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Salinity stress: Stress tolerance study in *Arachis hypogaea* L. genotypes

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Abstract
Crop plants that are tolerant to soil salinity are needed for a sustainable food production in areas where there is salt build-up caused by irrigation practices. Twenty-four groundnut (*Arachis hypogaea L.*) genotypes were screened in laboratory and field studies to identify those tolerant to soil salinity. Plants were irrigated with, 50, 100, and 300mM NaCl solution, prepared artificially from table salt. Germination rate and percentage germination decreased with increasing salinity. UG158, UG161, UG163 UG168 UG172, UG175, UG177, UG178, UG179 UG182 UG5, GG7 and PM2 germinate up to 300mM, while others germinated with low percentages. Agronomic characters were significantly reduced by salinity above 50mM NaCl. Biomass and growth decreased with increasing salinity. Data could not be taken for the genotypes UG169, UG175 and UG162 at 300mM as a result of complete mortality of genotypes. The traits from the salt tolerant genotypes could be a source for developing salt tolerant variants for improvement in groundnut production.

Keywords: Salinity, groundnut, mortality, genotypes, stress

1. Introduction

Salinity is an ever-increasing problem throughout the world and imposes major constraints to food production (Hasegawa *et al.*, 2000; Tester and Davenport, 2003). Globally, nearly 100 million ha of land is affected by salinity which accounts for 6-7% of the total arable land (Munns and James, 2003). Groundnut (*Arachis hypogaea* L.) is an annual plant belonging to Fabaceae family. It is grown in more than 100 countries around the world under different agro-climatic conditions, and India is one of the major producing countries. Groundnut is the 13th most important food crop of the world, the world's 4th most important source of edible oil and 3rd most important source of vegetable protein. Its seeds contain high quality edible oil (50%), easily digestible protein (25%) and carbohydrate (20%). Globally, 50% of groundnut produced is used for oil extraction, 37% for confectionery use and 12% for seed purpose. Its haulms (vegetative plant part) also provide excellent hay for feeding livestock. Groundnut world production (millions of tonnes), harvested area (millions ha) and yield (tonnes/ha) increased from 15 to 36, 17 to 24 and 0.9 to 1.5 respectively, between 1960s and 2007. Its production, harvested area and yield are projected to increase to 68 million tonnes, 35 million ha and 2.0tonnes/ha in 2050 respectively, to meet up with increasing human population for food and industrial use. Unfortunately, its production is decreasing while its demand is increasing. Groundnut as an oil crop in oil equivalent growth rate has decreased from 2.8 in 1990 to 1.5 in 2005, which may further decrease to 1.4 in year 2030. This decrease is largely due to climatic variations, biotic influence and abiotic stresses. In arid and semi-arid regions of the world, yield relies largely on irrigation practices for crop production. Unfortunately, some of the water for irrigation purposes contains salt, which builds up in soil over a period of time. Soil salinization is a fast-growing problem of agriculture, with about 23% of the world's cultivated land being saline, causing reduction in crop productivity and loss of arable land. Soil salinity has been widely reported to negatively affect germination, growth and yield of many crop plants. Unfortunately, this problem will continue as long as irrigation is being practiced, and it is expected that there would be about 30% arable land loss within the next 25 years due to salinity. Therefore, selection of salt tolerant genotypes has become a necessity for sustainable crop production. Salinity has been reported to negatively affect germination of many groundnut genotypes. Also, soil salinity has negatively affected growth and yield in most genotypes of groundnut. Soil salinity is a global environmental challenge, limiting crop production over 800 million hectares worldwide.

The majority of the saline land has arisen from natural events and human intervention, such as release of soluble salts of various types during weathering of parental rocks, the deposition of oceanic salts by wind and rain as well as irrigation containing trace amounts of sodium chloride. Groundnut is an important oil crop that provides 50–80% of daily calorie intake for more than 3 billion people. Groundnut plants are moderately sensitive to salt stress, particularly at the seedling and reproductive stages. However, groundnut genotypes differ in their sensitivity to salt stress, and some groundnut genotypes tolerant to salt stress have been reported. Elucidating of the molecular and physiological mechanisms by which groundnut genotypes respond and adapt to salt stress are pivotal for selecting and breeding groundnut genotypes capable of growth in the saline soils.

Plants suffering from high salt stress often display symptoms of Na^+ toxicity due to accumulation of Na^+, which in turn reduces nutrient acquisition, leading to nutritional imbalances, and oxidative damage. In addition, plants exposed to salt stress can also suffer from osmotic stress. Therefore, plants have to equip with capacity to tolerate osmotic stress under saline conditions. Salt stress limits plant growth by increasing the osmotic potential of the soil and, thus, decreasing water uptake by the roots. Accumulation of compatible osmolytes in the cytosol, lowering osmotic potential to sustain water absorption from saline soil solutions, is an important salinity tolerance mechanism. Many attempts to molecular breeding plants tolerant to drought have been made by introducing genes that encode key enzymes for biosynthesis of compatible solutes.

Increases in activities of enzymes that detoxify reactive oxygen species also contribute to plant tolerance to salinity. For example, Mishra *et al*. (2013) reported that salt tolerant rice seedlings have a better protection against reactive oxygen species (ROS) by increasing the activities of antioxidant enzymes under salt stress. Transgenic plants over-expressing genes encoding antioxidant enzymes are more tolerant to salt stress than their wild-type counterparts.

Soil is often referred to as saline one when the electrical conductivity (equivalent to the concentration of salts in saturated soil or in a hydroponic solution) is greater than 4 dS m^{-1}, which is equivalent to approximately 40 mM NaCl and yields of most crops are suppressed when grown in such saline soils. However, higher levels of concentration of NaCl (150–300 mM) have been frequently used to study the physiological and molecular mechanisms to saline stress in the literature. Therefore, elucidating the mechanisms underlying response and tolerance to moderate salt stress that is similar to natural saline soils will be of practical implications regarding for breeding crops capable of growing in saline soils. Only few of groundnut genotypes can endure the salinity stress and also yield satisfactorily. Those genotypes with high survival and seed yield are categorized as salinity tolerant genotypes. Thus, soil salinity must have contributed to reduced areas of groundnut cultivation and total productivity; hence selection of salt tolerant genotypes becomes a necessity to cope with its demand for food and industrial use. This selection will alleviate salinity stress and bring about more areas suitable for groundnut cultivation. This research is aimed at selecting salt tolerant accessions by investigating germination, growth and survival of 24 groundnut genotypes from India.

2. Material and method

The experimental material comprised of 24 groundnut genotypes including three checks namely UG-5 (Pratap Raj Mungphali), PM-2 and GG-7. The 24 genotypes were procured from Department of Plant Breeding and Genetics, RCA Udaipur. Details of the source and pedigree of material used are given in Table 1.

Table 1: List of genotypes used in present study and their pedigree

Sr. No.	Name of genotypes	Pedigree	Source
1.	UG-158	J 63 × TPG 41	DGR, Junagarh
2.	UG-160	GG 2 × B 95	DGR, Junagarh
3.	UG-161	GG 8 × TKG 19 A	DGR, Junagarh
4.	UG-162	GG 2× TPG 41	DGR, Junagarh
5.	UG-163	GG 20 × PBS 24030	DGR, Junagarh
6.	UG-164	ICGX 090018	ICRISAT
7.	UG-165	GG 21 × R-2001-3	DGR, Junagarh
8.	UG-167	GG 2 × TG 26	DGR, Junagarh
9.	UG-168	GG 20 × TAG 24	DGR, Junagarh
10.	UG-169	GG 20 × ICGV 86325	DGR, Junagarh
11.	UG-170	GG-7 × R-2001-3	DGR, Junagarh
12.	UG-172	TG-37 A × GG 20	DGR, Junagarh
13.	UG-173	GG 2 × ICGV 91114-1	DGR, Junagarh
14.	UG-174	TG 40 × ICGV 86325	DGR, Junagarh
15.	UG-175	PBS 24030 × TG 37 A	DGR, Junagarh
16.	UG-177	J 11 × TPG 41	DGR, Junagarh
17.	UG-178	ICGV 76 × ICGV 86305	DGR, Junagarh
18.	UG-179	ICGV 86564 × TPG 41	DGR, Junagarh
19.	UG-181	ICGV 86590 × PBS 24030	DGR, Junagarh
20.	UG-182	UG 20 × ALR-3	DGR, Junagarh
21.	UG-184	GG 5 × TPG 41	DGR, Junagarh
22.	PM -2	ICGV- 86055 × ICG- (FDRS 10)	DGR, Junagarh
23.	UG-5	Selection from ICGV-98223	DGR, Junagarh
24.	GG-7	S 206 × FEFR 81-1-9-B-B	DGR, Junagarh

2.2 Preparation of pot and sowing of seed

Sun dried plastic pots were weighed and lined with polyethylene sheet so that water could not leak. Thereafter, it was filled with soil mixture, prepared with sandy loam soil and vermin-compost in a 1:1 ratio. The fertilizer needed for each pot was determined following the Fertilizer Recommendation Guide-2005 (BARC, 2005). These were mixed thoroughly with the soil in each pot before sowing. Two pre germinated seeds of each genotype were sown each pot.

2.2 Preparation of saline stock solution

The saline water was prepared by using different mille-molar concentration of salt NaCl that is 50mm and 100 mm and 300 mm.

2.3 Morphological/Agronomical Characters

All the morphological characters were analysed on the basis of their survival in salt stress. The selected plants were tagged and data on individual plant will be recorded for the following characters-

1. Shoot length (cm).
2. Root length (cm).
3. Hypocotyls length (cm).
4. Number of branches in plant
5. No. of main root

Data recorded for following characteristics with different salt concentration are describe below (Table 2, 3 and 4) and graphical presentation is given in figure 1,2 and 3

Table 2: 50Mm concentration of salt

S. No.	Genotypes	Shoot height (cm)	Root length (cm)	Hypocotyls length (cm)	Number of branches in plant	No. of main root
1.	G1	16.1	25.9	5.2	6	5
2.	G2	16.9	27.2	6.0	6	4
3.	G3	17.8	25.0	5.9	6	5
4.	G4	14.9	13.9	7.5	6	5
5.	G5	15.9	24.8	5.5	5	3
6.	G6	17.6	29.3	10.2	7	5
7.	G7	19.8	20.2	2.0	7	2
8.	G8	16.1	25.0	4.5	5	5
9.	G9	14.5	37.6	6.3	5	5
10.	G10	16.6	18.5	6.2	6	5
11.	G11	12.9	14.9	10.3	5	5
12.	G12	15.6	17.9	7.5	6	3
13.	G13	16.2	18.2	8.0	5	5
14.	G14	12.9	20.2	7.1	5	5
15.	G15	16.0	17.2	2.2	6	5
16.	G16	20.0	17.6	2.3	5	7
17.	G17	12.6	20.2	5.5	6	5
18.	G18	18.3	18.5	3.5	4	5
19.	G19	17.5	20.2	4.5	6	5
20.	G20	19.2	30.6	2.5	6	3
21.	G21	20.2	18.5	4.2	4	6
22.	G22	22.4	17.5	8.2	6	6
23.	G23	23.2	15.9	8.6	5	6
24.	G24	19.6	20.2	6.5	6	7

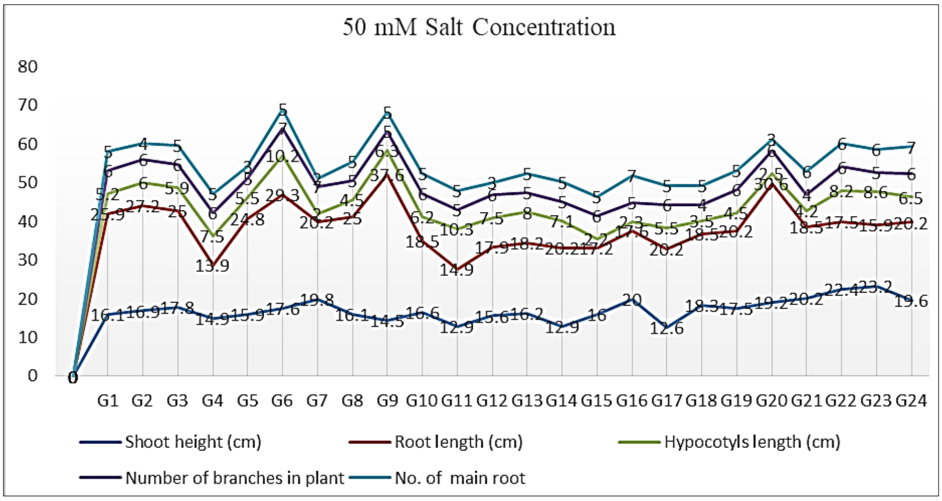

Fig 1: Effect of salt concentration of groundnut genotypes

Table 3: 100 Mm concentration of salt

S. No.	Genotypes	Shoot height (cm)	Root length (cm)	Hypocotyls length (cm)	Number of branches in plant	No. of main root
1.	G1	15.3	24.6	4.1	6	5
2.	G2	14.2	25.2	5.2	6	4
3.	G3	17.0	23.8	4.9	5	5
4.	G4	14.4	12.3	6.8	6	5
5.	G5	15.8	21.9	4.0	4	3
6.	G6	16.6	28.1	9.8	7	5
7.	G7	18.2	18.9	1.0	6	2
8.	G8	14.3	22.2	4.0	4	5
9.	G9	13.2	35.2	6.1	5	5
10.	G10	14.3	16.2	5.9	6	5
11.	G11	11.8	12.8	7.3	4	3
12.	G12	14.0	15.9	7.8	6	5
13.	G13	13.9	15.4	6.9	4	5
14.	G14	11.0	18.1	2.9	6	1

15.	G15	15.0	15.6	2.0	4	5
16.	G16	18.8	15.2	5.2	5	5
17.	G17	9.89	17.9	3.2	6	3
18.	G18	17.6	16.4	4	4	8
19.	G19	16.4	18.9	2.1	5	7
20.	G20	18.8	28.3	3.2	6	7
21.	G21	19.2	16.2	7.8	4	8
22.	G22	20.3	15.8	7.9	6	6
23.	G23	19.1	14.9	8.9	5	4
24.	G24	17.2	13.6	6.1	6	6

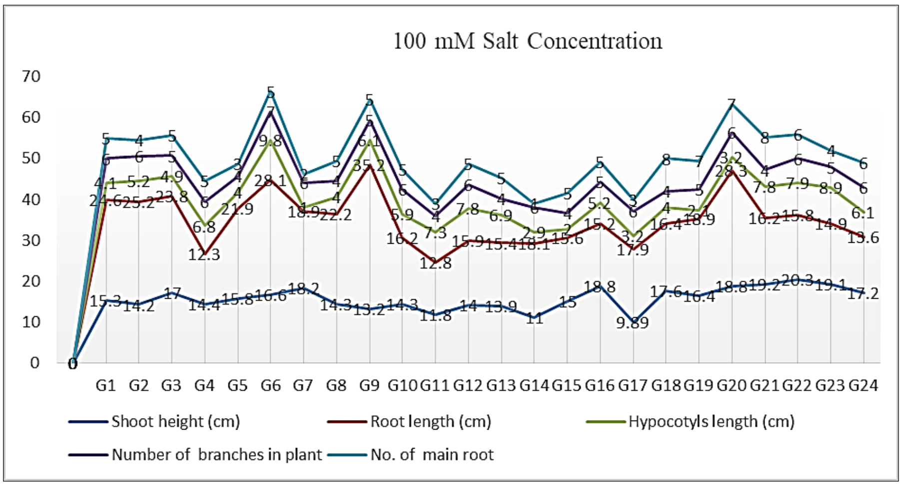

Fig 2: Effect of salt concentration of groundnut genotype

Table 4: 300 Mm concentration of salt

S. No.	Genotypes	Shoot height (cm)	Root length (cm)	Hypocotyls length (cm)	Number of branches in plant	No. of main root
1.	G1	14.0	22.0	3.8	5	3
2.	G2	12.1	19	3.1	5	1
3.	G3	15.2	18	2.2	3	3
4.	G4	-	-	-	-	
5.	G5	11.4	17	2.2	2	4
6.	G6	13.3	18	5.2	5	2
7.	G7	14.3	14.9	0.2	5	2
8.	G8	12.1	14.1	2.2	2	2
9.	G9	9.8	17.2	3.1	4	4
10.	G10	-	-	-	-	-
11.	G11	8.8	10.9	5.5	3	1
12.	G12	10.2	13	5.4	4	2
13.	G13	11.6	12	4.2	2	2
14.	G14	7.6	11.2	0.9	5	5
15.	G15	-	-	-	-	-
16.	G16	13.6	9.8	3.1	3	4
17.	G17	13.9	5.6	1.3	2	5
18.	G18	14.3	12.9	2.4	4	3
19.	G19	11.3	15.2	0.8	5	4
20.	G20	15.2	20.1	1.6	5	3
21.	G21	13.9	9.3	5.1	3	4
22.	G22	15.6	11.2	6.0	5	1
23.	G23	10.2	10.1	5.2	4	4
24.	G24	15.7	8.8	5.9	4	4

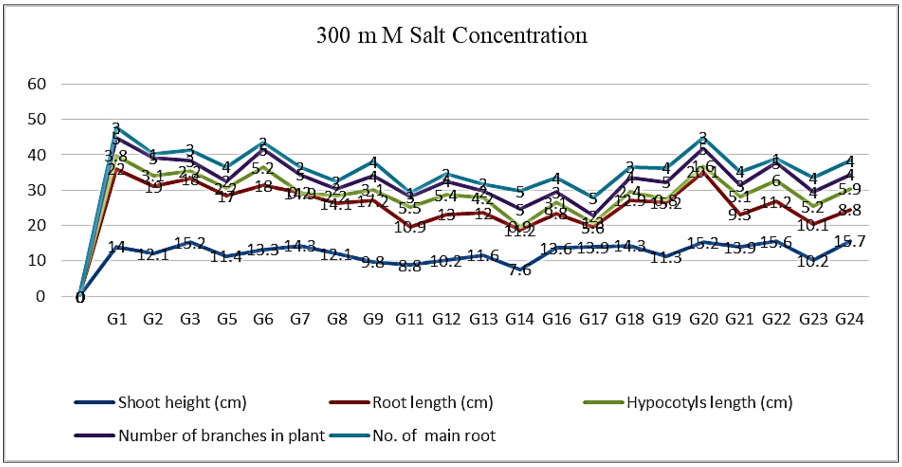

Fig 3: Effect of salt concentration of groundnut genotype

3. Result and Discussion

Salinity stress, unlike drought, is an intricate phenomenon which includes osmotic stress, specific ion effect, nutrient deficiency etc. thereby affecting various physiological and biochemical mechanisms associated with plant growth and development (Sairam *et al*., 2002). The results indicated that salinity delayed germination and reduced germination percentage with large genotypic variations. Salinity of 25-50mM did not delay germination but higher concentrations delayed germination by 1-2 days depending on the genotype. Salinity considerably reduced percentage germination in the genotypes. However, UG158, UG161, UG163 UG168 UG172, UG175, UG177, UG178, UG179 UG182 UG5, GG7 and PM2 germinate up to 300mM, others germinated but with low percentages.

Plant mortality increased with an increase in salinity in water used for irrigation. Mortality varied with genotypes. Salinity below 50mM did not cause mortality in any of the genotypes but higher level did, which increased with an increasing salinity. In addition, vegetative growth of remaining all genotype reduced day by day at 300mM.

Plant shoot length, root length, hypocotyls length, branches and main root were lower under salt stress than in low salt concentration (Tables 2, 3 and 4). The decrease in the growth parameters occurred in all the genotypes, with increase in the concentration of salt in water of irrigation. Data could not be taken for the genotypes UG169, UG175 and UG162 at 300mM as a result of complete mortality of genotypes.

Reduction in percentage germination and delay in germination at high salt concentration in this study is in line with the result on some groundnut genotypes at electrical conductivities greater than 2.60mS/cm. Seed germination of most non-halophytes may be inhibited by 0.5% salt. Sodium chloride (NaCl) at osmotic tension of 0.0625 and 0.125MPa did not have significant effects on germination of *Sesamum indicum*, but higher osmotic tensions of 0.250 and 0.500MPa significantly reduced the percentage germination compared to the control. The few seeds that germinated at 0.500MPa were weak and chlorotic and did not proceed with radicle elongation and shoot development. Negative effect of salinity on germination was also observed in guar. Salinity influences seed germination primarily by lowering the osmotic potential of the soil solution sufficiently to retard water absorption by seeds. Destruction and weakness of embryo by salt toxicity must have been responsible for the delay and a reduction in percentage germination recorded in this study.

Salinity caused accumulation of salt in the root zone and hence, its effect started with an imbibition of seed as soon as it came in contact with saline water. The tolerance is a relative term depending mainly upon the intensity of salinity and relative performance of genotypes.

Soil salinity has been earlier reported to cause death in several plants; *Hordeum vulgare* and *Coriandrum sativum*. Reduced plant survival in this study also agrees with the previous research that revealed that rye (*Secale cereale*) growth was reduced in the presenceof salt, but 110mmol/L NaCl was the highest concentration allowing its growth to the three-leaf stage. When the percentage of dead leaves in Hordeum species reached about 20% of the total, the rate of leaf production slowed down dramatically and some plants died. All these might be responsible for mortality in *Arachis hypogaea*. Growth reduction under salinity stress has been reported in Triticum dicoccum farrum, *Oryza sativa*, *Vigna subterranea* and *Coriandrum sativum*. Many nutrients have essential roles in the process of cell division and cell extension and those would cease soon after the supply were halted, especially in tissues with little nutrient storage. The growth reduction in *A. hypogaea* can therefore be attributed to disturbed/imbalance nutrition. Plant height was also reduced by salinity in Nigella sativa, which was attributed to suppression of internode growth. Decrease in plant height might be due to a reduced photosynthesis, which in turn limited the supply of carbohydrate needed for growth. Growth decrease might have resulted from reduced turgor in expanding tissues caused by reduced water potential in root growth medium. A decrease in the number of leaves under salt stress was due to leaf abscission, senescence and defoliation, coupled with an inhibition of lateral branches bearing the leaves, caused by Na+ and Cl- toxicity. The reduction in the whole-plant photosynthesis under high salinity was accompanied by both reduced photosynthetic potential and smaller total leaf area. Salinity inhibits plant growth by exerting low water potentials, ion toxicity and ion imbalance. Decreased dry mass of plant parts by salinity was probably due to the integrated reduction of the number of leaves and components of the leaf, which eventually resulted in poor overall plant biomass, since the leaf is the major source of carbohydrates required for growth. Soil salinity negatively affected yield in the groundnut genotypes similar to what has been reported in *Brassica napus*, *Hordeum vulgare* and a local variety of *Arachis hypogaea*. Also, the yield characters including 100 seed weight, number of pods and seeds per plant were reduced by salinity, and that the characters were controlled by additive genes which could be improved by selection. They stated that plants tend to record low yields under salinity stress because of adverse effects of salinity on such parameters as relative water content, total dry weight, plant height and number of leaves per plant. A decrease in pod number was associated with an increase in ABA and pollen death. Pod size was reduced, leading to a decrease in the number of seeds. It was observed that the significant decrease of yield components under salt stress in cowpea was partly related to a significant reduction of foliar chlorophyll contents, more than 50% essential for fruit production. Thus, a reduction in yield can be attributed to low chlorophyll content. Plants which are stressed by salinity had lower carbohydrate concentrations in their leaves than in control, which usually leads to reduced food storage in the seed, with resultant negative effect on yield. A decrease in the number of leaves limited surface area available for light interception for photosynthetic activities, with consequential effect on growth and productivity. Large genotypic variation in plant mortality and yield clearly indicated that there was an ideal salinity condition for screening and identification of salinity tolerant and sensitive genotypes. There was plant mortality as well as pod bearing depending upon the salinity levels and genotypic variations in this study. It has earlier been reported that there are a few genotypes of groundnut that can endure the salinity stress and also yield satisfactorily. Those genotypes with high survival and seed yield are categorized as salinity tolerant. The seed yield in a unit area (gm-2), a resultant of plant survival and yield parameters were identified as the best criterion for selecting the salinity tolerant genotypes in groundnut. They ranked groundnut genotypes based on lesser mortality and better yield and top 10 genotypes were grouped as being salinity tolerant.

4. Conclusion

The research revealed that UG158, UG161, UG163 UG168 UG172, UG175, UG177, UG178, UG179 UG182 UG5, GG7 and PM2 genotypes of *Arachis hypogaea* can withstand salt stress and produce good yield if grown in soil with salinity up to 150mM NaCl, while other genotypes are highly susceptible to salt stress. Those that can withstand salt stress hold immense promise to be grown in the coastal saline areas, and can be used in breeding programmes for developing salt-tolerant variants of groundnut.

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