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# Effect of sulphate type of salinity on some metabolic drifits in germinating groundnut (*Arachis hypogae*) varieties

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## SUMMARY

Groundnut varieties, JL-24, GG-2, GAUG-10 (tolerant group) and GG-7, GG-13, GG-20 (susceptible group) were germinated under sulphate dominant salinity ranging from 0, 20, 40, 80 m eq/L. Sulphate salinity decreased the seedling vigour index of all groundnut varieties, and the decrease was found more in susceptible varieties at 1st and 4th days after germination (DAG). With increasing salinity regimes, various metabolites like free amino acid, protein, total phenol and free proline contents were deposited at higher rate in seedlings of tolerant varieties compared to susceptible ones for better osmotic adjustment. However, sulphate salinity decreased the accumulation of total sugars, starch and free fatty acid contents in the seedlings of all groundnut varieties during 1st and 4th DAG. The decrease in sugar content was found more in susceptible varieties than tolerant once. Activities of alpha-amylase decreased but that of protease and peroxidase increased under salt stress at 1st and 4th DAG in all varieties of groundnut.


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

Groundnut (*Arachis hypogaea* L.) is an important oilseed and is emerging as a food crop in India, grown in an area of 6.45 million ha with a total production of 6.57 million tons based on an average of the last five years (FAO, 2005). This contributes to 26.6% of world's groundnut area and 18.5% of world's groundnut production. Groundnut occupies nearly 28.3% of the cultivated area and contributes 31.7% of the production of the total oilseeds in the country. Groundnut is mainly grown in the states of Andhra Pradesh, Gujarat, Tamil Nadu, Karnakata, Maharastra and Rajasthan in summer (January–June) and rainy season (June–October). It is widely used as cooking oil, digestible protein, minerals and vitamins in many countries and contributes significantly to food security and alleviating poverty. About 80% of India's groundnut production is crushed for oil, 12% for using as seed, 5% for food and 2% for export.

Among many reasons ascribed for the lower productivity of groundnut, salinity is an important abiotic stress which significantly affects seedling, vegetative and reproductive growth, seed quality and yield. Root zone salinity increases as a result of continuous use of saline water for irrigation because of limited or non-availability of good quality water in majority of groundnut growing areas. It can rapidly inhibit root growth and in turn their capacity to uptake water and essential mineral nutrients from the soil (Neumann, 1995). Groundnut yields were severely affected with an increase in soil and water salinity (Patel *et al.*, 1992).

Survival of plants in adverse environment depends on its ability to withstand extreme stresses, affecting the developmental, physiological and biochemical processes. To achieve this, understanding of the physiology and mechanism of salt tolerance in plants is highly essential. Present work was, therefore, designed to find out the effect of sulphate type of salinity on carbohydrate and protein metabolisms of germinating groundnut varieties differing in relative salt sensitivity.

## MATERIALS AND METHODS

The sulphate 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 3.66 : 9.34 : 0.5 : 4.0 : 2.5 for 20 m eq/l which comprises 13.3 m eq $SO_4$/l and 6.7 m eq Cl /l. Accordingly, 40 and 80 m eq/L sulphate dominant salt solutions were also prepared. Thus, the concentration of saline solutions- 20, 40 and 80 m eq /l were used for salt stress. Total four treatments were arranged for sulphate type of 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.

Seeds of uniform size of six varieties ($V_1$ - 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

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selected, sterilized for 2 min in 0.1 % sodium hypochlorite solution and thoroughly washed with deionized water. Fifty seeds of each varieties were germinated in control and sulphate dominant salt solutions in sterilized Petri dishes (15 cm diam) lined with blotting papers and kept at 28 ± 2 $^{O}$C in seed germinator under controlled conditions. The seeds were considered germinated when radicle and plumule have clearly protruded after five days of incubation . Seedling of 1$^{st}$ and 4$^{th}$ days after germination (DAG) 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).

For determination of vigour index, germination per cent and shoot length were recorded at 1$^{st}$ and 4$^{th}$ DAG and 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 and starch contents were determined by the method described by Dubois *et al*. (1956) and MeCreddy *et al*. (1958), respectively. Free amino acid, protein, free fatty acid and total phenol estimations were carried out as methods out lined by Lee and Takahashi (1966), Lowry *et al*. (1951), Cox and Pearson (1962) and Bray and Thorpe (1954), 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.

## RESULTS AND DISCUSSION

The vigour index in terms of groundnut germination per cent and shoot length showed a gradual decrease under sulphate dominant salinity ranging from 20 to 80 m eq/L in all varieties at 1$^{st}$ and 4$^{th}$ DAG (Fig. 1A, 1B). The maximum seedling vigour was observed in JL-24 followed by GG2, GAUG-10, GG-13, GG-20 and GG-7 at 4$^{th}$ DAG under control and saline conditions. At 4$^{th}$ day, it was observed that highest vigour index (430) was recorded in $T_1$ (00 m eq / L) of JL-24 which was decreased with increasing salinity level up to 135 at $T_4$ (80 m eq / L) of GG-20. Thus, interaction effect between varieties and treatments indicated that a variety JL-24 was found superior followed by GG-2 under sulphate 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).

Biochemical investigation revealed that there was significantly increase in free amino acid, protein, total phenol and free proline contents with increasing salinity levels during 1$^{st}$ and 4$^{th}$ DAG (Table 1), however, reverse trend was observed for total sugar, starch, free fatty acid contents during both the stages. Total sugars decreased in seedling of all varieties under sulphate based salt stress, and the depletion of sugar content was higher in susceptible varieties. The decrease in sugar content under NaCl stress was also observed in sugarbeet (Iyengar and Pandya, 1973) and wheat (Bharadwaj, 1958). A comparative account indicated that accumulation of total sugars (Table 1A) in response to salinity was more in tolerant varieties (Jl-24, GG-2, GAUG-10) than in susceptible ones. Such difference in sugar accumulation was attributed to salt tolerance of the varieties (Rathert, 1984). Accumulation of sugars during 1$^{st}$ to 4$^{th}$ DAG in all groundnut varieties 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). Starch content declined with increasing salinity (Table 1B), and, also decreased with increasing time period (1$^{st}$ to 4$^{th}$ DAG) in all groundnut varieties. Decrease in starch in response to higher salt concentration was more in GG-2 variety compared to other varieties. It appears from the results that sulphate salinity decreased the accumulation of starch and total sugars by inhibiting the activity of the enzymes of carbohydrate metabolism. The free fatty acid content was significantly decreased in all groundnut varieties under salt stress during both the stages (Table 1E) which might be due to increase in salinity decreases the mobilization of lipid which resulted to decrease 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).

Increasing salinity regimes gradually increased free amino acid, protein, total phenol and free proline contents in all groundnut varieties. The response of varieties under different levels of salinity with respect to these biochemical parameters studied, elicited that the highest amounts of free amino acids accumulation (Table 1C) were found in varieties GAUG-10 and JL-24 in response to salt stress. The free amino acids content of the seedlings were inferior at 1$^{st}$ day than it was increased at 4$^{th}$ day after

**Table 1 : Effect of sulphate based salinity on contents of various metabolites at 1st and 4th DAG in groundnut varieties**

| Varieties of groundnut | 1st DAG | | | | | 4th DAG | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | Vx | $T_1$ | $T_2$ | $T_3$ | $T_4$ | Vx |
| **[A] Total sugars (mg/g, Fr. Wt.)** | | | | | | | | | | |
| JL-24 | 30.8 | 26.7 | 18.9 | 15.8 | 23.0 | 62.7 | 58.4 | 53.8 | 50.8 | 56.4 |
| GG-2 | 30.0 | 21.0 | 17.2 | 14.9 | 20.8 | 57.6 | 56.0 | 52.3 | 49.1 | 53.8 |
| GG-7 | 23.4 | 19.4 | 16.5 | 13.4 | 18.2 | 58.4 | 54.5 | 51.6 | 49.1 | 53.4 |
| GAUG-10 | 25.0 | 21.8 | 17.6 | 12.8 | 19.3 | 60.1 | 56.8 | 52.6 | 49.4 | 54.8 |
| GG-13 | 23.4 | 19.9 | 17.2 | 13.3 | 18.5 | 59.8 | 56.9 | 52.3 | 50.2 | 54.8 |
| GG-20 | 25.0 | 21.0 | 16.9 | 15.5 | 19.6 | 60.1 | 56.0 | 51.9 | 49.8 | 54.5 |
| Tx | 26.3 | 21.6 | 17.4 | 14.3 | | 59.8 | 56.5 | 52.4 | 49.7 | |
| C.D.(P=0.05) | V = 0.80; T = 0.70; V x T = 1.60 | | | | | V = 0.40; T = 0.30; V x T = 0.80 | | | | |
| **[B] Starch content (mg/g, Fr. Wt.)** | | | | | | | | | | |
| JL-24 | 9.58 | 8.70 | 7.07 | 6.55 | 7.98 | 7.26 | 6.38 | 4.75 | 4.23 | 5.66 |
| GG-2 | 8.56 | 7.33 | 6.81 | 5.81 | 7.13 | 6.24 | 5.01 | 4.49 | 3.49 | 4.81 |
| GG-7 | 8.42 | 7.33 | 6.68 | 5.93 | 7.09 | 6.10 | 5.01 | 4.36 | 3.61 | 4.77 |
| GAUG-10 | 8.99 | 7.87 | 6.30 | 6.05 | 7.30 | 6.67 | 5.55 | 3.98 | 3.73 | 4.98 |
| GG-13 | 8.68 | 7.87 | 6.68 | 6.05 | 7.32 | 6.36 | 5.55 | 4.36 | 3.73 | 5.00 |
| GG-20 | 8.42 | 7.87 | 7.07 | 6.30 | 7.41 | 6.10 | 5.55 | 4.75 | 3.98 | 5.09 |
| Tx | 8.78 | 7.83 | 6.77 | 6.12 | | 6.46 | 5.51 | 4.45 | 3.80 | |
| C.D.(P=0.05) | V = 0.21; T = 0.17; V x T = 0.41 | | | | | V = 0.03; T = 0.03; V x T = 0.07 | | | | |
| **[C] Free amino acids (mg /g, Fr. Wt.)** | | | | | | | | | | |
| JL-24 | 2.32 | 2.58 | 2.82 | 3.26 | 2.75 | 8.74 | 9.00 | 9.24 | 9.68 | 9.17 |
| GG-2 | 2.33 | 2.62 | 2.82 | 3.13 | 2.73 | 8.75 | 9.04 | 9.24 | 9.55 | 9.15 |
| GG-7 | 2.23 | 2.43 | 2.61 | 3.04 | 2.58 | 8.65 | 8.85 | 9.03 | 9.46 | 9.00 |
| GAUG-10 | 2.50 | 2.63 | 2.85 | 3.10 | 2.77 | 8.92 | 9.05 | 9.27 | 9.52 | 9.19 |
| GG-13 | 1.82 | 2.26 | 2.56 | 3.06 | 2.43 | 8.24 | 8.68 | 8.98 | 9.48 | 8.85 |
| GG-20 | 2.35 | 2.44 | 2.80 | 3.01 | 2.65 | 8.77 | 8.86 | 9.22 | 9.43 | 9.07 |
| Tx | 2.26 | 2.50 | 2.75 | 3.10 | | 8.68 | 8.92 | 9.17 | 9.52 | |
| C.D.(P=0.05) | V = 0.17; T = 0.14; V x T = NS | | | | | V = 0.19; T = 0.15; V x T = NS | | | | |
| **D] True protein (mg/g, Fr. Wt.)** | | | | | | | | | | |
| JL-24 | 18.3 | 20.2 | 20.6 | 21.4 | 20.1 | 12.0 | 13.9 | 14.2 | 15.1 | 13.8 |
| GG-2 | 17.9 | 19.8 | 20.8 | 22.7 | 20.3 | 11.6 | 13.5 | 14.4 | 16.4 | 14.0 |
| GG-7 | 19.5 | 21.3 | 22.6 | 24.0 | 21.9 | 13.2 | 15.0 | 16.3 | 17.7 | 15.5 |
| GAUG-10 | 18.8 | 20.8 | 22.2 | 25.1 | 21.7 | 12.4 | 14.4 | 15.9 | 18.8 | 15.4 |
| GG-13 | 17.9 | 20.3 | 21.8 | 23.9 | 21.0 | 11.6 | 13.9 | 15.5 | 17.6 | 14.7 |
| GG-20 | 19.1 | 19.7 | 21.4 | 23.3 | 20.9 | 12.7 | 13.4 | 15.1 | 17.0 | 14.5 |
| Tx | 18.6 | 20.4 | 21.6 | 23.4 | | 12.3 | 14.0 | 15.2 | 17.1 | |
| C.D.(P=0.05) | V = 0.29; T = 0.24; V x T = 0.58 | | | | | V = 0.38; T = 0.31; V x T = 0.77 | | | | |
| **[E] Free fatty acids (mg/g, Fr. Wt.)** | | | | | | | | | | |
| JL-24 | 74.6 | 67.4 | 57.8 | 46.6 | 61.6 | 139 | 132 | 122 | 111 | 126 |
| GG-2 | 80.2 | 73.5 | 69.0 | 53.8 | 69.1 | 145 | 138 | 133 | 118 | 133 |
| GG-7 | 74.6 | 69.0 | 57.8 | 52.2 | 63.4 | 139 | 133 | 122 | 117 | 128 |
| GAUG-10 | 74.6 | 63.4 | 51.4 | 40.9 | 57.6 | 139 | 128 | 116 | 105 | 122 |
| GG-13 | 87.7 | 74.6 | 57.8 | 46.6 | 66.7 | 152 | 139 | 122 | 111 | 131 |
| GG-20 | 78.5 | 70.7 | 52.7 | 42.1 | 61.0 | 143 | 135 | 117 | 106 | 125 |
| Tx | 78.4 | 69.8 | 57.7 | 47.0 | | 143 | 134 | 122 | 111 | |
| C.D.(P=0.05) | V = 1.30; T = 1.00; V x T = 2.50 | | | | | V = 2.00; T = 1.60; V x T = 3.90 | | | | |

*Table 1 contd….*

*Contd….. Table 1*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **[F] Total phenols (mg/g, Fr. Wt.)** | | | | | | | | | | |
| JL-24 | 0.21 | 0.22 | 0.27 | 0.29 | 0.25 | 0.29 | 0.30 | 0.35 | 0.36 | 0.32 |
| GG-2 | 0.20 | 0.22 | 0.25 | 0.28 | 0.24 | 0.28 | 0.30 | 0.33 | 0.36 | 0.31 |
| GG-7 | 0.19 | 0.21 | 0.23 | 0.27 | 0.22 | 0.27 | 0.29 | 0.31 | 0.35 | 0.30 |
| GAUG-10 | 0.20 | 0.23 | 0.25 | 0.30 | 0.25 | 0.28 | 0.30 | 0.33 | 0.38 | 0.32 |
| GG-13 | 0.21 | 0.23 | 0.26 | 0.28 | 0.24 | 0.29 | 0.30 | 0.33 | 0.35 | 0.32 |
| GG-20 | 0.20 | 0.24 | 0.26 | 0.27 | 0.24 | 0.28 | 0.31 | 0.34 | 0.35 | 0.32 |
| Tx | 0.20 | 0.22 | 0.25 | 0.28 | | 0.28 | 0.30 | 0.33 | 0.36 | |
| C.D.(P=0.05) | | V = 0.013; T = 0.010; V x T = NS | | | | V = 0.004; T = 0.003; V x T = 0.009 | | | | |
| **[G] Free proline content (µg/g, Fr. Wt.)** | | | | | | | | | | |
| JL-24 | 35.8 | 52.3 | 74.3 | 95.8 | 64.6 | 49.1 | 65.6 | 87.5 | 109.0 | 77.8 |
| GG-2 | 30.9 | 48.5 | 69.7 | 84.4 | 58.4 | 44.1 | 61.7 | 83.0 | 97.7 | 71.6 |
| GG-7 | 29.1 | 46.2 | 67.4 | 84.0 | 56.7 | 42.4 | 59.5 | 80.6 | 97.3 | 69.9 |
| GAUG-10 | 33.8 | 49.6 | 72.9 | 89.2 | 61.4 | 47.1 | 62.9 | 86.2 | 102.5 | 74.6 |
| GG-13 | 28.9 | 50.3 | 71.1 | 92.2 | 60.6 | 42.2 | 63.6 | 84.3 | 105.5 | 73.9 |
| GG-20 | 29.5 | 47.0 | 61.6 | 86.5 | 56.1 | 42.8 | 60.2 | 74.9 | 99.8 | 69.4 |
| Tx | 31.3 | 49.0 | 69.5 | 88.7 | | 44.6 | 62.2 | 82.7 | 102.0 | |
| C.D.(P=0.05) | | V = 0.86; T = 0.70; V x T = 1.71 | | | | V = 0.15; T = 0.12; V x T = 0.30 | | | | |

DAG=Days After Germination; 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]

germination. Similarly, Shrma *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 varieties (JL-24, GAUG-10) in response to salt treatments (Table 1G). The rate of accumulation of proline content in the seedlings was higher at 4th day as compare to 1st day after germination. The rate of increase in proline content was found more in tolerant than in susceptible varieties enabling the former varieties to cope with the salt stress condition comparatively in an efficient manner. Thus, varieties under test showed tolerance because of better osmoregulation due to higher accumulation of proline. Similar increase in proline content has also been found in chickpea by Sharma *et al*. (1990) and Varshney and Sangeeta (1992).

Total phenol significantly increased with salt stress (Table 1F). The higher amount of total phenol content was found in tolerant varieties JL-24 and GAUG-10 in response to salt treatments. The deposition of total phenol content of the seedlings was higher at 4th DAG compared to 1st DAG. This pronouncement confirms with finding of Singh *et al*. (2005) who reported that, increase in salt stress resulted in increase the amount of phenols content in tolerance genotype as compare to susceptible genotype of *Brassica campestris*.

Protein content was found significantly higher in variety GG-7 (21.9 mg $g^{-1}$ fr. wt.) which was at par with GAUG-10 (21.7 mg $g^{-1}$ fr. wt.). The lowest protein value was found in JL-24 (20.1 mg g-1 fr. wt). On 4th DAG, there was declined in the protein content compared to 1st DAG (Table 1D). Protein content was increased with increasing salinity level in seedlings of all groundnut varieties. This was supported by Ali (1998) who reported that maize seeds were germinated on filter paper moistened with solutions 0, 50, and 150 ml molar NaCl salt, and resulted to significantly increase in protein content in the seedling.

Activities of alpha-amylase decreased (Fig. 2) but that of protease (Fig. 3) and peroxidase (Fig. 4) increased under salt stress at 1st and 4th DAG in all the varieties of groundnut. Sulphate salinity decreased alpha-amylase activity and decrease was more in seedlings of susceptible varieties. This decrease was, perhaps, due to substrate limitation in the seedling under salt stress. Reduction in alpha-amylase has been reported in chick pea and rice seedlings (Prakash *et al.*, 1988). The salt tolerant varieties Jl-24 and GG-2 showed less reduction in alpha-amylase activity with progress of salt stress compared to susceptible varieties GG-20, GG-7. Similarly, Krishnamurthy *et al*. (1987) found lower alpha-amylase activity at higher salt stress in rice seedlings. Dubey (1983) observed that tolerant varieties of rice had higher amylase activity compared to susceptible varieties. Salinity caused decrease in water uptake followed by inhibition in the activity of hydrolytic enzyme- alpha amylase (Dubey,

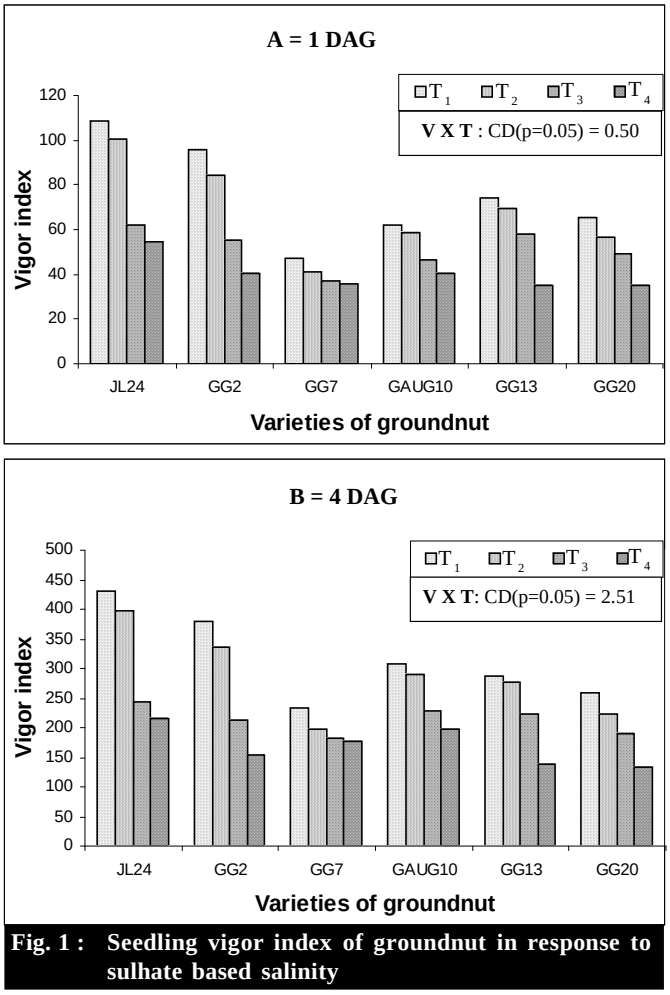


**Fig. 1 : Seedling vigor index of groundnut in response to sulhate based salinity**

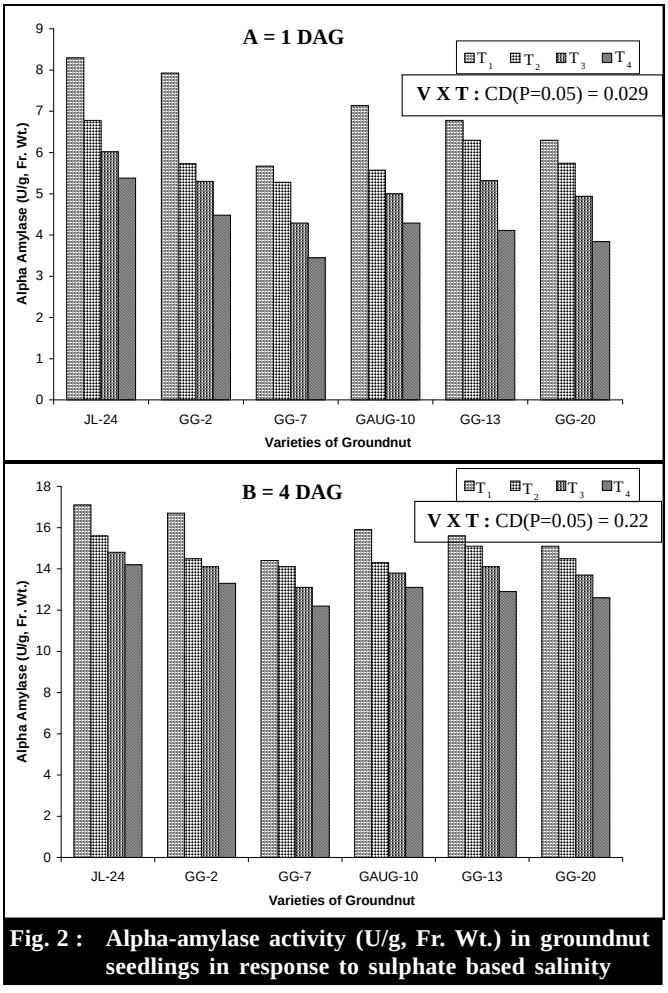


**Fig. 2 : Alpha-amylase activity (U/g, Fr. Wt.) in groundnut seedlings in response to sulphate based salinity**

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 varieties 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 varieties at 1st and 4th DAG except GAUG-10, GG-7 and GG-13 where fluctuations in protease activity at 4th DAG were observed. 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 varieties (GG-13, GG-20, GG-7) compared to tolerant varieties (Jl-24, GG-2, GAUG-10) at 1st DAG. These differences between the tolerant and susceptible varieties across salinity stress were highly significant.

Peroxidase activity was increased relatively at higher rate in tolerant cultivars compared to susceptible once with increasing salt stress (Fig. 4). The more increase in scavenging enzyme – peroxidase under salt stress in tolerant varieties may prevent the degradation of membrane integrity of the cells against the free radicals formed under salt stress. Total peroxidase activity was increased under salinity and the degree of elevation in the activity was dependent on salt concentration (Sreenivasulu *et al.*, 1999).

From these results, it seems that tolerant varieties (Jl-24, GG-2, GAUG-10) showed better vigour index of groundnut seedlings with less disturbances in metabolites compared to susceptible ones (GG-13, GG-20, GG-7) under sulphate type of salinity regimes. There were a higher alpha-amylase and peroxidase activities and lower protease activity under salt stress conditions in groundnut seedlings. Among various metabolites, higher free amino acid content, particularly, proline was attributed to salt tolerance mechanism through osmotic adjustment.

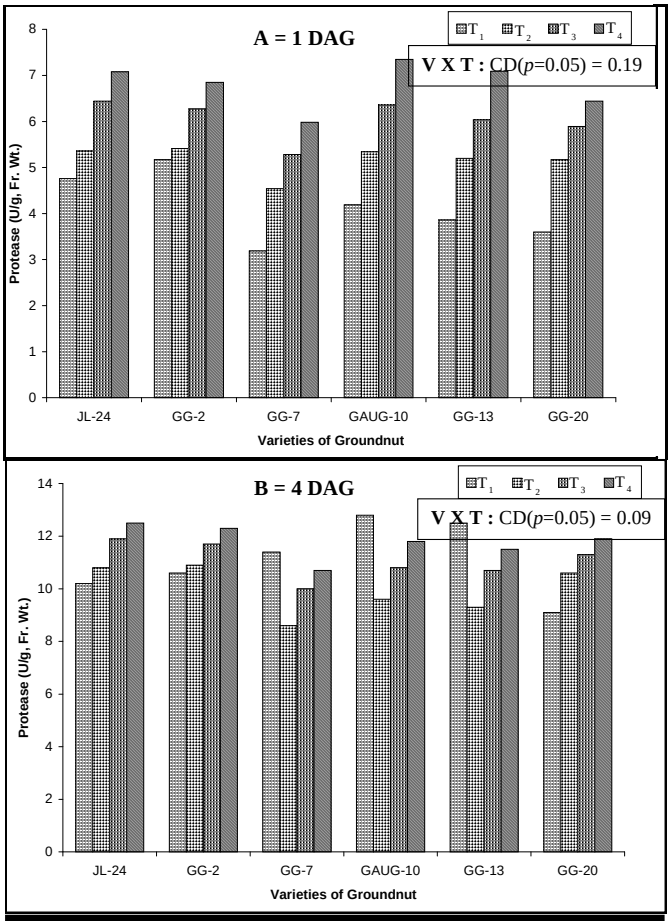


**Fig. 3 : Protease activity (U/g, Fr. Wt.) in groundnut seedlings in response to sulphate based salinity**

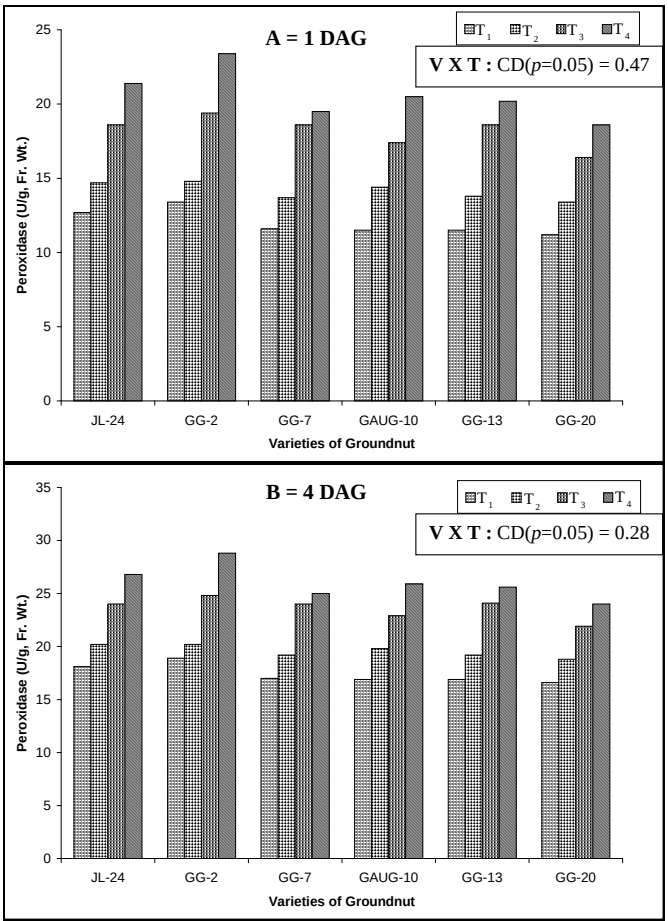


**Fig. 4 : Peroxidase activity (U/g, Fr. Wt.) in groundnut seedlings in response to sulphate based salinity**

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