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PROTEIN PROFILE OF CLUSTERBEAN [CYAMOPSIS TETRAGONOLOBA (L.)TAUB.] GENOTYPE UNDER SALINITY STRESS

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Abstract

Clusterbean [Cyamopsis tetragonoloba (L.) Taub.] genotype HG-884 seeds were grown under chloride dominated salinity levels (0, 4, 8, 12, 16dSm^{-1}). Radicle and plumule proteins profile was analyzed by SDS-PAGE. In SDS-PAGE the protein profile of radicle of HG-884 genotype showed the de novo synthesis of three, (79.45, 72.46 and 29.86 kDa) one (59.58 kDa) and one (7.50 kDa) protein bands at 4, 8 and 12 dSm^{-1} level of salinity respectively observed and at 16 dSm^{-1} level protein bands remain the same as 12 dSm^{-1} level. In plumule the de novo synthesis of three (92.28, 79.45 and 60.96 kDa) one (15.30 kDa) and one (7.50 kDa) protein bands at 4, 8 and 12 dSm^{-1} salinity level respectively was observed and at 16 dSm^{-1} level proteins bands remains the same as 12 dSm^{-1} level.

Key words: Clusterbean, (Cyamopsis tetragonoloba), HG-884, salinity, SDS-PAGE, protein profile

I. Introduction

Soil salinity is one of the major abiotic stresses which restricts the distribution and productivity of crops. No climatic zone in the world is free from salinization although the general perception is focused on arid and semi-arid region. Total global area of salt affected soils, including saline and sodic soils is 831 million hectare (Martinez-Beltran and Manzur, 2005). In India about 6744968 hectares of land is affected with salinity, whereas, it accounts for 232556 hectares in state of Haryana (CSSRI, ICAR, Govt. of India, 2012). Therefore, it poses serious problem to food security in developing countries like India due to high rate of population growth and stagnation due to declining crop productivity in high productivity areas (Kamaludin and Abdin, 2006). The excessive presence of these salts in the soils adversely affects several plant processes that in turn affect crop yields.

Clusterbean [Cyamopsis tetragonoloba (L.)Taub.] locally called guar, is a summer annual legume introduced from tropical Africa to India. Guar belonging to the family Papillionaceae/Fabaceae, is a robust annual with long tap root and well developed laterals. Guar pods are very good source of vitamin a, calcium, iron, phosphorus and ascorbic acid, therefore adding to its nutritive value. Seeds of guar are also an important source of glactomannan gum which is extracted from the endosperm which constitutes 25-30%of whole seed (Bhadoria et at.,1997). In India, guar is grown as a vegetable for human consumption, as a forage for cattle feed and as a green manure crop. It has emerged as an important industrial crop. Therefore, improvement in the quality of guar seed will be future deciding fate for this crop to gum industry in India. Although the best known use of this gum is as a stabilizer in a number of food products, in various industries like textiles, papers, cosmetics, pharmaceuticals, oil industry and photography. A by product of guar gum industry is also of considerable value because of its high protein content (Francois et al., 1990).

Several new proteins which are synthesized in response to an altered environment have been reported as 'stress proteins' or shock proteins in plants (Ericson and Alfinito, 1984; Kanabus et al., 1984; Oliver and Bewley, 1984). However, only a few of these proteins have been found to be involved in known physiological or metabolic processes (Hanson and Jacobson, 1984;

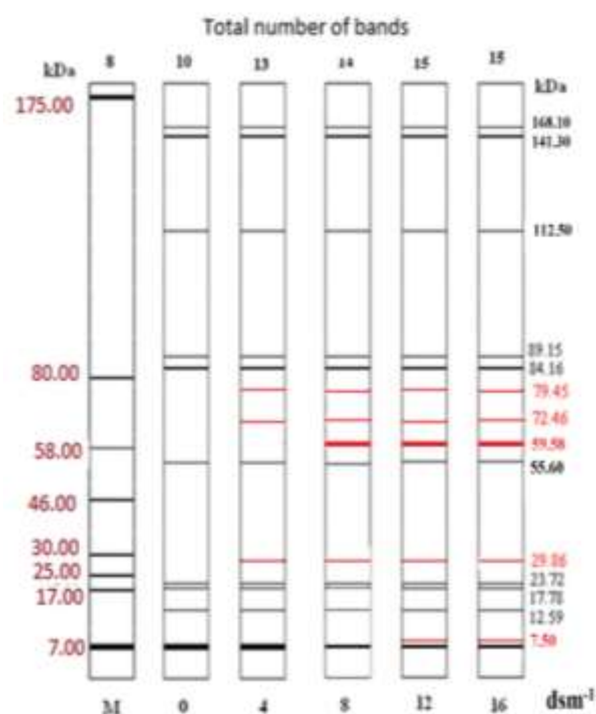
Hasegawa et al., 1984). Most of these proteins appear as an immediate response by the organism to an altered environment and many of them are associated with increased growth and survival of the plants in the new environment.

Several salt induced proteins have been identified in plant species and have been classified into two distinct groups (Mansour, 2000): (i) salt stress proteins, which accumulate only due to salt stress, and (ii) stress associated proteins, which also accumulate in response to heat, cold, drought, water logging, and high and low mineral nutrients. Proteins may be synthesized de novo in response to salt stress or may be present constitutively at low concentration and increase when plants are exposed to salt stress (Pareek et al.,1997).

II. Material and methods

Seeds of clusterbean [Cyamopsis teragonoloba (L.) Taub.] genotype HG-884, were surface sterilized in 0.1٪ sodium hypochloride solution for 2 min subsequently washed twice with distilled water and then 0.2٪ bavistin solution for 2 min subsequently washed twice with distilled water. Fifteen healthy seeds of uniform size were sown in Petri plates (9cm dia) having a filter paper at the bottom socked with 5ml solution of desired salinity (0, 4, 8, 12, 16 dSm^{-1}). Petri plates were kept at the room temperature (May-June months) and natural photoperiod. Each Petri plate was supplied with 2ml distilled water daily up to seven days. Sampling was carried out after 7 days of sowing. Samples were prepared by crushing one g of radicle and plumule in 2.5 ml of chilled tris buffer (0.1 M, pH 8.0) containing 0.1% polyvinyl pyrrolidone (PVP) with the help of chilled pestle and mortar (rinsed with double distilled water and dried). These were then centrifuged at 10,000 rpm at 4°C for 15 min in a refrigerated centrifuge. The supernatant containing the proteins was taken in a chilled test tube and pellet was discarded. Supernatant was stored at 20°C. The protein quantification was done by the method of Bradford (1976). The protein extract was transferred to an equal volume of 2X sample buffer (Laemmli's 2X sample buffer) heated at 100°C for 3 min, cooled and used for SDS-PAGE. Protein quantification was done according to the method given by Bradford (1976). One dimentional sodium dodecyl sulfate polyacrlamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970) by using dual midi vertical slab gel electrophoresis apparatus of Genei. The protein extract was transferred to an equal volume of 2x sample buffer (Laemmili`s 2x sample buffer) heated at 100°c for 3min, cooled and used for SDS-PAGE. An aliquot containing 50µg of sample protein was loaded in each well and marker proteins in separate well. After completion of electrophoresis, staining and background destaining, relative mobility values were calculated for each of marker proteins.

III. Results and discussion



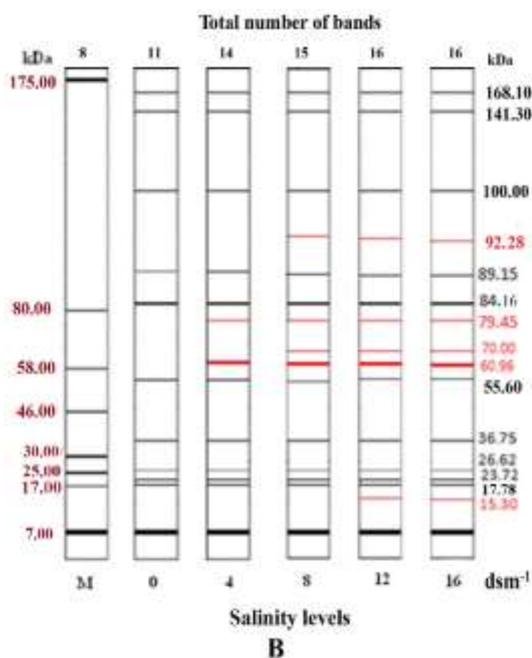


Figure 1: Protein profile of (A) radicle and (B) plumule of guar genotype HG-884 at 0, 4, 8, 12 and 16 dsm^{-1} level of salinity through SDS-PAGE.

M – Protein molecular weight marker

— -- Newly appeared treatment specific bands

It was concluded that protein bands of 79.45 kDa was synthesized de novo at all levels of salinity in radicle and plumule could be important in the adaptation of plant to salt stress.

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synthesized de novo that presumably are critical of plant adaptation to stress condition. Similar results were obtained in tobacco where a 26 kDa polypeptide was found due to salt stress and suggested its possible role in osmoregulation (Singh et al., 1985). In the Protein profile of leaves of the isabgol genotype GI-2 three new protein bands of the MW 29.59, 54.95 and 55.72 kDa and in the genotype HI-96 two new protein bands of the MW 29.59 and 55.72 kDa were

In radicle of guar genotype HG-884 under non-saline (control) treatment a total number 10 protein bands were observed ranges from 168.10 to7.00 kDa (fig.1A). At 4dSm^{-1} 3 new proteins were synthesized (i.e. 79.45, 72.46 and 29.86 kDa) in comparison to control. At 8 and 12 dSm^{-1} level of salinity one new protein at each level i.e. 59.58 and 7.50 kDa respectively were synthesized and at 16 dSm^{-1} level total number of bands 15 remain the same as 12 dSm^{-1} level. Protein profile of plumule of genotype HG-884 showed total number of 11 protein bands under non-saline (control) treatment ranges from 168.10 to 7.00 kDa (fig. B). In plumule also 3 new proteins were synthesized at 4 dSm^{-1} level (i.e. 92.28, 79.45 and 60.96 kDa) in comparison to the control. One each i.e. 70.00 and 15.30 kDa protein was synthesized at 8 and 12 dSm^{-1} level of salinity respectively. At 16 dSm^{-1} level the total number of proteins bands 16 remains the same as 12 dSm^{-1} level.

Depressed protein synthesis and acceleration its degradation in plants in response to salt stress has been reported by number of workers (Chandershekar et al., 1986, Lal and Bhardwaj, 1987). Amzallag and Lerner (1994) reported 76.3 to 14.6 kDa protein bands were apparently.

V. Conclusion

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detected under salt stress. While in the genotype PB-80 one protein band of MW 30.06 kDa was disappeared and one new protein band of MW 43.65 kDa was appeared under salt stress (Suraj Kala and Varshney, 2014). Genotype HI-5 showed three new protein bands of MW 43.65, 55.72 and 66.07 kDa and disappearance of one protein band of MW 32.36 kDa under salt stress. Bishnoi et al. (2006) have clearly shown that NaCl treatment induced 95.6 kDa proteins in

plumule and 67.5 kDa proteins in radicle of salt tolerant cultivar of Cajanus cajan. Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is re-utilized when stress in over (Singh et al., 1987) and may play a role in osmotic adjustment

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