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Differential Responses on Chlorophyll Content, Osmolyte Accumulation and Membrane Damage Parameters under Salinity Stress on Tolerant and Susceptible Genotypes of Groundnut

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ABSTRACT

Salinity can affect different physiological activity of plant in various ways. A controlled study was conducted to screen 26 genotypes of groundnut under 200mM NaCl salinity stress. The salt tolerance index or STI of the genotypes ranged from 47.57% to 96.40%. Out of all the genotypes KDG-197 (STI= 96.40%) was found to be the most tolerant under a salinity stress of 200 mM NaCl and it was closely followed by R 2001-2 (STI=87.92%), VG 315 (STI=84.05%), TCGS 1157 (STI=77.59%) and TG 51 (STI=73.67%). While the genotypes Girnar 3 (STI= 47.57%), OG 52-1 (STI=49.09%), TVG 0856 (STI= 49.28%) and J 86 (STI= 50.66%) were the most susceptible genotypes based on their relative performance under stress in respect of total dry weight. It has been noted further that out of the nine genotypes, KDG 197 registered the minimum reduction (4.51% over control, 2.70% over control) in total chlorophyll and sugar accumulation respectively under NaCl stress whereas, Girnar 3 recorded the highest reduction in both parameters (60.00%, 70.32% over control) respectively, under saline condition. The genotype KDG 197 and R 2001-2 accounted for the highest increase in soluble protein and proline content in their leaves (144.02%, 780.16% over control) respectively than Girnar 3. KDG 197 recorded the minimum (3.39%) increase in lipid peroxidation under stress followed by R 2001-2 with an increase of 13.04% over control plants. In contrast, Girnar 3 registered the highest increase of TBARS content and electrolyte leakage (44.44%, 31.47% over control respectively) indicating maximum membrane damage but R 2001-2 recorded the minimum (3.00%) increase in electrolyte leakage percentage than Girnar 3 (31.47% over control) followed by OG 52-1 (26.14% over control) under stress. So, better osmotic adjustment through accumulation of proline, less membrane damage the leaves helped the tolerant genotypes to sustain under salinity stress in a better way than the susceptible genotypes.

Key words: Compatible solute, Electrolyte leakage, Peroxidation, Salinity, Salt tolerance index.

INTRODUCTION

In saline soils plant faces prime problem of obtaining water from a soil of negative osmotic potential and its consequent effects was decreasing of germination and seedling growth, dry matter production (Janila *et al.,* 1999) and inducing Ca, K and Fe deficiencies in groundnut (Singh *et al.,* 2004) causing yield losses (Hunshal *et al.,* 1991). Groundnut is an important oilseed, food and feed crop of our country whose productivity is curtailed due to salinity stresses. Soil salinity is one of the most important abiotic factors affecting the groundnut productivity in the major groundnut growing states of India (Chhabra and Kamra, 2000). Groundnut yields have been reported to be severely affected with an increase in soil and water salinity (Girdhar *et al*, 2005 and Nithila *et al*., 2013). One of the most effective ways to overcome salinity problems is the introduction of salt tolerant crops. It has been reported that differences in salt tolerance exist, not only among different species, but also within certain species (Fooland and Lin, 1997). Success of selection of salt tolerant types depends upon the amount of genetic variation present in the population. Some research works have been carried out so far to screen groundnut genotypes for evolving tolerant genotypes (Joshi *et al.,* 1990; Nautiyal *et al.,* 2000; Mensah *et al*., 2006; Badigannavar *et al*., 2007; Singh *et al.,* 2007 and Nithila *et al*., 2013). However, information on tolerance of this crop to salinity at different growth stage as well as the physiological basis of tolerance is still meager. Based on these backgrounds, the present study was formulated with the objective to evaluate the physiological basis of salt tolerance among 26 genotypes

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of groundnut under 200mM salinity stress and to select the salt tolerant genotypes for higher productivity. Further, these genotypes are used in improving programmes; it seems to be effective and economic improvement.

MATERIALS AND METHODS

Experimental site

The laboratory experiment was carried out in Departmental Laboratory of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia and West Bengal in the year 2017.

Plant material

Evaluation for salinity tolerance was done with 26 genotypes of groundnut [*Arachis hypogaea* L] (Table 1). The seeds of all the genotypes were collected from AICRP (All India Co-ordinated Research Project) on Groundnut, Kalyani Centre, West Bengal.

Plant culture

For studies on seedling growth, the seeds of 26 genotypes of groundnut were surface sterilized with 0.1% $HgCl_2$ (w/v) solution for 3 minutes followed by thorough washing in distilled water. Twenty seeds of each of the genotypes were arranged in petridish of 9 cm diameter on Whatman No.1 filter paper moistened with normal distilled water. The seeds were then allowed to germinate for 72 hours at a temperature of 28±1°C and relative humidity around 80±1%. The germinated seeds were then transferred to plastic beakers of capacity one litre containing neutral sand. Full strength Hoagland solution prepared as per modification of Epstein (1972) (Table 2), was supplemented to each beaker as nutrient medium maintain the pH 6.3. Growing of groundnut genotypes in sand culture has been shown in plate no I.

Treatment application

Fourteen-day old seedlings were subjected to salinity treatment. For this, the modified Hoagland nutrient solution containing 200 mM NaCl (osmotic potential of about -0.8 MPa) was applied in each case and the pH was adjusted to 6.3. The treatments were repeated on every third day. Control set without salinity stress was also maintained similarly in each case for comparison of results. Observations on different dry weight and biochemical parameters were recorded on 40-day old seedlings.

Stress response index

The stress response index (SRI) in respect of comparative growth performances under salinity stress and unstressed control condition in each genotype was calculated as per Chen *et al*. (2007) using the following formula:

$$SRI\ \% = \frac{\text{Mean value of the genotype under stress}}{\text{Mean value of the genotype under non-stress condition}} \times 100$$

Table 1: List of genotypes used in the experiment.

Sl. No.	Genotype	Sl. No.	Genotype	Sl. No.	Genotype
1	AK 335	10	VG 09221	19	TG 75
2	KDG 197	11	ICGV 07038	20	ICGV 03042
3	ICGV 05155	12	ICGV 06138	21	OG 52 -1
4	AK 343	13	TG 74	22	Girnar 3
5	ICGV 03043	14	TVG 0856	23	CGMG 2010
6	Dh 235	15	JCG 3005	24	R 2001-2
7	ICGV 06420	16	LGN 163	25	CTMG 11
8	J 86	17	VG 315	26	TG 51
9	JCG 2141	18	TCGS 1157		

Table 2: Composition of modified Hoagland nutrient solution (Epstein, 1972).

Compound	Concentration of stock solution (mM)	Volume of stock solution per litre of final solution (ml)
	Macronutrient	
KNO_3	1,000	6.0
$Ca(NO_3)_2$, $4H_2O$	1,000	4.0
$NH_4H_2PO_4$	1,000	2.0
$MgSO_4$,$7H_20$	1,000	1.0
	Micronutrient	
KCl	25	2.0
H_3BO_3	12.5	
$MnSO_4$, H_2O	1.0	
$ZnSO_4$, $7H_2O$	1.0	
$CuSO_4$, $5H_2O$	0.25	
H_2MoO_4	0.25	
Fe-EDTA	53.7	1.0

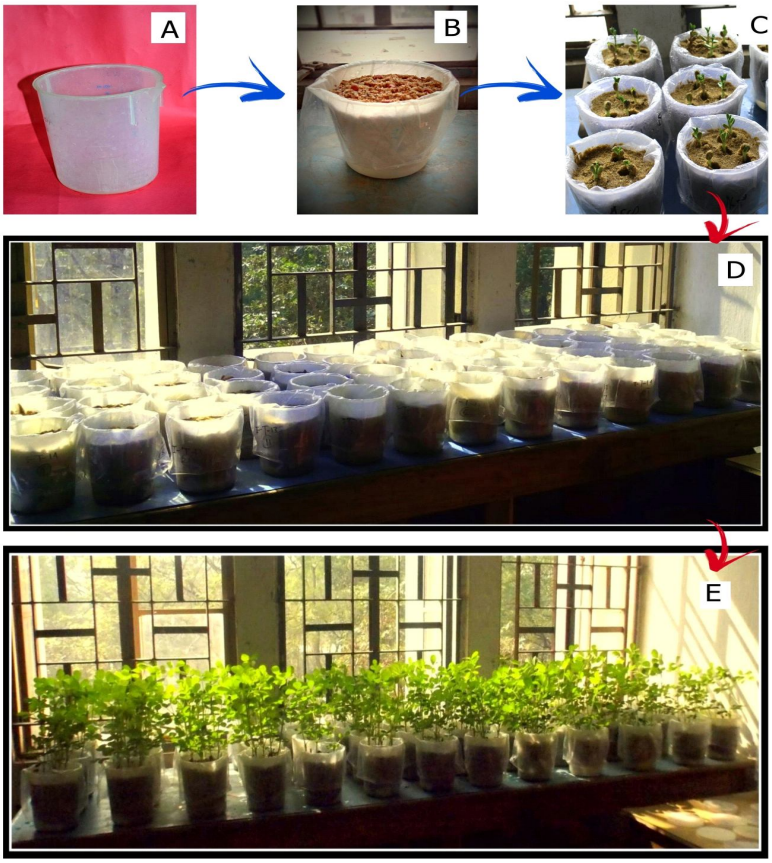

Plate I: Growing of groundnut genotypes in sand culture under salinity stress.

Salt tolerance index

The stress response index of total dry weight of the seedling was expressed as salt tolerance index (STI) for each of the genotypes.

Estimation of biochemical character

The chlorophyll content in the leaf sample was estimated as per Arnon (1949). Absorbance was read at 645 and 663 nm wavelengths in systronics-105 spectrophotometer against a blank containing only 80% acetone.

The soluble protein content in the leaf was estimated following the methods of Lowry *et al.* (1951). Absorbance was read at 660 nm against a reagent blank. Total soluble protein was estimated from a standard curve of BSA.

Extraction and estimation of total soluble sugar was done following the method of Yoshida *et al*. (1972). Amount of sugar was estimated from standard curve of glucose.

Proline content was determined from the leaf sample as per the method of Mohanty and Sridhar (1982).

Membrane damage was estimated in terms of lipid peroxidation and electrolyte leakage .The level of lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) content, a product of lipid peroxidation following the method of Heath and Packer (1968). The Electrolyte leakage was determined as per the method described method by Guo *et al.* (2006).

Statistical analysis

The mean data in all the cases were subjected to statistical analysis following two factor factorial designs with three replications using INDOSTAT version 7.1 software.

RESULTS AND DISCUSSION

Effect of salinity stress on seedling total dry weight of twenty-six groundnut genotypes

In the present experiment, the effect of 200 mM NaCl stress on the growth parameters in 40-day old seedlings of twenty six genotypes of groundnut was studied (Table 3). Genotypic means for total dry weight ranged from 0.49 to 1.12 g under control condition and from 0.30 to 1.07 g under salinity stress. The minimum reduction has been noted in KDG 197(3.89% over control), R 2001-2 (12.45% over control), VG 315 (15.96% over control), TCGS 1157 (22.42% over control) and TG 51 (26.40% over control). On the contrary, the highest reduction in total dry weight under salinity stress was found in Girnar 3 (52.06%) followed by TVG 0856 (51.20%), OG 52-1 (50.91%) and J 86 (49.17%).Reduction in plant growth as a result of salt stress has already been reported in several other species (Cicek and Cakirlar, 2002; Ashraf and Harris, 2004; Bakht *et al*., 2006; Munns *et al*., 2006; Ashraf *et al*., 2008; Ashraf, 2009; Achakzai *et al.,* 2010 and Akram *et al*., 2010). Salinity has both osmotic and

Table 3: Effect of salinity stress on dry weight of 40-day old plant and its different parts in 26 genotypes of groundnut.

Genotype	Root dry weight (g)			Shoot dry weight (g)			Leaf dry weight (g)			Total dry weight (g)		
	Control	Treatment	Mean	Control	Treatment	Mean	Control	Treatment	Mean	Control	Treatment	Mean
AK 335	0.10	0.09(-12.88)	0.10ijkl	0.34	0.21(-38.83)	0.28ijklm	0.20	0.09(-54.11)	0.15hij	0.65	0.39(-39.49)	0.52klmn
KDG 197	0.16	0.21(33.31)	0.19a	0.61	0.58(-4.40)	0.59a	0.34	0.28(-19.40)	0.31a	1.11	1.07(-3.89)	1.09a
ICGV 05155	0.10	0.06(-40.00)	0.08 l	0.33	0.23(-29.30)	0.28hijklm	0.19	0.10(-45.63)	0.15hij	0.62	0.40(-35.48)	0.51klmno
AK 343	0.13	0.12(-5.15)	0.13defghij	0.44	0.31(-28.26)	0.38cdefgh	0.26	0.14(-45.46)	0.20cdef	0.83	0.58(-30.52)	0.70defghi
ICGV 03043	0.09	0.07(-28.51)	0.08 l	0.37	0.20(-46.36)	0.28hijklm	0.21	0.09(-57.81)	0.15ghij	0.68	0.35(-49.01)	0.51klmno
Dh 235	0.12	0.08(-33.33)	0.10hijkl	0.41	0.23(-43.55)	0.32efghijk	0.25	0.11(-56.75)	0.18efgh	0.78	0.42(-45.50)	0.60ijkl
ICGV 06420	0.15	0.15(0.00)	0.15bcde	0.39	0.22(-42.25)	0.31fghijkl	0.23	0.10(-55.89)	0.16fghi	0.76	0.47(-38.43)	0.62hijk
J 86	0.20	0.13(-35.00)	0.17abc	0.51	0.26(-48.69)	0.38cdefg	0.30	0.12(-59.56)	0.21bcde	1.00	0.51(-49.17)	0.76cdef
JCG 2141	0.20	0.15(-25.00)	0.18ab	0.46	0.26(-44.59)	0.36defghi	0.27	0.12(-57.30)	0cdef.20	0.94	0.52(-44.33)	0.73defgh
VG 09221	0.10	0.10(0.00)	0.10hijkl	0.41	0.27(-33.87)	0.34defghij	0.24	0.12(-49.32)	0.18defgh	0.75	0.50(-33.33)	0.63ghijk
ICGV 07038	0.10	0.11(13.75)	0.10ghijkl	0.36	0.23(-35.52)	0.29ghjklm	0.21	0.10(-51.57)	0.16fghij	0.66	0.44(-34.16)	0.55jklm
ICGV 06138	0.12	0.08(-33.33)	0.10hijkl	0.29	0.19(-34.48)	0.24klm	0.15	0.09(-40.00)	0.12jk	0.56	0.36(-35.93)	0.46mno
TG 74	0.09	0.08(-11.11)	0.09kl	0.25	0.19(-23.69)	0.22lm	0.15	0.09(-40.00)	0.12jk	0.49	0.36(-26.35)	0.43no
TVG 0856	0.13	0.08(-39.46)	0.10hijkl	0.35	0.18(-48.12)	0.27ijklm	0.21	0.08(-61.90)	0.15hijk	0.69	0.34(-51.20)	0.51klmno
JCG 3005	0.24	0.08(-66.67)	0.16abcd	0.48	0.37(-22.23)	0.43cd	0.28	0.17(-41.16)	0.23bc	1.00	0.62(-38.00)	0.81cd
LGN 163	0.13	0.06(-52.64)	0.09jkl	0.24	0.16(-34.24)	0.20m	0.14	0.07(-50.00)	0.11k	0.51	0.30(-41.82)	0.40o
VG 315	0.13	0.12(-5.29)	0.12efghij	0.60	0.55(-8.83)	0.58a	0.36	0.25(-30.84)	0.30a	1.09	0.91(-15.96)	1.00a
TCGS 1157	0.17	0.13(-23.53)	0.15bcde	0.50	0.43(-14.66)	0.46bc	0.30	0.19(-35.96)	0.24b	0.97	0.75(-22.42)	0.86bc
TG 75	0.17	0.11(-37.24)	0.14cdef	0.47	0.33(-30.00)	0.40cdef	0.27	0.14(-47.57)	0.21bcde	0.91	0.58(-36.40)	0.74cdefg
ICGV 03042	0.17	0.06(-64.01)	0.11fghijkl	0.28	0.22(-21.43)	0.25jklm	0.16	0.10(-36.74)	0.13ijk	0.61	0.38(-37.50)	0.50lmno
OG 52-1	0.19	0.09(-53.56)	0.14cdefg	0.46	0.25(-44.93)	0.36defghi	0.27	0.11(-59.26)	0.19cdefg	0.92	0.45(-50.91)	0.68efghi
Girnar 3	0.16	0.10(-40.78)	0.13defghi	0.46	0.23(-50.36)	0.34defghij	0.27	0.10(-62.96)	0.19cdefgh	0.89	0.43(-52.06)	0.66efghij
CGMG 2010	0.13	0.10(-23.08)	0.12fghijk	0.39	0.30(-24.56)	0.35defghij	0.23	0.13(-42.86)	0.18defgh	0.76	0.53(-29.96)	0.64 fghij
R 2001-2	0.11	0.10(-6.09)	0.11fghijkl	0.44	0.41(-5.36)	0.43cd	0.26	0.18(-28.59)	0.22bcd	0.80	0.70(-12.45)	0.75 cdef
CTMG 11	0.17	0.09(-45.12)	0.13cdefgh	0.49	0.34(-30.82)	0.41cde	0.29	0.15(-47.68)	0.22bcd	0.94	0.58(-38.16)	0.76 cde
TG 51	0.15	0.10(-31.13)	0.13defghij	0.61	0.50(-17.94)	0.56ab	0.36	0.23(-37.60)	0.30a	1.12	0.83(-26.40)	0.98ab
Mean	0.14a	0.10b		0.42a	0.29b		0.25a	0.13b		0.81a	0.53b	
	S.E. m(±)	C.D.(P= 0.05)		S.E. m(±)	C.D.(P= 0.05)		S.E. m(±)	C.D.(P= 0.05)		S.E. m(±)	C.D.(P= 0.05)	
Genotype(G)	0.017	0.04		0.05	0.10		0.02	0.04		0.06	0.12	
Treatment(T)	0.005	0.01		0.01	0.03		0.01	0.01		0.02	0.03	
G × T	0.025	0.05		Non significant			Non significant			Non significant		

Means showing the same letters in a column do not differ significantly at 5% probability level.

Data in parentheses indicate percentage increase (+) or decrease (-) over control.

specific ionic effects on plant growth (Dioniso-Sese and Tobita, 2000). Addition of salt keeps changing the osmotic potential of soil solution. This fluctuation in osmotic potential adversely influences the physiological availability of water (Suarez and Lebron, 1993) as a result of which plants can't maintain turgor and thus suffers reduction in their growth and development. Moreover, the reduction in plant shoot and root dry matter is due to combined effects of osmotic causes and toxicity caused by Cl^- and Na^+ ions (Hajer *et al.,* 2006).

Ranking of genotypes

In the present experiment, the salinity stress caused reduction in dry weight of 40-day old plant as well as its different parts except for root in few genotypes. The response of genotypes varied as indicated by different mean values of SRI. Among all the genotypes, KDG 197, VG 09221 and R 2001-2 recorded SRI > 100% for dry weight of root. Other four genotypes, *viz*., AK 343, TG 74, VG 315 and CGMG 2010 also registered SRI exceeding 100% for very high SRI for root dry weight. Thus, all these genotypes had a tendency to increase root biomass under osmotic shock condition in salinity stress. On the contrary, OG 52-1, Girnar 3 and ICGV 03042 had very low mean values of SRI for dry weight of root. The root biomass was very adversely affected by salinity stress in these three genotypes. The genotype KDG 197 exhibited the highest mean SRI for fresh and dry weight of leaf and total plant under stress in the present experiment. In contrast, the lowest SRI for dry weight of shoot, leaf and total plant was recorded by Girnar 3.

The salt tolerance index (expressed as SRI for total plant dry weight) of the genotypes ranged from 47.57% to 96.40% (Table 4). The genotypes scoring STIs around or above 75% were considered to be the most tolerant types while those scoring STIs around 50% or less were identified as the most susceptible genotypes. Out of all the genotypes KDG-197 (STI= 96.40%) was found to be the most tolerant under a salinity stress of 200 mM NaCl and it was closely followed by R 2001-2 (STI=87.92%), VG 315 (STI=84.05%), TCGS 1157 (STI=77.59%) and TG 51 (STI=73.67%). While the genotypes Girnar 3 (STI= 47.57%), OG 52-1 (STI=49.09%), TVG 0856 (STI= 49.28%) and J 86 (STI= 50.66%) were the most susceptible genotypes based on their relative performance under stress in respect of total dry weight. On the basis of salt tolerance index five most tolerant and four most susceptible genotypes were selected in the present experiment for studies on few physiological and biochemical characters to have an idea about the physiological basis of salt tolerance in these genotypes of groundnut.

Effect of salinity stress on chlorophyll content in the leaves of tolerant and susceptible genotypes of groundnut

In the present experiment, the content of chlorophyll a, chlorophyll b and total chlorophyll along with the ratio of chlorophyll a and b for salt tolerant and susceptible genotypes were determined under salinity stress and unstressed control condition. Genotypes as well as treatments showed highly significant differences among them for chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio. Genotype x treatment interaction also showed significant differences for all these characters. Salinity stress significantly reduced the content of chlorophyll a, b and total chlorophyll in the leaves of all the genotypes (Table 5). The results corroborated the early findings of Tort and Turkyilmaz (2004), Turan *et al*. (2007), Taffouo *et al*. (2010) and Mafakheri *et al*. (2010). This decrease might be attributed to the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments (Murkute *et al*., 2006). However, the genotypes varied in their responses in respect of deleterious effects of salinity stress on leaf chlorophyll in the present experiment (Table 5). The susceptible genotypes showed greater decrease in chlorophyll a as well as chlorophyll b content in their leaves than the tolerant ones under salinity in all the cases.

Genotypic means for chlorophyll a, chlorophyll b and total chlorophyll varied from 0.66 to 0.95, 0.33 to 0.59 and 0.99 to 1.48 mg g^{-1} fresh weight, respectively, under unstressed control condition, while the corresponding mean

Table 4: Salt tolerance indices (STI) of twenty six genotypes of groundnut.

Genotype	Salt tolerance index (STI) or stress responsive index (SRI)
KDG 197	96.40
R 2001-2	87.92
VG 315	84.05
TCGS 1157	77.59
TG 51	73.67
TG 74	73.65
CGMG 2010	70.04
AK 343	69.76
VG 09221	66.37
ICGV 07038	66.33
ICGV 05155	64.52
ICGV 06138	64.29
TG 75	63.60
JCG 3005	62.00
ICGV 03042	61.96
ICGV 06420	61.84
CTMG 11	61.05
AK 335	60.51
LGN 163	58.17
JCG 2141	55.87
Dh 235	54.51
ICGV 03043	51.47
J 86	50.66
TVG 0856	49.28
OG 52-1	49.09
Girnar 3	47.57

Table 5: Effect of salinity stress chlorophyll content in the leaf of tolerant and susceptible genotypes of groundnut.

Genotype	Chlorophyll a (mg g^{-1} fresh weight)			Chlorophyll b (mg g^{-1} fresh weight)			Total Chlorophyll (mg g^{-1} fresh weight)			Chlorophyll a/b		
	Control	Treatment	Mean	Control	Treatment	Mean	Control	Treatment	Mean	Control	Treatment	Mean
KDG 197	0.73	0.70(-3.94)	0.72f	0.59	0.57(-2.39)	0.58a	1.33	1.27(-4.51)	1.30a	1.24	1.22(-1.58)	1.23f
R 2001-2	0.78	0.70(-10.26)	0.74de	0.38	0.35(-7.89)	0.37d	1.16	1.05(-9.48)	1.11c	2.05	2.00(-2.56)	2.03d
VG 315	0.78	0.67(-14.10)	0.73ef	0.43	0.31(-27.91)	0.37d	1.21	0.99(-18.18)	1.10c	1.81	2.16(19.15)	1.99d
TCGS 1157	0.95	0.74(-22.52)	0.85a	0.52	0.43(-18.41)	0.47b	1.48	1.17(-21.07)	1.32a	1.83	1.74(-5.04)	1.78e
TG 51	0.92	0.70(-24.54)	0.81b	0.44	0.33(-25.82)	0.38c	1.37	1.02(-25.31)	1.19b	2.10	2.13(1.72)	2.12c
J 86	0.92	0.65(-28.86)	0.78c	0.44	0.24(-44.09)	0.34e	1.35	0.91(-32.77)	1.12c	2.09	2.66(27.23)	2.38b
TVG 0856	0.94	0.56(-40.32)	0.75d	0.40	0.19(-52.56)	0.29g	1.32	0.75(-43.06)	1.05d	2.37	2.98(25.78)	2.68a
OG 52-1	0.66	0.38(-42.44)	0.52h	0.33	0.17(-49.34)	0.25h	0.99	0.55(-44.74)	0.77f	2.01	2.28(13.62)	2.14c
Girnar 3	0.82	0.36(-56.18)	0.59g	0.48	0.16(-66.54)	0.32f	1.29	0.52(-60.00)	0.90e	1.71	2.24(30.94)	1.98d
Mean	0.83a	0.61b		0.44a	0.31b		1.28a	0.91b		1.91b	2.16a	
	S.E. m(±)	C.D.(P=0.05)		S.E. m(±)	C.D. (P=0.05)		S.E. m(±)	C.D. (P=0.05)		S.E. m(±)	C.D. (P=0.05)	
Genotype (G)	0.010	0.020		0.006	0.011		0.016	0.032		0.029	0.059	
Treatment (T)	0.004	0.010		0.003	0.005		0.007	0.015		0.014	0.028	
G×T	0.014	0.029		0.018	0.016		0.022	0.045		0.041	0.084	

Means showing the same letters in a column do not differ significantly at 5% probability level.

Data in parentheses indicate percentage increase (+) or decrease (-) over control.

values under salinity ranged from 0.36 to 0.74, 0.16 to 0.57 and from 0.52 to 1.27 mg g^{-1} fresh weight, respectively. Out of the nine genotypes, KDG 197 registered the minimum (4.51% over control) reduction in total chlorophyll under NaCl stress followed by R 2001-2 (9.48% over control). In contrast, Girnar 3 recorded the highest reduction (60.00% over control) under saline condition. The same trend was noted in case of chlorophyll a and chlorophyll b as well. Two genotypes, KDG 197 and R 2001-2 recorded the minimum reduction in chlorophyll a and b content when they were exposed to salinity stress. The corresponding values were 3.94% and 10.26% over control, respectively, for KDG 197 and R 2001-2 in case of chlorophyll a and 2.39% and 7.89% over control, in case of chlorophyll b content. Girnar 3 showed a reduction of 56.18% and 66.54% over control, respectively, for chlorophyll a and chlorophyll b as the seedlings were grown in saline media for 40 days. Results in the present study were consistent with the earlier reports by Saha *et al*. (2010) and Dutta and Bera (2014). In the present experiment, the ratio of chlorophyll a to chlorophyll b increased under stressed condition as compared to control in all the genotypes except KDG 197, R 2001-2 and TCGS 1157. This indicated that salinity stress, in general, caused more drastic damage to chlorophyll b than chlorophyll a.

Effect of salinity stress on total soluble sugar in the leaves of tolerant and susceptible genotypes of groundnut

Data on the content of total soluble sugar in the leaves of 40-day old seedlings under salinity stress as well as in unstressed control have been presented in Table 6. Highly significant differences among genotypes and between treatments were seen whereas interaction effects also exhibited highly significant variations. The mean values ranged from 63.20 to 354.20 mg g^{-1} dry weight under control and from 56.80 to 144.00 mg g^{-1} dry weight under 200 mM NaCl stress. The salinity stress significantly reduced the sugar content in leaves of in all the genotypes. Such decrease might be the consequence of inhibition of photosynthetic activity by salinity stress. It might be noted further that the susceptible genotypes were more severely affected than the tolerant ones for this character in the present experiment. Genotype KDG 197 registered the minimum reduction (2.70% over control) in sugar content followed by TCGS 1157 (10.13% over control), while the genotype Girnar 3 showed the highest reduction (70.32% over control). A salt-induced reduction in the amount of sugar has also been reported earlier by Singh and Singh (1999), Gupta *et al*. (1999), Promila and Kumar (2000), Patel *et al*. (2007) and Mousavi *et al*. (2008) in different crops.

Effect of salinity stress on soluble protein in the leaves of tolerant and susceptible genotypes of groundnut

The mean values of soluble protein content in the leaves of nine genotypes have been presented in the Table 6. The analysis of variance indicated highly significant variation among genotypes as well as between treatments for soluble

Table 6: Effect of salinity stress on total soluble sugar, soluble protein and proline content in the leaves of tolerant and susceptible genotypes of groundnut.

Genotype	Sugar (mg g^{-1} dry weight)			Protein(mg g^{-1} fresh weight)			Proline (µmol g^{-1} fresh weight)		
	Control	Treatment	Mean	Control	Treatment	Mean	Control	Treatment	Mean
KDG 197	148.00	144.00(-2.70)	146.00e	79.99	195.19(144.02)	137.59f	146.64	1166.97(695.81)	656.805a
R 2001-2	136.00	104.80(-22.94)	120.40f	80.36	179.87(123.83)	130.11g	126.2	1110.76(780.16)	618.48b
VG 315	122.40	97.60(-20.26)	110.00g	94.76	220.30(132.48)	157.53b	144.08	1149.08(697.53)	646.58a
TCGS 1157	63.20	56.80(-10.13)	60.00i	125.59	168.61(34.25)	147.10de	146.64	1166.97(695.81)	656.81a
TG 51	226.40	113.60(-49.82)	170.00d	106.76	196.30(83.87)	151.53cd	89.16	563.05(531.51)	326.11e
J 86	131.20	63.20(-51.83)	97.20h	169.72	200.92(18.38)	185.32a	133.86	735.49(449.45)	434.68c
TVG 0856	320.00	127.20(-60.25)	223.60b	103.62	147.75(42.59)	125.68g	109.59	566.88(417.27)	338.24e
OG 52-1	295.20	124.80(-57.73)	210.00c	125.22	164.18(31.11)	144.70e	154.3	666.25(331.79)	410.28d
Girnar 3	354.40	105.2(-70.32)	229.80a	152.36	154.76(1.58)	153.56bc	325.46	573.27(76.14)	449.37c
Mean	199.64a	104.13b		115.38b	180.88a		152.88b	855.41a	
	S.E. m(±)	C.D.(P=0.05)		S.E. m(±)	C.D.(P=0.05)		S.E. m(±)	C.D.(P=0.05)	
Genotype (G)	1.85	3.75		2.33	4.72		11.72	23.78	
Treatment (T)	0.87	1.77		1.10	2.22		5.53	11.21	
G×T	2.61	5.30		3.29	6.67		16.58	33.63	

Means showing the same letters in a column do not differ significantly at 5% probability level.
Data in parentheses indicate percentage increase (+) or decrease (-) over control.

protein. The interaction effects of genotype and treatment also exhibited significant differences for this character. Genotypic means for soluble protein ranged from 79.99 to 169.72 mg g^{-1} fresh weight and from 147.75 to 220.30 mg g^{-1} fresh weight, under control condition and salinity stress, respectively. Salinity stress increased the protein content in leaves of all the genotypes. However, the genotypes differed in their responses. Such an increase in leaf protein in response to stress exposure might be attributed mainly to the increased synthesis of stress proteins (Jiang and Huang, 2002 and Sibole *et al*., 2003). Results of a recent study by Kapoor and Srivastava (2010) in *Vigna mungo* (L.) also corroborated well the results of the present experiment. However, decrease in total soluble protein content under NaCl stress was reported earlier by Al-aghabary *et al*. (2004), Parida and Das (2005) and Parvaiz and Satyavati, (2008). In general, the tolerant genotypes registered higher increase in soluble protein content in their leaves than the susceptible ones in the present experiment. The genotype KDG 197 accounted for the highest increase (144.02% over control) followed by VG 315 (132.48% over control), whereas genotype Girnar 3 managed to score only a slight increase (1.58% over control) under salinity stress.

Effect of salinity stress on proline content in the leaves of tolerant and susceptible genotypes of groundnut

The accumulation of osmolytes especially that of proline, is a common phenomenon in plants under osmotic shock. Besides its role as an osmolyte, proline contributes to scavenging ROS, stabilizing subcellular structures, modulating cell redox homeostasis, supplying energy and functioning as a signal (Kavi-Kishor *et al.,* 2005; Verbruggen and Hermans 2008; Szabados and Savouré 2010; Sharma *et al.,* 2011).

In the present study, mean values of proline content in leaf have been presented in the Table 6. Perusal of data revealed that all the nine genotypes showed significant variation among them in respect of leaf proline content. The treatments as well as genotype and treatment interaction also registered significant diggerences for this character. However, the genotypes differed in their responses to salinity treatment in respect of this character. Genotypic means for proline content ranged from 89.16 to 325.46 µmol g^{-1} fresh weights and from 563.05 to 1166.97 µmol g^{-1} fresh weight under control condition and saline stress, respectively. Salinity caused significant increase in leaf proline over control in all the genotypes with tolerant ones showing much higher range of increase than the susceptible genotypes. This increase in level of proline might attribute for maintenance of osmotic balance between cytoplasm and vacuole during osmotic shock induced by salinity (Flowers and Yeo, 1981). The non-enzymatic antioxidant proline might also help in the mitigation of adverse effect of ROS as suggested by Chen and Dickman (2005). Out of all the genotypes, the highest increase over control was recorded by R 2001-2 (780.16%) and it was closely followed by VG 315 (697.53%). The minimum increase over control was

recorded by Girnar 3 (76.14%). These results conformed to the early findings in groundnut by Girija *et al.* (2002) and Nithila *et al*. (2013). An increase in proline content under stress condition might be due to breakdown of proline-rich protein or *de novo* synthesis of proline. It could also be due to prevention of feedback inhibition of the biosynthetic enzyme caused by sequestering proline away from its site of synthesis or by relaxed feedback inhibition of the regulatory step enzyme or by decreased activity of enzymes involved in degradation of proline such as proline dehydrogenase and proline oxidase (Girija *et al*., 2002).

Effect of salinity stress on lipid peroxidation and electrolyte leakage in the leaves of tolerant and susceptible genotypes of groundnut

The lipid peroxidation in both cellular and organelle membranes takes place when above-threshold levels of ROS are reached, thereby not only directly affecting normal cellular functioning, but also aggravating the oxidative stress through production of lipid-derived radicals. The extent of leaf membrane damage under stress was measured by determining the level of lipid peroxidation estimated in terms of concentration of thiobarbituric acid-reactive substances (TBARS) and by electrolyte leakage percentage. Lipid peroxidation (LPO) refers to the oxidative degradation of lipids. Peroxidation of lipid results when polyunsaturated fatty acids (PUFA) in membrane undergo oxidation by hydroxyl radicals and singlet oxygen, giving rise to complex mixtures of lipid hydroperoxides. Lipid peroxidation decreases the fluidity of membrane, increases the leakiness and causes secondary damage to membrane proteins (Moller *et al*., 2007).

The statistical analysis indicated that the treatments, genotypes as well as treatment x genotype interaction had highly significant differences for both TBARS content and electrolyte leakage of membrane in the present experiment (Table 7). In general, all the nine genotypes showed higher content of TBARS in their leaves under salinity stress than unstressed control indicating oxidative stress-induced membrane damage. Comparison of data indicated that the susceptible genotypes registered higher increase of TBARS content over control than tolerant ones. The observed increase in TBARS concentration in stressed plants might indicate extensive lipid peroxidation of cell membrane components caused by ROS generated by the oxidative stress. The mean value of the genotypes ranged from 11.61 to 25.49 µmol of TBARS content g^{-1} fresh weight under unstressed condition and from 13.12 to 31.77 µmol of TBARS content g^{-1} fresh weight under stress. Out of all the genotypes, KDG 197 recorded the minimum (3.39%) increase in lipid peroxidation under stress which was closely followed by R 2001-2 with an increase of 13.04% over control plants. In contrast, Girnar 3 registered the highest increase of TBARS content (44.44% over control) indicating maximum membrane damage. Thus, the tolerant genotypes suffered less membrane damage induced by oxidative stress as compared to the susceptible ones. The result in the present experiment was well consistent with that of Panda (2001), Kukreja *et al.* (2005) and Khan and Panda (2008) who also reported increase in lipid peroxidation under salinity stress.

The trend of lipid peroxidation under salinity stress was also reflected in the electrolyte leakage (EL%) of cell membrane in the leaves of the nine genotypes under study (Table 7). The percentage of EL was a manifestation of membrane stability. The nine genotypes exhibited considerable increase in electrolyte leakage under NaCl stress in comparison with the corresponding control condition. This indicated impairment of membrane integrity

Table 7: Effect of salinity stress on lipid peroxidation and electrolyte leakage in the leaves of tolerant and susceptible genotypes of groundnut.

Genotype	Lipid peroxidation (µM of TBRAS content g^{-1} fresh weight)			Electrolytic Leakage (%)		
	Control	Treatment	Mean	Control	Treatment	Mean
KDG 197	14.89	15.39(3.39)	15.14g	69.67	74.18(6.47)	71.925e
R 2001-2	11.61	13.12(13.04)	12.37h	65.10	67.05(3.00)	66.075f
VG 315	17.92	20.44(14.08)	19.18d	71.74	82.64(15.20)	77.19c
TCGS 1157	15.90	18.93(19.05)	17.41e	74.95	86.55(15.48)	80.75b
TG 51	21.20	25.99(22.62)	23.60b	76.44	84.97(11.16)	80.705b
J 86	25.49	31.77(24.64)	28.63a	57.96	69.19(19.38)	63.575g
TVG 0856	14.38	18.66(29.76)	16.52f	68.63	80.75(17.70)	74.69d
OG 52-1	18.93	25.45(34.47)	22.19c	75.05	94.67(26.14)	84.86a
Girnar 3	13.63	19.68(44.44)	16.66f	70.92	93.24(31.47)	82.08b
Mean	17.10a	21.05b		70.05a	81.47b	
	S.E. m(±)	C.D. (P=0.05)		S.E. m(±)	C.D.(P=0.05)	
Genotype(G)	0.27	0.55		1.18	2.38	
Treatment(T)	0.13	0.26		0.55	1.12	
G×T	0.38	0.78		1.66	3.37	

Means showing the same letters in a column do not differ significantly at 5% probability level.

Data in parentheses indicate percentage increase (+) or decrease (-) over control.

and structure as a consequence of stress. Like lipid peroxidation, the susceptible genotypes recorded higher increase ranging from 17.70 to 31.47% increase over control while the tolerant genotypes registered lower values of such increase and the range was from 3.00 to 15.48% over control. Out of all the genotypes, R 2001-2 recorded the minimum (3.00%) increase in electrolyte leakage percentage under stress which was closely followed by KDG 197 with an increase of 6.47% over control. The genotype Girnar 3 recorded the highest increase (31.47% over control) indicating maximum membrane damage followed by OG 52-1 (26.14% over control). Thus, in the present experiment, the salinity treatment of 200 mM NaCl induced oxidative stress and membrane injury in all the genotypes. However, the genotypes differed substantially in their responses. This result supported the early observations of Chen *et al.* (2007) and Cha-Um *et al.* (2009). Lipid peroxidation disrupts the membrane integrity and increase the leakiness of the membrane to substances that do not normally cross it other than through specific channels. It might be concluded that higher extent of lipid peroxidation in the salt susceptible genotypes resulted in increased leakiness of the membrane which was indicated by the substantial increase in relative electrolyte leakage percentage.

CONCLUSION

Salinity stress significantly affected dry weight of whole seedling as well as different plant parts. It also reduced the chlorophyll content and soluble sugar in leaves of all the genotypes. An increase in leaf protein was recorded in response to stress exposure in the present experiment. This increase might be attributed mainly to the increased synthesis of stress proteins. The tolerant genotypes also registered much greater accumulation of proline in their leaves than the susceptible genotypes and thus, showed better osmotic adjustment under salinity stress. Summarizing the data it might be concluded that better osmotic adjustment through accumulation of proline, less membrane damage the leaves helped the tolerant genotypes to sustain under salinity stress in a better way than the susceptible genotypes and these characters might be considered as important physiological indicators of salt tolerance in these genotypes of groundnut at seedling growth stage.

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