

## Physio-morphological changes of cowpea (*Vigna unguiculata* Walp.) and jack bean (*Canavalia ensiformis* (L.) DC.) in responses to soil salinity

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### Abstract

Remediation of salt affected soil using organic matter (OM) chelating agent is an effective procedure with low cost, simple and sustainable techniques. We investigated the physio-biochemical responses of two legume species viz. cowpea (*Vigna unguiculata* Walp.) and jack bean (*Canavalia ensiformis* [L.] DC.) to salt stress with a view to select model plant species for salt-remediation. Seeds of jack bean and cowpea were germinated, planted in the pot culture then the various salt concentrations (0, 50, 100, 150 and 200 mM NaCl) were treated and physiological and morphological data were collected. Sodium ions (Na<sup>+</sup>), free proline and relative electrolyte leakage in salt stressed seedlings of two legumes were significantly increased in response to increase salt stress. In contrast, potassium ions (K<sup>+</sup>), water use efficiency (WUE), chlorophyll a and chlorophyll b pigments in cowpea were sharply dropped when subjected to 200 mM NaCl salt stress for 66.81%, 86.14%, 61.35% and 65.62%, respectively, indicating the physiological declines in the salt stressed plants. The K<sup>+</sup> reduction, WUE reduction and pigment degradation in salt stressed seedlings of jack bean were lower than those in cowpea, indicating as salt tolerant indices. Also, the maximum quantum yield (F<sub>v</sub>/F<sub>m</sub>) and photon yield of PSII (Φ<sub>PSII</sub>) were decreased for 26.02% and 30.18% in cowpea when seedlings exposed to 200 mM NaCl salt stress, leading to reduce net photosynthetic rate (P<sub>n</sub>). A positive relation between Φ<sub>PSII</sub> and P<sub>n</sub> was demonstrated in both jack bean and cowpea legumes. In addition, overall growth performances in salt stressed seedlings of two legumes were considerably declined. Jack bean was a candidate legume to play a role as green manure for saline soil remediation upon to salt tolerant ability.

**Keywords:** chlorophyll fluorescence; pigment; proline; relative electrolyte leakage; sodium ion; water use efficiency.

**Abbreviations:** Cl<sub>-</sub>chloride, C<sub>x+c</sub>-total carotenoids, Chl<sub>a</sub>-chlorophyll a, Chl<sub>b</sub>-chlorophyll b, CRD-Completely Randomized Design, DMRT-Duncan's New Multiple Range Test, DW-dry weight, F<sub>v</sub>/F<sub>m</sub>-maximum quantum yield of PSII, FW-fresh weight, K<sub>-</sub>potassium, LA-leaf area, Na<sub>-</sub>sodium, NPQ-non photochemical quenching, OM-organic matter, P<sub>n</sub>-net photosynthetic rate, RL-root length, SH-shoot height, TC-total chlorophyll, WUE-water use efficiency, Φ<sub>PSII</sub>-photon yield of PSII.

### Introduction

Soil salinity is one of the major abiotic factors affecting crop yield in arid and semiarid irrigated areas. Though the soil salinity existed long before humans and agriculture, but the problem has aggravated due to agricultural practices such as irrigation. It is estimated that nearly 20 % of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Qadir et al., 2008). Soil salinity is categorized by excessive levels of contaminated salts, especially sodium chloride (NaCl) as dominant salt (Tanji, 2002). It is a small molecule, producing sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) toxic ions to induce ionic and osmotic stresses, especially in glycophyte species (Mansour and Salama, 2004; Chinnusamy et al., 2005). Plants exposure to salt stress results in degradation of photosynthetic pigments, destruction of chloroplast, diminution of chlorophyll fluorescence and reduction in net photosynthetic rate (Yamane et al., 2008; Cha-um et al., 2009). Legumes are one of the most important foods in the tropical and sub-tropical regions in Asia, Africa, southern Europe and Central and South America. Cowpea (*Vigna unguiculata*) and jack bean (*Canavalia ensiformis*) are the two legumes that grow well in poor soils under wide

range of pH, water deficit, soil salinity, less organic matter and low levels of phosphorus (Andrade et al., 2005; Taffouo et al., 2009). Cowpea is the major food legumes that serves as protein source (>25% protein in seed), and cultivated on a total area of 14 million ha world over, and especially in West Africa (9 million ha) (Singh et al., 2003). Jack bean is a rapid growing legume in tropical areas and is used as green manure (cover cropping and intercropping), animal fodder and for remediating soil from heavy metals such as copper (Andrade et al., 2010), zinc (Andrade et al., 2009), cadmium (Andrade et al., 2005) and lead (Gabos et al., 2009; Pereira et al., 2010). In leguminous species, saline conditions may limit the nodulation symbiosis affecting survival and proliferation of *Rhizobium* species in the soil and rhizosphere, inhibiting the infection process, directly affecting root nodule function, and reducing plant growth, photosynthesis, and demand for nitrogen. Since soil salinity may directly affect either symbiont or affect their interaction, it is essential to identify the processes most sensitive to salinity (Uyanöz and Karaca, 2011; Venterino et al., 2012). Efforts then may be directed toward improving the tolerance of the most sensitive

**Table 1.** Sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>) ions and a ratio of Na:K in the leaf tissues of cowpea and jack bean subjected to 0, 50, 100, 150 and 200 mM NaCl for 2-weeks.

NaCl (mM)	Na (mg g <sup>-1</sup> dry weight)	K (mg g <sup>-1</sup> dry weight)	Na:K
<b>Cowpea</b>			
0	7.90e	23.50a	0.34d
50	13.70d	23.80a	0.58d
100	16.70c	17.90b	0.94c
150	20.20b	12.40c	1.63b
200	27.00a	7.80d	3.45a
<i>ANOVA</i>	**	**	**
<b>Jack bean</b>			
0	5.90e	25.60a	0.23e
50	10.80d	22.40b	0.48d
100	14.80c	21.70b	0.68c
150	17.40b	19.80c	0.88b
200	20.00a	17.90d	1.12a
<i>ANOVA</i>	**	**	**

Different letters in each column show significant difference at  $p \leq 0.01$  (\*\*) by Duncan's New Multiple Range Test (DMRT).

symbiont or the process of symbiosis (Zahran, 2001). Previous studies have demonstrated that soil physical, chemical and biological properties in salt-affected areas are strongly improved upon the application of green manure, leading to enhanced crop growth and development (Choudhary et al., 2004; Wong et al., 2009). It is therefore pertinent to evaluate legume species with potential to grow in the saline soil. In addition, there is no report to find-out the candidate plant species for green manure production in the saline soil area. The aim of the present study was to compare the physiol-morphological attributes changes in two model legumes cowpea and jack bean in response to NaCl stress to be played as candidate species for green manure production.

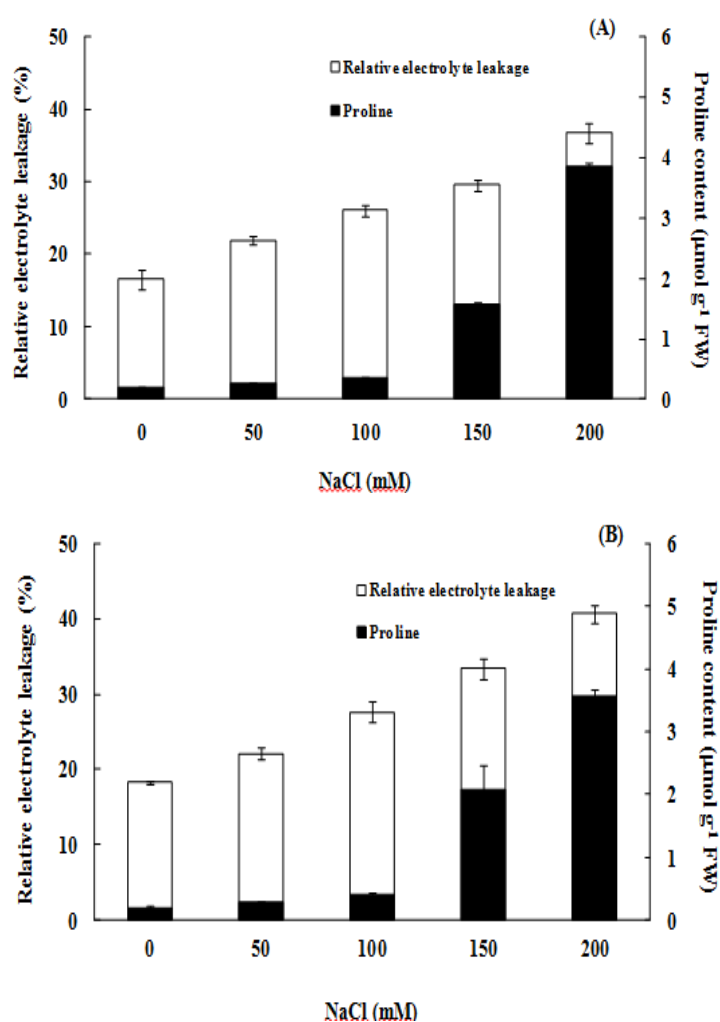
## Results and Discussion

### Sodium and potassium ions accumulation in the leaf tissues of legumes

Na<sup>+</sup> in the leaf tissues of two legumes was increased relating to a degree of NaCl in the soil solution, while K<sup>+</sup> was declined (Table 1). Under extreme salt stress (200 mM NaCl), Na<sup>+</sup> in cowpea was enriched to 27 mg g<sup>-1</sup> DW (3.42-folds) while K<sup>+</sup> was dropped to 7.8 mg g<sup>-1</sup> DW (67.00% reduction). In contrast, Na<sup>+</sup> in jack bean was reached to 20 mg g<sup>-1</sup> DW (3.39- folds) while K<sup>+</sup> was declined to 17.90 mg g<sup>-1</sup> DW (30.08% reduction) under 200 mM NaCl (Table 1). Further, the ratio of Na:K was significantly increased, especially at 150-200 mM NaCl, and the increase was rapid in cowpea seedlings than in jack bean seedlings (Table 1). The present observations are paralleled by earlier studies that Na<sup>+</sup> and proline are significantly increased under salt stress (10 dS m<sup>-1</sup>) in cowpea cultivars viz., Aksay-102, Gomti vu-89 and Pusa Falguni, whereas K<sup>+</sup> levels declined sharply (Patel et al., 2010). Na<sup>+</sup> accumulation is greater in salt sensitive cultivar "Pérola" than in salt tolerant "Pitiúba" cultivar of cowpea when subjected to 100 mM NaCl for 8 days (Maia et al., 2010a). Further, the decline in K<sup>+</sup> levels was greater in Pitiúba than in Pérola (Maia et al., 2010b).

### Proline content and relative electrolyte leakage in salt-stressed legumes

Both the legume species accumulated significant amount of free proline when exposed to 150 and 200 mM NaCl (Fig 1). Likewise, REL was increased in both the legumes depending



**Fig 1.** (A) Relative electrolyte leakage and proline content of cowpea and (B) relative electrolyte leakage and proline content of jack bean subjected to 0, 50, 100, 150 and 200 mM NaCl for two-weeks.

**Table 2.** Chlorophyll a (Chl<sub>a</sub>), chlorophyll b (Chl<sub>b</sub>), total chlorophyll (TC) and total carotenoids (C<sub>x+c</sub>) content in the leaf tissues of cowpea and jack bean subjected to 0, 50, 100, 150 and 200 mM NaCl for 2-weeks.

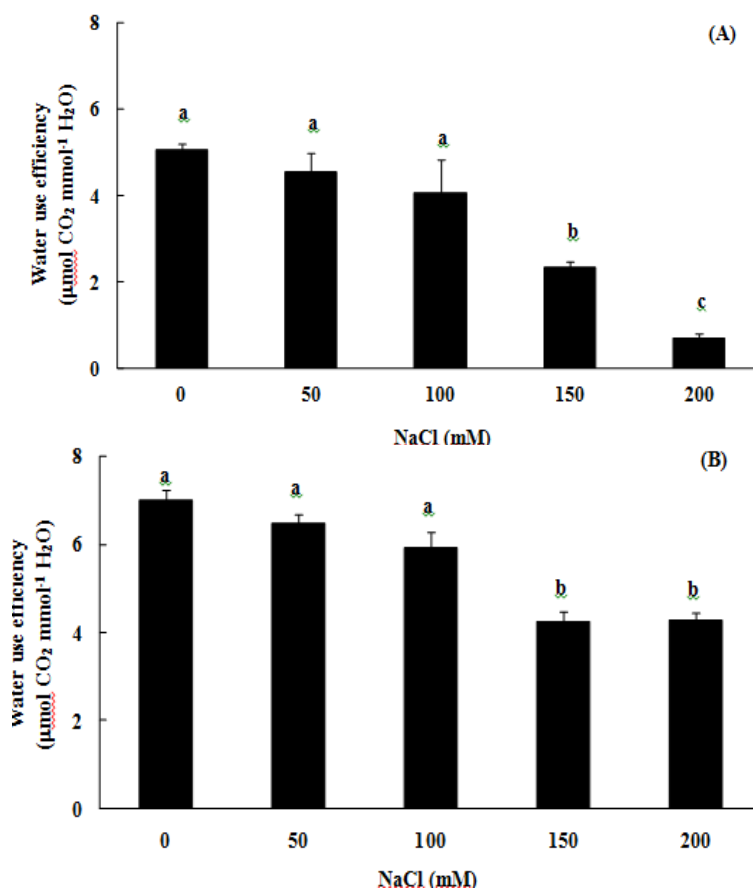
NaCl (mM)	Chl <sub>a</sub> (µg g <sup>-1</sup> FW)	Chl <sub>b</sub> (µg g <sup>-1</sup> FW)	TC (µg g <sup>-1</sup> FW)	C <sub>x+c</sub> (µg g <sup>-1</sup> FW)
<b>Cowpea</b>				
0	82.80a	60.50a	143.30a	18.00a
50	58.80b	57.70a	116.50b	17.20a
100	53.80b	44.20b	98.00b	17.00a
150	45.20c	30.20c	75.40c	15.50b
200	32.00d	20.80c	52.80d	12.50c
ANOVA	**	**	**	**
<b>Jack bean</b>				
0	85.30a	63.80a	149.10a	20.00a
50	74.30b	60.50b	134.80b	19.00b
100	68.80bc	59.00bc	127.80bc	18.00c
150	63.30c	57.30c	120.60c	17.50cd
200	51.50d	54.50d	106.00	17.00d
ANOVA	**	**	**	**

Different letters in each column show significant difference at  $p \leq 0.01$  (\*\*) by Duncan's New Multiple Range Test (DMRT).

on the salt concentration in the soil solution (Fig. 1). In contrast, WUE declined in both the legume species, particularly at  $\geq 150$  mM NaCl; however, WUE was maintained at a higher level in jack bean than in cowpea (Fig. 2). It suggested that in cowpea WUE was a sensitive parameter when plants were subjected to 200 mM NaCl (Fig. 2). A positive relationship was observed between REL and Chl<sub>a</sub> degradation in cowpea (Fig 3A) and jack bean (Fig. 3B). Enhanced REL and lipid peroxidation have been demonstrated as the symptoms of Na<sup>+</sup> toxicity in cowpea (Maia et al., 2010a; Maia et al., 2010b; Cavalcanti et al., 2007). Previous studies have documented that proline (Patel et al., 2010; Taffouo et al., 2009) and sugar levels (Praxedes et al., 2011) are increased in salt stressed legumes and play a key role as osmolytes to maintain the water relation. Proline is a good indicator to identify the salt tolerant abilities in leguminous species. For example, proline content in salt tolerant legumes, *Mucuna poggei*, *Vigna unguiculata* and *Phaseolus adenanthus*, was significantly enhanced relating to a degree of NaCl concentration, while it declined in salt-sensitive *Glycine max* and *P. vulgaris* (Taffouo et al., 2009). Leaf relative water content (RWC) in salt tolerant cowpea (cv. Pitiúba) was maintained better than in salt susceptible cowpea (cv. Pérola) (Maia et al., 2010b).

#### Photosynthetic abilities in salt stressed legumes

Amount of photosynthetic pigments, chlorophyll a (Chl<sub>a</sub>), chlorophyll b (Chl<sub>b</sub>), total chlorophyll (TC) and total carotenoids (C<sub>x+c</sub>), declined in the leaf tissues of both cowpea and jack bean, especially at  $\geq 150$  mM NaCl (Table 2). However, the level of pigment degradation was lesser in jack bean than in cowpea. In cowpea, degradation percentage of Chl<sub>a</sub>, Chl<sub>b</sub> and TC in the plants subjected to 150 mM NaCl was 45.40%, 50.10% and 47.40%, respectively, whereas in jack bean it was 25.80%, 10.20% and 19.10%, respectively (Table 1). Under extreme salt stress (200 mM NaCl), C<sub>x+c</sub> in cowpea was significantly declined (by 30.60%) compared to that in jack bean (only 15.00%) (Table 2). A positive relation was observed between Chl<sub>a</sub> and maximum quantum yield of PSII ( $F_v/F_m$ ) (Fig. 3C-D) and TC content and photon yield of PSII ( $\Phi_{PSII}$ ) in both cowpea and jack bean (Fig. 4A-B) under salt stress. Maximum quantum yield of PSII ( $F_v/F_m$ ) and photon yield of PSII ( $\Phi_{PSII}$ ) in two legume plants was significantly diminished when subjected to 100 mM NaCl (Table 3). In contrast, non-photochemical quenching (NPQ) in salt stressed plants was exhibited (Table 3). A positive



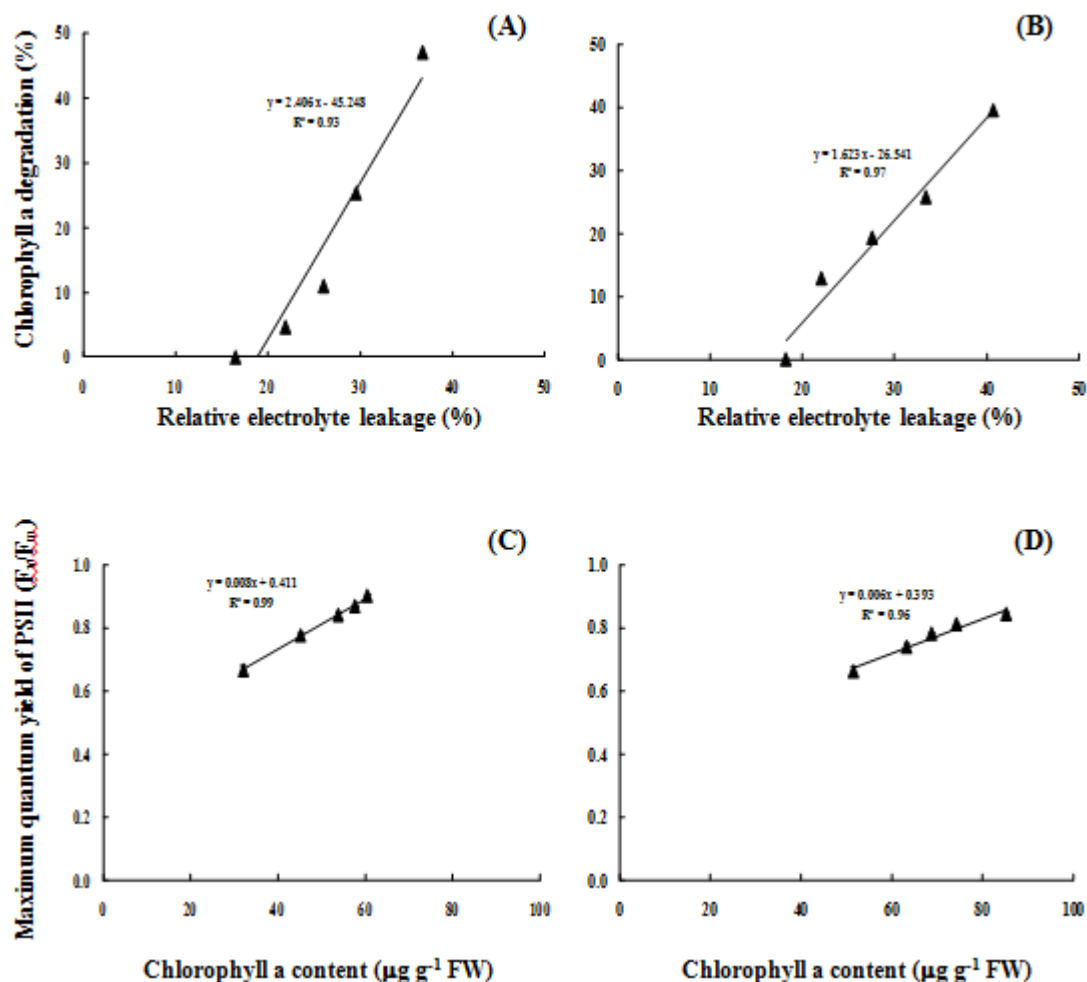
**Fig 2.** (A) Water use efficiency of cowpea and (B) water use efficiency of jack bean subjected to 0, 50, 100, 150 and 200 mM NaCl for two-weeks.

correlation between  $\Phi_{PSII}$  and net photosynthetic rate ( $P_n$ ) was observed in cowpea (Fig. 4C) and jack bean (Fig. 4D). Under 100 mM NaCl, the  $P_n$  in cowpea and jack bean was reduced by 91.00% and 70.90%, respectively (Table 3), whereas nearly 92.00% reduction was observed in  $P_n$  in both legumes at  $\geq 150$  mM NaCl. Earlier, a parallel reduction in photosynthetic pigments has been observed in castor bean (200–300 mM NaCl; Li et al., 2010) and green bean (50–100 mM NaCl; Yasar et al., 2008). However, our results are in sharp contrast to an earlier study of Lacerda et al., 2006) who

**Table 3.** Maximum quantum yield of PSII ( $F_v/F_m$ ), photon yield of PSII ( $\Phi_{PSII}$ ), non-photochemical quenching (NPQ) and net photosynthetic rate ( $P_n$ ) in the leaf tissues of cowpea and jack bean subjected to 0, 50, 100, 150 and 200 mM NaCl for two-weeks.

NaCl (mM)	$F_v/F_m$	$\Phi_{PSII}$	NPQ	$P_n$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
Cowpea				
0	0.90a	0.82a	0.03c	14.50a
50	0.87ab	0.77ab	0.05c	5.20b
100	0.84b	0.74bc	0.06c	1.30c
150	0.78c	0.70c	0.10b	0.50c
200	0.67d	0.57d	0.19a	0.10c
ANOVA	**	**	**	**
Jack bean				
0	0.85a	0.78a	0.03b	7.60a
50	0.81ab	0.76ab	0.05b	4.08b
100	0.78b	0.72b	0.07b	2.21c
150	0.74c	0.65c	0.13ab	0.60d
200	0.66d	0.56d	0.59a	0.30d
ANOVA	**	**	**	**

Different letters in each column show significant difference at  $p \leq 0.01$  (\*\*) by Duncan's New Multiple Range Test (DMRT).

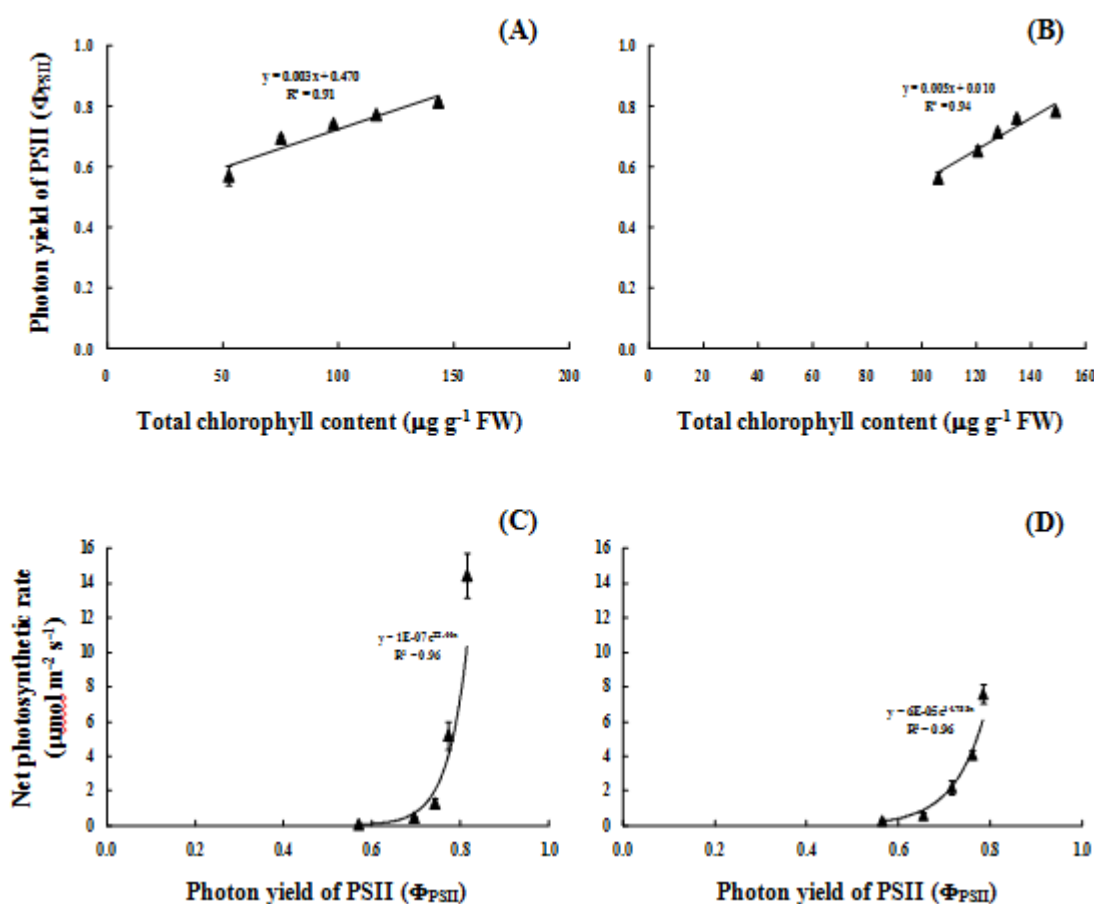


**Fig 3.** (A) Relationships between relative electrolyte leakage and chlorophyll a degradation in cowpea (B) relationships between relative electrolyte leakage and chlorophyll a degradation in jack bean, (C) relationships between chlorophyll a content and maximum quantum yield of PSII in cowpea and (D) relationships between chlorophyll a content and maximum quantum yield of PSII in jack bean (D) subjected to salt stress for two-weeks.

**Table 4.** Shoot height (SH), root length (RL), fresh weight (FW), dry weight (DW) and leaf area (LA) of cowpea and jack bean subjected to 0, 50, 100, 150 and 200 mM NaCl for two-weeks.

NaCl (mM)	SH (cm)	RL (cm)	FW (g plant <sup>-1</sup> )	DW (g plant <sup>-1</sup> )	LA (cm <sup>2</sup> plant <sup>-1</sup> )
Cowpea					
0	25.80a	13.60a	8.38a	0.78a	163.50a
50	23.70b	11.90b	6.31b	0.59b	116.00b
100	21.80c	10.80bc	4.43c	0.38c	61.10c
150	19.80d	9.80c	3.67cd	0.31c	37.80cd
200	16.20e	7.20d	2.64d	0.20d	21.20d
ANOVA	**	**	**	**	**
Jack bean					
0	29.50a	33.70a	21.50a	2.80a	424.00a
50	27.50a	27.00b	16.30b	2.50a	342.00a
100	24.40b	24.90bc	13.10c	1.80b	219.00b
150	21.70c	21.70cd	10.90c	1.30c	107.00c
200	17.50d	17.70d	6.20d	0.80d	25.00c
ANOVA	**	**	**	**	**

Different letters in each column show significant difference at  $p \leq 0.01$  (\*\*) by Duncan's New Multiple Range Test (DMRT).



**Fig 4.** (A) Relationships between total chlorophyll content and photon yield of PSII in cowpea (B) Relationships between total chlorophyll content and photon yield of PSII in jack bean, (C) photon yield of PSII and net photosynthetic rate in cowpea and (D) photon yield of PSII and net photosynthetic rate in jack bean subjected to salt stress for two-weeks.

recorded an increase in chlorophyll concentration in cowpea leaves after 24-days under 75 mM NaCl salt-stress. Our findings are supported by earlier studies reporting a decline in  $F_v/F_m$  and  $\Phi_{\text{PSII}}$  and increase in NPQ in cow pea under salt stress (200-300 mM NaCl (Li et al., 2010). Low NPQ representing  $C_{x+c}$  concentrations has been suggested to play a key role in plant protection against photo-oxidative damage under salt stress (Müller et al., 2001; Omasa and Takayama, 2003). The reduction in photosynthetic pigments and

diminishing of chlorophyll fluorescence has a direct effect on net photosynthetic rate ( $P_n$ ). Earlier, Wilson et al. (2006a) reported a sharp reduction in  $P_n$  in salt stressed vegetative and reproductive plants of cowpea genotypes.

#### Growth attributes in salt stressed legumes

Growth performance of both the legumes was declined in response to NaCl stress; however, the effect was greater in

cowpea than in jack bean. In cowpea, shoot height (SH), root length (RL), fresh weight (FW), dry weight (DW) and leaf area (LA) significantly decreased by 8.10%, 12.50%, 24.70%, 24.40% and 29.10%, respectively, when exposed to 50 mM NaCl (Table 4). In contrast, in jack bean the SH, DW and LA in jack bean declined by 17.30%, 35.70% and 48.40%, respectively, at 100 mM NaCl. Our results are in agreement with earlier studies reporting growth reduction in salt stressed legume plants, particularly cowpea (Patel et al., 2010, Cavalcanti et al., 2007, Wilson et al., 2006b, Maia et al., 2010a). Previous studies have reported a reduction in plant height and plant dry weight (Patel et al., 2010), leaf dry mass and root dry mass (Cavalcanti et al., 2007), leaf relative growth rate, leaf dry weight and leaf area (Wilson et al., 2006b; Maia et al., 2010a) of cowpea under salt stress.

## Materials and Methods

### Plant materials

Seeds of cowpea (*Vigna unguiculata* Walp.) and jack bean (*Canavalia ensiformis* (L.) DC.) were purchased / procured locally from the market. These were surface-sterilized with NaOCl followed by washing with distilled water three times. Seed were germinated on the plastic tray and one hundred seedlings (7-day-old) of both cowpea and jack bean were transplanted to earthenware pots ( $\phi = 15$  cm; height 30 cm) containing 2 kg of clay soil (EC = 2.687 dS m<sup>-1</sup>; pH = 5.5; organic matter = 10.36%; total nitrogen = 0.17%; total phosphorus = 0.07%; total potassium = 1.19%) in the greenhouse. After two weeks, the plants were given different treatments of NaCl, 0 mM (Control), 50, 100, 150 and 200 mM NaCl using salt solution. Two weeks after treatment, sodium and potassium ions, proline content, relative electrolyte leakage, water use efficiency, photosynthetic pigments, photosynthetic abilities and growth characters were analyzed.

### Sodium, potassium ions and proline contents

One hundred milligram of leaf tissue was ground in liquid nitrogen. Na<sup>+</sup> and K<sup>+</sup> in plant materials were extracted by acidic methods (HNO<sub>3</sub> and HClO<sub>4</sub>) and assayed according to Dionisio-Sese and Tobita (1998) using an Atomic absorption Spectrophotometer (AA, Model M16, Thermo Elemental, MA, USA). Proline in the leaf tissues was extracted and analyzed according to the method of Bates et al. (1973). Fresh material (50 mg) was ground with liquid nitrogen in a mortar. The homogenate powder was mixed with 1 mL aqueous sulfosalicylic acid (3%, w/v) and filtered through filter paper (Whatman #1, England). The extracted solution was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M H<sub>3</sub>PO<sub>4</sub>), and incubated at 95°C for 1 h. The reaction was stopped by placing the container in an ice bath. Then, the reaction mixture was mixed vigorously with 2 mL toluene. After cooling to 25°C, the chromophore was measured by UV-visible spectrophotometer (model DR/4000, HATCH, Loveland, Colorado, USA) at 520 nm using L-proline as a standard.

### Relative electrolyte leakage (REL)

REL was determined according to the method of Dionisio-Sese and Tobita (1998). Leaf tissue (100 mg) was collected, cut into a small size (~0.5 cm in diameter), then incubated in distilled water at 25°C for 2 h in test tubes and initial

conductivity (EC<sub>1</sub>) measurement of the solution was measured. The tubes were then boiled for 30 min to release all the electrolytes. These were cooled to 25°C and the conductivity (EC<sub>2</sub>) was measured. The electrolyte leakage was calculated as follows: REL (%) = (EC<sub>1</sub>/EC<sub>2</sub>) × 100.

### Photosynthetic abilities

Net photosynthetic rate (P<sub>n</sub>; μmol m<sup>-2</sup>s<sup>-1</sup>) and transpiration rate (E; mmol m<sup>-2</sup>s<sup>-1</sup>) were measured using a Portable Photosynthesis System fitted with an Infra-red Gas Analyzer (Model LI 6400, LI-CORR Inc., Lincoln, Nebraska, USA). The E was measured continuously by monitoring H<sub>2</sub>O of the air entering and existing in the IRGA headspace chamber. Water use efficiency (WUE) was calculated as the ratio of P<sub>n</sub> to E according to Cha-um et al. (2007). Chlorophyll a (Chl<sub>a</sub>), chlorophyll b (Chl<sub>b</sub>), and total chlorophyll (TC) concentrations were determined following the method of Shabala et al. (1998), and total carotenoid (C<sub>x+c</sub>) concentrations were determined following the method of Lichtenthaler (1987). One hundred milligrams of flag leaf material was placed in a 25 mL glass vial, along with 10 mL of 95.5% acetone, and blended using a homogeniser. The glass vials were sealed with Parafilm™ to prevent evaporation and then stored at 4°C for 48 h. The extract was centrifuged at 14,000×g for 5 min and the absorbance was read at 662 nm and 644 nm for Chl<sub>a</sub> and Chl<sub>b</sub> and at 470 nm for C<sub>x+c</sub> using a UV visible spectrophotometer against a blank of 95.5% acetone. Chlorophyll fluorescence emission from the adaxial surface of the leaf was measured using a fluorescence monitoring system (model FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode, as previously described by Loggini et al. (1999). A leaf, adapted to dark conditions for 30 min using leaf-clips, was initially exposed to the modulated measuring beam of far-red light (LED source with typical peak at wavelength 735nm). Original (F<sub>0</sub>) and maximum (F<sub>m</sub>) fluorescence yields were measured under weak modulated red light (<0.5 μmol m<sup>-2</sup> s<sup>-1</sup>) with 1.6 s pulse of saturating light (>6.8 μmol m<sup>-2</sup> s<sup>-1</sup> PAR) and calculated using FMS software for Windows®. The variable fluorescence yield (F<sub>v</sub>) was calculated by the equation F<sub>v</sub> = F<sub>m</sub> - F<sub>0</sub>. The ratio of variable to maximum fluorescence (F<sub>v</sub>/F<sub>m</sub>) was calculated as maximum quantum yield of PSII photochemistry. The photon yield of PSII (Φ<sub>PSII</sub>) in the light was calculated by Φ<sub>PSII</sub> = (F<sub>m</sub>' - F)/F<sub>m</sub>' after 45s of illumination, when steady state was achieved. In addition, non-photochemical quenching (NPQ) was calculated as described by Maxwell and Johnson (2000).

### Growth performances

Shoot height (SH), root length (RL), fresh weight (FW), dry weight (DW) and leaf area (LA) of legume seedlings were measured. Legume seedlings were dried at 80°C in a hot-air oven for 2 days, and then incubated in desiccators before the measurement of dry weight. The leaf area of legume seedlings was measured using a Leaf Area Meter DT-scan (Delta-T Scan®, Delta-T Devices Ltd., Cambridge, UK).

### Experimental design

The experiment was arranged as Completely Randomized Design (CRD) with eight replicates (n = 8). The mean values obtained were compared by Duncan's New Multiple Range Test (DMRT) and analyzed at p ≤ 0.01 using SPSS software ver. 11.5.

## Conclusion

Our study demonstrates the two legumes grown under salt stress,  $\text{Na}^+$ , free proline and REL were enhanced, whereas  $\text{K}^+$  and WUE declined. Among the two legumes tested the cowpea was more sensitive to salt stress whilst jack bean was comparatively tolerant. The study suggests the use of jack bean as a novel plant species for remediating salt-affected soils.

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## References

- Andrade SAL, Gratão PL, Schiavinato MA, Silveira APD, Azevedo RA, Mazzafera P (2009) Zn uptake, physiological response and stress attenuation in mycorrhizal jack bean growing in soil with increasing Zn concentrations. *Chemosphere*. 75:1363–1370
- Andrade SAL, Gratão PL, Azevedo RA, Silveira APD, Schiavinato MA, Mazzafera P (2010) Biochemical and physiological changes in jack bean under mycorrhizal symbiosis growing in soil with increasing Cu concentrations. *Environ Exp Bot*. 68:198–207
- Andrade SAL, Jorge RA, Silveira APD (2005) Cadmium effect on the association of jack bean (*Canavalia eniformis*) and arbuscular mycorrhizal fungi. *Sci Agric* 62:389–394
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil*. 39:205–207
- Cavalcanti FR, Lima JPMS, Ferreira-Silva SL, Viégas RA, Silveira JAG (2007) Roots and leaves display contrasting oxidative response during salt stress and recovery in cowpea. *J Plant Physiol*. 164:591–600
- Cha-um S, Charoenpanich A, Roytrakul S, Kirdmanee C (2009) Sugar accumulation, photosynthesis and growth of two indica rice varieties in response to salt stress. *Acta Physiol Plant*. 31:477–486
- Cha-um S, Supaibulwatana K, Kirdmanee C (2007) Glycinebetaine accumulation, physiological characterizations, and growth efficiency in salt tolerant and salt sensitive lines of indica rice (*Oryza sativa* L. spp. *indica*) response to salt stress. *J Agron Crop Sci*. 193:157–166
- Chinnusamy V, Jagendorf A, Zhu JK (2005) Understanding and improving salt tolerance in plants. *Crop Sci*. 45:437–448
- Choudhary OP, Josan AS, Bajwa MS, Kapur L (2004) Effect of sustained sodic and saline-sodic irrigation and application of gypsum and farmyard manure on yield and quality of sugarcane under semi-arid conditions. *Field Crops Res*. 87:103–116
- Dionisio-Sese ML, Tobita S (1998) Antioxidant responses of rice seedlings to salinity stress. *Plant Sci*. 135:1–9
- Gabos MB, de Abreu CA, Coscione AR (2009) EDTA assisted phytoremediation of a Pb contaminated soil: Metal leaching and uptake by jack beans. *Sci Agric* 66:506–514
- Lacerda CF, Júnior JOA, Filho LCAL, Oliveira TS, Guimarães FVA, Gomes-Filho E, Prisco JT, Bezerra MA (2006) Morpho-physiological responses of cowpea leaves to salt stress. *Brazilian J Plant Physiol*. 18:455–465
- Li G, Wan S, Zhou J, Yang Z, Qin P (2010) Leaf chlorophyll fluorescence, hyperspectral reflectance, pigment content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. *Ind Crop Prod* 31:13–19
- Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol*. 148:350–380
- Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999) Antioxidant defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiol*. 119:1091–1099
- Maia JM, de Macedo CEC, Voigt EL, Freitas JBS, Silveira JAG (2010a) Antioxidative enzymatic protection in leaves of two contrasting cowpea cultivars under salinity. *Biol Plant*. 54:159–163
- Maia JM, Voigt EL, Macédo CEC, Ferreira-Silva SL, Silveira JAG (2010b) Salt-induced changes in antioxidative enzyme activities in root tissues do not account for the differential salt tolerance of two cowpea cultivars. *Brazilian J Plant Physiol*. 22:113–122
- Mansour MMF, Salama KHA (2004) Cellular basis of salinity tolerance in plants. *Environ Exp Bot* 52:113–122
- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. *J Exp Bot*. 51:659–668
- Müller P, Li XP, Niyogi KK (2001) Non-photochemical quenching. A response to excess light energy. *Plant Physiol*. 125:1558–1566
- Omasa K, Takayama K (2003) Simultaneous measurement of stomatal conductance, non-photochemical quenching, and photochemical yield of photosystem II in intact leaves by thermal and chlorophyll fluorescence imaging. *Plant Cell Physiol*. 44:1290–1300
- Patel PR, Kajal SS, Patel VR, Khristi SM (2010) Impact of salt stress on nutrient uptake and growth of cowpea. *Brazilian J Plant Physiol*. 22:43–48
- Pereira BFF, de Abreu CA, Herpin U, de Abreu MF, Berton RS (2010) Phytoremediation of lead by jack beans on Rhodic Hapludox amended with EDTA. *Sci Agric*. 67:308–318
- Praxedes SC, de Lacerda CF, Ferreira TM, Prisco JT, da Matta FM, Gomes-Filho E (2011) Salt tolerance is unrelated to carbohydrate metabolism in cowpea cultivars. *Acta Physiol Plant*. 33:887–896
- Qadir M, Tubeileh A, Akhtar J, Larbi A, Minhas PS, Khan MA (2008) Productivity enhancement of salt-affected environments through crop diversification. *Land Degrad Develop* 19:429–453
- Shabala SN, Shabala SI, Martynenko AI, Babourina O, Newman IA (1998) Salinity effect on bioelectric activity, growth,  $\text{Na}^+$  accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. *Aust J Plant Physiol*. 25:609–616
- Singh BB, Hartmann P, Fatokun C, Tamo M, Tarawari SA, Ortiz R (2003) Recent progress in cowpea improvement. *Chron Hort*. 43:8–12
- Taffouo VD, Meguekam L, Kenne M, Magnitsop A, Akoa A, Ourry A (2009) Salt stress effects on germination, plant growth and accumulation of metabolites in five leguminous plants. *Afri Crop Sci Conf Proc*. 9:157–161
- Tanji KK (2002) Salinity in the soil environment, pp 21–51, In: A. Läuchli and U. Lüttge, eds., *Salinity environment-plant-molecules*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

- Uyanöz R, Karaca Ü (2011) Effects of different salt concentrations and *Rhizobium* inoculation (native and *Rhizobium tropici* CIAT899) on growth of dry bean (*Phaseolus vulgaris* L.). *Europ J Soil Biol.* 47:387–391
- Ventorino V, Caputo R, de Pascale S, Fagnano M, Pepe O, Moschetti G (2012) Response to salinity stress of *Rhizobium leguminosarum* bv. *viciae* strains in the presence of different legume host plants. *Ann Microbiol.* 62:811–823
- Wilson C, Liu X, Lesch SM, Suarez DL (2006a) Growth response of major USA cowpea cultivars II. Effect of salinity on leaf gas exchange. *Plant Sci.* 170:1095–1101
- Wilson C, Liu X, Lesch SM, Suarez DL (2006b) Growth response of major USA cowpea cultivars I. Biomass accumulation and salt tolerance. *HortSci.* 41:225–230
- Wong VNL, Dalal RC, Greene RSB (2009) Carbon dynamics of sodic and saline soil following gypsum and organic material additions: A laboratory incubation. *Appl Soil Ecol.* 41:29–40
- Yamane K, Kawasaki M, Taniguchi M, Miyake H (2008) Correlation between chloroplast ultrastructure and chlorophyll fluorescence characteristics in the leaves of rice (*Oryza sativa* L.) grown under salinity. *Plant Prod Sci.* 11:139–145
- Yassar F, Ellialtioglu S, Yildiz K (2008) Effect of salt stress on antioxidant defense systems, lipid peroxidation, and chlorophyll content in green bean. *Russ J Plant Physiol.* 55:782–786
- Zahran HH (2001) Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. *J Biotechnol.* 91:143–153