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Selection of Salt Tolerant Cowpea Genotypes Based on Salt Tolerant Indices of Morpho-biochemical Traits

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Abstract

Soil salinity is one of the abiotic factors affecting crop production worldwide. Introducing salt tolerant genotypes is considered as an economical strategy for increasing the production of agricultural crops in salt affected soils. In this study, morphological and biochemical changes of twenty three cowpea genotypes from India were evaluated for salt tolerance using seven days old seedlings treated with 0 and 75mM sodium chloride solutions. Chlorophyll, sugar, proline, soluble protein, sodium (Na^+), potassium (K^+), K^+/Na^+ ratio, shoot length, root length and fresh weight were determined and all observations were converted to salt tolerance indices (STI). Chlorophyll content, K^+/Na^+ ratio, and growth parameters decreased on exposure to salt stress; whereas sugar, proline and soluble protein contents increased. To improve the selection efficiency of salt tolerant genotypes, STI values of all parameters were subjected to hierarchical clustering and the 23 genotypes were grouped into three clusters. Nine salt tolerant genotypes (KBC2, IVT-VCP-09-013, VBN1, VBN2, CO(CP)7, VCP-09-001, DC15, PGCP5 and VCP-09-030) were identified by hierarchical clustering. Correlation studies showed that soluble protein content and K^+/Na^+ ratio positively correlated with all the growth parameters indicating their major contribution towards salt tolerance.

Keywords: Cowpea, salt tolerance, proline, K^+/Na^+ ratio, cluster analysis

Introduction

Cowpea (*Vigna unguiculata* (L) Walp), is an important pulse crop in semi-arid tropics covering Asia, Africa, southern Europe and Central and South America. It is grown throughout India for its long, green vegetable pods, grains, and foliage for fodder. The grain contains about 25% protein and 64% carbohydrate (1) and serves as a source of cheap protein for both rural and urban consumers. In India, it is grown in an area of about 3.9 million hectares with productivity of 567 kg per hectare (2). But, the average cowpea yield is very much less than the estimated potential yield. The major reasons for low yield are climatic, biotic, abiotic, and technological problems. Soil salinity is one of the most severe abiotic stresses affecting production of legumes worldwide (3). Cowpea is grouped as a moderately salt tolerant crop (4).

Salinity is one of the major hazards, usually confined to arid and semi-arid regions of the world (5). Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (6). Soil salinity is a serious threat worldwide for sustainable agricultural production (7, 8). In the Indian context, salt affected soils occupy about 6.73 million hectares affecting production and productivity across a number of states (9). In addition to this, use of poor quality irrigation water also causes salt stress to the agricultural crops (10). Though chemical amelioration can reduce productivity losses, the availability of soil amelioration chemicals is often limiting in interior villages and

also chemical amelioration is costly and thus out of reach for small and marginal farmers. Hence, introducing salt tolerant genotypes is considered as an eco-friendly, economical and socially acceptable feasible strategy for increasing the production in salt affected soils (11).

Salinity affects seed germination, plant growth, nutrient uptake, and metabolism owing to osmotic inhibition of water availability, ion imbalance, toxic effect of salt ions, and their effects on cellular gene expression machinery. Different plant species have developed different mechanisms to cope up with salinity stress effects. It is accepted that non-toxic compatible organic solutes accumulate in the cytoplasmic compartment of cells and inorganic ions toxic to metabolic process are restricted to the vacuoles. The progress in developing salt tolerant crop varieties has been very slow due to our incomplete knowledge of the mechanism of salt damage and the complex nature of salt tolerance. The differential response of plants to salt stress at different growth stages has added further problems in this direction (12, 13).

To improve the reliability and selection efficiency for salt tolerance, it is necessary to identify the salt induced characteristic changes in multiple traits among different genotypes. This study compared seedling growth parameters and biochemical traits, as well as their relationship with salt tolerance. The STI value was considered as the indicator for salt tolerance. Through comparing genetics and correlation of STI, we have obtained critical information about salt tolerance in different cowpea genotypes. Findings of this study will be useful for screening salt tolerant cowpea genotypes from germplasm and also provide information for breeding tolerant cultivars.

Materials and Methods

***Seed materials*:** Seeds of twenty three cowpea genotypes obtained from different places in India were used in this study. Twelve genotypes (CPD121, PGCP6, KBC5, CoVu702, PGCP5, GC3, NBC5, GC0817, PGCP12, DC15,GC521 and KBC2) were obtained from Central Arid Zone Research Institute, Jodhpur; three genotypes ACM002, CP16 and CO(CP)7 from Agricultural College and Research Institute, Madurai. The remaining eight genotypes (VBN1, VBN2, VCP-09-001, IVT-VCP-09-013, VCP-09-016, VCP-09-030, VCP-09-019 and VCP-09-035) were from National Pulses Research Centre, Vamban.

***Selection of salt concentration*:** Five cowpea genotypes (COCP7, CP16, CPD121, DC15, GC0817) were chosen on random basis and used for selection of salt concentration that will be ideal for carrying out further screening of salt tolerant genotypes. Germination was conducted by the standard roll towel method (14). Seeds were first surface sterilized in 70% ethanol for 2 minutes and rinsed thoroughly with sterile distilled water. Fifteen sterilized seeds were placed on a pre-soaked germination paper placed over a polythene sheet. The seeds were held in position by another pre-soaked germination paper strip and gently pressed. This was rolled separately for each treatment and kept vertically inside troughs containing 0 (distilled water), 50, 75, 100 and 125 mM NaCl solutions in germination room maintained at 28 ± 1°C and 80% of relative humidity. A completely randomized design with five cowpea genotypes subjected to five treatments and five replications was adopted. Shoot length and root length of five seedlings from each replication were measured on the 7th day after sowing. Based on this experiment, 75 mM NaCl concentration was selected for carrying out further experiments.

***Screening for salt tolerant genotypes*:** Seeds of twenty three different cowpea genotypes were first surface sterilized and allowed to germinate by roll towel method as described above and subjected to 0 mM (distilled water) and 75 mM NaCl solutions. The temperature was maintained at 28 ± 1°C with relative humidity of 80%. A completely randomized design with 23 cowpea genotypes subjected to two treatments and three replications was adopted. Seedlings were harvested on the 7th day after sowing and morphological and biochemical observations were recorded.

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***Morphological parameters*:** Morphological parameters such as shoot length, root length and fresh weight of the seedlings were measured and the mean value worked out.

***Mineral Constituents*:** Sodium (Na^+) and Potassium (K^+) contents were analysed in primary leaf alone to ensure measurements were focussed on the effects of these ions on the primary photosynthetic tissues. The leaves were harvested, dried at 65°C and the dry weights were determined. The dried plant samples were powdered. About 0.5g of the sample was wet digested with 10 ml of nitric acid: perchloric acid (4:1) mixture. After completion of digestion, the solution was transferred to a 25 ml volumetric flask and made up to the mark with deionized water. Na^+ and K^+ contents were determined by flame photometry (15). From this, K^+/Na^+ ratio was calculated.

Biochemical analysis: All biochemical analysis was conducted in fresh primary leaf samples. Photosynthetic pigments such as chlorophyll a, b and total chlorophyll were estimated by spectrophotometric method (16). About 0.2 g of leaf sample was extracted with 80% acetone and made up to 25 ml and read the absorbance at 645 and 663 nm in a spectrophotometer. Chlorophyll (Chl) contents were determined using the following equations where A_{645} and A_{663} are the absorbance at 645 and 663 nm respectively.

$$\text{Chl a (mg L}^{-1}) = (12.7 \times A_{663}) - (2.69 \times A_{645})$$
$$\text{Chl b (mg L}^{-1}) = (22.9 \times A_{645}) - (4.68 \times A_{663})$$
$$\text{Total Chl (mg L}^{-1}) = (20.2 \times A_{645}) + (8.02 \times A_{663})$$

Total soluble sugar was estimated by anthrone method (17) using glucose as standard. Weighed 0.2 g of the fresh leaf sample and extracted with 80% ethanol and the supernatant evaporated to dryness in a hot water bath at 80°C. The residue was dissolved and made up to 25 ml with distilled water. To 1.0 ml of the extract, 4 ml anthrone reagent (0.2% anthrone in 95% H_2SO_4) was added and cooled in ice, then kept in boiling water bath for 8 minutes and absorbance measured at 630 nm.

Proline was estimated by acid ninhydrin method using proline as standard (18). Fresh leaves (0.5 g) were extracted with 10 ml 3% sulphosalicyclic acid and filtered. To 2 ml of the filtrate, 2 ml of acid ninhydrin reagent (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml orthophosphoric acid) and 2 ml glacial acetic acid were added and kept in boiling water bath for 1 hour and cooled in ice bath. Toluene (4 ml) was added and mixed vigorously and the upper coloured toluene layer was separated and absorbance measured at 520 nm against toluene as blank using spectrophotometer.

Total soluble protein content was estimated by Lowry's method using bovine serum albumin as standard (19). Leaf sample (0.2 g) was homogenised with 0.1 M phosphate buffer (pH 7.0) and centrifuged. To 0.1 ml supernatant, 2.4 ml distilled water and 5 ml of alkaline copper reagent were added. Incubated at 37°C for 10 minutes and added 0.5 ml of Folin Ciocalteau reagent and again incubated at 37°C for 20 minutes. Absorbance was measured against reagent blank at 620nm.

***Data processing and statistical analysis*:** All observed data were converted to salt tolerance indices (STI). A salt tolerance index is defined as the observation at salinity divided by the average of the controls (20, 21). The data were analysed for significance by ANOVA using SPSS v.20 software. The STI values of the different traits were subjected to correlation analysis (Pearson) using SPSS v.20 software. After conversion of STI values into non-dimensional data matrices using the subordination method in fuzzy mathematics (22), hierarchical cluster analysis based on squared Euclidian distance and Ward's linkage was performed using SPSS v.20 software.

Results

Effect of different salt concentrations on growth: The preliminary study showed that increasing the salt concentration reduced both shoot and root length in cowpea seedlings as shown in figure-1. At 75 mM NaCl concentration,

CPD121 showed 50.83% reduction in shoot length as given in table-1. At 100 mM NaCl concentration, all genotypes except GC0857 showed more than 50% reduction in shoot length. All genotypes showed greater than 64.72% reduction in shoot length when exposed to 125 mM NaCl.

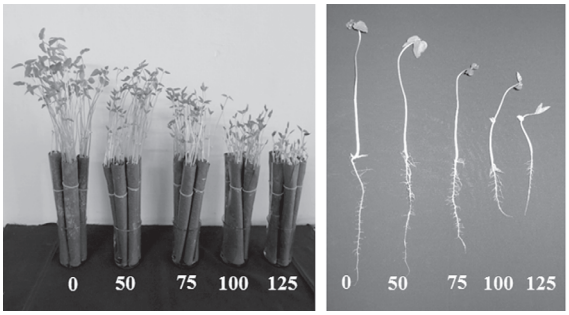

Fig. 1. Effect of salt stress on shoot and root growth. Reduction of shoot and root length observed in DC 15 cowpea seedlings subjected to 0, 50, 75, 100, and 125 mM NaCl solutions.

NOTE: Each value for shoot length represents five replications ± Standard deviation

Root length also decreased with increasing NaCl concentration (table-2). At 75 mM NaCl concentration, reduction in root length ranged between 18.31 and 48.94%. Root length reduced drastically above 100 mM NaCl concentration. All genotypes showed more than 56.47% reduction in root length when subjected to 100 mM NaCl concentration.

Salt tolerance indices for different traits: Biochemical and growth parameters were determined for 23 cowpea genotypes and the STI values were calculated. The relative STI for all 12 measured parameters varied among genotypes (table-3). Seedlings of VBN2 and GC0817 had significantly higher STI value for shoot length. VBN2 showed highest STI for root length, but was on par with that of VCP-09-035, PGCP5, DC15, VBN1 and VCP-09-030. Lowest STI for shoot length was observed in PGCP12. Genotypes CP16, PGCP12 and GC521 showed statistically lower STI for root length. Seedlings of PGCP6, PGCP5, NBC5, KBC2, VCP-09-030, VCP-09-035, VBN2, VCP-09-019, IVT-VCP-09-013, GC521 and VBN1 showed minimal reduction in fresh weight and STI values were high ranging between 0.94 and 0.82; whereas, fresh weight reduction was higher in CPD121, PGCP12 and ACM02 with lower STI values ranging between 0.59 and 0.69.

Salt stress caused significant reduction in chlorophyll contents which is reflected in the STI values (table-3); especially chlorophyll a decreased more obviously than chlorophyll b. KBC2 and CO(CP)7 had higher chlorophyll a and total chlorophyll contents; PGCP5 and VCP 09 030 had higher chlorophyll b contents. STI values for total soluble sugar varied between 1.69 and 1.03 among genotypes. However, the proline indices showed wide variation between genotypes and ranged between 2.46 and 1.06. Proline indices for VBN1 and DC15 were two

Table 1. Effect of salt stress on shoot length of cowpea genotypes

	Shoot length (cm)					Reduction (%)			
Genotype	Salinity levels (mM NaCl)								
	0	50	75	100	125	50	75	100	125
CO(CP)7	23.56±0.94	18.14±0.67	16.34±0.69	9.72±0.31	6.24±0.29	23.00	30.64	58.74	73.51
CP16	23.22±0.95	20.10±0.37	11.78±0.59	9.02±0.59	3.94±0.27	13.44	49.27	61.16	83.03
CPD121	20.50±0.81	14.14±0.48	10.08±0.48	7.14±0.58	4.86±0.13	31.02	50.83	65.17	76.29
DC15	24.24±0.77	18.72±0.67	16.64±0.59	10.88±0.78	6.88±0.30	22.77	31.35	55.12	71.62
GC0817	21.12±0.47	18.98±0.32	17.68±0.78	11.38±0.41	7.86±0.57	13.86	19.57	48.92	64.72

times higher than NBC5 and PGCP12. Total soluble protein indices also showed wide variation with values between 1.29 and 2.74. VBN1 had two times higher protein index than ACM02 and CP16.

Salinity affected Na^+ and K^+ contents of cowpea genotypes. Increase in Na^+ content was observed and STI values ranged between 1.59 and 3.45. CP16 accumulated more Na^+ and PGCP5 showed least accumulation. High K^+ content and K^+/Na^+ ratio was observed in CO(CP)7, DC15, VCP-09-030 and VCP-09-001 genotypes. PGCP5 and VCP-09-035 also showed higher K^+/Na^+ ratio. Lower values for K^+/Na^+ ratio was observed in CP16, CPD121 and PGCP12 genotypes. K^+ content decreased in three genotypes PGCP6, PGCP5 and PGCP12 under salt stress, whereas, increased in other genotypes. STI values for K^+ ranged between 0.92 and 1.61.

Cluster analysis

Hierarchical clustering using squared Euclidian distance and Ward's linkage formed three clusters (I, II and III) to characterize salt tolerance of cowpea genotypes at seedling stage (figure-2). Cluster I represented the salt sensitive genotypes (CPD121, ACM002, CP16 and PGCP12) which showed lower STI values for the different traits estimated. Moderately salt tolerant genotypes were grouped in cluster III and tolerant genotypes in II (KBC2, IVT-VCP-09-013, VBN1, VBN2, CO (CP)7, VCP-09-001, DC15, PGCP5 and VCP-09-030). Cluster means of STI values for all the traits were calculated and were represented graphically (figure-3). Generally tolerant genotypes in cluster II showed higher cluster mean values of STI for the morphological and biochemical parameters. It was observed that cluster means for proline and total soluble protein contents were comparatively high in

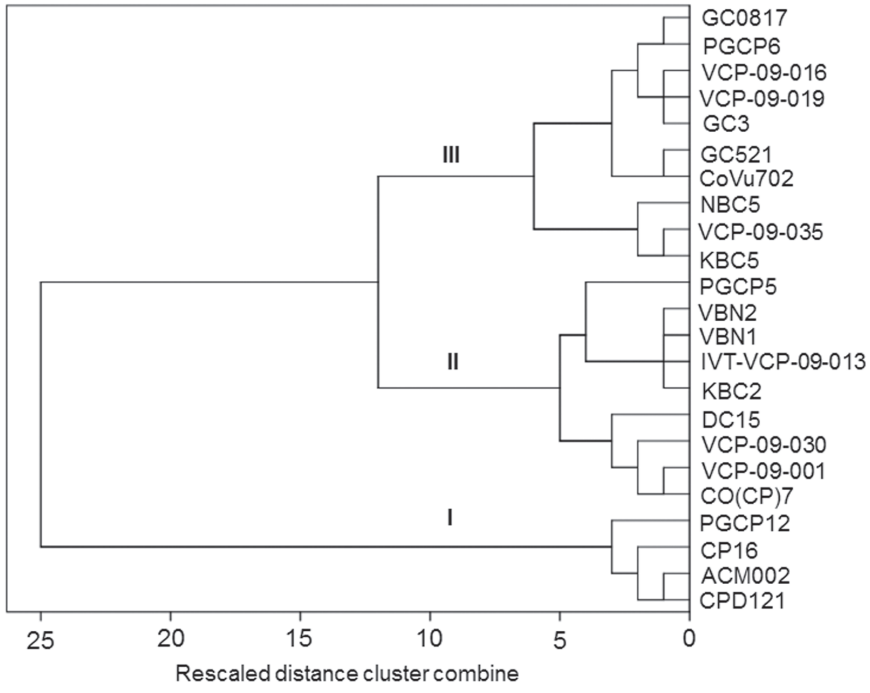

Fig. 2. Dendrogram of cowpea genotypes clustered for salt tolerance using Ward linkage. The 23 cowpea genotypes were grouped into three clusters. Cluster I represents the salt sensitive genotypes; cluster II, salt tolerant and cluster III, moderately salt tolerant.

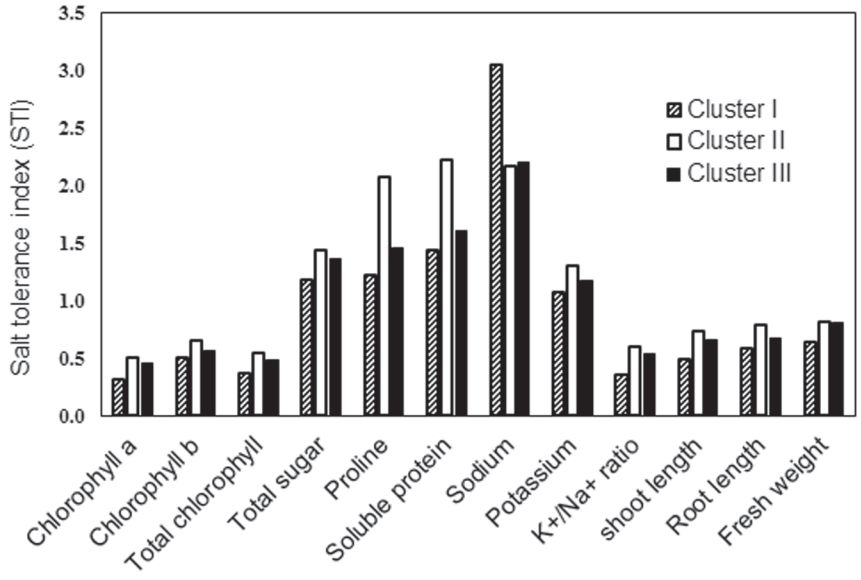

Fig. 3. Cluster means for the biochemical and growth traits. Proline and soluble protein contents are relatively higher in the salt tolerant cluster II, whereas salt sensitive cluster I has higher accumulation of sodium ions.

Table 2. Effect of salt stress on root length of cowpea genotypes

Genotype	Root length (cm)					Reduction (%)			
	Salinity levels (mM NaCl)								
	0	50	75	100	125	50	75	100	125
CO(CP)7	22.06±1.15	19.82±0.31	14.68±0.34	9.50±0.31	6.78±0.48	10.15	33.45	56.94	69.26
CP16	17.90±1.10	15.78±0.22	9.14±0.63	7.06±0.27	4.82±0.31	11.84	48.94	60.56	73.07
CPD121	22.08±0.36	16.92±0.71	15.06±0.84	5.90±0.46	1.80±0.42	23.37	31.79	73.28	91.85
DC15	16.82±0.57	15.10±0.27	13.74±0.23	6.90±0.51	6.26±0.15	10.23	18.31	58.98	62.78
GC0817	23.48±0.95	20.04±0.54	14.68±0.70	10.22±0.60	6.84±0.69	14.65	37.48	56.47	70.87

NOTE.- Each value for root length represents five replications ± Standard deviation

cluster II, whereas Na^+ accumulation was higher in cluster I.

Discussion

Reduction in growth: The present study showed that salinity reduced shoot length, root length and fresh weight of cowpea seedlings; salt-sensitive genotypes CPD121, CP16, PGCP12 and GC521 showed comparatively more reduction in growth parameters than tolerant genotypes. Previous studies also reported growth reduction during salt stress (23). This study showed that Na^+ content was negatively correlated with shoot and root length as well as fresh weight. Generally, growth reduction occurs due to salt-induced osmotic stress (24), hardening of the cell wall (25) and a decrease in conductance of the plasma membrane (26, 27). Growth reduction might be partly due to stomatal closure which limits CO_2 assimilation and

Table 3. Salt tolerance indices for individual traits in 23 cowpea genotypes

Genotype	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
CPD121	0.28	0.46	0.31	1.23	1.30	1.54	3.03	1.08	0.36	0.50	0.67	0.59
PGCP6	0.52	0.60	0.54	1.40	1.31	1.78	2.06	0.94	0.46	0.70	0.71	0.94
ACM002	0.28	0.43	0.31	1.19	1.18	1.29	3.22	1.31	0.41	0.57	0.69	0.67
KBC5	0.36	0.48	0.39	1.20	1.65	1.48	2.07	1.20	0.58	0.69	0.75	0.78
CoVu702	0.46	0.66	0.51	1.21	1.84	1.49	2.60	1.10	0.42	0.70	0.70	0.74
PGCP5	0.34	0.80	0.44	1.59	1.95	1.99	1.59	0.99	0.62	0.74	0.85	0.91
GC3	0.50	0.62	0.54	1.47	1.29	1.60	2.15	1.26	0.59	0.68	0.64	0.75
NBC5	0.38	0.51	0.42	1.03	1.10	1.74	2.05	1.17	0.57	0.61	0.60	0.88
GC0817	0.52	0.65	0.55	1.69	1.31	1.86	2.28	1.04	0.45	0.84	0.66	0.78
CP16	0.29	0.59	0.37	1.31	1.37	1.38	3.45	1.00	0.29	0.50	0.49	0.69
PGCP12	0.43	0.57	0.48	1.03	1.06	1.54	2.47	0.92	0.37	0.40	0.50	0.63
VBN1	0.55	0.62	0.57	1.44	2.46	2.74	1.89	1.07	0.57	0.71	0.84	0.82
VBN2	0.52	0.66	0.56	1.31	2.26	2.20	2.34	1.09	0.47	0.86	0.88	0.84
DC15	0.44	0.52	0.46	1.28	2.46	1.66	2.44	1.56	0.64	0.71	0.85	0.73
GC521	0.52	0.67	0.55	1.24	1.64	1.45	2.44	1.41	0.58	0.55	0.56	0.83
KBC2	0.63	0.67	0.64	1.42	1.99	2.40	2.18	1.16	0.53	0.73	0.73	0.87
CO(CP)7	0.58	0.67	0.60	1.51	1.81	2.58	2.28	1.61	0.70	0.71	0.68	0.73
VCP-09-001	0.54	0.55	0.55	1.62	1.99	2.12	2.30	1.45	0.63	0.65	0.69	0.79
IVT-VCP -09-013	0.54	0.72	0.58	1.27	2.06	2.33	2.32	1.32	0.57	0.68	0.73	0.83
VCP-09-016	0.50	0.54	0.51	1.63	1.79	1.52	2.00	1.03	0.52	0.55	0.66	0.77
VCP-09-030	0.44	0.75	0.52	1.55	1.72	1.96	2.17	1.50	0.69	0.79	0.83	0.85
VCP-09-019	0.42	0.55	0.45	1.58	1.48	1.68	2.22	1.25	0.56	0.64	0.68	0.84
VCP-09-035	0.41	0.46	0.42	1.27	1.27	1.53	2.15	1.40	0.65	0.70	0.85	0.85

NOTE.- X_1- Chlorophyll a, X_2- Chlorophyll b, X_3- Total chlorophyll, X_4- Total soluble sugar, X_5-Proline, X_6-Soluble protein, X_7-Sodium, X_8-Potassium, X_9-K+/Na+ ratio, X_{10}-Shoot length, X_{11}-Root length, X_{12}-Fresh weight.

reduced photosynthetic rate which in turn limited the supply of carbohydrate needed for growth (28, 29). Our study showed the reduction of chlorophyll pigments under salt stress which also contributed to less photosynthetic rate and hence growth reduction.

Contribution of biochemical traits to salt tolerance: Salt stress cause altered metabolism which leads to accumulation or depletion or changes in biochemical constituents. It was observed that salt tolerant genotypes had relatively higher chlorophyll a content (KBC2 and CO(CP)7) and chlorophyll b contents (PGCP5 and VCP-09-030) than salt sensitive genotypes. Correlation study showed positive correlation of chlorophyll a, b and total chlorophyll contents with shoot length and fresh weight. This showed that higher the chlorophyll content during salt stress, more will be its salt tolerant capacity. Decrease in chlorophyll content under salt stress had already been reported in different crops (30).

Accumulation of soluble sugars was observed in our study in all genotypes with varied concentration. Soluble sugars play a lead role in osmoprotection, carbon storage and radical scavenging indicating its partial role in salt

Table 4. Correlation of STI for 12 traits in cowpea genotypes

Trait	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
X_1	1											
X_2	.468**	1										
X_3	.973**	.654**	1									
X_4	.360**	.364**	.386**	1								
X_5	.443**	.374**	.467**	.252*	1							
X_6	.626**	.493**	.661**	.334**	.617**	1						
X_7	-.450**	-.354**	-.474**	-.333**	-.294*	-.438**	1					
X_8	.197	-.033	.146	.080	.247*	.143	.012	1				
X_9	.375**	.226	.368**	.305*	.367**	.389**	-.657**	.719**	1			
X_{10}	.402**	.407**	.442**	.401**	.476**	.497**	-.463**	.195	.444**	1		
X_{11}	.110	.146	.118	.226	.571**	.390**	-.407**	.240*	.460**	.670**	1	
X_{12}	.343**	.368**	.384**	.209	.215	.303*	-.595**	.002	.418**	.529**	.373**	1

NOTE.- X_1- Chlorophyll a, X_2- Chlorophyll b, X_3- Total chlorophyll, X_4- Total soluble sugar, X_5-Proline, X_6-Soluble protein, X_7-Sodium, X_8-Potassium, X_9-K+/Na+ ratio, X_{10}-Shoot length, X_{11}-Root length, X_{12}-Fresh weight. Correlation coefficient significant at **P < 0.01 and *P < 0.05

tolerance (12). Sugar content positively correlated with chlorophyll content indicating the role of photosynthesis in sugar accumulation.

The accumulation of nitrogen-containing compatible solutes including proline is known to function in osmotic adjustment, protection of cellular macromolecules from damage by salts, storage of nitrogen and scavenging of free radicals. The present study showed accumulation of proline in cowpea seedlings under salt stress. Tolerant genotypes were able to accumulate twice the amount of proline when subjected to salt stress. Proline accumulation was previously observed under salinity in many plants including maize (31), sorghum (32), green gram (33) and mulberry (34). Proline content showed positive correlation with both shoot and root length, but not with fresh weight. Thus, proline content showed more contribution towards salt tolerance than sugar content. Also the high cluster mean value of proline for the salt tolerant cluster confirms its contribution to salt tolerance.

Salt stressed cowpea seedlings expressed higher total soluble protein levels with variation among genotypes. Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is re-utilized when stress is over and may play a role in osmotic adjustment (12). Proteins may be synthesized by *denovo* in response to salt stress or may be present constitutively at low concentration and increase when plants are exposed to salt stress. Our study showed that salt tolerant genotypes had higher protein content than salt sensitive genotypes. The cluster mean value for salt tolerant cluster was markedly higher for protein. Also, protein content positively correlated with all growth parameters andour study proved its major contribution in overcoming salt stress.

Changes in mineral contents

In most of the genotypes Na^+ content increased to more than two times, but was much higher in sensitive genotypes. This study proves the deleterious effect of sodium accumulation. We observed decrease in K^+ content in 3

genotypes, whereas increased in other genotypes. It was observed that K^+/Na^+ ratio decreased under salt stress. Increase in K^+ content was observed in leaves of *Butea monosperma* seedlings exposed to salt stress (35). Several studies reported that salt tolerant plants showed higher K^+/Na^+ ratio compared to salt sensitive plants (36, 37). Our study also showed that some tolerant genotypes such as DC15, CO(CP)7 and VCP-09-030 and a moderately tolerant genotype VCP-09-035 had higher K^+/Na^+ ratio compared to sensitive genotypes under salt stress. Positive correlation of K^+/Na^+ ratio with all growth and biochemical parameters showed that K^+/Na^+ ratio had a major contribution towards salt tolerance.

Conclusion

Salt tolerance is a complex phenomenon contributed by multiple biological parameters in plants. The current study on salt tolerance and associated traits of cowpea seedlings suggested that soluble protein content and K^+/Na^+ ratio contributed more towards salt tolerance since these traits positively correlated with the growth parameters. Moreover this study enabled us to identify nine salt tolerant cowpea genotypes *viz.* KBC2, IVT-VCP-09-013, VBN1, VBN2, CO (CP) 7, VCP-09-001, DC15, PGCP5 and VCP-09-030. Detailed studies on gene expression patterns associated with the above traits will be useful to strengthen our knowledge on biochemical basics of the salt tolerance in cowpea.

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