3^{-2}$ (meq/L)	$HCo3^{-}$ (meq/L)	Cl (meq/L)	Ca^{2+} (meq/L)	Mg^{2+} (meq/L)	Na^{+} (meq/L)	S.A.R. (Meq/lit)
Salin water	5.5	7.2	0.0	1.0	21.0	10.4	10.0	14.3	4.5

Protein extraction

Before and after salinity stress, some leaves were picked up for protein extraction. Leave proteins were extracted by using Damerval method [15] with some modification as follow. 0.5 gr leaf tissues of each cultivar were ground to powder under liquid nitrogen. The proteins denatured and precipitated in a mixture of 2-mercaptoethanol (2ME) and trichloroacetic acid (TCA) in cold acetone (solution 1). The samples were incubated in -20°C for 45 min and mixed by vortex in 10 min intervals. Then samples were centrifuged for 15 min at 13000 rpm at 4°C. The supernatant was removed and solution 2 (0.7% 2ME, 1Mm PMSF, 2Mm EDTA in acetone) was added into each tube. The samples were mixed by inverting the tubes. Afterwards, samples were kept in in -20°C for 45 min and mixed ever 10 min intervals by inverting, followed by centrifugation for 15 min at 13000 rpm at 4°C. The supernatant was removed and solution 3 (7 mol urea, 2 mol thiourea, CHAPS 2% W/V and dithiotritol 2% W/V in distilled water) was added and mixed by vortexing. Then samples were incubated in 30°C for 60 min with vortexing every 5 min, followed by centrifugation for 15 min at 13000 rpm in room temperature. Because the urea is crystallized in low temperature. Finally, the supernatant was collected and kept in -20°C for future experiments.

Protein assay

The amount of total proteins was determined according the method of Bradford assay [2] by used of BSA as standard solution. After uniformity of

protein concentration, they were loaded on SDS-PAGE.

SDS-PAGE

SDS-PAGE was carried out in 12.5% separating gel and 5% stacking gel according to the method of Hames and Rickwood [9]. Gels were run at a pretty low temperature (13˚C) for 6 h at constant power of 40 mA until the tracking dye reached the end of the gel. After electrophoresis, the gel was stained with coomassie blue according to method of Reed *et al.* (1998). Relative molecular weight of proteins was determined using a standard curve generated from the standard proteins [1, 8]. The standard proteins were as follows: Ovalbumin from chicken egg (45KDa), Glyceraldehydes-3-phosphate Dehydrogenase (36KDa), Carbonic Anhydrase from bovine erythrocytes (29KDa), Trypsinogen from bovine pancreas (24 KDa), Aprotinin from bovine lung (14.2 KDa) and Aprotinin from bovine lung (6.5 KDa).

RESULTS

In order to assess the relative levels of salinity tolerance, particularly during germination, we germinated treated seeds in vases with saline water (EC = 5.5 dSm^{-1}) and control seeds in vases with city water (EC = 0.6 dSm^{-1}). Appearance of the control and treated seedlings at the end of experiment is shown in fig. 1.

Before and after salinity stress, some leaves were picked up for protein extraction. After protein extraction Bradford assay showed that the total protein content of leaves decreased in all cultivars as result of salinity treatment (table2).

Fig. 1. The effect of saline water on seedling appearance (left: salt treated & right: controle)

Table 2. The amount of total proteins (µg/ml) according Bradford assay

cultivars	Before salinity	After salinity
khandani	0.612	0.492
Shahpasand	0.694	0.587
Badami-zarand	0.582	0.554
Ebrahimi	0.608	0.451
M4	0.481	0.410
M21	0.575	0.475

40 µg extracted proteins were loaded on SDS-PAGE gel. Table 3 shows abbreviation symbols used in figures. The most striking changes in extracted proteins of seedlings yield from female cultivars were a significant increase of a 40 KDa band and decrease of 25 KDa proteins (figures 2 and 3). Besides some other bands increased or decreased after salinity stress. In seedlings yield from M4 male genotypes (figures 4), a 45 KDa protein was disappeared. In contrast some new polypeptide with 41 and 34 KDa molecular weights were appeared. It was found that the intensity of bands proteins for 40 & 37 KDa bands were increased and for 38.5 & 35 KDa bands were decreased. In seedlings yield from M21 male genotypes (figures 5), a 45 KDa protein was disappeared. In contrast 39 KDa protein was appeared. It was found that the intensity of bands proteins for 24-40 KDa range was decreased after salinity except for 40 KDa band.

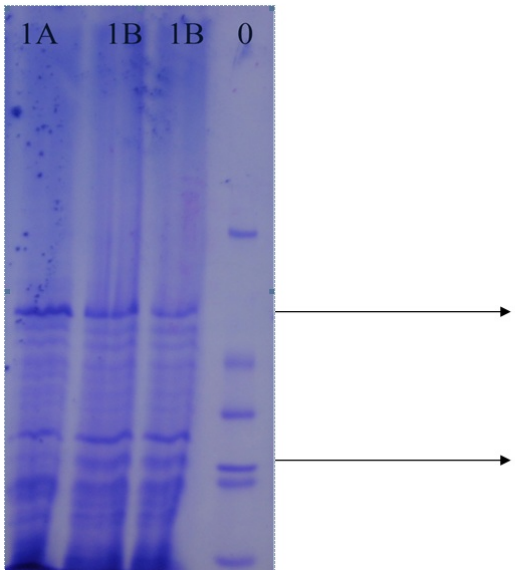

Table 3. Abbreviation symbols

khandani	1
Shahpasand	2
Badami-zarand	3
Ebrahimi	4
M4	5
M21	6
Marker	0
Before salinity	B
After salinity	A

Fig. 2. Changes in protein profile of Khandani cultivar before (1B) and after (1A) salt stress

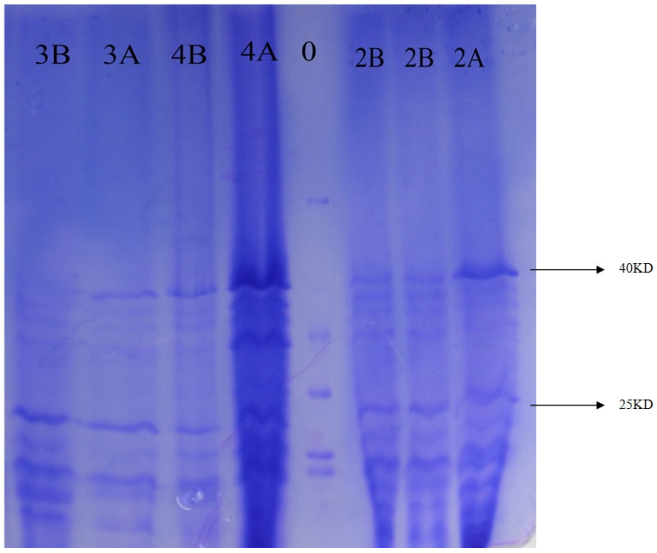

Fig. 3. Changes in protein profile of Shahpasand, Badami-zarand, and Ebrahimi cultivars before (B) and after (A) salt stress

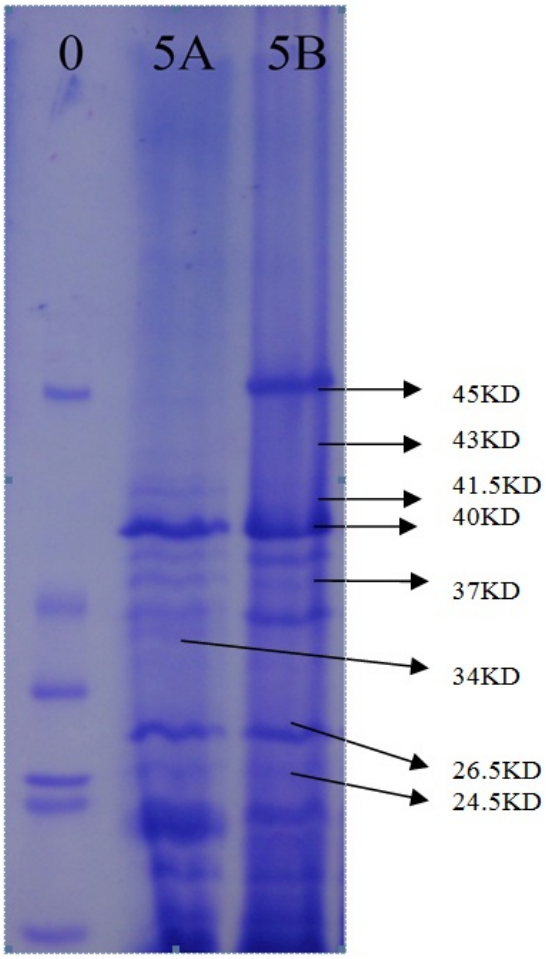

Fig. 4 Changes in protein profile of M4 genotype before (5B) and after (5A) salt stress …….

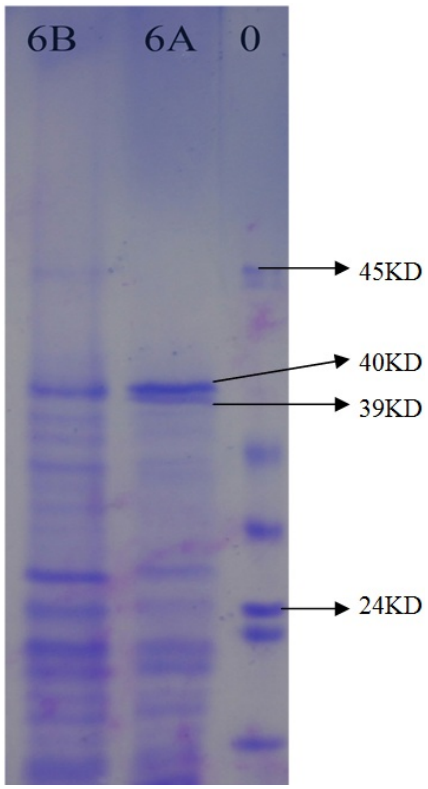

Fig. 5. Changes in protein profile of M21 genotype before (6B) and after (6A) salt stress ……

DISCUSSIONS

Plants species may change their response to salt stress by alter their gene expression and protein accumulation to reduce the effects of salt stress. In an attempt to understand the molecular basis of salt tolerance in pistachio, a SDS-PAGE method was used to identify proteins involved in the process. It is widely known that there are numerous transient responses to environmental shocks and that many of these genes are common to several types of stresses, such as cold, salinity, heat and osmotic stress [7].

Overall after salt stress the intensity of protein expression changed to different degrees which was depend on the pistachio cultivars. For example, a 40 KDa protein band in all cultivars, some bands with 37, 26.5 and 24.5 KDa in m4 genotype were increased after salt stress as well as appearing 43, 41.5 and 34 KDa bands in m4 genotype and 39KDa band in m21 genotype. Jain [12] in *Brassica Juncea L.II*: 93.8KDa, De Souza *et al.* (2003) in rice: 14.2 KDa, Garcia *et al.* (2004) in Olive: 40 and 24 KDa, Hurkman and Tanaka [11] in barley: 26 and 27 KDa, demonstrated increase after salt stress.

In our result, some proteins were found to express at a lower level or completely disappeared after salt stress. Such as a 45 KDa band was disappeared in male samples (Fig. 4 and 4) and a 25 KDa band in female cultivars and some bands for 24- 40 KDa range in male genotypes were decreased. This could be due to the inhibitory effects of salt stress on transcriptional process. This result was similar to a previous work [3, 12]. Several reasons [4] may explain the protein content decrement is attributed to the decreased in the rate of protein synthesis, the increased activities of hydrolyzing enzymes, the decreased availability of amino acids, or the denaturation of the enzymes involved in amino acid and protein synthesis. However, there is no explanation concerning decreased in the level of

proteins content in this study which should be investigated.

Generally, after salt stress, the total protein content of leaves, as quantified by Bradford assessments, was decreased. This decrease in protein content might be due to the increasing activity of acid and alkaline protease [17]. Parida [16] reported that the levels of free amino acid increased as a result of salt stress in *Bruguiera parviflora*, thus the NaCl treatments on *Bruguiera parviflora* at high salt concentration showed an increase in total amino acid pool by decreasing protein content which reflects the model of adjustment to salinity stress. Proteins are hydrolyzed by proteases to release amino acids for storage and/or transport and for osmotic adjustment during NaCl stress. Osmotic adjustment, protection of cellular macromolecules, storage form of nitrogen, maintaining cellular PH, detoxification of the cells, and scavenging of free radicals are proposed functions of free amino acid accumulation.

In conclusion, the data presented here revealed that salinity induced changes in protein pattern and decreasing of total proteins in leaves of Pistachio. Additionally a 40 KDa band was increased in all samples that Garcia's experiment [5] on Olive tissues showed same result, some bands were decreased, increased, appeared or disappeared after salinity. Moreover, this investigation reported the molecular weights of some salt responsive proteins. Changes in protein pattern in inflorescence of Pistachio in Hosseini's experiment are too obvious under cold stress. Regard to Pistachio is known as a salt tolerant plant It is necessary to future study the structural and functional roles of these salt stress-responsive polypeptides to enhance our understanding of the salt responses in Pistachio trees. A better understanding of the role that altered gene expression plays in salt tolerance can be achieved when the genes coding are identified for the salt induced polypeptides [11].

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Hokmabadi, Dr Moradi and Dr Nooraeen for their valuable advices.

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