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Agronomy/Physiology

Standardization of a Protocol to Screen for Salinity Tolerance in Groundnut

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Salinity is an ever-increasing problem, especially in areas where lands are irrigated with water containing salts. Worldwide, about 100 million ha of arable land is affected by salinity, which accounts for about 6–7% of the total arable land (Munns and James 2003). Salinity adversely affects plant growth at all stages and at seedling and reproductive stages in particular, dramatically reducing the crop yield (Munns et al. 2002).

Groundnut (*Arachis hypogaea*) is an important commodity in many developing countries, particularly in India where the nitrogen (N)-rich crop residues are also used as fodder. The production of groundnut in India needs to be increased from the current 8 million t to about 14 million t by 2020 to meet the increasing demand of the oil and confectionery industry (Girdhar 2004). This increase will have to be partially achieved by growing groundnut in lands considered so far as unsuitable for agriculture, like rice (*Oryza sativa*) fallow affected by salinity during the postrainy season.

Little is known about the salinity tolerance of groundnut and no attempt has been made to breed salinity tolerant groundnut varieties. A protocol is a prerequisite for understanding the response to salinity stress, assessing genetic variability and identifying surrogate traits and mechanisms contributing to tolerance. Therefore, the first step to this work is to standardize a screening protocol to use for the selection of tolerant materials. Although this protocol will be used to test large number of genotypes for their yield response under salinity, its standardization can be done on the basis of the vegetative biomass reduction under salt treatment.

In this article, we report the results of two experiments that were carried out to standardize a protocol to screen groundnut for salinity tolerance. Our objectives were: (i) to identify an optimum NaCl treatment; (ii) to explore the potential tolerance mechanisms; and (iii) to assess the genotypic variation for salinity tolerance in groundnut.

Materials and methods

Growth conditions and salt application. Two experiments were carried out in a glasshouse, with day/night temperature of 28/22°C. In both experiments, six genotypes belonging to different botanical types [ICG (FDRS) 10, ICGS 44, ICGS 76, ICGV 86031, JL 24 and TAG 24] were grown in 15-cm diameter pots filled with 2 kg of Alfisol, collected from the experimental station at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The soil was fertilized with diammonium phosphate (DAP) at 300 mg kg^{-1} soil, and also treated with carbofuran to prevent thrips infestation and thereby peanut bud and stem necrosis incidence. Four seeds were planted per pot and later thinned to two seedlings per pot. Five replicated pots per treatment and genotype were grown. In both the experiments, NaCl was applied at a fixed rate in g kg^{-1} of soil. The required salt was dissolved in water needed to saturate the soil to field capacity (23% w/w). Plants were grown for seven weeks in both the experiments and then harvested.

Experiment 1 (Exp 1). Four salt treatments were imposed, 0, 0.67, 1.34 and 2.02 g kg^{-1} of soil. They corresponded to a solution concentration of 0, 50, 100 and 150 mM NaCl, in the amount sufficient to saturate the soil at field capacity. In this experiment, salt was applied in three split doses, within the initial 10 days after sowing, to avoid a rapid build-up of salt in the soil. Plants were sown on 18 August and harvested on 6 October 2004. At harvest, the plants were separated into leaves, stems, roots, pods and nodules and dried to constant weight in a hot air oven at 70°C. Since pod weight was negligible in the different salt treatments, pod weight was not considered in the analysis.

Table 1. Ratio of biomass of groundnut under salinity to biomass under control in different NaCl treatments.

NaCl (mM) treatment	Exp 1[1]	Exp 2[1]
0	1	1
50	0.84±0.08	–
100	0.59±0.08	0.61±0.09
125	–	0.39±0.07
150	0.33±0.04	0.25±0.02

1. Data are the average ratios of 6 groundnut genotypes (±SD). Mean biomass across genotypes in 0 mM treatment was 10.6 g $plant^{-1}$ in Exp 1 and 6.3 g $plant^{-1}$ in Exp 2.

Experiment 2 (Exp 2). Four salt treatments were imposed, 0, 1.34, 1.68 and 2.02 g kg^{-1} soil, corresponding to an application of solution of 0, 100, 125 and 150 mM concentrations. Salt treatments were applied all in one dose at sowing. Plants were sown on 19 February and harvested on 13 April 2005. At harvest, leaves, stems and pods were separated and dried as in Exp 1.

Criteria to assess salt tolerance. Salt tolerance was assessed on the basis of total biomass (shoot + roots) in Exp 1 and on shoot biomass alone in Exp 2 as shoot biomass and total biomass in Exp 1 were found to be very closely associated ($r^2 = 0.93$, data not shown). The total biomass or shoot biomass is hereafter referred as biomass for brevity. Also the ratio between the biomass produced under salinity to that of control was used to assess salt tolerance (Krishnamurthy et al. 2003a, 2003b).

Measurement of plant traits. Leaf size: A few days before harvest, the two most fully expanded leaves in the main stem were collected for the leaf area measurement. The ratio of the replication-wise values under salinity divided by mean control value for each genotype and treatment gave an estimate of the relative reduction in leaf size due to salinity.

Stem/leaf ratio: After harvest, stems and leaves were separated and their ratio computed for each individual plant.

Nodulation: In Exp 1, at harvest, the nodule number and nodule dry mass were measured and their relative decreases under salinity were computed (replication-wise values under salinity divided by mean control value for each genotype and treatment).

SCMR: In Exp 2, the chlorophyll content of leaves at 49 days after sowing was assessed using the SPAD (Soil and Plant Analysis-Development) chlorophyll meter reading (SCMR). The SPAD readings were recorded on 4 leaflets of the top two most fully expanded leaves of the main stem, and averaged. The ratio of replication-wise values under salinity divided by mean control value for each genotype and treatment gave an estimate of the relative reduction in chlorophyll.

Na concentration in leaves: In Exp 1, 150 mg of finely ground leaf sample was digested in 4 ml of concentrated sulfuric acid with 0.5% selenium powder at 360°C for 75 min on a block digester and the digest was diluted to 75 ml. Using this digest K and Na were estimated (Sahrawat et al. 2002) using an atomic absorption spectrophotometer (Varion model 1200, Australia).

Results

Biomass response to salinity. In Exp 1, plants were little affected by 50 mM NaCl treatment, although there were significant genotypic differences (Fig. 1). Similarly, in both Exp 1 and Exp 2 the genotypic response for biomass production at 150 mM was minimal. In Exp 1, 100 mM NaCl appeared to induce large genotypic biomass differences, with genotypes ICGS 44, ICGS 76 and JL 24 having higher biomass than ICG (FDRS) 10, ICGV 86031 and TAG 24 (P = <0.001) (Fig. 1). In Exp 2, although the 100 mM concentration induced some differences between the genotypes, ie, ICGS 44 and ICGS 76 also had a high biomass compared to ICGV 86031 and TAG 24 (P = 0.042), the 125 mM concentration brought about larger contrast between genotypes, with ICGS 44 reaching the highest biomass whereas JL 24 and TAG 24 were the lowest (P = 0.003) (Fig. 1). Across experiments, it appeared that ICGS 44 achieved consistently the highest biomass at 100 mM whereas ICGV 86031 and TAG 24 had the lowest biomass.

While the ratio of biomass production under salinity to that of control was little affected at 50 mM concentration (0.84), the ratio decreased to a value as low as 0.59 and 0.61 at 100 mM concentration in Exp 1 and Exp 2, respectively (Table 1), and 0.39 at 125 mM in Exp 2. In both experiments, the ratio of biomass production was severely decreased at 150 mM NaCl (0.33 in Exp 1 and 0.25 in Exp 2). The consistent results across experiments clearly indicated significant genotypic differences. Therefore, we used the treatment from the two experiments giving the most genotypic contrast, ie, the 100mM treatment in Exp 1 and 125 mM treatment in Exp 2, to identify surrogate traits and mechanisms contributing to salinity tolerance.

Plant morphology and salinity tolerance. Leaf size reduction: In Exp 2, genotypes showing good growth under 125 mM treatment seemed to maintain leaf size close to that of control (Table 2). The regression of the relative leaf size reduction at 125 mM treatment on the ratio of shoot biomass under salinity revealed a significant association (r^2 = 0.45, P = <0.01), showing that tolerant plants were able to maintain the leaf size closer to that of control (data not shown).

Ratio of stem to leaves: Stem portion in groundnut represent a substantial part of the dry matter (Table 2). The ratio of stem to leaves dry weight and the ratio of shoot biomass under salinity were correlated with a highly significant relationship (r^2 = 0.56, P = <0.01) (data not shown). This shows that although Na accumulation in stems in relation to leaves was not measured, a larger stem proportion may serve as a Na sink and confer higher tolerance to salinity.

N status and salinity tolerance. Nodulation: Nitrogen fixation is very sensitive to salinity (Rao et al. 2002). In Exp 1 the number and dry mass of nodules reduced drastically with increasing salinity, especially at concentrations above 100 mM NaCl (Table 2). A highly significant positive relationship (r^2 = 0.40, P = <0.05) was found between the relative nodule biomass reduction and the ratio of shoot biomass under salinity, indicating that the more sensitive genotypes suffered a relatively larger decrease in nodulation compared to their respective controls (data not shown).

Ratio SCMR: Since nodulation was decreased in Exp 1, there was an interest to measure SCMR as an indirect measure of shoot N status. Although there was a trend to have plants with relatively less decrease in the SCMR values compared to control being also more tolerant (Table 2), this trend was not significant (r^2 = 0.24, P = 0.29). Several SCMR measurements recorded at various dates after sowing failed to show any significant trend (data not shown).

Na accumulation in leaves: In most plants, the accumulation of Na in shoot brings about deleterious effect, and the plant strategy is to limit the Na build-up in the shoot tissues. Although it was found that the Na concentration in shoot increased with the salt treatment (Table 2), there was no relationship between the shoot Na concentration and the relative sensitivity of plants to salt treatment (data not shown).

Discussion

We have shown that the 100–125 mM range of NaCl treatments was suitable to screen for salinity tolerance in groundnut. The material screened in this study was very limited, but large differences could be shown for response to salinity stress. So, there is a good scope for identifying genotypes with higher level of tolerance from larger screening of diverse sets of materials.

Certain aspects of the plant morphology, ie, the reduction in leaf size and the stem/leaves ratio in response to salinity stress provided interesting insights. The reduction in leaf area in sensitive plants under salinity stress indicated arrest of leaf expansion, which eventually limits the area available for photosynthesis. Further research

Table 2. Mean (±SE) values of nodule dry mass, Na concentration in leaves, ratio of stem/leaves, leaf area and SCMR in different NaCl treatments tested against six groundnut genotypes.

Genotype	Control	50 mM	100 mM	125 mM	150 mM
Nodule dry mass (g) (Exp 1)					
ICG (FDRS) 10	0.168±0.010	0.096±0.008	0.075±0.023		0.018±0.003
ICGS 44	0.168±0.023	0.134±0.015	0.132±0.020		0.056±0.003
ICGS 76	0.204±0.030	0.139±0.021	0.163±0.031		0.091±0.021
ICGV 86031	0.221±0.013	0.136±0.023	0.084±0.013		0.036±0.000
JL 24	0.160±0.010	0.136±0.017	0.106±0.024		0.048±0.018
TAG 24	0.131±0.007	0.132±0.011	0.074±0.015		0.041±0.006
Na concentration (%) (Exp 1)					
ICG (FDRS) 10	0.12±0.01	0.24±0.04	0.21±0.03		0.55±0.06
ICGS 44	0.13±0.02	0.20±0.04	0.23±0.04		0.73±0.13
ICGS 76	0.11±0.02	0.15±0.03	0.17±0.01		0.41±0.05
ICGV 86031	0.15±0.02	0.15±0.03	0.23±0.03		0.33±0.04
JL 24	0.12±0.01	0.14±0.02	0.28±0.05		0.80±0.22
TAG 24	0.19±0.03	0.28±0.04	0.27±0.05		0.57±0.08
Stem/leaves ratio (Exp 1)					
ICG (FDRS) 10	0.84±0.04	0.96±0.04	0.83±0.08		0.86±0.05
ICGS 44	0.99±0.04	1.08±0.07	1.08±0.04		0.80±0.04
ICGS 76	0.98±0.04	0.94±0.01	0.98±0.05		0.84±0.08
ICGV 86031	0.87±0.07	0.83±0.06	0.83±0.08		0.99±0.01
JL 24	0.86±0.05	0.87±0.06	0.97±0.08		0.86±0.05
TAG 24	0.82±0.11	0.90±0.10	0.88±0.12		0.88±0.03
Stem/leaves ratio (Exp 2)					
ICG (FDRS) 10	0.78±0.07		0.73±0.04	0.62±0.03	0.61±0.02
ICGS 44	0.83±0.05		1.06±0.27	0.84±0.08	0.69±0.02
ICGS 76	0.72±0.03		0.62±0.04	0.64±0.06	0.59±0.04
ICGV 86031	0.65±0.03		0.77±0.17	0.59±0.04	0.58±0.03
JL 24	0.68±0.02		0.59±0.04	0.56±0.05	0.56±0.03
TAG 24	0.70±0.04		0.81±0.05	0.73±0.04	0.69±0.02
Leaf area (of 8 leaflets) (cm^2) (Exp 2)					
ICG (FDRS) 10	47.4±3.3		38.7±2.9	33.2±3.8	26.2±2.4
ICGS 44	25.7±2.8		24.5±2.0	21.3±1.6	16.7±1.4
ICGS 76	30.1±2.8		24.9±3.0	20.1±1.8	14.9±1.4
ICGV 86031	40.0±3.7		25.3±2.3	24.1±1.8	19.3±2.0
JL 24	47.9±6.2		37.7±2.9	28.3±2.3	26.2±3.0
TAG 24	18.8±1.5		17.1±2.1	13.9±0.9	11.2±1.2
SCMR[1] (Exp 2)					
ICG (FDRS) 10	39.4±0.6		41.4±1.3	35.0±3.6	32.6±4.1
ICGS 44	46.5±2.4		36.9±1.4	40.3±2.0	36.8±1.3
ICGS 76	50.1±2.5		45.2±2.4	43.7±2.8	42.6±2.9
ICGV 86031	46.0±5.2		40.1±2.3	33.8±1.1	32.1±1.2
JL 24	42.3±3.3		39.6±2.2	30.7±1.9	33.1±1.4
TAG 24	39.5±1.8		38.6±0.8	33.1±2.0	31.2±2.3

1. SCMR = SPAD chlorophyll meter reading.

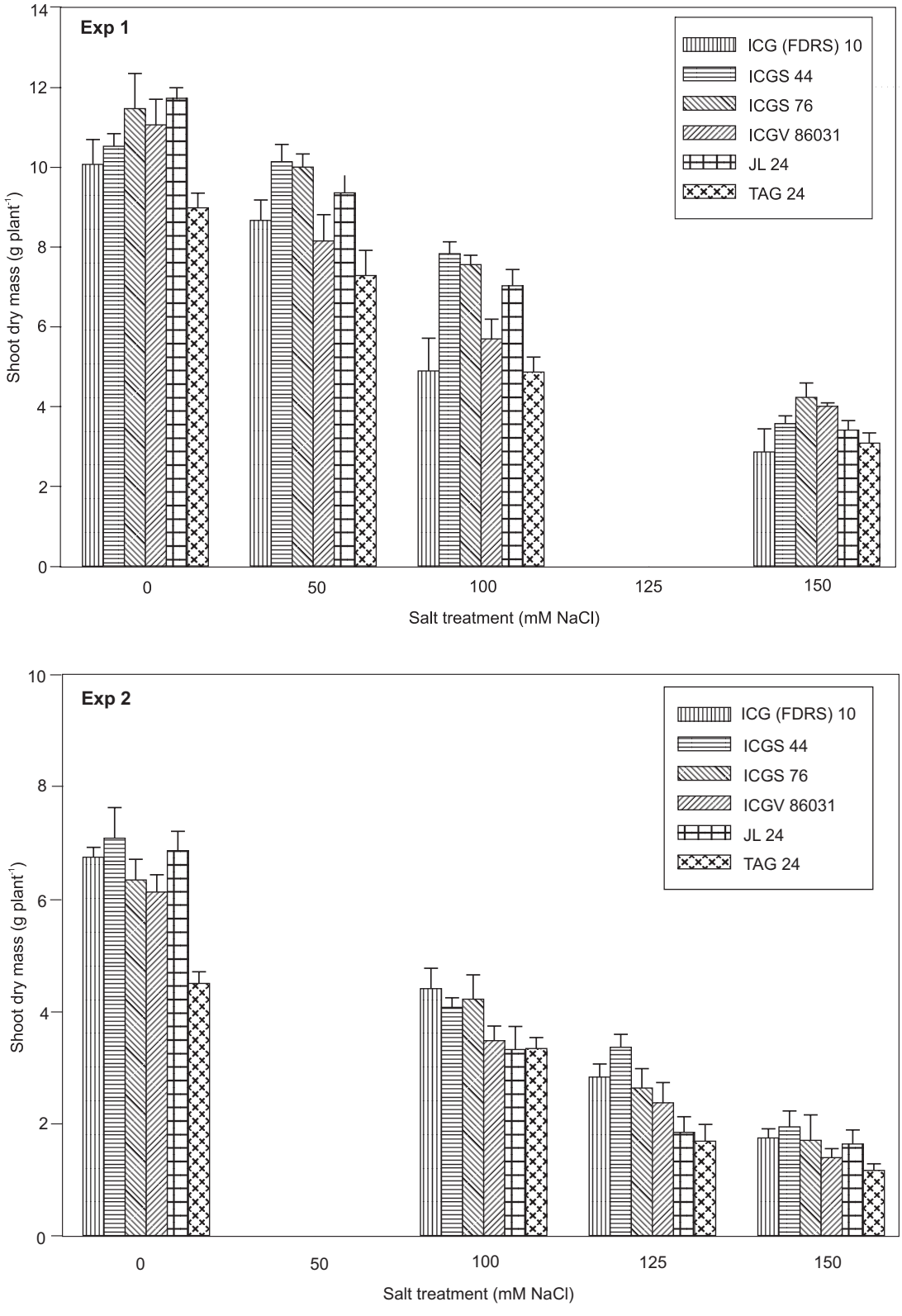

Figure 1. Shoot dry mass of groundnut under different salt treatments in Exp 1 and Exp 2. (Note: Data are means of five replicated plants per genotype and treatment and the vertical bars denote SE.)

is therefore needed to compare the leaf expansion of tolerant and sensitive genotypes under salinity stress and to assess the potential role played by abscisic acid. The ratio of stem/leaves was also an interesting aspect related to the possible storage of Na. It has been found in sorghum (*Sorghum bicolor*) that plants under salinity store a large amount of Na in the stem, as compared to leaves and young leaves (Netondo et al. 2004). We found in sorghum that there was a highly significant correlation between the salinity tolerance and the stem/leaves ratio (our on-going unpublished work in sorghum). The same turned out to be true in groundnut, where stems could be used as Na storage. Further investigation is needed to dissect the precise localization of Na in the shoot parts of tolerant and sensitive groundnut genotypes.

The N status of plants under salinity appeared to be severely affected along with a drastic reduction in leaf size. It is too early to conclude that nodulation reduction was the cause for the reduced production of biomass under salinity in sensitive genotypes, as nodulation is an endogenous variable (nodulation affects shoot growth but shoot growth in turn also affects nodulation). Further work would be needed to explore whether the N_2-fixation process is the most sensitive physiological mechanism in groundnut exposed to salinity.

Now that this protocol is set up, further work is needed to investigate the range of yield response to 100–125 mM NaCl treatment, using a large range of genotypes.

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