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Physiological and Biochemical changes during *in vitro* germination under salinity stress in groundnut (*Arachis hypogaea* L.)

Harsh B Purohit, HP Gajera, DD Savaliya and Darshna G Hirpara

Abstract
An experiment comprised of ten different groundnut varieties (V_1=GG-4, V_2=GG-5, V_3=GG-6, V_4=GAUG-10, V_5=GG-11, V_6=GG-13, V_7=GG-20, V_8=GJG-22, V_9=GJG-31, V_{10}=GJG-32) and six levels (T_0=Distilled water or <2 EC, T_1=2 EC, T_2=4 EC, T_3=6 EC, T_4=8 EC, T_5=10 EC) of salinity stress treatments for changes in physiological parameters (germination percentage, relative water content, salt tolerance index-STI) and biochemical parameters (chlorophyll content, free proline content, glycine betaine content and lipid peroxidation) during *in vitro* germination. The physiological parameters were decreased under salinity stress condition particularly in susceptible variety (GG-4). Salinity stress influences biochemical parameters like free proline, glycine betaine and lipid peroxidation. Proline and glycine betaine content is increased and lipid peroxidation decreased in salinity tolerant varieties compared to susceptible varieties under salinity stress. SDS-PAGE protein profiling showed that banding pattern of protein under 10 EC salinity stress different from other treatment condition. Among ten groundnut variety, GJG-31 was found to be the most salinity tolerant and GG-4 was found to be susceptible salt tolerance index (STI). The STI was found to be highly positively correlated with chlorophyll content followed by glycine betaine and free proline as biochemical markers and lipid peroxidation was negatively correlated to STI.

Keywords: Groundnut, salinity stress, salt tolerance index, protein profiling, biochemical markers

Introduction

Groundnut (*Arachis hypogaea* L.) is an annual prostrate herbaceous leguminous oilseed crop. Groundnut is native of South America (Hammons, 1982) [7]. After cereals, oilseeds are the second largest agricultural commodity sharing 14 per cent of country's gross cropped area and account for nearly 5 per cent of gross national products and 10 per cent of the value of all agricultural products. The major groundnut producing countries are India, China, Nigeria, U.S.A., Taiwan, Indonesia, Senegal, Ghana, Argentina and Brazil. It is the most important commercial oilseed crop mostly grown in the semi-arid tropical region like India. The crop can be grown successfully on the areas receiving rainfall from 600 to 1250 mm. The best soil for the groundnut crop is sandy loam, loamy and medium black. Groundnut oil is edible and therefore, it is extensively used as a cooking medium both as refined oil and Vanaspati ghee. Groundnut is a good source of minerals such as phosphorus (P), calcium (Ca), magnesium (Mg) and potassium (K) as well as the vitamins A, B and B_2 group. Groundnut oil is considered as stable and nutritive as it contains just the right proportion of oleic (40-50%) and linoleic acids (25-35%) (Mathur and Khan, 1997) [17]. Increase crop salt tolerance is a highly attractive approach to overcome the salinity threat. The need of the hour is to explore and select salt-tolerant genotypes within a species in comparison to relatively salt-sensitive ones through conventional selection and breeding techniques. Soil salinity adversely affects plant growth and development. Worldwide, about one-third of irrigated arable land is already affected and that level is still rising (Lazof and Bernstein, 1999) [13].

Materials and Methods

Present study examined biochemical and physiological changes in different groundnut varieties influenced by salinity. Ten groundnut varieties (V_1-GG-4, V_2-GG-5, V_3-GG-6, V_4-GAUG-10, V_5-GG-11, V_6-GG-13, V_7-GG-20, V_8-GJG-22, V_9-GJG-31 and V_{10}-GJG-32) were grown in petriplate for *in-vitro* study along with different salinity stress treatments T_0 to T_5 [T_0: Control (Distilled water), T_1: 2 EC, T_2: 4 EC, T_3: 6 EC, T_4: 8 EC and T_5: 10 EC].

Saline water collected from Arabian sea and different salinity treatments were formulated by making appropriate (2, 4, 6, 8, 10 EC) dilution. Ten seeds of groundnut were kept in each petriplate for germination at 20 ºC ± 2 ºC under incubator (Nova En-500). Groundnut seedlings were collected at 15 DAS for physiological and biochemical analysis with 3 replications.

Physiological parameters
Germination
In vitro germination percent was recorded as per I.S.T.A., (1976).

Relative water content (RWC)
Relative water content (RWC) was measured on the basis of fresh weight, turgid weight and dry weight according to the method given by Weatherley, (1962) [30].

Salt tolerance index (STI)
Salt tolerance index (STI) was calculated on the basis of root and shoot length using formula given by Rahman *et al*. (2008) [22].

Biochemical parameters
Total chlorophyll content
The fresh leaves of groundnut weighed (0.1 g) were cut into small pieces and crushed into 5 ml DMSO. The whole paste was filtered with whatman no. 1 filter paper. Absorbance was measured in spectrophotometer at 645 nm and 663 nm for determination of total chlorophyll given by Hiscox and Israelsta (1979) [10].

Free proline
The free proline was measured by acid ninhydrin method given by Bates *et al*. (1973) [2]. About 0.1 g seedlings were crushed in 5 ml of 3% sulfosalicylic acid followed by centrifugation at 10,000 rpm for 10 min and supernatant was collected for free proline assay. Then 2 ml glacial acetic acid and 2 ml acid ninhydrin reagent (1.25 g ninhydrin + 30 ml glacial acetic acid + 8 ml 6 M phosphoric acid in 12 ml distilled water) were added. Then tubes were kept in boiling water bath for 1 hrs. The tubes were cooled in running water at room temperature. After that 4 ml toluene was added. The absorbance was recorded from toluene phase at 520 nm in spectrophotometer. The content was expressed as mg.g^{-1} fresh weight.

Glycine betaine
Glycine betaine was done from fresh leaves of groundnut per the method of Hendawey (2015) [9]. Finely ground leaf material (0.5 g) was mechanically shaken with 20 ml of distilled water for 16 hrs. at 25 °C. The samples were then filtered and the filtrate was stored in freezer until analysis. Thawed extracts were diluted 1:1 with 2 N sulphuric acid. Aliquot (0.5 ml) was measured into test tube and cooled in ice for 1 hour 0.2 ml of cold potassium iodide-iodine reagent [Iodine (15.7 g) and potassium iodide (20 g) were dissolved in 100 ml of water and kept in fridge at 4 °C] was added and the mixture was gently mixed with vortex mixture. The samples were stored at 0 to 4 °C for 16 hr. After the expiration of the period samples were transferred to centrifuge tubes and then centrifuged at 10,000 g for 15 min at 0 °C. The supernatant was carefully aspirated with 1 ml micropipette. The peridotite crystals were dissolved in 9 ml of 1,2-dichloroethane. Vigorous vortex mixing was done to effect complete solubility in developing solvent. After 2.0-2.5 hrs. an absorbance was measured at 365 nm. Reference standards of glycine-betaine (50-200 µgml^{-1}) were prepared in 2 N sulphuric acid and the amount of glycine betaine present in the sample was calculated by appropriate formula.

Lipid peroxidation
Lipid peroxidation was measured using thiobarbituric acid (TBA) method (Heath and Packer, 1968) [8]. The level of lipid peroxidation was measured in the terms of malondialdehyde (MDA) content. One gram groundnut seedlings were ground in liquid nitrogen and homogenized in 0.1% (w/v) trichloroacetic acid (TCA) and 2% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000 rpm for 15 min followed by addition of 2% PVP to supernatant of homogenate to remove excess polysaccharide, and again centrifuged at 10,000 rpm for 15 min. One ml of supernatant was taken and added into 4 ml of 20% TCA containing 1% thiobarbituric acid (TBA). The mixture was heated at 95ºC for 30 min and then quickly cooled in an ice bath. The resulting mixture was centrifuged at 10,000 rpm for 15 min. The absorbance of MDA content was measured at 532 nm and 600 nm for the correction of non-specific turbidity. The level of lipid peroxidation was expressed in nano mol of MDA formed g^{-1} fr. wt using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Protein profiling by SDS-PAGE
Groundnut seedlings (0.5 g) crushed in 5 ml of 0.2 M phosphate buffer pH (7.0) with mortar and pestle. The homogenate were centrifuged at 6000 rpm for 10 min and supernatant was used as crude extract for isolation of proteins by resolving on electrophoresis. The concentration of protein in groundnut seedling was estimated by method of Lowry *et al*. (1951) [14] using bovine serum albumin (BSA) as the standard. For one dimensional separation, proteins (300 µg per lane) were electrophoresed on 12% linear sodium dodecyl sulphate (SDS)-polyacrylamide slab gels overlayed with 4% stacking gel (Laemmli, 1970) [12]. Electrophoresis was carried out at 50 V for 1 h followed by 100 V for 2 h. Proteins and molecular mass markers were visualized by staining with Coomassie Brilliant Blue R-250 followed by destaining (Methanol: Acetic acid: Distilled water in 40: 10: 50 ratio).

Statistical analysis
The physiological and biochemical data were analyzed using 2FCRD (Factorial Complete Randomized Design) for detection of level of significance among varieties influenced by salinity. The first factor as ten varieties and second factor as six treatments were used for the statistical analysis (Panse and Sukhatme, 1985) [21].

Results and Discussion
Physiological parameters
Germination
The influence of salinity treatments on germination percent of groundnut varieties is depicted in Table 1. The mean highest (98.89%) of germination was recorded in variety GJG-31 (V_9) followed by GAUG-10 (V_4) (94.44%). However, significantly mean lower germination percent examined in variety GG-4 (V_1) (83.89%). The interaction effect between varieties and salinity treatments indicated that the higher germination

percentage was recorded in V_9 variety (GJG-31) under T_5 treatment. The susceptible GG-4 (V_1) was examined with lowest percent of germination (56.67%) under T_5 treatment (10 EC). The rate of decreasing germination percentage was found lower in GJG-31 (V_9) compared to other varieties, when salinity treatment explored up to 10 EC (T_5).
Mensah *et al.* (2006) [18] observed that lower levels of salinity delayed germination whereas higher levels reduced the final percentage of seed germination and vegetative plant growth is suppressed under saline conditions. The effect of salinity on germination of seeds can be either by creating osmotic potential which prevent water uptake or by toxic effects of ion on embryo viability of the groundnut seeds. Singh *et al.* (2008) [27] indicated that plants are particularly susceptible to salinity during early seedling and early vegetative growth stage as compared to germination in groundnut.

Table 1: Effect of salinity on germination percentage (%) in groundnut varieties at 5 DAS

Varieties (V)	Salinity treatments (T)						
	T_0	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**Mean V**
V_1	100.00	100.00	90.00	83.33	73.33	56.67	83.89
V_2	100.00	100.00	90.00	83.33	76.67	60.00	85.00
V_3	100.00	100.00	90.00	90.00	83.33	80.00	90.56
V_4	100.00	100.00	96.67	93.33	90.00	86.67	94.44
V_5	100.00	100.00	90.00	90.00	86.67	76.67	90.56
V_6	100.00	100.00	93.33	86.67	80.00	76.67	89.44
V_7	100.00	100.00	93.33	86.67	80.00	76.67	89.44
V_8	100.00	96.67	93.33	90.00	93.33	90.00	93.89
V_9	100.00	100.00	100.00	100.00	96.67	96.67	98.89
V_{10}	100.00	96.67	96.67	90.00	86.67	83.33	92.22
Mean T	100.00	99.33	93.33	89.33	84.67	78.33	
	S.Em. ±	C.D. at 5%	CV %	5.06			
V	1.083	3.033					
T	0.839	2.349					
V X T	2.653	7.428					

V_1=GG-4, V_2=GG-5, V_3=GG-6, V_4=GAUG-10, V_5=GG-11, V_6=GG-13, V_7=GG-20, V_8=GJG-22, V_9=GJG-31, V_{10}=GJG-32. T_0=Distilled water or <2 EC, T_1=2 EC, T_2=4 EC, T_3=6 EC, T_4=8 EC, T_5=10 EC

Relative water content (RWC)

The different varieties of groundnut significantly influenced RWC at seedlings stage (15 DAS). The maximum and minimum mean RWC content was recorded for variety GJG-31 (88.60%) and GG-4 (76.38%), respectively (Table 2). The control treatment (T_0) gave significantly higher RWC (89.46%), however, 10 EC salinity gave the lowest value of RWC (73.70%). The interaction effect between varieties and salinity treatments indicated that the higher RWC was recorded in V_9 variety (GJG-31) under T_0 treatment. The susceptible GG-4 (V_1) was examined with lowest RWC (69.09%) under T_5 treatment (10 EC). The combined effect of variety and treatment was found significant for RWC in groundnut seedlings. The rate of decreasing RWC was found minimum in GJG-31 (V_9) compare to other varieties, when salinity level increased up to 10 EC.
Vakharia *et al.* (1997) [29] also reported the decreased in leaf RWC from 81.72% to 75.92% in groundnut imposed to abiotic stress. The relative water content decreases under salinity stress conditions. The main reason behind that is shoot and leaf water concentrations of plants under optimum conditions is significantly greater than those of plants under high salinity conditions. The water intake of plants is limited based on salinity. Under these conditions plants try to overcome water stress by increasing the concentrations of their intracellular osmotic compounds (Srivastava *et al.* 1998) [28]. Leaf relative water content is a criterion that is frequently used to define the water content of plants. It is thought that plants with high leaf relative water content have a more stable osmotic balance (Schonfeld *et al.* 1988) [24].

Table 2: Effect of salinity on relative water content (%) in groundnut varieties at 15 DAS

Varieties (V)	Salinity treatments (T)						
	T_0	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**Mean V**
V_1	82.51	81.01	78.56	75.09	72.03	69.09	76.38
V_2	88.23	86.74	83.97	82.12	79.76	77.40	83.04
V_3	89.07	87.01	85.08	82.66	78.85	72.94	82.60
V_4	88.15	86.36	85.08	81.05	78.24	75.16	82.34
V_5	86.73	84.12	80.47	76.52	72.88	70.50	78.53
V_6	86.52	84.98	80.97	76.55	73.05	71.61	78.95
V_7	92.29	91.02	85.69	81.88	78.59	73.93	83.90
V_8	90.60	89.15	84.62	80.98	77.13	73.27	82.63
V_9	96.47	93.59	91.30	86.85	83.38	80.01	88.60
V_{10}	94.00	91.11	87.38	81.18	77.69	73.08	84.07
Mean T	89.46	87.51	84.31	80.49	77.16	73.70	
	S.Em. ±	C.D. at 5%	CV %	1.89			
V	0.172	0.482					
T	0.133	0.373					
V X T	0.421	1.180					

V_1=GG-4, V_2=GG-5, V_3=GG-6, V_4=GAUG-10, V_5=GG-11, V_6=GG-13, V_7=GG-20, V_8=GJG-22, V_9=GJG-31, V_{10}=GJG-32. T_0=Distilled water or <2 EC, T_1=2 EC, T_2=4 EC, T_3=6 EC, T_4=8 EC, T_5=10 EC

Salt tolerance index

The salt tolerance index of groundnut varieties was presented in Table 3. The data revealed that different varieties of groundnut significantly influenced salt tolerance index at seedlings stage (15 DAS). The mean value of salt tolerance index was recorded significantly higher (79.27%) in variety GJG-31. The control treatment gave significantly higher salt tolerance index (100%), however 10 EC salinity gave lowest value of salt tolerance index (40.98%). The interaction effect between varieties and salinity treatments indicated that the higher (54.60%) STI was recorded in V_9 variety (GJG-31) under T_5 treatment (10 EC). The susceptible variety GG-4 (V_1) was examined with lowest (30.92%) STI in T_5 treatment (10 EC). The combined effect of variety and salinity was found significant for salt tolerance index in groundnut seedlings. Shrimali *et al.* (2015) [26] carried out research on effects of salinity on ten groundnut cultivars (GG-2, GG-4, GG-6, GG-7, GG-20, TG-37A, TMV-13, DRG-17, TPG-41 and Girnar-2). Seeds of all the cultivars treated with 40 mM NaCl, 80 mM NaCl, 120 mM NaCl and 160 mM NaCl along with control (DW) were kept for germination at room temperature up to 120 hrs. Physiological parameters *viz.*, shoot length, root + hypocotyls length, no. of secondary root, germination percentage and seed vigour index were decreased. Based on result was found, salinity tolerant and susceptible cultivars were identified.

Table 3: Effect of salinity on salt tolerance index (%) in groundnut varieties at 15 DAS

Varieties (V)	Salinity treatments (T) T_0	T_1	T_2	T_3	T_4	T_5	Mean V
V_1	100.00	73.72	62.58	51.51	45.32	30.92	60.67
V_2	100.00	85.08	74.60	61.36	48.51	40.13	68.28
V_3	100.00	84.30	74.15	58.32	43.32	35.80	65.98
V_4	100.00	85.61	76.81	64.72	48.81	37.09	68.84
V_5	100.00	86.57	73.33	67.73	48.37	38.68	69.11
V_6	100.00	84.62	73.86	58.94	47.63	35.89	66.82
V_7	100.00	87.49	78.29	65.73	55.44	45.46	72.07
V_8	100.00	89.33	79.25	70.47	57.36	44.24	73.44
V_9	100.00	95.85	83.36	76.07	65.77	54.60	79.27
V_{10}	100.00	91.21	79.92	70.81	57.54	46.97	74.41
Mean T	100.00	86.38	75.62	64.57	51.81	40.98	
	S.Em. ±	C.D. at 5%	CV %	1.47			
V	0.243	0.679					
T	0.188	0.526					
V X T	0.594	1.663					

V_1=GG-4, V_2=GG-5, V_3=GG-6, V_4=GAUG-10, V_5=GG-11, V_6=GG-13, V_7=GG-20, V_8=GJG-22, V_9=GJG-31, V_{10}=GJG-32. T_0=Distilled water or <2 EC, T_1=2 EC, T_2=4 EC, T_3=6 EC, T_4=8 EC, T_5=10 EC

Biochemical parameters

Total chlorophyll content

The total chlorophyll content was recorded at 15 days after sowing (DAS) and showed statistically significant differences. The different salinity treatment influence total chlorophyll content of groundnut varieties was depicted in Table 4. The mean value of total chlorophyll content was recorded significantly higher (0.163 mg.g^{-1} of fr. wt.) in GJG-31 variety and it was statistically at par with variety GJG-22 (0.139 mg.g^{-1} of fr. wt.). The control treatment gave significantly higher total chlorophyll content (0.153 mg.g^{-1} of fr. wt.) however 10 EC salinity gave lower value of total chlorophyll content (0.087 mg.g^{-1} of fr. wt.). With increasing the salinity, total chlorophyll content was decreasing. The interaction effect between varieties and salinity treatments indicated that higher total chlorophyll content (0.132 mg.g^{-1} of fr. wt.) was recorded in V_9 variety (GJG-31) and lower total chlorophyll content (0.048 mg.g^{-1} of fr. wt.) was recorded in V_1 variety (GG-4) under T_5 treatment (10 EC). These results were in agreement with Chakraborty *et al.* (2016) [4] reported that decrease in chlorophyll a & b and total chlorophyll in response to salinity stress. Decrease in chlorophyll content under salinity stress is observed more in salt sensitive genotypes in comparison to cultivars with low tolerance (Alzahrani *et al.* 2019) [1]. Salwa *et al.* (2010) [23] were carried out through the successive growth seasons to study salinity tolerance of two peanut cultivars namely Gregory and Giza 6 and the soil salinity level effect on plant growth, yield, leaf water content, chemical composition and other parameters. Salinity levels of the three used soils were 7.55, 9.20 and 12.5 dSm^{-1}. The obtained results cleared that all studied characters of growth, total water content, free water content, relative water content, leaf water potential, photosynthetic pigments, total carbohydrates and non-soluble carbohydrates showed a significant decrease by increasing salinity levels.

Table 4: Effect of salinity on total chlorophyll content (mg.g^{-1} of fr. wt.) in groundnut varieties at 15 DAS

Varieties (V)	Salinity treatments (T) T_0	T_1	T_2	T_3	T_4	T_5	Mean V
V_1	0.125	0.123	0.112	0.095	0.070	0.048	0.095
V_2	0.134	0.128	0.114	0.106	0.072	0.064	0.103
V_3	0.139	0.135	0.115	0.106	0.084	0.063	0.107
V_4	0.161	0.158	0.145	0.124	0.108	0.092	0.131
V_5	0.145	0.133	0.125	0.113	0.105	0.080	0.117
V_6	0.145	0.134	0.125	0.118	0.108	0.090	0.120
V_7	0.162	0.154	0.140	0.135	0.118	0.098	0.134
V_8	0.164	0.163	0.159	0.140	0.115	0.096	0.139
V_9	0.187	0.187	0.175	0.161	0.136	0.132	0.163
V_{10}	0.166	0.160	0.139	0.132	0.118	0.104	0.137
Mean T	0.153	0.148	0.135	0.123	0.103	0.087	
	S.Em. ±	C.D. at 5%	CV %	2.99			
V	0.001	0.003					
T	0.001	0.002					
V X T	0.002	0.006					

V_1=GG-4, V_2=GG-5, V_3=GG-6, V_4=GAUG-10, V_5=GG-11, V_6=GG-13, V_7=GG-20, V_8=GJG-22, V_9=GJG-31, V_{10}=GJG-32. T_0=Distilled water or <2 EC, T_1=2 EC, T_2=4 EC, T_3=6 EC, T_4=8 EC, T_5=10 EC

Free proline content

The accumulation of proline, under biotic or abiotic stress is a common phenomenon in plants. Besides its role as an osmolyte, proline contributes to scavenging reactive oxygen species (ROS), stabilizing sub cellular structures, modulating cell redox homeostasis, supplying energy and functioning as a signal. Proline was accumulated in the seedling of tolerant compared to the sensitive genotype. The proline is accumulated for maintaining chlorophyll level and cell turgor to protect photosynthetic activity under drought stress. Present study demonstrated the trends of data (Table 5) connecting to different varieties and various level of salinity as well as its combined effect on free proline content of groundnut seedlings recognized at 15 DAS. The free proline content in seedlings of groundnut at 15 DAS was significantly affected by different salinity treatments and higher free proline content (0.320 mg.g^{-1} of fr. wt.) was noted with tolerant variety GJG-31. The proline content increased with increasing level of salinity. Mean value of salinity treatments indicated significantly higher proline content (0.567 mg.g^{-1} of fr. wt.) under application of 10 EC salinity stress (T_5). The interaction effect between varieties and salinity treatments indicated that the higher free proline content (0.661 mg.g^{-1} of fr. wt.) was recorded in tolerant V_9 variety (GJG-31) and lower (0.469 mg.g^{-1} of fr. wt.) was recorded in susceptible GG-4 (V_2) under T_5 treatment (10 EC).

These results were in agreement with Nithila *et al.* (2013) [20] who reported that salinity increase proline content in groundnut seedling. Girija *et al.* (2001) [6] also revealed same trend. Proline is a particular osmolyte in plants, increasing rapidly under reduced water levels and assists the plants to preserve cell turgor (Bidabadi *et al.* 2012) [3]. This osmolyte is a compatible solute, which can be considered as protective response in terms of osmotic adjustment (OA) in abiotic stress condition (Mahajan and Tuteja, 2005) [15]. Plants synthesize proline under arid and salinity stress conditions in order to protect themselves and to regulate their physiological status (Edreva, 2005) [5]. Hence, it can be stated that plants and their cultivars which synthesize large amounts of proline are more tolerant to stress conditions.

Table 5: Effect of salinity on free proline content (mg.g^{-1} of fr. wt.) in groundnut varieties at 15 DAS

	Salinity treatments (T)						
Varieties (V)	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**Mean V**
V_1	0.072	0.118	0.221	0.273	0.360	0.469	0.252
V_2	0.055	0.112	0.246	0.302	0.376	0.571	0.277
V_3	0.047	0.119	0.247	0.305	0.367	0.518	0.267
V_4	0.061	0.123	0.285	0.317	0.451	0.552	0.298
V_5	0.058	0.108	0.250	0.312	0.497	0.587	0.302
V_6	0.088	0.132	0.243	0.299	0.370	0.482	0.269
V_7	0.090	0.116	0.209	0.234	0.607	0.600	0.309
V_8	0.087	0.123	0.197	0.270	0.442	0.629	0.291
V_9	0.090	0.125	0.284	0.309	0.451	0.661	0.320
V_{10}	0.089	0.123	0.221	0.300	0.441	0.596	0.295
Mean T	0.074	0.120	0.240	0.292	0.436	0.567	
	S.Em. ±	C.D. at 5%	CV %	5.01			
V	0.003	0.010					
T	0.003	0.007					
V X T	0.008	0.023					

V_1=GG-4, V_2=GG-5, V_3=GG-6, V_4=GAUG-10, V_5=GG-11, V_6=GG-13, V_7=GG-20, V_8=GJG-22, V_9=GJG-31, V_{10}=GJG-32. T_0=Distilled water or <2 EC, T_1=2 EC, T_2=4 EC, T_3=6 EC, T_4=8 EC, T_5=10 EC

Glycine betaine content: Glycine betaine is a quaternary ammonium compound that accumulates in a large variety of species in response to different types of stress. Glycine betaine counteracts adverse effects caused by abiotic factors, preventing the denaturation and inactivation of proteins. Thus, its determination is important, particularly for scientists focused on relating structural, biochemical, physiological, and/or molecular responses to plant water status. Present study demonstrated the trends of data (Table 6) connecting to different varieties and various level of salinity as well as its combined effect on glycine betaine content of groundnut seedlings recognized at 15 DAS. The glycine betaine content in seedlings of groundnut at 15 DAS was significantly affected by different salinity treatments and higher glycine betaine content (0.259 mg.g^{-1} of fr. wt.) was noted with tolerant variety GJG-31. The glycine betaine content increased with increasing level of salinity. Mean value of salinity treatments indicated significantly higher glycine betaine content (0.337 mg.g^{-1} of fr. wt.) under application of 10 EC salinity stress (T_5). The interaction effect between varieties and salinity treatments indicated that the higher glycine betaine content (0.441 mg.g^{-1} of fr. wt.) was recorded in tolerant V_9 variety (GJG-31) and lower (0.245 mg.g^{-1} of fr. wt.) was recorded in susceptible GG-4 (V_2) under T_5 treatment (10 EC). These results were in agreement with Shaddad *et al.* (2013) [25] studied effect of salinity stress, and found that the regulation of osmotic pressure, protection of membrane that increased due to accumulation of glycine betaine.

Table 6: Effect of salinity on glycine betaine content (mg.g^{-1} of fr. wt.) in groundnut varieties at 15 DAS

	Salinity treatments (T)						
Varieties (V)	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**Mean V**
V_1	0.115	0.120	0.123	0.150	0.211	0.245	0.160
V_2	0.122	0.131	0.151	0.182	0.212	0.323	0.187
V_3	0.081	0.110	0.116	0.186	0.246	0.333	0.178
V_4	0.146	0.123	0.094	0.182	0.155	0.320	0.170
V_5	0.114	0.131	0.181	0.174	0.235	0.368	0.200
V_6	0.079	0.115	0.119	0.237	0.265	0.341	0.193
V_7	0.176	0.126	0.149	0.192	0.248	0.371	0.210
V_8	0.154	0.109	0.174	0.221	0.265	0.318	0.207
V_9	0.116	0.160	0.174	0.293	0.370	0.441	0.259
V_{10}	0.125	0.151	0.173	0.207	0.256	0.311	0.204
Mean T	0.123	0.127	0.145	0.202	0.246	0.337	
	S.Em. ±	C.D. at 5%	CV %	4.72			
V	0.002	0.006					
T	0.002	0.005					
V X T	0.005	0.015					

V_1=GG-4, V_2=GG-5, V_3=GG-6, V_4=GAUG-10, V_5=GG-11, V_6=GG-13, V_7=GG-20, V_8=GJG-22, V_9=GJG-31, V_{10}=GJG-32. T_0=Distilled water or <2 EC, T_1=2 EC, T_2=4 EC, T_3=6 EC, T_4=8 EC, T_5=10 EC

Lipid peroxidation

The data on lipid peroxidation of groundnut varieties with different salinity treatments were recorded from whole seedlings tissues and depicted in Table 7. The lipid peroxidation was found to be significant among all the treatments. The mean value of highest lipid peroxidation was found 38.61 nmol.g^{-1} fr. wt in variety GG-4 and lowest lipid peroxidation was found 25.35 nmol.g^{-1} fr. wt in variety GJG-31. Higher lipid peroxidation (44.45 nmol.g^{-1} fr. wt.) was recorded under application of 10 EC (T_5) salinity stress. Minimum lipid peroxidation (25.58 nmol.g^{-1} fr. wt.) was recorded under control condition (T_0). The lipid peroxidation was found significantly higher in variety susceptible GG-4 and lower in tolerant GJG-31 as compared to other varieties under the influence of salinity. The interaction effect between varieties and salinity treatments indicated that the higher lipid peroxidation (56.33 nmol.g^{-1} fr. wt.) was recorded in V_1 variety (GG-4) under T_5 treatment (10 EC).

Malviya (2015) [6] studied effect of salt stress on growth parameter, lipid peroxidation, antioxidant enzymes and lignans of sesame. Their results show that, under increasing salinity, MDA content increased significantly during the experimental period in all varieties as compared to control groups. Lipid peroxidation was higher at both 50 and 100 mM NaCl treatment in all varieties to control groups. They concluded that membrane damage in term of lipid peroxidation, which was much more than the control plant groups in variety Uma, indicates that it is the most sensitive variety of the three accession of *Sesamum indicum* tested. TMV-3 showed very less lipid peroxidation which correlate that this varieties is more tolerant to salt stress. Neto *et al.* (2006) [19] observed increase in MDA content in maize genotypes roots and leaves under salinity stress. LPO has enhanced only in salt-stressed leaves of the salt sensitive genotypes (BR5011) of maize. These results indicate that oxidative stress by salt stress in roots and leaves increase LPO in salt-sensitive genotype (BR5011) to maintain or increase the activity of antioxidant enzymes of salt tolerant genotypes (BR5033), reduce the level of LPO.

Table 7: Effect of salinity on lipid peroxidation (nmol.g^{-1} of fr. wt.) in groundnut varieties at 15 DAS

	Salinity treatments (T)						
Varieties (V)	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**Mean V**
V_1	26.67	27.82	34.50	40.38	45.95	56.33	38.61
V_2	24.83	28.50	34.87	37.57	40.75	46.00	35.42
V_3	24.33	29.08	34.93	37.79	41.46	46.75	35.72
V_4	28.29	30.90	34.83	37.00	40.36	42.75	35.69
V_5	27.91	31.75	38.16	40.83	42.33	45.42	37.74
V_6	25.41	28.33	34.55	38.50	41.49	46.00	35.71
V_7	24.87	29.29	35.40	38.73	41.29	43.50	35.51
V_8	27.88	30.50	38.33	40.37	43.29	45.67	37.67
V_9	21.21	22.15	24.17	25.33	28.33	30.93	25.35
V_{10}	24.33	25.83	30.00	33.00	38.67	41.17	32.17
Mean T	25.58	28.42	33.97	36.95	40.39	44.45	
	S.Em. ±	C.D. at 5%					
V	0.214	0.599	CV %	2.61			
T	0.166	0.464					
V X T	0.524	1.468					

V_1=GG-4, V_2=GG-5, V_3=GG-6, V_4=GAUG-10, V_5=GG-11, V_6=GG-13, V_7=GG-20, V_8=GJG-22, V_9=GJG-31, V_{10}=GJG-32. T_0=Distilled water or <2 EC, T_1=2 EC, T_2=4 EC, T_3=6 EC, T_4=8 EC, T_5=10 EC

Protein profiling by SDS-PAGE

Buffer soluble proteins from different groundnut varieties are treated with different saline water treatments and were separated by SDS-PAGE (Sodium dodecyl sulphate-polyacrylamide gel electrophoresis) (Table 8, Figure 1).

In GG-4 (V_1), GG-5 (V_2), GG-6 (V_3), GG-13 (V_6), GJG-22 (V_8) and GJG-32 (V_{10}) varieties total four bands of proteins were separated with different Rf value ranged from 0.102 to 0.918 and molecular weight ranged from 6.94 to 213.33 kDa. In GAUG-10 (V_4), GG-11 (V_5) and GJG-31 (V_9) varieties total five bands of proteins were separated with Rf value ranged from 0.147 to 0.894 and molecular weight ranged from 9.86 to 245.98 kDa. In GG-20 (V_7) variety total six bands of protein were separated with different Rf value ranged from 0.196 to 0.801 and molecular weight ranged from 12.55 to 209.24 kDa.

Jaccard's similarity coefficient and UPGMA method were used to develop a dendrogram (Figure 2) which divided into two main clusters-A and B with an average similarity of 58%. Cluster A further divided into two groups, A1 and A2. A1 comprises of total 3 treatments in that T_0 and T_1 (2 EC) was found in same group while T_2 (4 EC) was separated from this group. In A2 cluster, T_3 (6 EC) and T_4 (8 EC) were shown similar protein banding pattern. Cluster B consisted with T_5 (10 EC) which was totally different from other treatments and shown different banding pattern due to effect of higher salinity treatments.

Table 8: Densitometric analysis for SDS protein (buffer soluble fraction) of groundnut seedling with different saline water treatments

Varieties	**Total bands**	**Rf**	**MW (kDa)**	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
V_1 (GG-4)	1	0.102	213.33	-	+	-	-	-	-
	2	0.186	153.71	-	+	+	-	-	+
	3	0.265	113.20	-	-	-	-	-	+
	4	0.791	14.73	+	+	+	+	+	+
V_2 (GG-5)	1	0.113	208.80	+	+	+	+	-	-
	2	0.240	119.15	+	+	+	-	-	+
	3	0.748	12.85	+	+	+	+	+	+
	4	0.824	9.19	+	+	+	+	+	+
V_3 (GG-6)	1	0.250	137.20	+	+	+	+	-	+
	2	0.304	114.70	+	+	+	+	+	+
	3	0.824	20.16	+	+	+	+	+	+
	4	0.918	14.69	+	+	+	+	-	+
V_4 (GAUG-10)	1	0.230	142.59	+	-	+	+	+	+
	2	0.322	97.44	+	+	+	+	+	-
	3	0.489	48.88	-	+	+	+	+	-
	4	0.759	15.97	+	+	+	+	+	+
	5	0.845	11.18	+	-	+	-	-	+
V_5 (GG-11)	1	0.226	163.65	-	-	+	-	-	+
	2	0.293	119.77	+	+	+	+	-	-
	3	0.343	95.21	+	+	+	+	+	+
	4	0.675	20.75	+	+	+	+	+	+
	5	0.836	9.86	+	+	+	+	+	+
V_6 (GG-13)	1	0.264	120.15	+	-	+	-	-	-
	2	0.341	82.04	+	+	+	+	+	-
	3	0.487	39.74	-	-	+	+	-	-
	4	0.717	12.77	+	+	+	+	+	+
V_7 (GG-20)	1	0.196	209.24	+	-	+	+	+	-
	2	0.236	173.91	-	-	+	+	+	-
	3	0.310	123.36	+	+	-	+	+	-
	4	0.477	56.59	+	+	-	-	-	-
	5	0.739	16.79	+	+	+	+	+	+
	6	0.801	12.55	+	+	+	+	-	-
V_8 (GJG-22)	1	0.222	181.25	+	+	+	-	-	+
	2	0.322	107.26	+	+	+	+	+	+
	3	0.503	41.72	+	-	-	+	-	-
	4	0.793	9.18	+	+	+	+	+	+
V_9 (GJG-31)	1	0.147	245.98	+	+	-	-	+	+
	2	0.296	131.61	+	+	+	+	+	+
	3	0.485	59.51	+	+	+	-	-	-
	4	0.582	39.54	+	+	+	+	+	-
	5	0.894	10.66	+	+	+	+	+	+
V_{10} (GJG-32)	1	0.155	191.59	-	-	+	-	-	-
	2	0.331	77.51	+	+	+	+	+	+
	3	0.505	31.74	+	-	+	-	-	-
	4	0.801	6.94	+	+	+	+	+	+

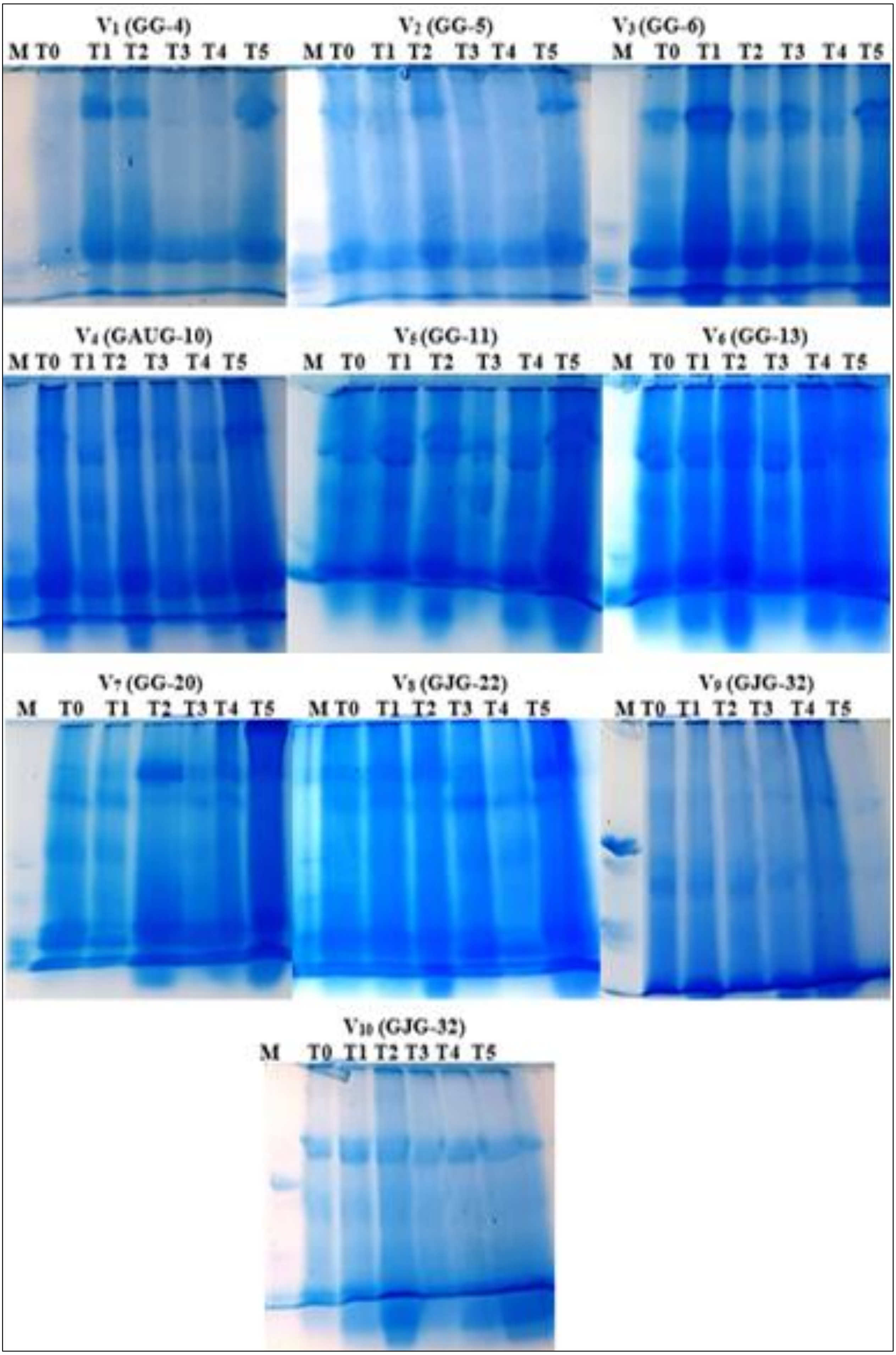

Fig 1: Banding pattern of groundnut seedling with different saline water treatments by SDS protein

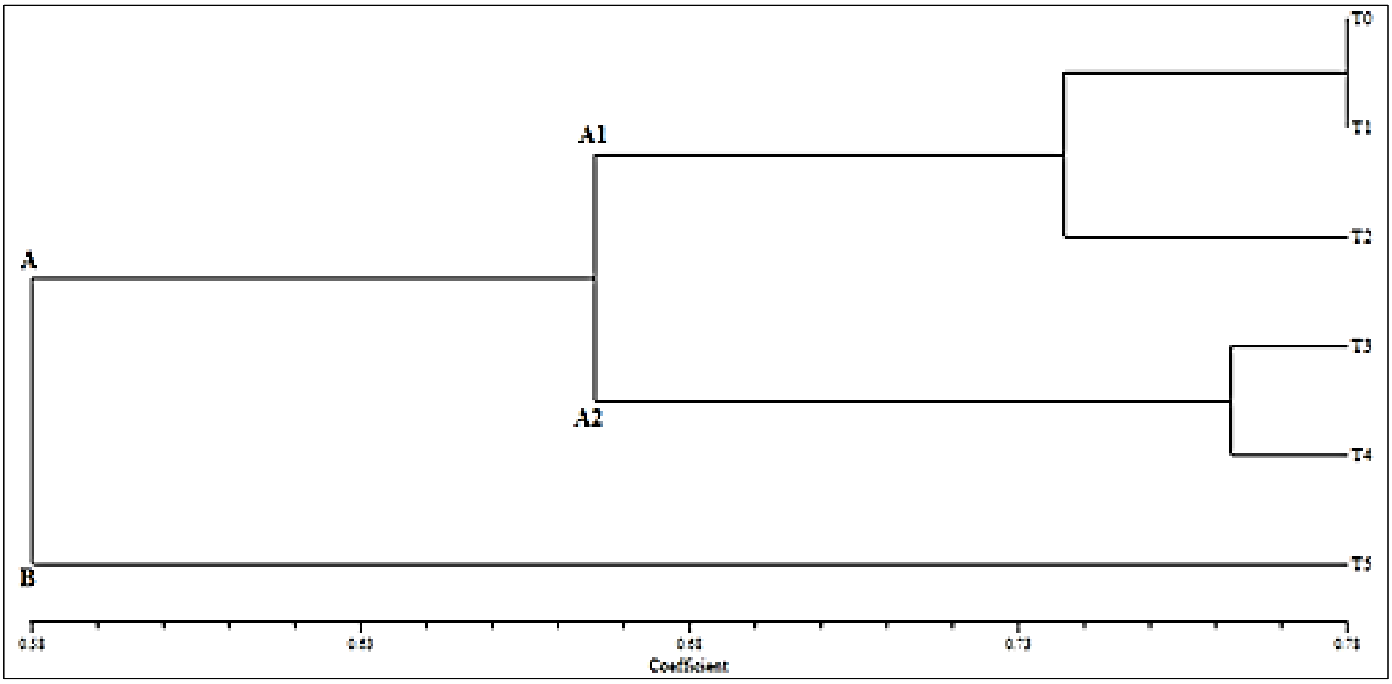

Fig 2: Dendrogram depicting the treatments variations in groundnut based on the SDS PAGE data

Correlation

The correlation of different parameters and salinity influenced of ten groundnut varieties treated under salinity stress condition is given in Table 9. All biochemical parameters are positively correlated with Salt tolerance index. The STI has highly significant positive relationship with total chlorophyll (0.932), glycine betaine (0.902), free proline (0.871), RWC (0.859) and germination percentage (0.804) while significant negative relationship with lipid peroxidation (0.760). The highest positive correlation was observed between salt tolerance index and chlorophyll content followed by glycine betaine and free proline content. It indicated that glycine betaine and free proline are biochemical markers and STI and RWC was physiological marker against salinity stress in groundnut. However, lipid peroxidation was significantly negative correlation with salinity tolerance index and it was considered as negative marker under salinity stress.

Table 9: Correlation of salt tolerance index compared with physiological and biochemical parameters

	STI	GP	RWC	Total Chll	Free Proline	GB	LP
STI	1.000						
GP	0.804^b	1.000					
RWC	0.859^c	0.692^a	1.000				
Total Chll	0.932^c	0.901^c	0.769^b	1.000			
Free Proline	0.871^c	0.744^b	0.700^a	0.847^c	1.000		
GB	0.902^c	0.689^a	0.748^b	0.841^b	0.771^b	1.000	
LP	-0.760^b	-0.668^a	-0.829^b	-0.735^b	-0.589^a	-0.798^b	1.000
n=10, ^{a}P(0.05)=0.576, ^{b}P(0.01)=0.708, ^{c}P(0.001)=0.847							

(STI-Srought tolerant index; GP-Germination percentage; RWC-Relative water content; Total Chll-Total chlorophyll; GB-Glycine betaine; LP-Lipid peoxidation).

Conclusion

Groundnut was highly affected by salinity stress. Different six salinity treatments and ten varieties were used for this study. On the basis of results, the groundnut variety GJG-31 showed significantly higher values in physiological parameters *viz.*, germination percentage, relative water content, salt tolerance index; and biochemical parameters such as total chlorophyll, free proline, glycine betaine, lipid peroxidation and protein profiling under various salinity treatments. However, lipid peroxidation negatively correlated due to salinity stress particularly in susceptible varieties. It can be concluded that the variety GJG-31 was found to be tolerance against salinity stress. This variety GJG-31 performed better under different salinity level and demonstrated better tolerance criteria. The order of groundnut varieties with respect to salt tolerance are GJG-31 > GJG-32 > GJG-22 > GG-20 > GG-11 > GAUG-10 > GG-5 > GG-13 > GG-6 > GG-4.

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