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Alterations in metabolites during germination and seedling growth of groundnut (*Arachis hypogaea* L.) genotypes in response to chloride based salt stress

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SUMMARY

Groundnut genotypes were germinated *in vitro* under chloride dominant salt stress (0, 20, 40, 80 m eq/l) in seed germinator at 28 ± 2^0 C. Chloride based salinity decreased the seedling vigour index of all groundnut genotypes, and the decreases were found more in GG-7, GG-20, GG-2 genotypes (susceptible group) at 4 and 8 days after sowing (DAS). With increasing salinity regimes, various metabolites like free amino acid and free proline contents were deposited at higher rate in seedlings of JL-24, GAUG-10, GG-13 genotypes (tolerant group) compared to susceptible ones for better osmotic adjustment. However, chloride based salinity decreased the accumulation of total sugars and free fatty acid contents in the seedlings of all groundnut genotypes at 4 and 8 DAS. The decrease in sugar content was found more in susceptible genotypes than tolerant once. SDS-PAGE of soluble proteins at 8 DAS indicated five protein bands in control (0 m eq/l) of all groundnut genotypes, and, it increased to ten bands in tolerant genotypes with higher salinity regimes (40, 80 m eq/l). Activities of alpha-amylase decreased but that of protease and peroxidase increased under salt stress at both the stages in all groundnut genotypes. This increase or decrease of enzymes activities due to salt stress reflected the level of its respective metabolites in the seedlings of groundnut genotypes.

Key words : Groundnut, Chloride based salinity, Vigour index, Metabolites, Alpha-amylase, Protease, Peroxidase, Salt tolerance

Salt stress is a worldwide problem. In arid and semi-arid regions, soil salinity is a common occurrence. The use of poor irrigation water and salt water encroachment is also increasingly threatening agriculture in humid regions (Syvertsen *et al.,* 1989) From an agricultural point of view, salinity is the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth (Gorham, 1992). Salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. Estimates vary, but approximately 7% of the world's total land area is affected by salinity (Flowers *et al.,* 1997). Furthermore, there is also a dangerous trend of a 10 % per year increase in the saline area throughout the world (Pannamieruma, 1984).

Salinity is known to induce imbalance of metabolism in crop plants (Levitt, 1980). Constraints on growth of glycophytic plants in saline environments are categorized into three major factors – water deficit; effect on energy balance; ion toxicity and nutrition imbalance (Gorham *et al.*, 1985; Pasternak, 1987; Ashraf, 1994). Alterations of various cellular processes such as activity of enzymes, photosynthesis and degradation of macromolecules by NaCl are well documented (Levitt, 1980; Saha and Gupta, 1993). Furthermore, general effects of salinity on carbohydrate metabolism, water relations, proteins, enzymatic systems and other physiological aspects have been proved to be controversial and inclusive especially in different types of salinity.

Groundnut (*Arachis hypogaea* L.) is an important oilseed, food and feed crop of India. Salinity is one of the important abiotic stresses which affect all stages of groundnut growth and finally the yield. Understanding the biochemistry of salinity response in groundnut plants may help in identifying genotypes that are better adapted to saline conditions. Hence, the present investigation was undertaken to study the effect of chloride based salinity, created by mixing different salts, on various metabolites, enzymes and proline content during early seedling growth in six genotypes (JL-24, GG-2, GG-7, GAUG-10, GG-13 and GG-20) of groundnut to assess the relative tolerance of these genotypes.

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MATERIALS AND METHODS

Seeds of six genotypes (V_1 - JL-24, V_2 – GG-2, V_3 – GG-7, V_4 – GAUG-10, V_5 – GG-13 and V_6 – GG-20) of groundnut (*Arachis hypogaea* L.) were surface sterilized by soaking in 0.1 % sodium hypochlorite for 2

min and thoroughly washed with deionized water to avoid fungal infection. Fifty seeds of each genotypes were germinated in control and chloride dominant salt solutions in sterilized Petri dishes (15 cm diam) lined with blotting papers and kept at $28 \pm 2^{O}C$ in seed germinator under controlled conditions. The chloride dominant salt solution was prepared by taking 1N of NaCl : Na_2SO_4 : $MgCl_2.6H_2O$: $MgSO_4.7H_2O$: $CaCl_2.2H_2O$ in the ratio of 7.33 : 4.67 : 1.0 : 2.0 : 5.0 for 20 m eq/l which comprises 13.3 m eq Cl/l and 6.7 m eq SO_4 /l ions concentration. Accordingly, 40 and 80 m eq/l chloride dominant salt solutions were also prepared. Thus, the concentrations of saline solutions- 20, 40 and 80 m eq /l were used for salt stress. Total four treatments were arranged for chloride based salt stress as $-T_1$ - 00 m eq /l (*i.e.* Distilled water, Control), T_2 - 20 m eq /l, T_3 -40 m eq /l, T_4 -80 m eq /l.

Seedling of 4 and 8 days after sowing (DAS) were taken out, washed with distilled water and placed between filter paper for removing external moisture. Entire seedling including cotyledon was used for determination of various physiological and biochemical parameters. Experiment was conducted in three replications of Completely Randomized Design with Two Factors (I – Varieties; II - Treatments of salt stress) to interpret the data (Snedecor and Cochran, 1967).

Seeds of groundnut genotypes were allowed to germinate as described previously and shoot length were recorded at 4 and 8 DAS. Vigour index was calculated as germination per cent multiplied with shoot length (ISTA, 1976). Plant material (0.5g) was used for the estimation various biochemical parameters. Total sugars was determined by the method described by Dubois *et al*. (1956). Free amino acid and free fatty acid estimations were carried out as methods out lined by Lee and Takahashi (1966), and Cox and Pearson (1962), respectively. The method developed by Bates *et al.* (1973) was used for quantification of proline. The enzyme activities were assayed following the modified method of Bernfeld *et al*. (1955) for alpha-amylase (E.C. 3.2.1.1), Malik and Singh (1980) for protease (E.C. 3.4.21.25) and peroxidase (E.C. 1.11.1.7). The enzymatic activity was expressed as U/g Fr. Wt. and Unit activity was defined as mg maltose released per 20 min for alpha-amylase, mg peptides released per 20 min for protease and OD per min for peroxidase. Groundnut seedlings at 8 DAS were homogenized in 0.1M phosphate buffer (pH 7.2) to extract soluble proteins. SDS-PAGE was carried out for soluble proteins according to method described by Laemmi (1970). After electrophoresis at 30 mA, gel was stained with coomassie brillient blue R – 250 to visualize the bands, and the relative mobility of the different protein bands were photographed.

RESULTS AND DISCUSSION

The vigour index in terms of germination per cent and shoot length of groundnut seedlings showed a gradual decreased under chloride dominant salinity ranging from 20 to 80 m eq/l in all genotypes at 4 and 8 DAS (Fig. 1A, 1B). The maximum seedling vigour was observed in JL-24 followed by GAUG-10, GG-13, GG-20, GG-7 and GG-2 at 8 DAS under control and saline conditions. Interaction effect between genotypes and treatments indicated that genotype JL-24 was found superior followed by GAUG-10 under chloride based salinity for their vigour index. Mondal *et al.* (1988) observed plumule and radicle length of rice gradually decreased with increasing salinity. Higher salinity also decreased the seedling growth of barley (Kumar *et al.*, 1988).

Various biochemical parameters studied during the present experimentation showed that there was significantly increase in free amino acid and free proline contents with increasing salinity levels during 4 and 8 DAS (Table 1), however, reverse trend was observed for total sugars and free fatty acid contents during both the stages. Total sugars decreased in seedling of all groundnut genotypes under chloride based salt stress, and the depletion of sugar content was higher in susceptible genotypes. The decrease in sugar content under NaCl stress was also observed in sugarbeet (Iyengar and Pandya, 1973), mustard (Singh *et al.*, 2005) and wheat (Bharadwaj, 1958)). A comparative account indicated that accumulation of total sugars (Table 1A) in response to salinity was more in tolerant genotypes (Jl-24, GAUG-10) than in susceptible ones during 4 to 8 DAS. Such difference in sugar accumulation was attributed to salt tolerance of the genotypes (Rathert, 1984). Accumulation of sugars during 4 to 8 DAS in all groundnut genotypes may be due to their less utilization in biosynthesis leading to reduction in growth of seedlings and may be used for osmotic adjustment (Yeo, 1981; Yang *et al.*, 1990). The free fatty acid content was significantly decreased in all groundnut genotypes under salt stress during both the stages (Table 1C) which might be due to increase in salinity decreases the mobilization of lipid which resulted to decreased in amount of free fatty acids composition in the seedlings. Similar pattern was also observed in sunflower seedlings under salt stress conditions (Maiti *et al.*, 2005).

Chloride salinity regimes gradually increased free amino acid and free proline contents in all groundnut genotypes. The response of genotypes under different

Table 1 : Effect of chloride dominant salt stress on contents of various metabolites at 4 and 8 DAS in groundnut genotypes

Groundnut Genotypes	4 DAS					8 DAS				
	T_1	T_2	T_3	T_4	Vx	T_1	T_2	T_3	T_4	Vx
[A] Total sugars (mg/g, Fr. Wt.)										
JL-24	25.2	21.1	17.5	15.1	19.7	60.9	56.8	53.7	50.8	55.6
GG-2	27.0	21.9	17.3	13.4	19.9	62.7	57.6	53.0	49.1	55.6
GG-7	25.9	20.7	17.3	12.7	19.2	59.2	55.6	53.0	49.1	54.2
GAUG-10	24.4	22.1	17.7	13.3	19.4	60.1	56.4	53.4	49.4	54.8
GG-13	22.7	20.7	20.0	12.6	19.0	58.4	56.4	55.7	51.7	55.6
GG-20	27.0	21.9	18.0	14.5	20.4	5.92	57.6	53.7	49.8	55.1
Tx	25.4	21.4	18.0	13.6		6.01	56.7	53.8	50.0	
C.D. (P=0.05)		V = 0.50; T = 0.40; V x T = 0.90					V = 0.40; T = 0.30; V x T = 0.70			
[B] Free amino acids (mg /g, Fr. Wt.)										
JL-24	2.64	2.31	2.78	3.07	2.70	7.31	7.79	8.08	8.81	8.00
GG-2	2.14	2.35	2.64	3.01	2.53	7.15	7.36	7.65	8.02	7.54
GG-7	2.02	2.27	2.38	2.92	2.39	7.03	7.28	7.39	7.93	7.40
GAUG-10	2.25	2.40	2.64	2.98	2.56	7.25	7.41	7.65	7.99	7.58
GG-13	2.01	2.34	2.54	2.01	2.22	7.02	7.35	7.55	7.98	7.47
GG-20	1.98	2.37	2.64	2.94	2.48	6.99	7.38	7.66	7.93	7.49
Tx	2.17	2.34	2.60	2.82		7.13	7.43	7.66	8.11	
C.D. (P=0.05)		V = 0.16; T = 0.13; V x T = 0.33					V = 0.13; T = 0.11; V x T = NS			
[C] Free fatty acids (mg/g, Fr. Wt.)										
JL-24	71.0	59.7	54.7	48.5	58.5	134	123	118	111	121
GG-2	82.5	69.3	59.7	57.0	67.2	145	132	123	120	130
GG-7	81.2	65.4	65.4	54.1	66.5	144	128	128	117	129
GAUG-10	82.2	71.0	59.7	42.9	64.0	145	134	123	106	127
GG-13	76.6	71.0	65.4	48.8	65.4	139	134	128	112	128
GG-20	83.3	78.8	71.9	41.2	68.8	146	142	135	104	132
Tx	7.95	6.92	6.28	48.8		142	132	126	112	
C.D. (P=0.05)		V = 3.80; T = 3.10; V x T = 7.70					V = 2.20; T = 1.80; V x T = 4.50			
[D] Free proline content (µg/g, Fr. Wt.)										
JL-24	38.0	55.0	75.0	94.2	65.5	50.6	67.6	87.6	106.8	78.1
GG-2	33.0	50.5	72.6	85.7	60.4	45.6	63.1	85.2	98.3	73.0
GG-7	31.3	49.4	70.2	86.3	59.3	43.8	62.0	82.8	98.9	71.9
GAUG-10	36.2	53.2	74.7	96.1	65.0	48.7	65.8	87.3	108.6	77.6
GG-13	31.3	53.2	75.0	92.0	62.9	43.8	65.8	87.6	104.6	75.5
GG-20	32.4	50.7	64.6	89.6	59.3	45.0	63.3	77.2	102.1	71.9
Tx	33.7	52.0	72.0	90.6		46.3	64.6	84.6	103.2	
C.D. (P=0.05)		V = 1.57; T = 1.28; V x T = 3.14					V = 1.13; T = 0.92; V x T = 2.25			

DAS=Days After Sowing; V= Varieties; T=Treatments [T_1=0 m eq/L, T_2=20 m eq/L; T_3=40 m eq/L; T_4=80 m eq/L]

levels of salinity with respect to these biochemical parameters studied, elicited that the highest amounts of free amino acids accumulation (Table 1B) were found in genotypes JL-24, GAUG-10 and GG-2 in response to salt stress. The free amino acids content of the seedlings were inferior at 4 DAS than it was increased at 8 DAS. Similarly, Sharma *et al.* (1986) reported that salinity increased the pool of free amino acids and proteolytic activity in germinating chickpea. The higher proline content was observed in tolerant genotypes (JL-24, GAUG-10, GG-13) in response to salt treatments (Table 1D). The rate of accumulation of proline content in the seedlings was higher at 8 DAS as compared to 4 DAS. The rate of increase in proline content was found more in tolerant than in susceptible genotypes enabling the former genotypes to cope with the salt stress condition comparatively in an efficient manner. Thus, genotypes under test showed tolerance because of better osmo-regulation due to higher accumulation of proline. Similar increase in proline content has also been found in chickpea

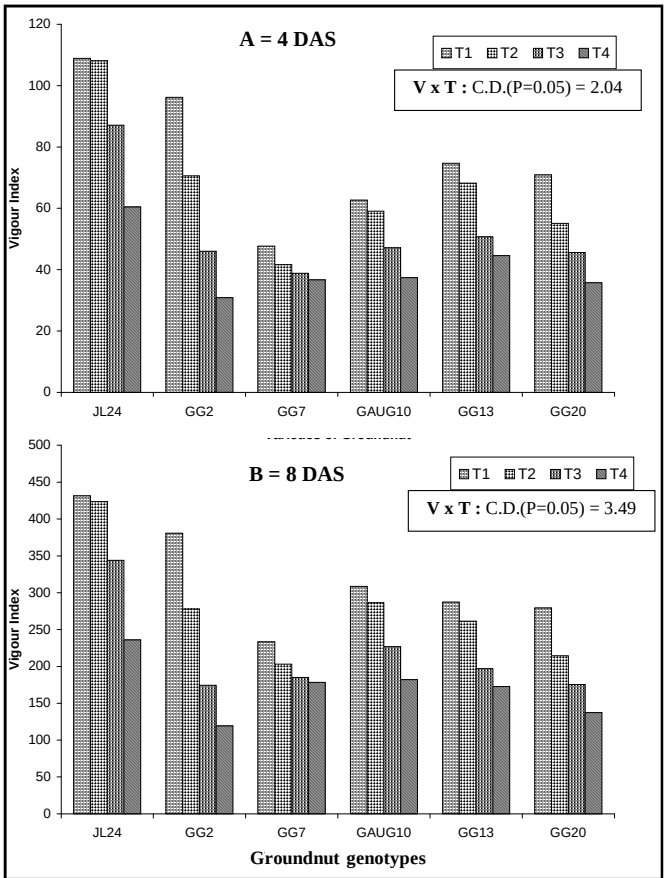

Fig. 1 : Seedling vigour index of groundnut at 4 DAS (A) and 8 DAS (B) in response to chloride dominant salt stress

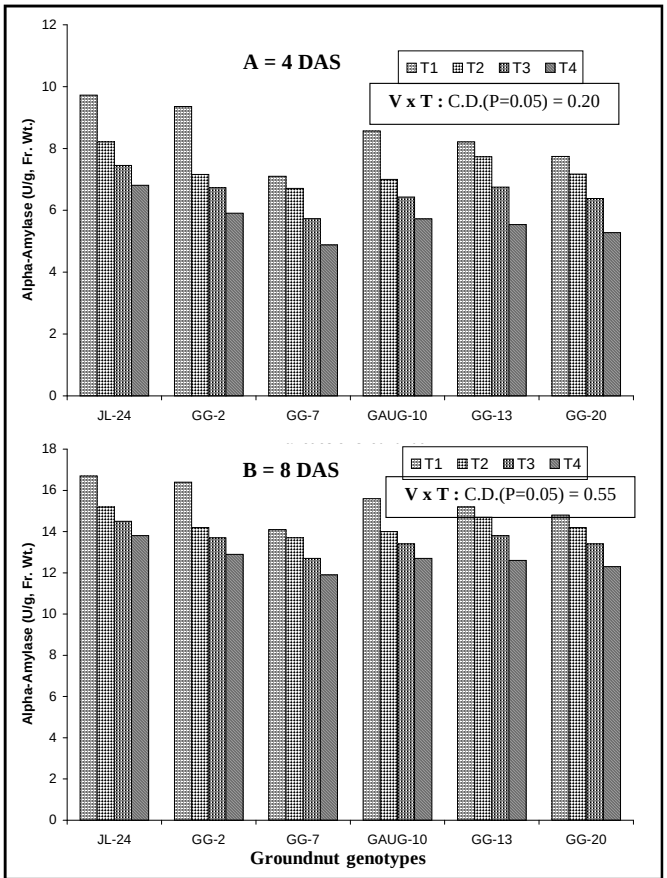

Fig. 2 : Alpha-amylase activity (U/g, Fr. Wt.) in groundnut seedlings at 4 DAS (A) and 8 DAS (B) in response to chloride dominant salt stress

by various workers (Sharma *et al.*, 1990; Varshney and Sangeeta, 1992).

Alpha-amylase activity decreased (Fig. 2) but that of protease (Fig. 3) and peroxidase (Fig. 4) increased under salt stress at 4 and 8 DAS in all the groundnut genotypes. Chloride based salinity decreased alpha-amylase activity and decrease was found more in seedlings of susceptible genotypes (GG-7, GG-20). This decrease was, perhaps, due to substrate limitation in the seedling under salt stress. Reduction in alpha-amylase has been reported in chickpea and rice seedlings (Prakash *et al.*, 1988). The salt tolerant genotypes (Jl-24, GAUG-10) showed less reduction in alpha-amylase activity with progress of salt stress compared to susceptible genotypes. Similarly, Krishnamurthy *et al.* (1987) found lower alpha-amylase activity at higher salt stress in rice seedlings. Dubey (1983) observed that tolerant genotype of rice had higher amylase activity compared to susceptible genotypes. Salt tolerant genotypes germinated more rapidly under saline conditions than susceptible genotypes and showed earlier induction of alpha-amylase activities. Thus, early commencement of alpha-amylase activity may be an adaptive mechanism for salt tolerance germination. Salinity caused decrease in water uptake followed by inhibition in the activity of hydrolytic enzyme- alpha amylase (Dubey, 1982). The decline in the free energy of water caused due to the presence of salts in the medium. Thus, it limits the amylase activity by reducing the water up take. In the present study, the decreased sugar levels with increasing salt stress in all genotypes might be due to less water uptake under salty medium and it also caused less mobilization of sugars from cotyledon to growing organs, such as shoots and roots, to maintain osmotic balance in stressed seedlings.

The increase in protease activity under salinization (Fig. 3) was obtained in all groundnut genotypes at 4 and 8 DAS. Similar pattern of increasing protease activity was also reported earlier (Shigong *et al.*, 1999; Reddy and Vora, 1986). Protease activity, on the other hand, increased relatively at higher rate in susceptible genotypes (GG-20, GG-7) compared to tolerant genotypes (Jl-24, GAUG-10) at 4 and 8 DAS. These differences between the tolerant and susceptible genotypes across salinity stress were highly significant. Increasing protease activity under

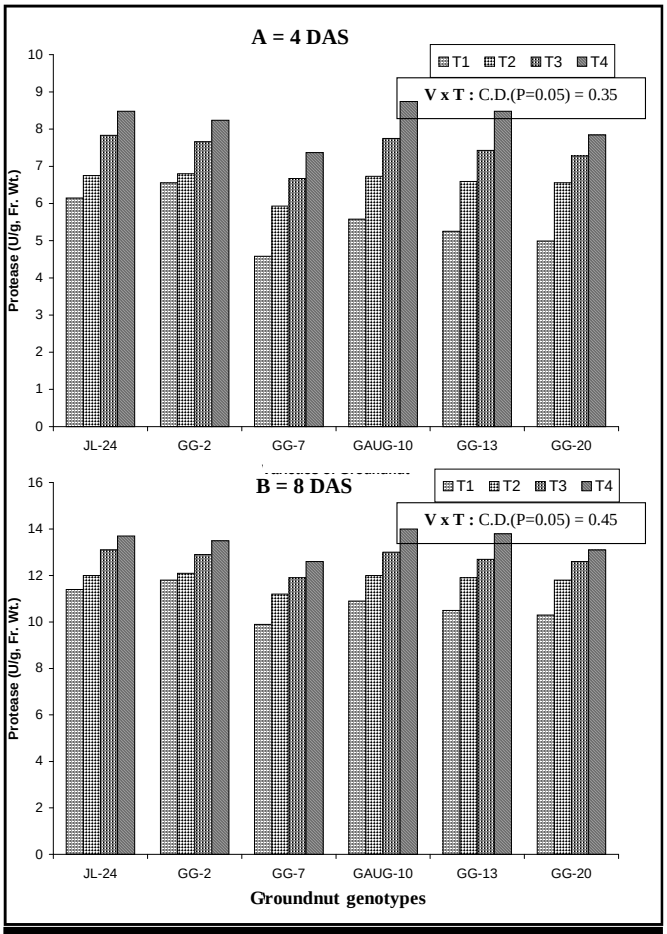

Fig. 3 : Protease activity (U/g, Fr. Wt.) in groundnut seedlings at 4 DAS (A) and 8 DAS (B) in response to chloride dominant salt stress

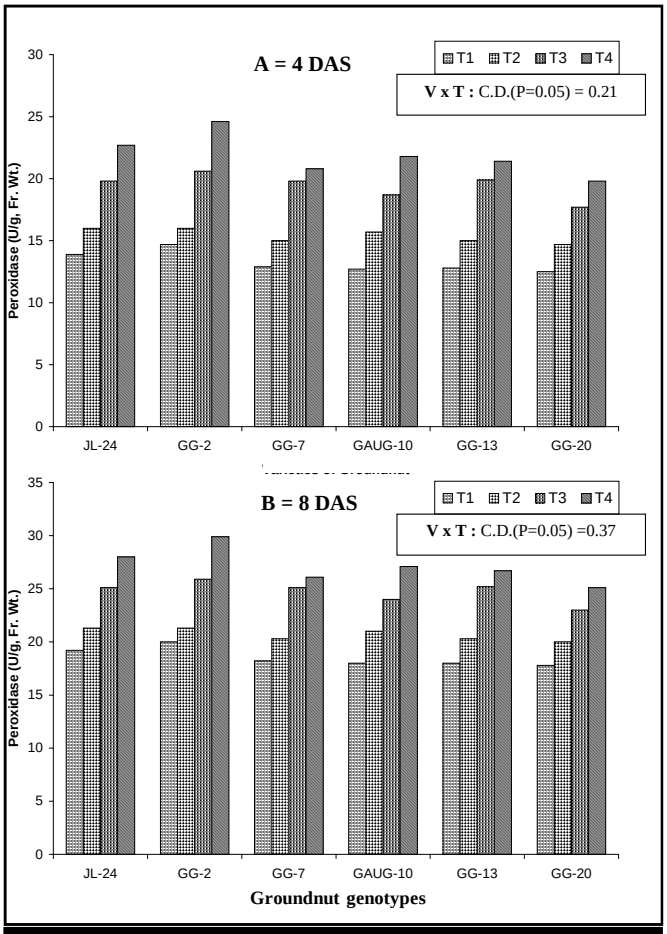

Fig. 4 : Peroxidase activity (U/g, Fr. Wt.) in groundnut seedlings at 4 DAS (A) and 8 DAS (B) in response to chloride dominant salt stress

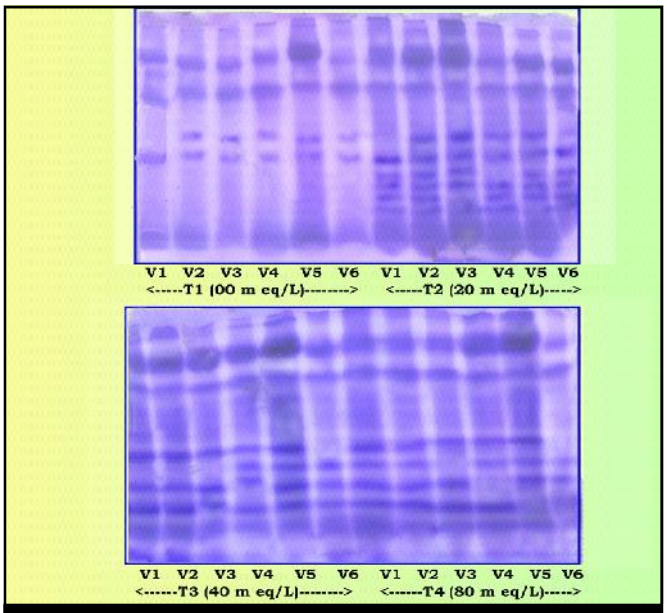

Fig. 5 : SDS-PAGE of proteins of groundnut seedling at 8 DAS in response to chloride dominant salt stress (V_1=JL-24, V_2=GG-2, V_3-GG-7, V_4=GAUG-10, V_5=GG-13, V_6 = GG-20

saline conditions was concomitant to the raise in free amino acid levels in all groundnut genotypes.

Peroxidase activity was increased relatively at higher rate in tolerant genotypes compared to susceptible once with increasing salt stress at both the stages (Fig. 4). The more increase in scavenging enzyme – peroxidase under salt stress in tolerant genotypes may prevent the degradation of membrane integrity of the cells against the free radicals formed under salt stress. Total peroxidase activity in millet was increased under salinity and the degree of elevation in the activity was dependent on salt concentration (Sreenivasulu *et al.*, 1999). Peroxidase enzyme may serve as the indicator for testing the stress tolerance (Deshmukh and Dhumal, 2005).

SDS-PAGE proteins of groundnut seedling at 8 DAS (Fig. 5) showed that the number of protein bands was increased with increasing salinity regimes. Total five phosphate buffer soluble protein bands were visualized in T_1 (00 m eq /L), while in salt stressed treatments T_2 (20 m eq /L), it was increased in seven numbers in all the

groundnut genotypes. The second and third bands were shown very dark in this treatment -T_2. In T_3 (40 m eq /L) and T_4 (80 m eq /L) treatments, total nine to ten bands were found in all genotypes except GG-20 (V_6). Tolerant genotypes visualized more bands with higher intensities compared to susceptible ones under high salinity regimes. Jiang and Ren (2004) observed additional protein band under stress condition in tolerant genotype of groundnut. Present result is also agree with Tawab *et al.* (1997) who reported that SDS-PAGE showed higher band intensities under salt treatments in rice genotypes.

The present study, thus, justifies the *in vitro* findings that chloride type of salinity has an adverse effect on the growth as reflected in decreased vigour index of groundnut seedlings, which is cumulative effect of the disturbances in metabolic activities as observed in various metabolites and their enzymes – alpha-amylase, protease, peroxidase activities. Tolerant genotypes (Jl-24, GAUG-10, GG-13) showed less disturbance in metabolic activities with higher accumulation in solutes (proline, amino acids, sugars) and hence found better seedling vigour index and a vice versa for susceptible genotypes (GG-7, GG-20, GG-2).

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