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Full Length Research Paper

Polypeptide electrophoretic pattern of *Matricaria chamomilla* and *Anthemis nobilis* under salt and Fe-deficiency stress

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In order to study the effects of salinity and Fe-deficiency on electrophoresis pattern of polypeptides in two chamomile genera (*Matricaria chamomilla* and *Anthemis nobilis*), a factorial experiment was conducted based on completely randomized block design with three replications, in 2010. The experimental factors were two chamomile genera *M. chamomilla* and *A. nobilis*, four levels of salinity (0, 50, 100 and 150 mM NaCl) and two levels of iron (0 and 100 mM). The patterns of proteins extracted from stress-treated leaves of *M. chamomilla* were not different from the control plants; except for new of 14 and 18 kDa polypeptides which appeared in stress-treated individuals. *M. chamomilla* produced fewer protein bands compared to *A. nobilis* and its electrophoretic profile had lower variation. *A. nobilis* lost its many bands under salinity stresses and iron deficiency. *A. nobilis* roots synthesized osmotin, (26 kDa MW) and their leaves under salinity stresses and iron deficiency synthesized protein bands of 42, 40, 37 and 15 kDa molecular weight. It was therefore suggested that the alteration in the pattern of protein synthesis in *A. nobilis* and *M. chamomilla* under stress condition is related with specific adaptive response to stresses.

Key words: Chamomile, *Matricaria chamomilla, Anthemis nobilis,* stress, salinity, Fe-deficiency.

INTRODUCTION

The world is replete with places that experience extreme environmental stresses (Taiz and Zeiger, 2006). Stresses caused damage on the plant species potential. This damage can be reversible or irreversible (Blum, 1986). Therefore, it is important to understand the physiological processes that cause stresses injury and the adaptation and acclimatization mechanisms of plants develop in response to environmental stresses.

Alkaline soils such as calcareous ones are commonly found in semi-arid and arid regions; they represent nearly 30% of the world's arable soils (Guerinot, 2001). It is known that the harmful effects of the high soil alkalinity are related to the nutrients' shortage; particularly iron (Lindsay, 1984). The most plants would show Fe deficiency when grown in an above medium pH= 5 (Lindsay, 1984). However, this alkalinity is not a characteristic of only calcareous soils, but also of some kinds of salt affected soils, such as sodic soils (Duchauffour, 1983; Mali et al., 2002). Soil salinity is an important issue for plant health. Worldwide, approximately 7% of the land area, or 930 million hectares, is negatively affected by elevated concentrations of salts (Sultana et al., 1999; Szabolcs, 1994; Wang et al., 2003). Salinity excess is toxic to plants, affecting plant growth. One of the important impacts of salinity is creating a physiological drought in plants (Munns et al., 1995). Furthermore, recent studies have demonstrated that salinity stress reduces the capacity of plants to absorb Fe from alkaline soils (Yousfi et al., 2007). Iron plays an important role because of its unique physico-chemical properties. One-third of the world's lands suffer from Fe deficiency and have production loss because of the paucity of readily available soluble Fe (Ma and Nomoto, 1996). Iron is an essential element for living organisms since it is required for many vital enzymes, such as the cytochromes in the

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electron transport chain as well as a wide range of other biological functions (Mori, 1999; Takagi, 1990). Furthermore, Fe is a requirement for plants for metabolic functions such as respiration, DNA synthesis, photosynthesis and nitrogen fixation (Sharma, 2006).

One strategy for understanding the ability of plants to environmental stress tolerance is the recognition of stress-induced changes in their protein profile, presuming that adaptation to stress induced changes of gene expression (Kawasaki and Borchert, 2001). In fact, gene expression and enzyme activity of various proteins (like nitrate reductase) is responsible to internal and external factors (Mahboobi et al., 2002). A deficient supply of one element such as iron usually produces correlative changes in the rate of protein biosynthesis or breakdown as described by Suzuki et al. (1998).

The specifically synthesized protein under salt stresses appears to have a role in providing tolerance adaptation to plants. However, the overall mechanism of how the proteins could provide adaptation is not clearly understood (Dubey, 1990). Elsamad and Shadad (1997) stated that the levels of protein differ in salt tolerant and sensitive genotypes under salinity stresses. Besides, the resistant cultivar showed extra protein bands, while it was absent in salt sensitive genotype. It is imperative that stress proteins may have a role in providing plants with ability to cross-adapt, at least in selected instance, that stress proteins play crucial role for assisting the cells to carry out their metabolic activities during adverse condition (Gomathi and Vasantha, 2006).

Considering the importance of chamomile as medicinal plant, such inference can be made that this plant can be a valuable subject for studies and pharmaceutical, medical and industrial food research is raised. Hence, identification of proteins that are involved in stress tolerance of plants and also comparism of these proteins in two sensitive and resistant genius of chamomilla plant in the headway inbreeding goals can be very effective.

MATERIALS AND METHODS

Plant growth

The experiment was laid-out in a factorial experiment based on completely randomized block design with three replicates, in 2010. Experimental factors were the two chamomile genera *Matricaria chamomilla* and *Anthemis nobilis*, four levels of salinity (0, 50, 100 and 150 mM NaCl) and two levels of iron (0 and 100 mM). Seeds of chamomilla (*A. nobilis* L. and *M. chamomilla* L.) were surface-sterilized by 1% (v/v) sodium hypochlorite for 10 min. Surface-sterilized seeds were germinated in the dark on moistened sand with distilled water and the resulting seedlings were transferred to containers with the following continuously aerated standard nutrient solution: 0.7 mM K_2SO_4, 0.1 mM KCl, 2 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 10 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.2 µM $CuSO_4$ and 0.01 µM $(NH_4)_6MO_7O_{24}$ (Mori and Nishizawa, 1987).

The plants were grown under greenhouse conditions with a photoperiod of natural daylight, maximum and minimum temperatures of 26 and 18°C, respectively and average relative humidity of 70%. Four salinity treatments (0, 50, 100 and 150 mM NaCl) were imposed to the nutrient solution after the plants were ten days old. Iron treatment was the same nutrient solution without 100 µM Fe and standard nutrient solution containing 100 µM Fe. The culture solution was weekly refreshed and its pH was initially adjusted to 6.5. After 30 days of salt treatment, the plants were harvested, the roots and shoots were separated and the specimen was prepared for protein extraction.

Protein extraction for SDS-PAGE

The protein extracts were prepared essentially according to Sambrook et al. (1989). In brief, at first 0.2 g of fresh leaf or root tissue was homogenized in liquid nitrogen. Then 900 µl of extraction buffer [50 mM Tris-HCl, 2%(w/v) SDS, 0/008%(w/v) Comassie brilliant blue (G250), 30% glycerol, 7% 2-mercapto-ethanol] was added to each sample and were transferred into 2 ml tubes. Tubes were vortexed for 1 min. The samples were heated for 5 min at 94°C and then were vortexed again for 1 min. Afterwards tubes were centrifuged at 14000 *g* for 10 min and the supernatants discarded and transferred into new tubes. Finally, the supernatant was prepared assay with the method of Bradford (1976) and SDS-PAGE.

SDS-PAGE

One-dimensional gel electrophoresis was performed according to Mostafaye (2004). The separation performed with 10% separating gel containing (20 ml separating gel buffer (1.5 M Tris-HCl pH= 8.8, 0.4% SDS), 25.97 ml acrylamid solution (30%:0.8[w/v] acrylamid: bisacrylamid), 33.3 ml H2O, 800 µl of 10% APS, 355.5 µl of 10% Temed and 4% stacking gel containing 2 ml stacking gel buffer (0.5 M Tris-HCl pH= 6.8, 0.4% SDS), 1.3 ml acrylamid solution (30%:0.8 [w/v] acrylamid:bisacrylamid), 6.1 ml H2O, 100 µl of 10% APS, 30 µl of 10% Temed. The gel was prerun for 15 min in Tris-glycin buffer (0.25 M Tris-HCl, 1% SDS, 2 M Glysin). Samples containing 150 µg proteins were boiled for 5 min at 100°C and then loaded on the gel. The gels were run at 50 mA for 6 h until the dye reached the end of the gel. After electrophoresis, the gel was stained with coomassie brilliant blue (G-250) according to Mostafaye (2004). The band profile was scored as presence (1) or absence (0) of protein polypeptide bands. Relative molecular weight of each protein was determined using standard protein marker (SM0661).

RESULTS AND DISCUSSION

Polypeptide electrophoretic pattern of *M. chamomilla* and *A. nobilis* leaves

The molecular weight of protein in *M. chamomilla* and *A. nobilis* leaves were presented between 14 to 48 kDa (Figure 1) and 15 to 66 kDa (Figure 2), respectively. The pattern of proteins extracted from*, M. chamomilla* leaves of stress-treated (salt and Fe-deficiency) had no difference with the control plant, except for the bands of 14 and 18 kDa molecular weight that appeared in salt and Fe-deficiency treatment (Figure 1). But, in *A. nobilis* of stress-treated (salt and Fe-deficiency) we observed significant changes compared with the control plant.

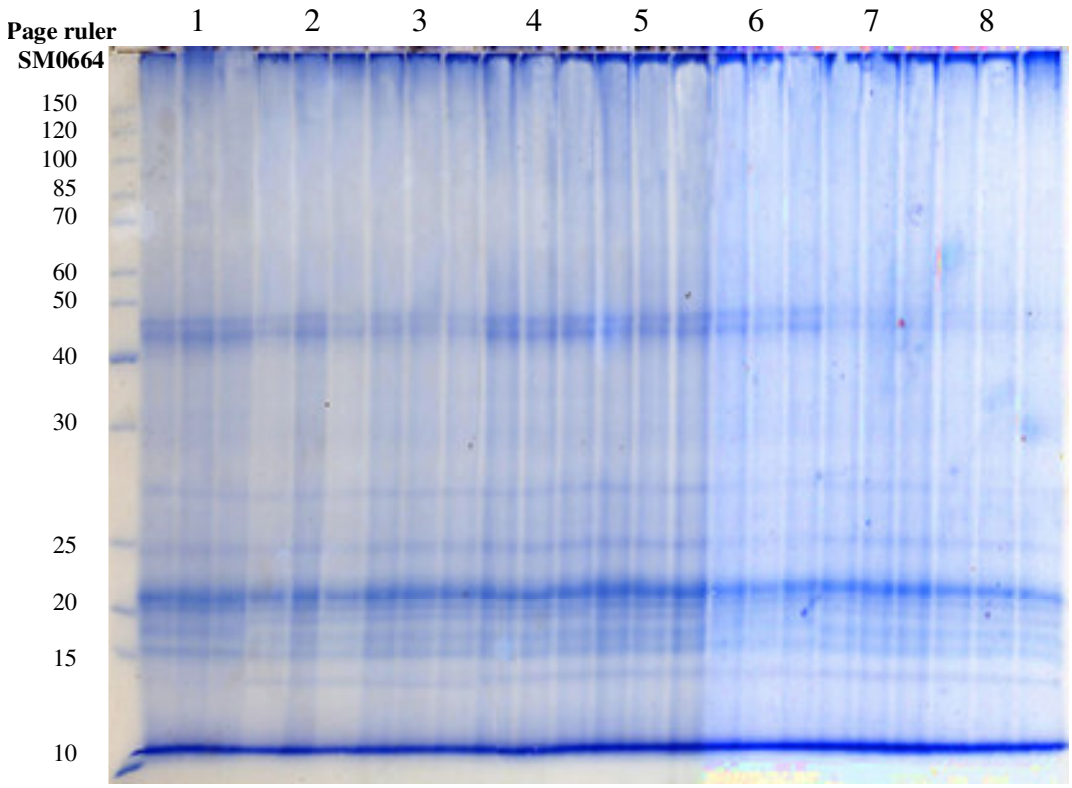

Figure 1. Electrophoretic pattern of protein bands of *M. chamomilla* leaves. Every three columns are corresponding to one treatment. 1: control plant; 2, 3 and 4: 50,100 and 150 mmol/l NaCl; 5: Fe-deficiency; 6, 7 and 8: 50,100 and 150 mmol/l NaCl and Fe-deficiency.

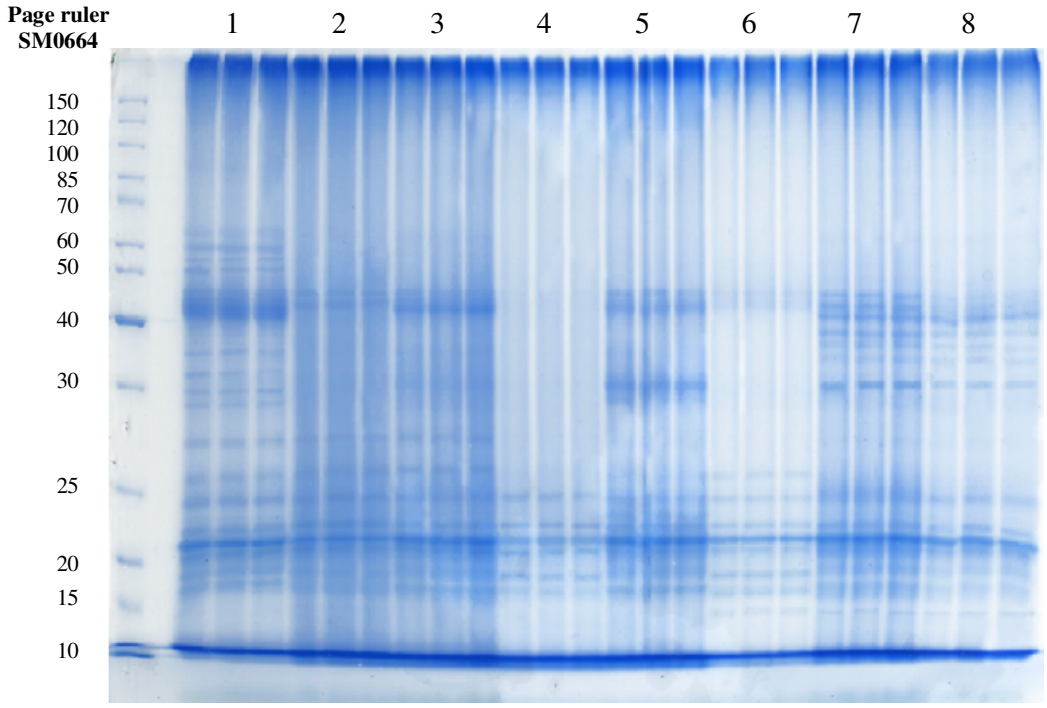

Figure 2. Electrophoretic pattern of protein bands of *A. nobilis* leaves. Every three columns are corresponding to one treatment. 1: control plant; 2, 3 and 4: 50,100 and 150 mmol/l NaCl; 5: Fe-deficiency; 6, 7 and 8: 50,100 and 150 mmol/l NaCl and Fe-deficiency.

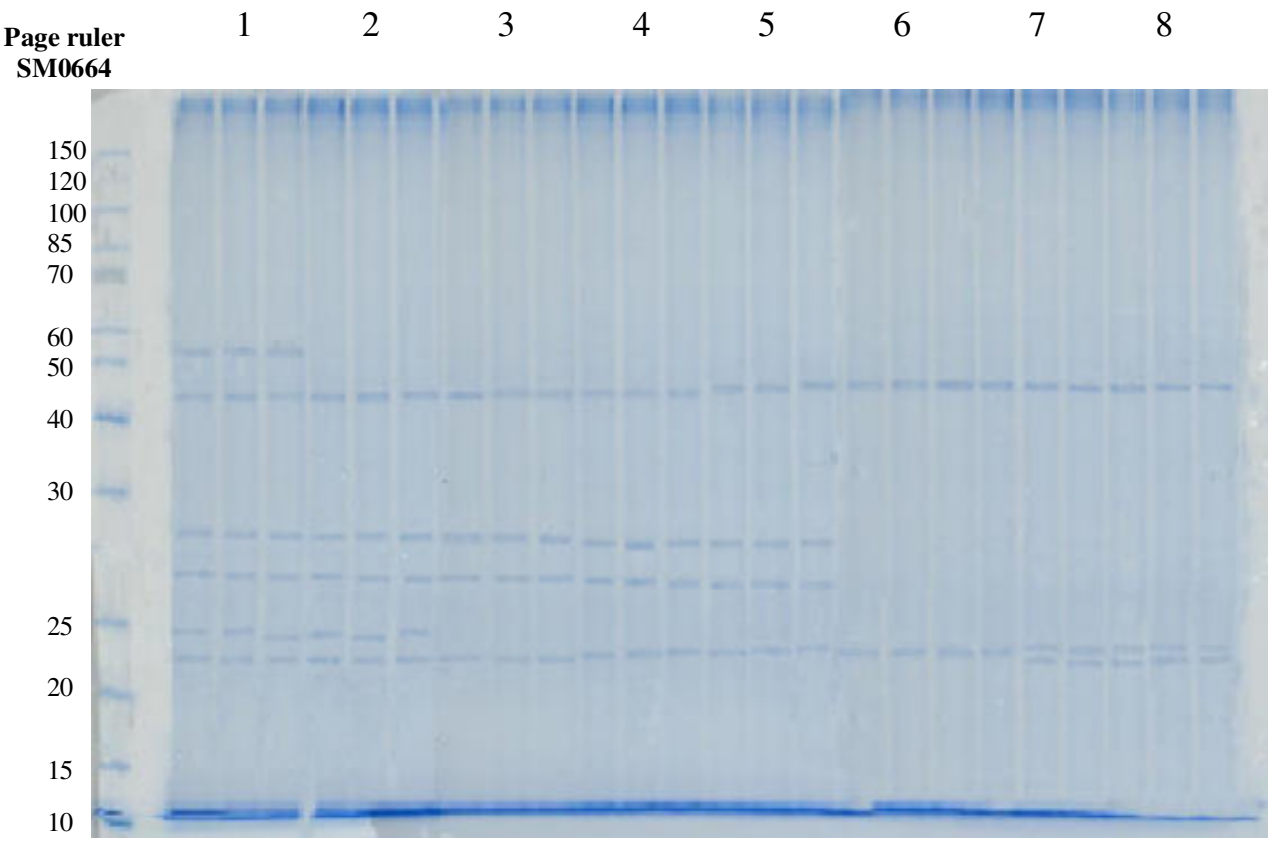

Figure 3. Electrophoretic pattern of protein bands of *M. chamomilla* roots. Every three columns are corresponding to one treatment. 1: Control plant; 2, 3 and 4: 50,100 and 150 mmol/l NaCl; 5: Fe-deficiency; 6, 7 and 8: 50,100 and 150 mmol/l NaCl and Fe-deficiency.

Many of the protein bands in the *A. nobilis* of stress effect disappeared. In the SDS-PAGE of *A. nobilis* treated with 50 and 100 mmol/l NaCl compared with the control plants, 6 protein bands were absent. Instead, a new band appeared in 42 kDa molecular weight (Figure 2). In 150 mmol/l NaCl, more of the protein bands and any protein synthesis disappeared (Figure 2). Therefore, in 150 mmol/l NaCl, plant resistance was broken. Under Fe-deficiency stress, 8 protein bands disappeared and a new protein (29 kDa MW) appeared (Figure 2). Under interaction between Fe-deficiency and 50 mmol/l NaCl, 15 and 42 kDa protein bands appeared. In Fe-deficiency and 100 and 150 mmol/l NaCl, 9 protein bands disappeared, but 15, 37, 40 and 42 kDa MW protein bands appeared (Figure 2).

Polypeptide electrophoretic pattern of *M. chamomilla* and *A. nobilis* roots

Molecular weight of *M. chamomilla* and *A. nobilis* roots proteins were between 22 to 57 and 15 to 47 kDa, respectively (Figures 3 and 4). Comparing the protein profiles between control plant and those treated with different salt concentrations and Fe-deficiency using SDS-PAGE showed that NaCl and Fe-deficiency treatment induced only few changes in the pattern of *M. chamomilla* roots proteins (Figure 3). It was found that in all treatments, the protein bands of 53 and 57 kDa and under interaction between salt and Fe-deficiency the protein band of 27 and 28 kDa that observed in the control plants, disappeared. While, new polypeptide of 21 kDa MW under interaction of salt and Fe-deficiency appeared (Figure 3). In *A. nobilis* roots, compared with the control plants under 50 mmol/l NaCl, 8 and under 100 and 150 mmol/l NaCl, 9 protein bands disappeared (Figure 4). Under Fe-deficiency, new polypeptides of 28 and 29 kDa MW appeared and 4 protein bands disappeared. In *A. nobilis,* 8 protein bands disappeared under interaction of NaCl and Fe-deficiency (Figure 4). In *A. nobilis* roots like leaves, more proteins disappeared and only under Fe-deficiency a new protein appeared.

In this study, we have shown that *A. nobilis* is a sensitive plant, since more proteins were lost and fewer proteins were synthesized compared to *M. chamomilla*. Ericson and Alfinito (1984) examined the protein pattern of NaCl adapted and non adapted tobacco cell lines and reported extra protein bands with 32 and 20 kDa mole-

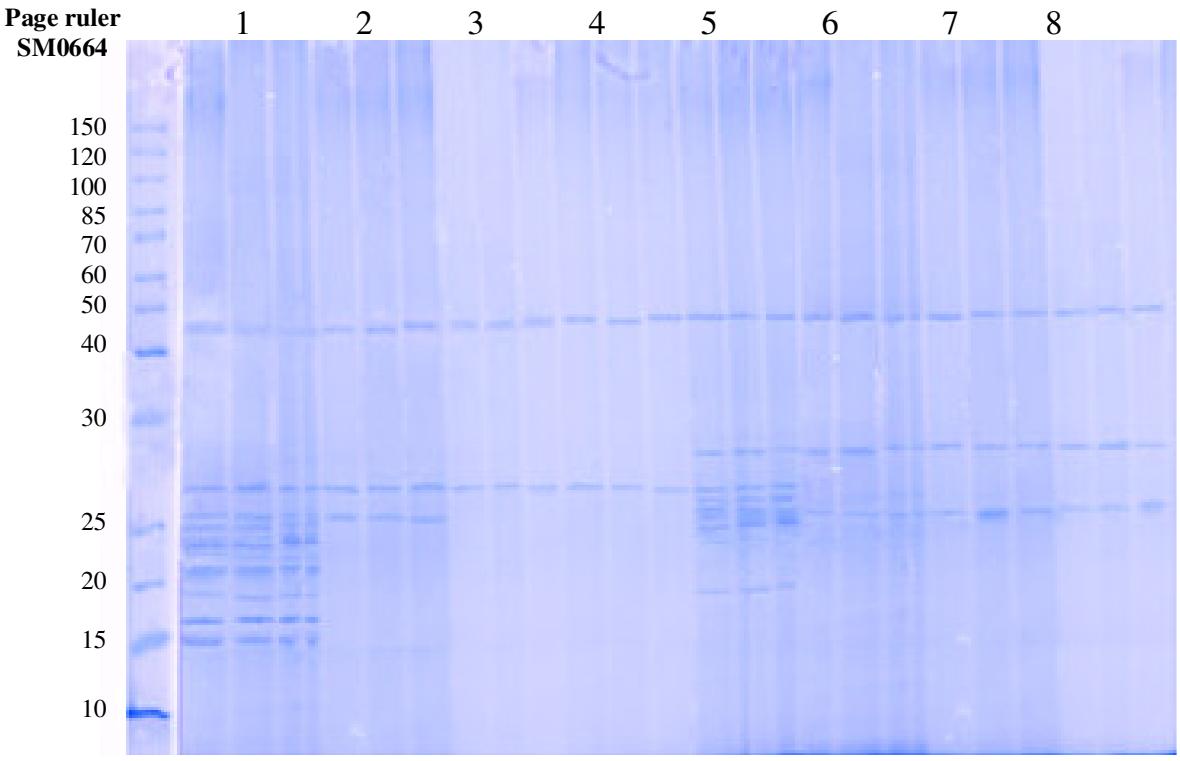

Figure 4. Electrophoretic pattern of protein bands of *A. nobilis* roots. Every three columns are corresponding to one treatment. 1: Control plant; 2, 3 and 4: 50,100 and 150 mmol/l NaCl; 5: Fe-deficiency; 6, 7 and 8: 50,100 and 150 mmol/l NaCl and Fe-deficiency.

cular weight, while it was absent in non adapted cell lines. The protein bands with 20 and 32 kDa MW was also presented in *M. chamomilla* and *A. nobilis* leaves but in *A. nobilis* 32 kDa band under whole stress and 20 kDa band under Fe-deficiency and 100 and 150 mmol/l NaCl was lost. Radha and Reddy (1994) observed the presence of additional 26 kDa protein band associated with salt tolerance in rice, named as osmotin. Other researchers also uphold this problem (Singh et al., 1985; Popova et al., 1995; Elennany, 1997). The role of osmotin is to provide osmotic adjustment to the cells either by facilitating the accumulation of solutes or by providing certain metabolic alteration in the cell and thus, might be responsible for the development of stress tolerance (Dubey and Pessarakali, 1995). 26 kDa protein band (Osmotin) was presented in the control plants, 50 mmol/l NaCl, Fe-deficiency treatment and interaction of Fe-deficiency and 50, 100 and 150 mmol/l NaCl in *A. nobilis* roots.

Protein profile showed differences between used genera. All two genus are clearly identifiable from the protein-banding pattern. An SDS-PAGE protein profile is, therefore, an efficient procedure for differentiating chamomile genotypes. Ahmad et al. (1986) believes that salt will decrease the rate of protein synthesis. It has negation effect involved destruction transcriptional mechanism and translation. Various investigators suggested that decrease of protein content is attributed to the decreased 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 (Dubey, 1994; Dubey and Rani, 1990). The obtained results are in agreement with the results of Schmidt and Buchout (1997), which an alteration in the pattern of protein synthesis in tomato roots grown under iron deficiency stress as a result of adaptive response specific to Fe-deficiency was reported. Suzuki et al. (1998) reported that Fe-deficiency stress specifically induced an appearance of several new proteins in barley roots. Rengel and Hawkesford (1997) also detected new polypeptides in wheat genotypes exposed to zinc deficiency, which may be possibly linked to a resistance mechanism. Conclusively, an alteration in the pattern of protein synthesis in *A. nobilis* and *M. chamomilla* under stress condition is due to specific adaptive response to stress.

Conclusion

Protein fingerprinting of tolerant genotypes is very important for the prediction of genotypes efficiency to salt and iron. It could be possible to combine tolerance with other desirable traits of crop to e volve new varieties much

better adapted to abiotic stress.

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