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Standardization of a screening technique for salinity tolerance in groundnut (*Arachis hypogaea* L.) and pigeonpea (*Cajanus cajan* L.)

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Abstract

Salinity affects plant growth, development and yield in approximately 100 M ha of arable land worldwide. Besides, various management options available the introduction of salinity tolerant varieties in such areas could partly ease the increasing global food demand. Here, six groundnut (ICG (FDRS) 10, ICGS 44, ICGS 76, ICGV 86031, JL 24, and TAG 24) and pigeonpea (ICPL 88039, ICPL 88034, ICPL 87119, ICPL 96058,ICP 7035 and ICPL 366) genotypes were screened by conducting two experiments in soil treated with five different NaCl (mM) concentrations (0, 50, 100, 125, 150) and (0, 50, 75, 100, 150) respectively for groundnut and pigeonpea, under controlled conditions. Salt concentrations of 100-125 mM were found to be critical to screen groundnut genotypes whereas 75 mM NaCl appeared most suited treatment for pigeonpea. There was a positive and significant correlation between the SPAD Chlorophyll Meter Reading (SCMR) under salinity and the ratio of biomass under salinity to that of control, our proxy for salinity tolerance, though this relation was better in pigeonpea. The sodium concentration in shoot was well correlated with the ratio of biomass in pigeonpea but not in groundnut. Finally, the nodule dry weight was positively and significantly related to the ratio of biomas in both the crops. Our results show a suitable protocol to screen salinity tolerant germplasm of groundnut and pigeonpea and propose few traits, SCMR, shoot Na accumulation, and nodulation, that could be used to understand better the mechanisms of tolerance, and/or possibly to screen for salinity tolerance.

Key words: Salinity, Groundnut, pigeonpea, SCMR, and Na accumulation.

Introduction

Salinity is an ever-increasing problem, especially in areas where lands are irrigated with water containing salts. Worldwide, about 100 million hectares of arable land are affected by salinity, which accounts for about 6-7% of the total (Munns and James, 2003). Salinity adversely affects plant growth at all stages, at seedling and reproductive stages in particular, dramatically reducing the crop yield (Munns *et al.,* 2002). Although there is now more and more knowledge about the genes involved in salinity response and tolerance in a few model plants such as arabidopsis or rice, little efforts have been made to breed for salinity tolerance in economically important crops (Flowers, 2004). More so, there has been no exhaustive assessment of the variability for salinity tolerance in many crops. Therefore, an initial assessment of the range of plant tolerance is required before undertaking a breeding program (Materon, 1988).

Legumes are not only the protein source in the diet of humans and livestock in poor areas, but they also play an important role for the fixation of atmospheric nitrogen to improve the physical and chemical structure of soil (Hoshikawa, 1991). Legumes tend to be a lot more sensitive to salinity than cereals. Yet, legumes are often grown on marginal lands like salinity-affected areas and legume crops with improved performance in such conditions are required. Groundnut and pigeonpea are important crops in many of the developing countries, particularly in India where the nitrogen rich crop residues are also used as fodder. In India alone, where 40 % and 90% of the world's groundnut and pigeonpea are produced respectively, around 13.3 million ha land is affected by salinity (Consortium for Unfavorable Rice Environment, IRRI, 2003). To meet the increasing food demand, the production of legumes needs to be increased, and this will be achieved to some extent by growing them in saline areas. Very little information is available about the salinity tolerance in groundnut and pigeonpea and no attempt has been made to breed tolerant lines in these two crops. To find genetic variation in salinity response is the prerequisite for improving crop salt tolerance (Shannon, 1985). Therefore, our first goal was to standardize a protocol to screen salt tolerant materials, to be used later for assessing genetic variability in a large number of genotypes and to identify potential mechanisms contributing to tolerance.

Although our long-term objective is to use this protocol for yield evaluation under saline conditions, this standardization has been done on the basis of vegetative biomass reduction under saline conditions to fasten the process. We have consciously avoided setting a protocol based on seedling growth evaluation under saline conditions because early growth under salinity shows very poor relation with growth at later stage (Munns *et al.,* 2003). In this paper, we report the results of two experiments that were carried out in groundnut and pigeonpea to standardize a screening protocol, where our objectives were to (i) identify an adequate NaCl treatment to identify genetic variability in the response to salt stress (ii) explore the potential tolerance mechanisms in each crop.

Material and methods

Growth conditions and salt application: Two experiments were conducted in a glasshouse, with day/night temperature of 28/22 ºC. In both experiments six genotypes of each crops [For groundnut ICG (FDRS) 10, ICGS 44, ICGS 76, ICGV 86031, JL 24, and TAG 24 and of pigeonpea two short duration (ICPL 88039 and ICPL 88034), two medium duration (ICPL 87119 and ICPL 96058) and two long duration (ICP 7035 and ICPL 366)] were grown in 6" pots filled with 2 kg of Alfisol, collected from the experimental station at ICRISAT. The soil was fertilized with diammonium phosphate (DAP) at 300 mg kg soil, and also treated with carbofuran to prevent fungal and trips infestation in soil. Five replicated pots per treatment and genotype were grown. In both the experiments, NaCl was applied at a fixed rate in g kg of soil. The required

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Table 1a. Ratio of biomass under salinity to biomass under control in different NaCl treatments in two experiments. Data are the average ratios of six groundnut genotypes (± SD).

NaCl (mM) treatment	Exp 1 *	Exp 2 *
0	1	1
50 (0.584 g kg^{-1} of soil)	0.84 ± 0.08	-
100 (1.168 g kg^{-1} of soil)	0.59 ± 0.08	0.61 ± 0.09
125 (1.46 g kg^{-1} of soil)	-	0.39 ± 0.07
150 (1.75 g kg^{-1} of soil)	0.33 ± 0.04	0.25 ± 0.02

Table 1 b. Ratio of biomass under salinity to biomass under control in different NaCl treatments in two experiments. Data are the average ratios of six pigeonpea genotypes (± SD).

NaCl (mM) treatment	Exp. 1*	Exp.2*
0	1	1
50 (0.584 g kg^{-1} of soil)	0.79±0.03	0.79±0.03
75 (0.876 g kg^{-1} of soil)	-	0.41±0.05
100 (1.168 g kg^{-1} of soil)	0.26±0.02	0.13±0.02
150 (1.75 g kg^{-1} of soil)	0.06±0.007	

* Mean biomass across genotypes in 0 mM treatment was 10.6 and 8.6 g $plant^{-1}$ in Exp 1 and 6.3 and 6.2 g $plant^{-1}$ in Exp. 2 for groundnut and pigeonpea, respectively.

Table 2a. Mean (±SE) values of nodule dry weight, Na^+ accumulation in shoot (Exp. 1), and SCMR (Exp. 2, data are average of the three measurements in each plant taken at 30, 35 and 42 DAS), at different NaCl treatments tested against six groundnut genotypes. Data are the mean of 5 replicated plants per genotype and treatment.

Genotypes	Control	50 mM	100 mM	125 mM	150 mM
Nodule dry weight (g) (Exp.1)					
ICG (FDRS)-10	0.16 ± 0.010	0.09 ± 0.008	0.07 ± 0.023	-	0.01 ± 0.003
ICGS 44	0.16 ± 0.023	0.13 ± 0.015	0.13 ± 0.020	-	0.05 ± 0.003
ICGS 76	0.20 ± 0.030	0.13 ± 0.021	0.16 ± 0.031	-	0.09 ± 0.021
ICGV 86031	0.22 ± 0.013	0.13 ± 0.023	0.08 ± 0.013	-	0.03 ± 0.000
JL 24	0.16 ± 0.010	0.13 ± 0.017	0.10 ± 0.024	-	0.04 ± 0.018
TAG 24	0.13 ± 0.007	0.13 ± 0.011	0.07 ± 0.015	-	0.04 ± 0.006
Na^+ accumulation (Exp. 1)					
ICG (FDRS)-10	0.12 ± 0.010	0.24 ± 0.040	0.21 ± 0.030	-	0.55 ± 0.060
ICGS 44	0.13 ± 0.020	0.20 ± 0.040	0.23 ± 0.040	-	0.73 ± 0.130
ICGS 76	0.11 ± 0.020	0.15 ± 0.030	0.17 ± 0.010	-	0.41 ± 0.050
ICGV 86031	0.15 ± 0.020	0.15 ± 0.030	0.23 ± 0.030	-	0.33 ± 0.040
JL 24	0.12 ± 0.010	0.14 ± 0.020	0.28 ± 0.050	-	0.80 ± 0.220
TAG 24	0.19 ± 0.030	0.28 ± 0.040	0.27 ± 0.050	-	0.57 ± 0.080
SCMR (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	-

Table 2b. Mean (±SE) values of nodule dry weight, Na^+ accumulation in shoot (Exp. 1), and SCMR (Exp. 2, data are average of the three measurements in each plant taken at 30, 35 and 42 DAS), at different NaCl treatments tested against six pigeonpea genotypes. Data are the mean of 5 replicated plants per genotype and treatment.

Genotypes	Control	50 mM	75 mM	100 mM	150 mM
Nodule dry weight (g) (Exp.1)					
ICP 7035	0.05 ± 0.02	0.27 ± 0.04	-	0.0004	*
ICPL 366	0.38 ± 0.06	0.21 ± 0.02	-	0.0006	*
ICPL 87119	0.55 ± 0.04	0.31 ± 0.01	-	0.0076	*
ICPL 88034	0.37 ± 0.03	0.20 ± 0.02	-	0.0110	*
ICPL 88039	0.41 ± 0.03	0.19 ± 0.02	-	0.0330	*
ICPL 96058	0.07 ± 0.05	0.39 ± 0.04	-	0.0160	*
Na^+ accumulation (Exp. 1)					
ICP 7035	0.11 ± 0.046	0.20 ± 0.028	-	1.66 ± 0.160	2.87 ± 0.260
ICPL 366	0.12 ± 0.020	0.15 ± 0.041	-	1.35 ± 0.190	2.66 ± 0.390
ICPL 87119	0.09 ± 0.009	0.10 ± 0.009	-	0.76 ± 0.160	2.09 ± 0.450
ICPL 88034	0.07 ± 0.006	0.15 ± 0.010	-	1.01 ± 0.120	2.18 ± 0.440
ICPL 88039	0.09 ± 0.015	0.08 ±0.009	-	0.35 ± 0.080	1.26 ± 0.170
ICPL 96058	0.08 ± 0.014	0.13 ± 0.019	-	0.48 ± 0.070	1.90 ± 0.370
SCMR (Exp. 2)					
ICP 7035	48.6 ± 1.30	38.4 ± 1.03	24.1 ± 3.20	22.0 ± 6.10	-
ICPL 366	46.7 ± 1.10	39.4 ± 1.82	32.9 ± 3.20	25.5 ± 9.90	-
ICPL 87119	47.1 ± 1.40	38.1 ± 1.24	38.5 ± 6.90	26.2 ± 6.80	-
ICPL 88034	47.8 ± 1.40	44.5 ± 0.61	34.9 ± 0.50	27.7 ± 8.90	-
ICPL 88039	47.7 ± 0.90	47.8 ± 0.62	44.7 ± 6.90	36.6 ± 7.70	-
ICPL 96058	49.5 ± 1.50	40.4 ± 1.49	39.0 ± 0.80	20.4 ± 5.90	-

* NA (Not available)

salt was dissolved in water needed to saturate the soil to field capacity (23% w/w). Therefore, treatments are expressed in mM NaCl of the solution that was used to saturate the soil profile. Plants were grown for seven weeks in both experiments and then harvested.

Experiment 1: In this experiment four salt treatments were given to both the crops in three split doses within the first 10 days after sowing to avoid the rapid build up of salt. These saline treatments were 0, 50, 100 and 150 mM NaCl. The experiment was planted on 18 August and harvested on 6 October 2004. At harvest, plants were separated into leaves, stems, roots, pods and nodules and oven dried for three days at 70^{0}C. Since pod weight was negligible in different saline treatments, it was not considered in the analysis.

Experiment 2: In this experiment, a different set of salt treatments were given for groundnut and pigeonpea, adjusted based on the results of the first experiment, focusing on the range of 100-150 mM in groundnut (0, 100, 125, and 150 mM), and in the range of 50-100 mM in pigeonpea (0, 50, 75, and 100 mM). These treatments were all applied in one dose at the time of sowing. This experiment was planted on 19 February and harvested on 13 April 2005. At the time of harvest, the plants were separated into leaves, stems and pods and dried as first experiment.

Criteria to assess the salt tolerance: Salt tolerance was assessed on the basis of total biomass (shoot + roots) in Exp 1 and on shoot biomass in Exp 2 because 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 hereafter referred as biomass for brevity. We also use the ratio of biomass (biomass under salinity/biomass under control). This ratio was well correlated to the biomass under salinity (Krishnamurthy *et al.*, 2003a, b), and controls for differences in genotype vigor.

Measurements of plant traits

Nodulation: Nitrogen fixation is very sensitive to salinity (Rao *et al.*, 2002), and any effect on the N supply from N_2 fixation might explain why biomass is reduced. In Exp.1, the nodule number and their dry weights were measured.

Na^+ concentration in shoot: In Exp. 1, 150 mg of finely ground shoot samples of groundnut and pigeonpea were digested in 4 ml of concentrated sulphuric acid with 0.5% selenium powder at 360^{0}C for 75 min on a block digester and the digest was diluted to 75 ml using distilled

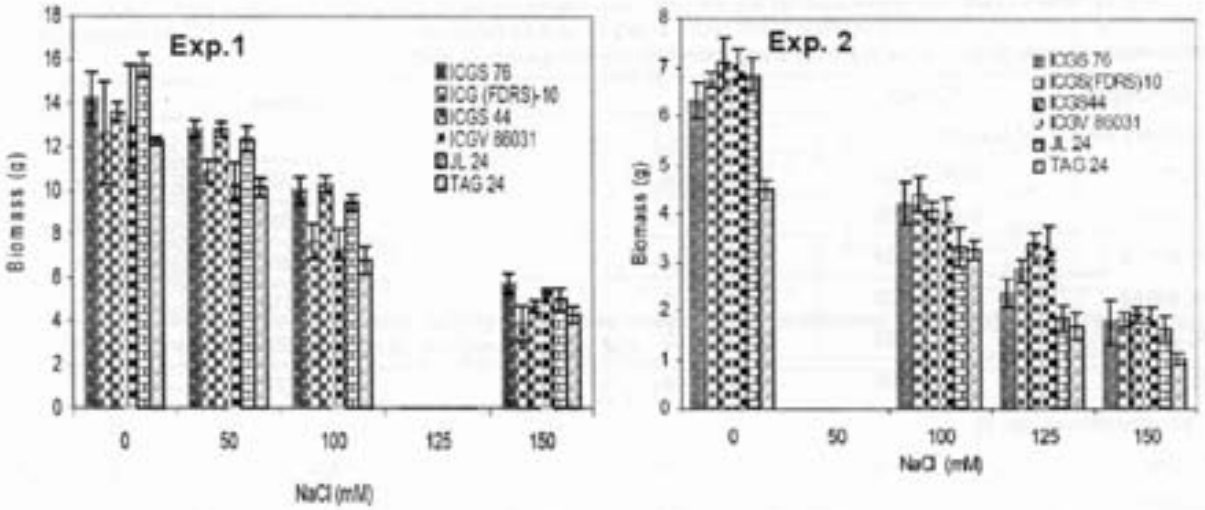

Figure 1. *Means of shoot dry weights (±SE) of six groundnut genotypes under different salt treatments across two experiments (data are the means of five replications).*

water. This dilution was used to estimate Na^+ (Sahrawat *et al.*, 2002) using an atomic absorption spectrophotometer (Varion model 1200, Australia).

SCMR In Exp. 2, SCMR (Spad Chlorophyll Meter Reading) was recorded. SCMR is a unit less measurement that proxies the amount of chlorophyll in the leaves by measuring the intensity of greenness. Spad Chlorophyll Meter Readings were recorded at 30, 35 and 42 DAS, on four leaflets of the top most fully expanded leaf (groundnut) and six leaflets of the two most fully expanded leaves of the main axis (pigeonpea) and averaged.

Results and discussion

In Exp. 1, groundnut and pigeonpea were little affected by 50 mM NaCl treatment, with biomass being 84 and 79% of control respectively (Table 1), although significant genotypic differences were found (fig 1 and 2). On the contrary, 150 mM NaCl imposed a very severe treatment on both the legumes, in particular in pigeonpea, and biomass was only 29 and 6% of control. On the whole, it appeared that pigeonpea was relatively more sensitive to salinity than groundnut, shown by lower ratio of biomass under similar treatments (Table 1).

In groundnut, the 100mM NaCl treatment revealed the largest genotypic differences in Exp. 1, with genotypes ICGS 44, ICGS 76 and JL 24 having higher biomass than ICGS (FDRS) 10, ICGV 86031 and TAG 24 ($P< 0.001$). At that treatment the ratio of biomass was 59 and 61% of control in Exp.1 and Exp.2 respectively. In Exp. 2, the largest genotypic differences were found at 125mM treatment, and there again,ICGS 44 had the highest biomass whereas ICGV 86031 and TAG 24 showed lowest biomass (Fig 1). At that treatment, the ratio of biomass was 39% (Table 1).

For pigeonpea, the 100 mM treatment in Exp. 1 was still fairly severe for all genotypes, i.e. biomass was only 13% of

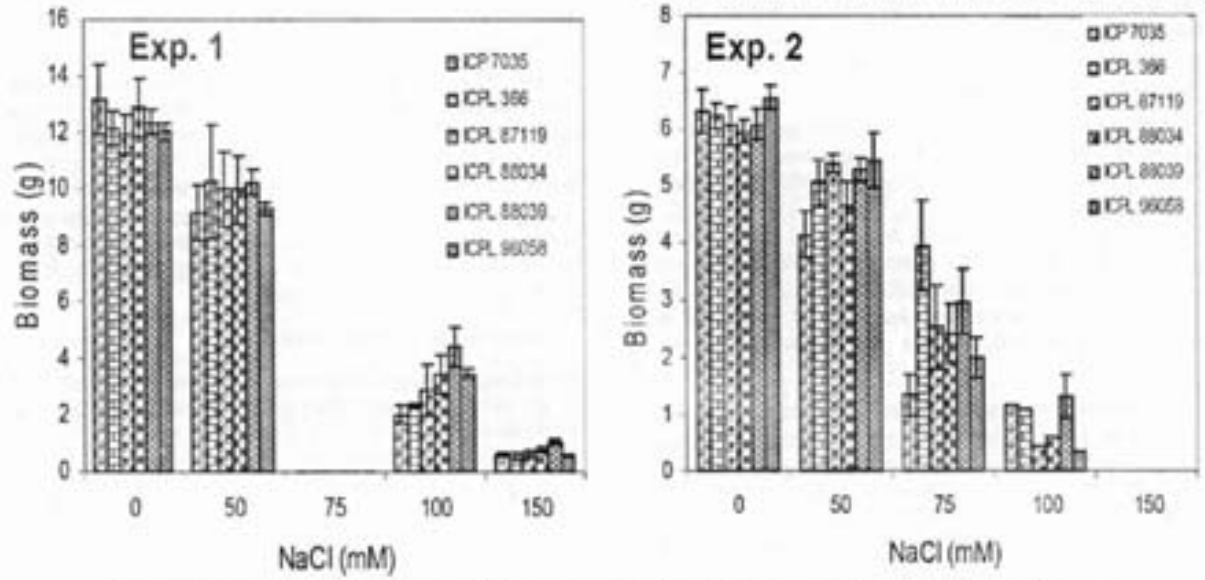

Figure 2. *Means of shoot dry weights (±SE) of six pigeonpea genotypes under different salt treatments across two experiments (data are the means of five replications).*

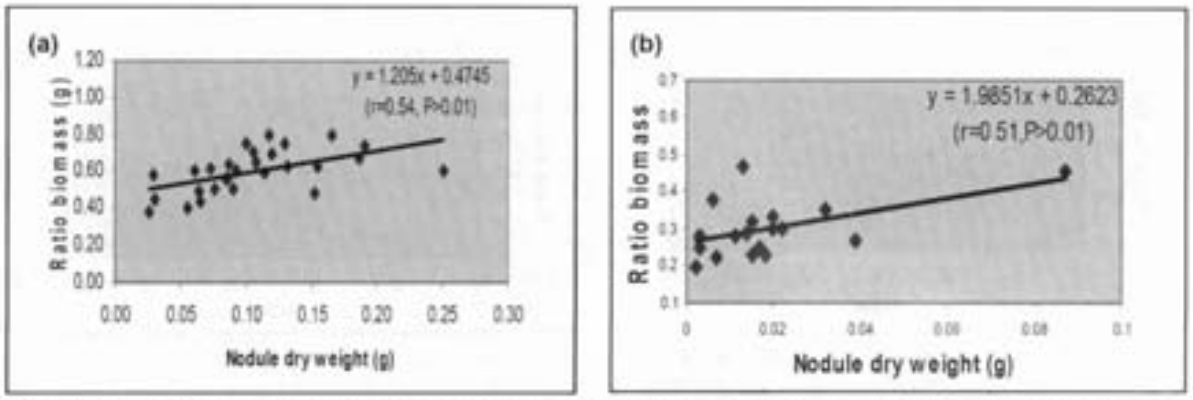

Figure 3. *Simple linear correlation between nodule dry weight (g) and the ratio biomass in (a) groundnut and (b) pigeonpea.*

control (Table 1), and there were no significant genotypic differences (Fig 2). In Exp. 2, the 75 mM NaCl appeared to be the most adequate concentration since it revealed the largest genotypic differences (Fig 2), and the biomass was up to 41% of control (Table 1). In this experiment, at 75 mM NaCl, ICPL 366 and ICPL 88039 had a higher biomass than ICP 7035, ICPL 87119, ICPL 88034 and ICPL 96058. In both the experiments ICPL 366 and ICPL 88039 had consistently higher biomass under saline conditions whereas ICP 7035 had the lowest biomass.

Therefore, 100 mM and 125 mM NaCl treatment for groundnut, and 75mM NaCl treatment for pigeonpea, revealed the largest contrast among the genotypes. These treatments conferred an adequate level of stress also because they were neither too severe, nor too mild, bringing about a biomass reduction in the range of 50%. Those can be used for the screening of large number of genotypes in both these crops (Srivastava *et. al.*, 2005). Using the recommended treatments, we also initiated the exploration of potential traits related to salinity tolerance in both crops.

Measurements of plant traits

Nodulation: In Exp. 1, nodules dry mass decreased with the increase of salinity levels in both the crops. A significant positive correlation (r= 0.54, P<0.01) for groundnut and (r=0.51, P>0.01) for pigeonpea were found between the nodule dry weight and the ratio shoot biomass under salinity, indicating that more sensitive genotypes showed larger decrease in nodule number compared to respective controls (Fig.3a & b).

Na^+ accumulation in shoot and salinity tolerance: In most plants, the accumulation of Na in shoot brings about deleterious effects and plant strategy is to limit the Na^+ build up in shoot tissues. In the case of groundnut, shoot Na^+ accumulation increased with salt concentration (Table 2a), but there was no significant correlation between the shoot Na^+ concentration and the ratio of biomass at 100 mM NaCl (Fig. 4a). On the contrary in pigeonpea, shoot Na concentration in leaves also increased with the increase of salt concentration but there was a negative and highly significant relationship (r=0.71, P>0.001) between the shoot Na^+ concentration and the ratio of shoot biomass at 100 mM NaCl, in Exp.1 (fig. 4b). Genotype ICPL 88039, which had the highest shoot biomass across salt treatments, showed the least Na^+ accumulation compare to other genotypes, which is likely to result from a dilution effect.

SPAD chlorophyll meter reading: Since we found a nodulation decrease with increased salt concentration in Exp. 1, which may affect the N status of plants, this created interest to measure the SCMR as an indirect measure of N status at different salt concentrations in Exp. 2. There was a significant and positive correlation between SCMR and the ratio of biomass in both crops. However, that relation was weak in the case of groundnut (r=0.41), and better in pigeonpea (r=0.62) (Fig. 5a & b).

We have shown that the 100-125 mM and 75 mM treatments of NaCl treatments were suitable to screen salinity tolerance in groundnut and pigeonpea respectively. Pigeonpea appeared a lot more sensitive to salinity than

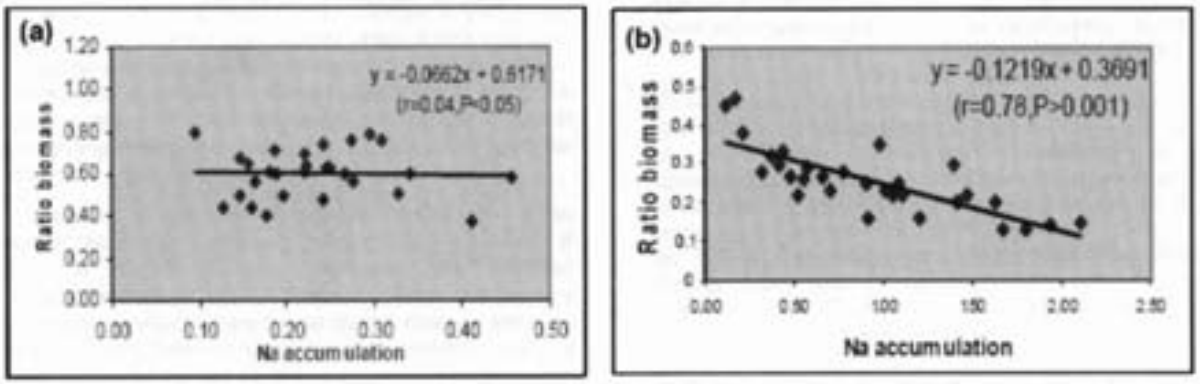

Figure 4. *Simple linear correlation between shoot Na+ accumulation and the ratio biomass in (a) groundnut and (b) pigeonpea.*

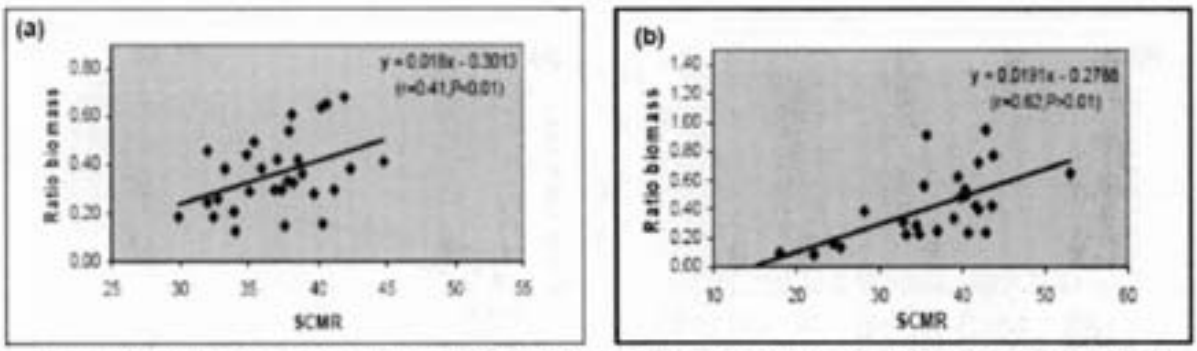

Figure 5. *Simple linear correlation between SCMR and the ratio biomass in (a) groundnut (SCMR are average value of measurements taken at 30,35 and 42 DAS) and (b) pigeonpea (SCMR data collected at 30 DAS)*

groundnut. The material screened in this study was very limited and spread across different botanical types in groundnut and across maturity groups in pigeonpea, but large differences could be shown for response to salinity stress at those treatments. These concentrations would therefore be suitable to identify a high level of tolerance from a large and diverse set of materials in both the crops. The response to salinity of some basic parameters like N status measured by nodulation and SCMR appeared well related to the degree of tolerance in both the crops. The accumulation of Na in shoot was also well related to the degree of tolerance in pigeonpea (Srivastava *et al.*, 2006) but not in groundnut, which is probably a novel report in the domain of salinity research. On-going work is in progress to screen large numbers of accessions in pigeonpea and groundnut using that protocol.

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