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Low Ca^{2+} Content and Ca^{2+}/Na^{+} Ratio in Leaf Tissues Determine Salinity Tolerance in Spanish Type Groundnut (*Arachis hypogeal* L.)

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Abstract: The study was performed with seven groundnut varieties/genotypes and F1s derived from crossing in all possible combinations without reciprocal among the mentioned varieties/genotypes. The objective was to assess whether low Ca^{2+} content and Ca^{2+}/Na^{+} ratio of leaf tissue or stem tissue determine salinity tolerance in terms of economic yield (kernel yield) in groundnut. It revealed that the varieties, "Binachinabadam-6", "Binachinabadam-5" and the F1 G2 × G3 were most tolerant based on kernel yield under 8 dS/m and 10 dS/m salinity stresses. These two tolerant varieties and the F1 also showed lower Ca^{2+} and Ca^{2+}/Na^{+} ratios in leaf tissue, which indicated lower Ca^{2+} and Ca^{2+}/Na^{+} ratio of leaf tissue determined salinity tolerance in terms of kernel yield in Spanish type groundnut. These findings could be applied in future plant breeding applications for screening salt tolerant Spanish type groundnut genotypes.

Key words: Salinity tolerance, groundnut, *Arachis hypogea*, Ca^{2+} content in leaf tissues, Ca^{2+}/Na^{+} ratio in leaf tissue.

1. Introduction

Salinity is certainly one of the most serious environmental factors limiting crop productivity [1]. This stress is complex and causes a number of detrimental effects: (i) reduces the ability of plants to absorb water, called water or osmotic stress; (ii) causes ionic imbalance; (iii) imposes hyper osmotic shock by decreasing chemical activity of water and causing loss of cell turgor; (iv) reduces chloroplast stromal volume and generates reactive oxygen species (ROS). Globally, nearly 100 million hectares of land is affected by salinity which accounts for 6%-7% of the total arable land [2]. In Bangladesh, in the coastal belt, about 1.02 million hectares of cultivated land is affected by different degrees of soil salinity and thus very limited or no crop can be grown particularly in the dry period during December to May [3, 4]. Groundnut can be grown under rainfed condition in Bangladesh because it needs only 350 mL water to complete life cycle [5] provided that the variety is salt tolerant. Salt tolerance of a plant is defined as the degree to which it can withstand the imposed salinity without significant adverse effects. Accordingly, salt tolerance of groundnut has been defined as the ability of maintaining higher, equal or the least reduction of biomass yield under salinized than to the non-salinized condition [6-9]. The authors considered pod yield and yield attributes rather than biomass yield to define salt tolerant groundnut genotype/variety [10]. They defined the salt tolerant groundnut genotype/variety that can perform better,

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equal or have least reduction in pod and kernel yield, and yield attributes under salinity stress compared to non-salinized condition. These authors also observed that the variety with higher, equal or least reduction in pod and kernel yield, and yield attributes under salinity stress compared to non-salinized condition had low Ca^{2+} and Ca^{2+}/Na^{+} ratio in the shoot tissues. As shoot tissues include both stem and leaf tissues and hence the question is raised whether low Ca^{2+} content and Ca^{2+}/Na^{+} ratio of leaf or shoot tissues determine salinity tolerance in groundnut. Therefore, this study was undertaken to elucidate whether low Ca^{2+} content and Ca^{2+}/Na^{+} ratio of leaf or stem tissues determine salinity tolerance in groundnut.

2. Materials and Methods

2.1 Plant Material and Growth Conditions

A population, obtained from crossing among five salt tolerant, a moderately tolerant and a sensitive variety of Spanish type groundnut (Table 1) in all possible combinations without reciprocals, exposed to 8 dS/m and 10 dS/m salinities along with a non-salinized treatment (tap water) during flowering to maturity stages following completely randomized design (CRD) with three replications. The salinity of the tap water was 0.40 dS/m. The experiment was conducted under rain out shelter in net house of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh from January to May 2013. The average minimum temperature in the net house during the experimental period was 18 °C and the maximum average temperature was 34 °C. The salt tolerant and sensitive varieties/genotypes were discriminated based on pod formation ability under salinity stress [11]. The crossing was made following the methods [12, 13]. Early formed buds close to the soil surface were used for hybridization so that the pegs could easily penetrate into the soil. The well developed buds close to the soil, of the recipient parents were emasculated during 4:30-6:30 pm. A small incision was made on the depressed side of the bud, at two-thirds of its length. Then pressing the top cone-like structure consisting of calyx and standard petal was detached, and afterwards wings, keel and anthers were removed. The emasculated buds were covered with green colored straw tube sealed on one side to avoid fertilization with undesirable foreign pollen. Before pollination, flowers from the entire male parents were collected early in the morning by 6:00-7:00 am to avoid setting and to ensure steady supply of male flowers. During 6:00-8:30 am, pollination was performed by collecting pollen from male parents. The standards and wings (petals) were removed and then the tubular keel petal was pressed with forceps. The extruded pollen was collected on the forceps and applied to the stigmatic end of the female flower. Finally, the stigma was further covered with red colored straw tube. After completion of crossing, the newly formed flowers were removed daily from the recipient parents.

Table 1 Salt tolerant, moderately tolerant and sensitive varieties of groundnut with sources.

Sl. No.	Code	Parent	Botanical group	Salinity tolerance class	Source*
1	G1	"Binachinabadam-6"	Spanish	Tolerant	BINA
2	G2	"Binachinabadam-5"	Spanish	Tolerant	BINA
3	G3	"Binachinabadam-2"	Spanish	Tolerant	BINA
4	G4	"BARI Badam-5"	Spanish	Tolerant	BARI
5	G5	"BARI Badam-6"	Spanish	Moderately tolerant	BARI
6	G6	"Dacca-1"	Spanish	Sensitive	BARI
7	G7	ICGV-00309	Spanish	Tolerant	ICRISAT

* Bangladesh Institute of Nuclear Agriculture (BINA); Bangladesh Agricultural Research Institute (BARI); International Crops Research Institute for the Semi-arid Tropics (ICRISAT).

2.2 Preparation of Pot and Sowing of Seed

Sun dried earthen pots, 27 × 22 cm size were weighed and lined with polyethylene sheet so that water could not leak. Thereafter, it was filled with 8 kg soil mixture, prepared with sandy loam soil and farm yard manure (FYM) in a 1:1 ratio. The fertilizer needed for each pot was determined following the Fertilizer Recommendation Guide-2005 [14]. The total amount of nitrogen, phosphorus, potassium, sulphur and zinc were applied in the form of urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum and zinc sulphate. These were mixed thoroughly with the soil in each pot before sowing. Five sprouted seeds of each variety/genotype/F1 were sown in each pot.

2.3 Estimate of Pot Capacity (PC) of Soil Mixture

For determination of pot capacity analogous to field capacity, three such empty pots were weighed and filled with same amount of soil, as above. Then these were watered until leaked through the hole at the bottom. Thereafter, these were covered with black polyethylene sheet and weighed after cessation of water leaking through the perforated hole. Finally, pot capacity was determined using Eq. (1) [15]:

$$\text{PC (\%)} = \begin{bmatrix} \text{Final weight (pot + soil + water) -} \\ \text{Initial weight (pot + soil)} \end{bmatrix} / \text{Soil weight} \times 100 \quad (1)$$

2.4 Estimation of Initial Moisture Content of Soil Mixture

Three brass cores with 5 cm height and diameter were properly filled with the soil mixture and weighed. These were then oven dried at 105 °C for 24 h. After cooling, these were again weighed and the dry soil removed. Weight of the blank cores was also recorded. Initial moisture content of the soil was calculated following Ref. [15] by Eq. (2):

$$\text{Initial moisture content (\%)} = \begin{bmatrix} \text{Initial weight (brass core + soil) -} \\ \text{oven dry weight (brass core + soil)} \end{bmatrix} \div \text{Oven dry weight of soil} \times 100 \quad (2)$$

2.5 Estimation of Initial Salinity of the Soil Mixture

Three random samples of mixed soil were taken each with 50 g, sun dried, pulverized and sieved. Twenty milliliters distilled water was added with 8 g of such sieved mixed soil and was stirred for 30 min at 250 rpm. The following day, it was stirred again and electrical conductivity was recorded using an EC meter (HI98304, HANNA, Philippines) in dS/m.

2.6 Intercultural Operations

When the plants were established, only three healthy plants were kept in each pot. The pots were kept free from weeds. The plants were protected from insect pest and diseases by spraying appropriate insecticides, fungicides and acaricide as and when necessary.

2.7 Preparation of Saline Stock Solution

The saline water was synthesized by using mixture of different salts: 50% NaCl, 15% Na_2SO_4, 10% $NaHCO_3$, $CaCl_2$ and $MgCl_2$ together with 5% $MgSO_4$ so that their composition was almost alike their average composition in the ground water of saline areas of Bangladesh [4]. Fifty grams of such salt was dissolved in 1 L tap water to prepare the stock solution. The salinity of the stock solution was 80 dS/m.

2.8 Salinity Imposition

The total amount of stock solution needed to raise the desired salinity of the soil mixture was estimated with Eq. (3):

$$V_1S_1 = V_2S_2 \quad (3)$$

where, V_1 = volume of soil mixture in a pot; S_1 = desired salinity – initial salinity of the soil; V_2 = volume of water at 70%-80% PC; S_2 = salinity of stock solution.

The estimated amount of stock solution was then diluted to the desired salinity levels by adding tap water and then imposed during the assigned stage till maturity. The total amount of saline water for the respective doses was applied at different installments. At each installment, 0.5-1.0 L saline water was applied so that the moisture content of the pots remained 70%-80% of PC. For the control, same amount of only fresh tap water was applied.

2.9 Harvesting

The plants in a pot were uprooted at full maturity and washed with running tap water. The leaves and stems were oven dried at 70 °C for 72 h. Number of pods and kernel of a plant were recorded after harvest and weighed on sun drying and cooling.

2.10 Determination of Na^+, K^+ and Ca^{2+} Contents

2.10.1 Digestion

One gram finely grinded powder of both leaf and stem tissues from all the treatments were digested separately following the procedure [16] with a mixture of HNO_3 and $HClO_4$ acids at the ratio of 5:3. One gram oven dried ground tissues of leaf and 1 g of stem from all treatments were taken into clean and dry 100 mL volumetric flasks and 5 mL concentrated HNO_3 added, kept overnight at room temperature for pre-digestion. The pre-digested material was then heated with agitation at 100-120 °C for 1 h on a hot plate within a fume hood to evolve the brown nitrous oxide fumes. Thereafter, 2.5 mL HNO_3 was added and further heated with agitation at 100-120 °C for 1 h. This step was repeated two times. Then it was cooled at room temperature and 3 mL $HClO_4$ added, heated at 120-150 °C and again cooled at room temperature. These steps were also repeated two times and heating at this temperature was continued till it became colorless. This step completed oxidation of all soluble inorganic forms. The digested sample was then made 50 mL by adding de-ionized water. To prepare working solution, 5 mL of the above solution both from leaf and stem tissue was taken and further diluted to 50 mL, separately.

2.10.2 Estimation

2.10.2.1 Na^+

Estimated directly from the working solution with a flame photometer together with standard solutions of 10, 20, 40 and 80 ppm Na^+.

2.10.2.2 K^+

Five milliliters of the working solution was taken and further diluted to 50 mL and readings were taken with a flame photometer along with standard solutions of 1, 2 and 4 ppm K^+.

2.10.2.3 Ca^{2+}

Two milliliters lanthanum oxide was added with 20 mL working solution and then reading was taken with a flame photometer along with standard solutions of 0, 20, and 30 ppm Ca^{2+}.

2.11 Data Analysis

The recorded data were analyzed following completely randomized design and the treatment means were compared by using least significant difference (LSD) at 5% level of probability [17].

3. Results

3.1 ANOVA and Mean Squares

Mean squares of pod/plant, pod and kernel yield/plant of seven parents and F1s of groundnut exposed to 8 dS/m, 10 dS/m and a non-salinized treatment (tap water) during flowering to maturity stages are presented in Table 2; Na^+, K^+ and Ca^{+2} contents in leaf are listed in Table 3 and those of stem tissues are listed in Table 4. Pod/plant, pod yield/plant and kernel yield/plant will be termed hereafter as pod number, pod yield and kernel yield, respectively. Results showed highly significant differences among the genotypes (both parental genotypes and F1s) for pod number, pod and kernel yield at all salinities except kernel yield at tap water and 10 dS/m salinity (Table 2). Kernel yield at tap water showed just significant difference ($p > 0.05$) among the genotypes while kernel yield at 10 dS/m failed to show any significant difference.

The parental genotypes also showed significant ($p > 0.05$) to highly significant differences ($p > 0.01$) for pod number, pod and kernel yield at all salinities except kernel yield at tap water, pod number, pod and kernel yield at 10 dS/m. Kernel yield at tap water and pod number, pod and kernel yield at 10 dS/m did not show significant difference among the parents. The F1s also showed significant to highly significant differences for pod number, pod and kernel yield at all salinities except kernel yield at 10 dS/m. Kernel yield at 10 dS/m salinity stress failed to show any significant difference among the F1s.

The parent versus F1s showed significant to highly significant differences for pod number, pod and kernel yield at all salinities except pod number at 8 dS/m and 10 dS/m, pod yield at tap water and 10 dS/m and kernel yield at 10 dS/m. Pod number at 8 dS/m and 10 dS/m, pod yield at tap water and 10 dS/m and kernel yield at 10 dS/m had not shown any significant difference for the parent versus F1s.

The genotypes, parents, F1s and parent versus F1s showed significant to highly significant differences for Na^+, K^+, Ca^{2+}, K^+/Na^+ and Ca^{2+}/Na^+ ratios in leaf and stem tissues at all salinity levels (Tables 3 and 4) except the genotypes, parents, F1s and parent versus F1s for Na^+ at 10 dS/m, parents for K^+ at 10 dS/m, F1s and parent versus F1s for Ca^{2+} at 10 dS/m and parents for Ca^{2+}/Na^+ ratio at tap water treatment (Table 4).

3.2 Parental Means

Means of pod number, pod and kernel yield of seven parents and 21 F1s of groundnut exposed to 8 dS/m, 10 dS/m and a non-salinized treatment (tap water) during flowering to maturity stages are presented in Table 5. The parent G2 had significantly the highest number of pods; pod and kernel yield at tap water treatment although kernel yield of the parents did not show significant difference with each other. By contrast, G7 had the lowest number of pod and the lowest pod and kernel yield at this control treatment. But when 8 dS/m salinity was imposed, G1 produced significantly the highest number of pod, and highest pod and kernel yield although much less compared to the tap water treatment. On the other hand, G4 and G6 produced significantly the lowest number of pod, G4 the lowest pod yield, and G4, G6 and G7 the lowest kernel yield. Interestingly, when 10 dS/m salinity was imposed, all the seven parents appeared indifferent statistically for pod number, pod and kernel yield. Three parents G3, G4 and G6 failed to produce any kernel at this salinity treatment.

3.3 F1 Means

Of the F1s, derived from hybridization between the parents, G2 × G5 produced significantly the highest number of pod, and the highest pod and kernel yield at tap water treatment (Table 5) while G3 × G7 produced the lowest number of pod, and the lowest pod and kernel yield. But when exposed to 8 dS/m salinity, G2 × G4 produced significantly the highest number of pod and the highest pod yield while G2 × G3 produced the highest kernel yield. The cross combinations G3 × G6, G4 × G7, G5 × G6, G5 × G7 and G6 × G7 produced the lowest number of pod, G5 × G6 the lowest pod yield and G1 × G5 failed to produce any kernel at 8 dS/m salinity treatment. At 10 dS/m salinity treatment, G2 × G3 produced significantly the highest number of pod; the highest pod and kernel yield although kernel yield at this treatment did not differ significantly among the F1s. In contrast, G1 × G6, G1 × G7, G3 × G5, G4 × G5, G5 × G6 and G6 × G7 failed to produce any pod and thus no pod and kernel yield. Moreover, G4 × G7 although produced some pods but the pods were not matured. Similarly, G1 × G5, G2 × G4, G3 × G6, G4 × G6, G4 × G7 and G5 × G7 failed to produce any kernel when exposed to 10 dS/m salinity treatment.

Means of Na^+, K^+, Ca^{2+}, Na^+/K^+ and Ca^{2+}/Na^+ ratio in leaf tissues of an F1 population of groundnut at different levels of salinity imposed during flowering till maturity stages are presented in Table 6. The parent G1 had significantly the highest accumulation

Table 2 Mean squares of pod number, pod and kernel yield in an F1 population of groundnut without reciprocal at different salinity stresses imposed during flowering to maturity stages.

Sources of variation	Degree of freedom (df)	Pod/plant			Pod yield/plant			Kernel yield/plant		
		Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m
Genotypes	27	28.29*	31.10**	8.35**	19.75**	0.78**	0.11**	9.27*	0.22**	0.02
Parents	6	23.44*	25.86**	3.89	13.72*	0.06**	0.06	7.53	0.02*	0.02
F1s	20	27.68*	34.04**	9.42**	20.93**	0.55**	0.13**	9.03*	0.05*	0.02
Parents vs. F1s	1	69.67*	3.57	13.58	32.32	0.24**	0.03	24.67*	0.07**	0.002
Error	56	10.02	6.97	3.93	5.80	0.02	0.05	5.11	0.01	0.013

* Significant at 5% level of probability; ** significant at 1% level of probability.

Table 3 Mean squares of Na^+, K^+ and Ca^{2+} contents in leaf tissues in an F1 population of groundnut without reciprocal at different salinity stresses imposed during flowering to maturity stages.

Sources of variation	df	Na^+			K^+			Ca^{2+}			K^+/Na^+			Ca^{2+}/Na^+		
		Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m
Genotypes	27	0.007**	0.56**	0.332**	1.783**	4.31**	0.773**	0.137**	1.44**	1.051**	362.98**	0.93**	0.207**	195.29**	0.11**	0.178**
Parents	6	0.011**	0.17**	0.408**	1.943**	0.11**	0.998**	0.052**	0.67**	0.865**	491.61**	0.03**	0.174**	98.64**	0.08**	0.086**
F1s	20	0.006**	0.63**	0.294**	1.697**	5.10**	0.691**	0.163**	1.53**	1.066**	335.43**	1.14**	0.225**	53.43**	0.11**	0.159**
Genotypes vs. F1s	1	0.002**	1.52**	0.635**	2.435**	13.54**	1.075**	0.112**	4.09**	1.867**	142.10**	2.30**	0.036**	110.46**	0.09**	1.108**
Error	56	0.0003	0.001	0.003	0.001	0.001	0.015	0.001	0.02	0.001	24.72	0.001	0.001	133.57	0.003	0.001

* Significant at 5% level of probability; ** significant at 1% level of probability.

Table 4 Mean squares of Na^+, K^+ and Ca^{2+} contents in stem tissues in an F1 population of groundnut without reciprocal at different salinity different stresses imposed during flowering to maturity stages.

Sources of variation	df	Na^+			K^+			Ca^{2+}			K^+/Na^+			Ca^{2+}/Na^+		
		Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m
Genotypes	27	0.001**	0.002	0.001	0.471**	0.39**	0.006**	0.6070**	1.77**	0.397*	1,035.95**	0.49**	0.014**	10,282.88**	2.33**	1.54**
Parents (G)	6	2.999**	0.05**	0.286	0.499**	0.42**	0.004	0.4876**	0.45**	0.611*	610.73**	0.79**	0.015**	1,943.02	0.70**	1.16**
F1s	20	0.0004**	0.23**	0.235	0.371**	0.06**	0.006**	0.6276**	0.73**	0.245	1,187.17**	0.05**	0.014**	7,809.07*	2.22**	1.72**
Genotypes vs. F1s	1	3.835**	0.17**	0.136	2.314**	7.01**	0.018**	0.9108**	30.45**	2.173	562.85**	6.61**	0.005**	109,798.36**	14.42**	0.22**
Error	56	0.0001	0.19**	0.243	0.0001	0.001	0.001	0.0106	0.002	0.001	241.97	0.003	0.001	3,701.68	0.02	0.005

* and ** Significant and highly significant at $p > 0.05$ and $p > 0.01$, respectively.

Table 5 Means of pod number, pod and kernel yield in an F1 population of groundnut as influenced by different salinity stresses imposed during flowering till maturity stages.

Parent/F1	Pod/plant (no.)			Pod yield/plant (g)			Kernel yield/plant (g)		
	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m
Parent									
G1	15.83	10.00	3.67	8.18	1.44	0.26	5.58	0.74	0.10
G2	21.67	6.17	1.67	11.92	0.29	0.17	9.22	0.14	0.10
G3	16.17	3.83	0.33	9.13	0.11	0.01	6.47	0.06	0.00
G4	15.17	2.00	1.50	10.98	0.03	0.07	7.15	0.01	0.00
G5	18.67	2.17	3.17	12.88	0.18	0.46	8.80	0.05	0.21
G6	16.67	2.00	2.83	9.02	0.04	0.14	6.30	0.01	0.00
G7	12.83	3.67	2.00	6.90	0.05	0.21	4.97	0.01	0.01
LSD (0.05)	2.59	2.16	NS	1.97	0.12	NS	NS	0.08	NS
F1									
G1 × G2	19.67	4.50	2.17	10.52	0.13	0.14	8.08	0.13	0.10
G1 × G3	15.50	3.17	2.17	9.67	0.13	0.22	7.32	0.01	0.02
G1 × G4	20.50	5.50	0.50	11.95	0.15	0.17	8.43	0.13	0.13
G1 × G5	18.83	5.33	0.33	12.18	0.11	0.02	8.13	0.00	0.00
G1 × G6	18.33	6.00	0.00	9.23	0.22	0.00	7.18	0.05	0.00
G1 × G7	15.67	5.17	0.00	10.55	0.09	0.00	7.70	0.01	0.00
G2 × G3	20.83	6.17	6.83	9.73	0.20	0.94	7.57	0.35	0.34
G2 × G4	21.83	15.50	0.50	13.33	0.65	0.10	9.15	0.05	0.00
G2 × G5	24.67	4.00	2.33	18.30	0.09	0.14	12.62	0.01	0.01
G2 × G6	18.33	6.67	4.83	7.98	0.18	0.33	6.10	0.14	0.10
G2 × G7	17.00	3.33	2.17	9.92	0.06	0.23	7.37	0.13	0.11
G3 × G4	18.00	2.00	0.83	13.57	0.03	0.18	9.20	0.02	0.02
G3 × G5	15.67	2.00	0.00	10.00	0.08	0.00	7.53	0.03	0.00
G3 × G6	21.83	0.67	0.17	9.87	0.16	0.01	7.52	0.08	0.00
G3 × G7	10.00	1.50	1.50	5.75	0.02	0.27	3.80	0.19	0.15
G4 × G5	20.67	3.00	0.00	13.93	0.02	0.00	9.68	0.01	0.00
G4 × G6	20.00	2.17	0.17	11.05	0.03	0.10	8.28	0.01	0.00
G4 × G7	21.17	0.67	0.17	11.18	0.02	0.00	7.70	0.01	0.00
G5 × G6	18.83	0.67	0.00	11.63	0.01	0.00	8.57	0.01	0.00
G5 × G7	18.17	0.67	1.33	15.15	0.02	0.19	11.02	0.01	0.00
G6 × G7	19.67	0.83	0.00	11.63	0.02	0.00	8.78	0.01	0.00
LSD (0.05)	4.49	3.74	2.80	3.41	0.20	0.32	3.20	0.14	NS

NS: not significant at 5% level of probability.

Table 6 Means of Na^+, K^+ and Ca^{2+} contents in leaf tissues in an F1 population of groundnut under different salinity stresses imposed during flowering till maturity stages.

Parent/F1	Na^+ (%)			K^+ (%)			K^+/Na^+ ratio			Ca^{2+} (%)			Ca^{2+}/Na^+		
	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m
Parent															
G1	0.24	1.77	1.79	1.29	0.84	1.70	5.42	0.49	0.95	1.20	1.51	1.79	5.05	0.86	1.00
G2	0.10	1.80	2.31	1.85	0.81	1.50	18.08	0.45	0.65	0.90	1.75	2.29	8.79	0.97	0.99
G3	0.08	1.95	2.75	2.09	0.72	1.05	25.51	0.37	0.38	1.07	2.33	2.29	13.05	1.20	0.83
G4	0.08	1.70	2.40	3.00	0.75	2.49	36.67	0.46	1.04	1.11	2.07	3.00	13.54	1.22	1.25
G5	0.07	1.52	1.86	0.73	0.79	0.95	9.67	0.54	0.51	0.83	1.82	1.50	11.54	1.20	0.80
G6	0.07	2.27	1.96	2.33	1.16	1.57	37.99	0.51	0.80	1.13	2.94	1.48	18.39	1.30	0.76
G7	0.07	1.78	2.57	1.01	1.18	2.29	14.01	0.66	0.89	1.00	1.86	2.20	13.93	1.05	0.86
LSD (0.05)	0.01	0.03	0.05	0.03	0.03	0.10	4.07	0.03	0.03	0.03	0.12	0.03	9.46	0.04	0.03
F1															
G1 × G2	0.06	2.15	1.81	1.09	1.07	1.25	18.88	0.50	0.69	0.96	2.48	2.67	16.68	1.15	1.48
G1 × G3	0.07	1.57	2.12	1.21	0.79	1.19	16.82	0.50	0.56	1.06	1.45	1.90	14.77	0.92	0.90
G1 × G4	0.07	1.60	2.02	1.17	1.52	1.08	16.26	0.95	0.53	0.78	1.86	3.06	10.84	1.16	1.52
G1 × G5	0.07	1.54	1.47	1.17	1.44	2.34	16.26	0.94	1.59	0.89	1.78	2.16	12.39	1.16	1.47
G1 × G6	0.07	1.69	2.29	1.63	1.10	2.50	26.49	0.65	1.09	1.26	2.22	2.74	20.52	1.31	1.20
G1 × G7	0.12	1.78	1.68	1.70	2.01	1.06	14.75	1.13	0.63	1.45	2.10	2.41	12.59	1.18	1.44
G2 × G3	0.11	2.11	1.72	4.01	0.92	1.09	36.95	0.44	0.63	1.47	1.50	1.29	13.53	0.71	0.75
G2 × G4	0.06	1.55	1.74	2.44	3.08	1.06	42.50	1.99	0.61	1.25	1.65	1.81	21.75	1.06	1.04
G2 × G5	0.06	2.03	2.10	1.47	1.54	1.42	25.53	0.76	0.68	0.97	2.55	1.98	16.94	1.26	0.94
G2 × G6	0.06	1.89	2.22	1.79	1.29	1.51	31.13	0.68	0.68	1.41	1.89	2.24	24.55	1.00	1.01
G2 × G7	0.07	2.25	1.87	1.40	6.13	1.50	22.71	2.73	0.80	0.98	2.70	1.89	15.92	1.20	1.01
G3 × G4	0.05	2.37	1.38	1.22	1.10	1.16	28.02	0.46	0.85	1.12	2.85	1.65	25.86	1.20	1.20
G3 × G5	0.07	2.26	2.25	0.83	4.33	2.36	11.49	1.92	1.05	0.86	2.58	2.89	11.97	1.14	1.28
G3 × G6	0.08	2.56	2.56	1.10	2.86	1.54	13.37	1.12	0.60	1.54	2.75	3.13	18.81	1.07	1.22
G3 × G7	0.17	2.62	1.86	0.53	0.81	1.06	3.16	0.31	0.57	1.26	3.04	1.98	7.56	1.16	1.06
G4 × G5	0.06	1.98	2.27	0.64	1.21	0.89	12.36	0.61	0.39	1.00	3.04	2.53	19.72	1.54	1.11
G4 × G6	0.17	2.05	2.39	1.25	1.68	1.64	7.49	0.82	0.69	1.20	3.32	2.94	7.20	1.62	1.23
G4 × G7	0.10	2.68	2.22	0.96	1.18	1.19	9.35	0.44	0.54	1.07	3.04	2.32	10.46	1.13	1.05
G5 × G6	0.07	3.08	2.26	0.89	1.36	0.87	14.34	0.44	0.39	1.30	3.92	3.36	21.18	1.27	1.49
G5 × G7	0.13	3.02	2.27	1.00	1.54	0.93	7.94	0.51	0.41	0.89	3.88	3.58	7.08	1.29	1.58
G6 × G7	0.21	2.09	2.22	0.67	1.31	1.53	3.22	0.63	0.68	0.77	2.95	2.35	3.69	1.41	1.07
LSD (0.05)	0.02	0.04	0.08	0.04	0.04	0.17	7.04	0.04	0.04	0.04	0.20	0.04	16.38	0.08	0.04

Table 7 Means of Na^{+}, K^{+} and Ca^{2+} contents in stem tissues in an F1 population of groundnut under different salinity stresses imposed during flowering till maturity stages.

Parent/F1	Na^{+} (%)			K^{+} (%)			K^{+}/Na^{+} ratio			Ca^{2+} (%)			Ca^{2+}/Na^{+}		
	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m	Tap water	8 dS/m	10 dS/m
Parent															
G1	0.11	1.08	1.39	0.53	0.75	0.22	5.52	0.70	0.16	2.40	3.55	3.18	22.48	3.30	2.29
G2	0.06	1.12	0.83	0.95	1.25	0.17	24.84	1.12	0.20	3.29	4.37	2.02	62.98	3.92	2.44
G3	0.04	1.04	0.98	1.14	0.68	0.15	29.80	0.66	0.15	3.34	3.62	1.97	87.13	3.49	2.02
G4	0.05	1.15	1.43	1.18	0.81	0.25	42.64	0.70	0.17	3.41	3.29	2.47	82.40	2.87	1.73
G5	0.05	0.81	1.25	1.60	1.63	0.19	32.92	2.04	0.15	2.91	3.43	2.27	59.76	4.29	1.82
G6	0.04	0.89	0.58	1.76	0.69	0.20	45.99	0.77	0.34	3.40	3.59	2.03	88.69	4.04	3.52
G7	0.03	1.10	1.02	1.23	0.66	0.15	44.44	0.60	0.15	2.67	4.12	1.89	96.28	3.77	1.86
LSD (0.05)	0.008	0.36	NS	0.008	0.02	NS	12.73	0.04	0.02	0.08	0.03	0.02	NS	0.12	NS
F1															
G1 × G2	0.03	1.05	1.32	0.75	0.60	0.20	29.17	0.58	0.15	3.54	3.69	2.24	137.39	3.51	1.70
G1 × G3	0.02	1.27	1.11	0.56	0.67	0.18	32.50	0.53	0.16	3.30	2.78	1.93	191.67	2.19	1.74
G1 × G4	0.02	0.77	1.08	0.66	0.29	0.17	40.33	0.38	0.16	2.70	2.30	1.94	164.72	3.03	1.80
G1 × G5	0.04	0.61	1.01	0.75	0.20	0.20	19.62	0.34	0.20	2.83	1.80	2.01	73.81	2.95	2.00
G1 × G6	0.03	0.61	1.25	1.30	0.17	0.14	46.94	0.28	0.11	3.06	1.94	1.76	110.22	3.19	1.41
G1 × G7	0.03	0.90	0.81	1.68	0.18	0.09	60.69	0.20	0.11	3.92	2.02	2.17	141.42	2.24	2.69
G2 × G3	0.02	0.88	0.60	0.46	0.20	0.12	30.50	0.23	0.20	2.50	2.55	1.52	165.83	2.89	2.55
G2 × G4	0.02	0.62	1.11	1.56	0.15	0.11	95.33	0.25	0.10	3.56	1.44	1.68	217.28	2.32	1.52
G2 × G5	0.02	0.34	1.14	0.66	0.16	0.11	40.33	0.47	0.10	3.14	1.84	1.66	191.61	5.43	1.46
G2 × G6	0.03	0.81	1.16	1.07	0.26	0.12	38.64	0.32	0.10	3.41	2.75	1.77	122.86	3.40	1.53
G2 × G7	0.03	0.57	1.09	0.61	0.30	0.13	22.06	0.52	0.12	2.53	1.74	2.26	91.22	3.05	2.08
G3 × G4	0.03	1.09	0.85	0.65	0.31	0.14	23.50	0.28	0.16	3.24	2.36	1.80	116.86	2.16	2.12
G3 × G5	0.02	0.98	0.53	0.66	0.31	0.11	40.33	0.32	0.21	3.08	2.37	1.99	187.94	2.42	3.78
G3 × G6	0.02	1.24	1.02	1.27	0.17	0.22	77.61	0.14	0.22	4.03	1.79	2.71	246.00	1.45	2.66
G3 × G7	0.02	1.21	1.01	0.57	0.21	0.23	34.83	0.18	0.23	3.10	2.28	1.60	189.17	1.89	1.59
G4 × G5	0.03	1.26	0.56	0.73	0.23	0.21	26.39	0.18	0.37	4.24	2.47	1.91	152.97	1.96	3.43
G4 × G6	0.03	0.71	0.95	0.57	0.19	0.15	20.61	0.27	0.16	3.32	2.61	1.71	119.75	3.67	1.80
G4 × G7	0.02	0.84	0.65	0.64	0.19	0.16	39.11	0.22	0.25	3.41	2.21	1.51	208.11	2.63	2.33
G5 × G6	0.02	1.19	0.55	0.82	0.19	0.13	36.44	0.16	0.24	3.76	2.38	2.01	166.83	2.00	3.68
G5 × G7	0.07	1.30	1.60	0.43	0.21	0.14	6.26	0.16	0.09	3.20	2.80	1.81	46.32	2.16	1.13
G6 × G7	0.02	1.09	1.09	0.70	0.19	0.22	42.78	0.17	0.20	3.45	2.57	1.70	210.56	2.36	1.56
LSD (0.05)	0.01	0.62	NS	0.01	0.04	0.04	22.04	0.08	0.04	0.15	0.06	NS	86.22	0.20	NS

NS: not significant at 5% level of probability.

of Na^+ and Ca^{2+}, G4 K^+/Na^+ ratio and G6 Ca^{2+}/Na^+ ratio at tap water treatment while the lowest accumulation of Na^+ in G5, G6 and G7 (Table 7), K^+ and Ca^{2+} in G5, K^+/Na^+ and Ca^{2+}/Na^+ ratios in G1. But when exposed to 8 dS/m salinity stress, the parent G6 accumulated significantly the highest percentage of Na^+, Ca^{2+} and Ca^{2+}/Na^+ ratio, G7 the highest K^+, G5 the highest K^+/Na^+ ratio. In contrast, G5 accumulated the lowest percentage of Na^+, G3 the lowest K^+ and K^+/Na^+ ratio, and G1 the lowest Ca^{2+} and Ca^{2+}/Na^+ ratio. When exposed to 10 dS/m salinity, G3 accumulated significantly the highest percentage of Na^+, G6 the highest Ca^{2+} and Ca^{2+}/Na^+ ratio; G7 accumulated the highest percentage of K^+ and G4 the highest K^+/Na^+ ratio. In contrast, G1 accumulated the lowest percentage of Na^+, G5 the lowest K^+ and K^+/Na^+ ratio, and G6 the lowest Ca^{2+} and Ca^{2+}/Na^+ ratio.

Of the F1s, derived from hybridization between the parents, G6 × G7 accumulated significantly the highest percentage of Na^+, G2 × G3 K^+, G1 × G5 K^+/Na^+ ratio, G3 × G6 Ca^{2+} and G3 × G4 had the highest Ca^{2+}/Na^+ ratio at tap water treatment (Table 7). On the other hand, the cross combination, G2 × G7 accumulated the lowest Na^+, G3 × G7 K^+ and K^+/Na^+ ratio, G6 × G7 Ca^{2+} and the lowest Ca^{2+}/Na^+ ratio. But when exposed to 8 dS/m salinity, G5 × G6 accumulated significantly the highest percentage of Na^+, G2 × G7 K^+ and K^+/Na^+ ratio, G1 × G3 Ca^{2+} and G4 × G5 the highest Ca^{2+}/Na^+ ratio. On the other hand, G1 × G5 accumulated the lowest Na^+, G1 × G3 K^+ and Ca^{2+}, G3 × G7 K^+/Na^+ and G2 × G3 Ca^{2+}/Na^+ ratio (Table 6). At 10 dS/m salinity treatment, G3 × G6 accumulated significantly the highest percentage of Na^+, G1 × G6 K^+, G1 × G5 K^+/Na^+ ratio, G5 × G7 Ca^{2+} and Ca^{2+}/Na^+ ratio (Table 6). On the other hand, G1 × G5 accumulated the lowest Na^+, G5 × G6 K^+, G4 × G5 K^+/Na^+ ratio, G2 × G3 Ca^{2+} and Ca^{2+}/Na^+ ratio.

Means of Na^+, K^+, Ca^{2+}, Na^+/K^+ and Ca^{2+}/Na^+ ratio in stem tissues of an F1 population of groundnut at different levels of salinity imposed during flowering till maturity stages are presented in Table 7. The parent G1 had the highest accumulation of Na^+ at tap water treatment among the parents. The parent G6 had significantly the highest percentage of K^+, K^+/Na^+ ratio and Ca^{2+} at tap water treatment. In contrast, the lowest accumulation of Na^+ was in G7, while K^+, K^+/Na^+ ratio, Ca^{2+} and Ca^{2+}/Na^+ in G1. But when exposed to 8 dS/m salinity stress, the parent G4 accumulated significantly the highest percentage of Na^+, G5 K^+ and K^+/Na^+ ratio, G2 Ca^{2+} and G5 had the highest Ca^{2+}/Na^+ ratio. On the other hand, G5 accumulated the lowest percentage of Na^+, G7 K^+ and K^+/Na^+ ratio, and G4 Ca^{2+} and Ca^{2+}/Na^+ ratio. When exposed to 10 dS/m salinity stress, G4 once again accumulated significantly the highest percentage of Na^+ and K^+, G6 K^+/Na^+ and Ca^{2+}/Na^+ ratios, G1 Ca^{2+} despite having no significant difference among the parents for Na^+ and K^+ at this salinity stress. By contrast, G6 accumulated the lowest percentage of Na^+, K^+, K^+/Na^+ ratio and Ca^{2+}, and G4 the lowest Ca^{2+}/Na^+ ratio.

Of the F1s, G5 × G7 accumulated significantly the highest percentage of Na^+ (Table 7) G1 × G7 K^+, G2 × G4 K^+/Na^+ ratio, G4 × G5 Ca^{2+} and G3 × G6 Ca^{2+}/Na^+ ratio in tap water treatment while 10 of the F1s accumulated the lowest percentage of Na^+, G5 × G7 K^+ apart from its lowest K^+/Na^+ and Ca^{2+}/Na^+ ratios. But when exposed to 8 dS/m salinity stress, G5 × G7 accumulated significantly the highest percentage of Na^+, despite having no significant difference with eight others, G1 × G3, G1 × G2 K^+ and K^+/Na^+ ratio, Ca^{2+} and G2 × G5 Ca^{2+}/Na^+ ratio. On the other hand, G2 × G5 accumulated the lowest Na^+ which appeared significantly different with 12 others including that accumulated significantly the highest Na^+, and G2 × G4 K^+, G3 × G6 K^+/Na^+ and G2 × G4 Ca^{2+} (Table 7). At 10 dS/m salinity stress, G5 × G7 accumulated significantly the highest percentage of Na^+, G3 × G7 K^+, G4 × G5 K^+/Na^+ ratio, G3 × G6 Ca^{2+} and G3 × G5 Ca^{2+}/Na^+ ratio although the F1s did not show any significant difference for Na^{+}, Ca^{2+} and Ca^{2+}/Na^{+} ratio. On the other hand, G3 × G5 accumulated the lowest Na^{+}, G1 × G6, G2 × G4, G2 × G5, G3 × G5 K^{+}, G5 × G7 K^{+}/Na^{+} ratio, G4 × G7 Ca^{2+} and G5 × G7 Ca^{2+}/Na^{+} ratio.

4. Discussion

The insight of reduced pod number, pod and kernel yield at different salt stresses compared to non-stress control treatment in the parents and F1s as well (Table 5) was reduced plant growth and development via osmotic stress followed by ion toxicity [10, 15, 18-20]. Osmotic stress induces various physiological changes like membrane instability, nutrient imbalance, inability to detoxify reactive oxygen species (ROS) and decreased photosynthetic activity [19, 21]. Plants exposed to high soil salinity cause hyperionic stress through accumulation of Na^{+} and Cl^{-} ions in tissues. High Na^{+} concentration inhibits uptake of K^{+} ions which are essential elements for growth and development that results into lower productivity and may even lead to death of the plant [19]. Moreover, salinity stress enhances the production of ROS like singlet oxygen, superoxide, hydroxyl radical, and hydrogen peroxide through leakage of electrons onto O_2 from the electron transport activities of chloroplasts, mitochondria, and plasma membranes or as a byproduct of various metabolic pathways localized in different cellular compartments [22-26]. ROS induced by salinity stresses leads to oxidative damages of cellular proteins, lipids, and DNA, interrupting vital cellular functions of plants [27].

However, there exist inter varietal genetic variations for salt tolerance in groundnut for yield and yield attributes [10, 15, 18]. This is because when exposed to salt stress, the salt tolerant variety/genotype of groundnut accumulates higher relative total sugar, non-reducing sugar, free amino acids, Na^{+}, K^{+} and Ca^{2+} even than control treatment which maintain turgor of guard cells [15]. This is called osmotic adjustment that ensures higher relative biomass production. Higher biomass production at vegetative stage and efficiency of assimilate translocation to the reproductive sinks result in higher relative pod number, pod and kernel yields in a salt tolerant genotype. As kernel is the ultimate product of groundnut, the variety or genotype that produced kernel under imposed salinity stresses could be termed as salt tolerant [15]. In this experiment, at 8 dS/m salinity, all the varieties/genotypes and F1s produced some kernel except the cross G1 × G5. This means at 8 dS/m salinity all the parents and F1s except the cross G1 × G5 are tolerant. But the amount of kernel produced by the parents and F1s if falls below the respective LSD values was not termed as tolerant. In that context, “Binachinabadam-6” (G1), “Binachinabadam-5” (G2) and three F1s like G2 × G3, G2 × G6 and G3 × G7 were tolerant at 8 dS/m salinity stress (Table 5). These results are not in full conformity with that reported earlier [11]. It was reported that of the seven parents five were tolerant, one moderately tolerant and the other was sensitive based on pod formation ability under salinity stress (Table 1). The insight will be discussed later. At 10 dS/m salinity “Binachinabadam-6” (G1), “Binachinabadam-5” (G2), “BARI Badam-5” (G5) and ICGV-0039 could produce some kernel with “Binachinabadam-6” being the highest producer of kernel followed by “Binachinabadam-5”. Therefore, these two varieties are undoubtedly tolerant to salinity stress. This result is supported by another study [28] in which it was reported that “Binachinabadam-6” and “Binachinabadam-5” are tolerant to salinity stress in their study with groundnut for salinity stress tolerant.

Unlike the parents, the F1s showed significant variation for kernel yield even at tap water treatment and for pod number and pod yield at 10 dS/m salinity, too (Table 5). This might be due to the additive effects of the genes from the two parents of the respective crosses controlling pod formation and maturity [29]. Kernel yield of the F1s, like the parents, did not show any significant difference at 10 dS/m salinity stress.

This might be due to the additive effect of the genes from the two parents of the respective crosses controlling pod formation and maturation under this salinity. At 8 dS/m salinity, almost all the F1s produced kernel except G1 × G5 while nine F1s produced kernel both at 8 dS/m and 10 dS/m salinities with G2 × G3 being the highest producer of kernel followed by G3 × G7, G1 × G4, G2 × G7 and G2 × G6. This result indicated that G2 × G3 is the most tolerant. But the tolerance of the others is relatively weak. These results could be further verified by the Ca^{2+} and Ca^{2+}/Na^+ contents of shoot tissues because Azad et al. [10] reported lower Ca^{2+} and Ca^{2+}/Na^+ ratio of shoot tissue determine salinity tolerance in groundnut. The tolerant parent varieties, "Binachinabadam-6", "Binachinabadam-5" and the F1 G2 × G3 really showed lower Ca^{2+} and Ca^{2+}/Na^+ ratio in leaf tissue (Table 6). Therefore, these results undoubtedly proved that lower Ca^{2+} and Ca^{2+}/Na^+ ratio of leaf tissue determine salinity tolerance in terms of kernel yield in Spanish type groundnut. The deviation in results in tolerance reported in Table 1 might be due to not considering the Ca^{2+} and Ca^{2+}/Na^+ ratio of leaf tissues.

In general, leaf tissues contain more Na^+ and K^+ (Tables 6 and 7) than shoot tissues, either in control (tap water treatment) condition or in salinity stressed conditions. Accordingly, K^+/Na^+ ratio was higher in leaf tissues. But from this study it is not clear whether there is any variation in the Na^+ content of younger and older leaves, which might be a future research interest. From the available literature it is found that under salinity stress younger leaves compartmentalize/sequester the excess Na^+ to the older leaves which ultimately sacrificed [30, 31]. Another important thing in this experiment is the content of K^+ at different salinity levels. In all glycophytes, usually K^+ content decreases with increased salinity levels. But here in this experiment, the parents "BARI Badam-5", ICGV-0039 and 13 F1s showed increased K^+ contents at 8 dS/m salinity in leaf tissues than the tap water treatment. But at 10 dS/m salinity level, three parents including the above two, "Binachinabadam-6" and 11 F1s showed increased K^+ contents. In contrast, in case of stem tissues, K^+ contents of the parents and F1s mostly decreased gradually with either 8 dS/m or 10 dS/m salinity levels compared to the tap water treatment except "Binachinabadam-6", "Binachinabadam-5", "BARI Badam-5" and the G1 × G3 of the F1s at 8dS/m salinity level. This can be explained in two ways: (i) groundnut might have activated a specific K^+ transporter or channel when subjected to salinity stress that maintained optimum concentration of K^+ required for sustaining normal enzymatic reactions; (ii) the excess calcium added as salt and fertilizers (mentioned in materials and methods) might have ameliorative effect and helped sustain uptake of K^+ even under higher salinity stress. However, the result of increased K^+ contents with increased salinity levels partially corroborate with another report [10] in which it was reported that gradual increase of K^+ content with increasing salinity level in shoot tissues of groundnut .

It is generally and widely accepted that a variety with the highest/higher K^+/Na^+ ratio will exhibit greater tolerance. But in this study, the variety with the highest/higher K^+/Na^+ ratio had not shown such tolerances in terms of economic yield (kernel yield). This could be due to the fact that the variety that can maintain highest/higher K^+/Na^+ ratio under salinity stress will exhibit tolerance as in terms of biomass yield. This is in full agreement with that of Azad et al. [10] who observed the variety that maintained highest/higher K^+/Na^+ ratio in shoot tissues had highest/higher tolerances in terms of relative biomass yield in his experiment with groundnut. Additionally, it was also reported that tolerance under salinity stress in terms of economic yield depends on the ability of the variety/genotype to translocate the reserved photosynthates from shoot tissues to the reproductive organs particularly kernel [10].

5. Conclusions

Finally, it could be concluded that lower Ca^{2+} and Ca^{2+}/Na^+ ratio in leaf tissue determine salinity tolerance in terms of kernel yield in groundnut. These findings could be used in future plant breeding applications for screening salt tolerant Spanish type groundnut genotypes. Additionally, further research is needed to unveil whether lower Ca^{2+} and Ca^{2+}/Na^+ ratio of older or younger leaf tissues contribute to the salinity tolerance of Spanish type groundnut.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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