

Full Length Research Paper

Effect of different levels of NaCl and Na₂SO₄ salinity on dry matter and ionic contents of cowpea (*Vigna unguiculata* L. Walp.)

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Salinity is an environmental stress that limits growth and development in plants. The present study was to assess the effect of salinity on dry matter and ionic contents in cowpea accessions. A pot experiment was carried out to investigate the effect of different levels of salinity on growth and ionic contents of cowpea plant. There were three levels of NaCl and Na₂SO₄ salinity which includes 0 (control), 50, 100 and 150 mM. Growth characters such as root and shoot dry weights decreased with increase in salinity levels. Na⁺, Cl⁻, PO₄⁻, SO₄⁻ and Na⁺/K⁺ concentrations increased with increase in salinity but Ca²⁺, K⁺ and Mg²⁺ concentrations were lower as salinity levels increased. It is concluded that with increase in salinity levels there was a significant reduction in biomass production in cowpea plant. Pattern of accumulation of ions varied significantly.

Key words: Salinity, concentrations, *Vigna unguiculata* (L.) Walp., stress.

INTRODUCTION

Salinity is an environmental stress that limits growth and development in plants. It is considered a significant factor affecting crop production and agricultural sustainability in arid and semi arid region of the world reducing the value and productivity of the affected land (Munns, 2002). Salinity causes not only differences between the mean yield and the potential yield, but also causes yield reduction from year to year. It affects the plant growth directly through its interaction with metabolic rates and pathways within the plants. In the simplest analysis of the response of a plant to salinity stress, the reduction in shoot growth occurs in two phases: A rapid response to

the increase in external osmotic pressure and a slower response due to accumulation of Na⁺ in leaves (Munn, 2002). In the first, osmotic phase which starts immediately after the salt concentration around the root increases to the threshold 40 mM NaCl for most plant which is equivalent to 4 dSm (George, 2008). As a result, the rate of shoot growth falls significantly. The second is the ionic specific phase of plant response to salinity which starts when salt accumulates to toxic concentration in the leaves causing necrosis and reducing photosynthesis area resulting in decline in growth (Munn, 2002; Bayuelo-Jimenez et al., 2012).

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Table 1. Effect of different concentrations of NaCl and Na₂SO₄ on biomass components of two accessions of *Vigna unguiculata* (L.) Walp.

Salt	Conc. (mM)	RDW (g)		SDW (g)		TDW (g)	
		TVu 11711	TVu 15245	TVu 11711	TVu 15245	TVu 11711	TVu 15245
NaCl	0	1.20 ^a	1.13 ^a	8.19 ^a	6.20 ^a	9.39 ^a	7.33 ^a
	50	0.59 ^b	0.3 ^b	2.57 ^b	2.35 ^b	3.15 ^b	2.65 ^b
	100	0.28 ^c	0.27 ^b	1.74 ^b	0.88 ^c	2.02 ^b	1.14 ^c
	150	0.29 ^c	0.15 ^b	1.85 ^b	0.67 ^c	2.14 ^b	0.82 ^c
Na ₂ SO ₄	0	0.94 ^a	0.96 ^a	8.23 ^a	6.52 ^a	9.15 ^a	7.49 ^a
	50	0.46 ^b	0.39 ^b	2.74 ^b	1.99 ^b	3.20 ^b	2.38 ^b
	100	0.38 ^b	0.21 ^b	2.00 ^{bc}	1.63 ^b	2.39 ^{bc}	1.84 ^b
	150	0.20 ^c	0.15 ^b	1.52 ^c	0.9 ^b	1.73 ^c	1.05 ^b

RDW, Root dried weight; SDW, shoot dried weight; TDW, Total dried weight. Each value is a mean of three replicates. Values with the same superscript are not significantly different at $P \geq 0.05$ using Duncan's multiple range test (DMRT).

Cowpea (*Vigna unguiculata* L. Walp.) is an important grain legume crop used as a fodder crop for livestock (Zahedi et al., 2012) and as a cheap source of vegetable. It is consumed both as green pod and as dry seed. The fresh green pods of cowpea contain 85.9% moisture, 4.6% protein, 0.2% fat, 0.8% minerals, 2.0% fibre and 8.5% carbohydrates. The high protein content of cowpea makes it an important supply to the diet of many African people (Giarni et al., 2001).

Many varieties are grown in tropical and sub-tropical agricultural areas of the world, where salinity is a yield-limiting factor (Zahedi et al., 2012). Cowpea is reported to have a good tolerance to heat and drought (Vasquez-Tello et al., 1990 cited in Zahedi et al., 2012), and it has a high yield potential under irrigation (Murillo-Amador et al., 2006). Salinity stress disturbs the uptake and accumulation of essential nutrients (Zhu, 2001).

In view of these studies, the principal objective to carry out the present study was to assess the effect of salinity on dry matter and ionic contents in cowpea accessions.

MATERIALS AND METHODS

This study was carried out from February to April, 2013 in the green house at the Department of Botany, Faculty of Science, University of Ibadan and seeds of cowpea cultivars (namely; TVu 11711 and TVu 15245) were obtained from the International Institute of Tropical Agriculture (IITA), Oyo State, Ibadan. Cowpea seeds were planted in pots of 14 cm diameter and 18.5 cm depth; each pot was filled with 3.0 kg soil. Four seeds of each cultivar were sown in each pot. Four levels of NaCl salt (0, 50, 100 and 150 mM) were applied after 15 days of germination. The experiment was laid in completely randomized designed in triplicates. When the seedlings were well established (after 10 days) that is, when the first trifoliolate leaf had reached its full size and the second trifoliolate leaf was starting its development, thinning was carried out, leaving two plants per pot. Salt treatment commenced 5 days after plants were thinned. These involved the application of NaCl and Na₂SO₄ with varied equimolar concentration (0, 50, 100 and 150 mM). The treatments were applied twice a week except for the control (0 mM) that was watered regularly. Plants were harvested four weeks after treatment. Plants were uprooted carefully and washed in running tap water.

Plant samples were placed in oven at 80°C. After 4-days shoot and root dry weights (g/pot) were estimated with the help of weighing balance at final harvest.

The dried ground plant material (0.1 g) was digested with sulphuric acid (H₂SO₄) and hydrogen peroxide H₂O₂ according to the method of Wolf (1982). The ionic contents (Na⁺, K⁺, Ca²⁺ and Mg²⁺) of the samples were determined by atomic absorption spectrophotometry. The PO₄ and SO₄ contents were estimated by colorimetric and turbidimetric methods, respectively.

The Chloride of the plant material (0.1 g) was extracted in 10 ml distilled water at 80°C for 4 h. The Cl⁻ content was analyzed by precipitation as AgCl and titrated according to Johnson and Ulrich (1959).

Data were subjected to analysis of variance, using Statistical Analysis System (SAS). Various treatment means were compared with Duncan's New Multiple Range (DMR) Test.

RESULTS

Effect of varying concentrations of NaCl and Na₂SO₄ on biomass of two cowpea accessions is presented in Table 1. It was observed that depending on the increasing salt concentration, the fresh and dry weights of root and shoot of both accessions decrease from 0 to 150 mM (Table 1). Salt stress significantly reduced dry matter production. Total dry matter at the highest salinity (150 mM) was lower than the dry matter of plants grown in the control pots after 4 weeks of saline treatment. At the highest salinity treatment, TVu 11711 had greater dry weight than TVu 15245. Shoot dry weight was significantly reduced by salinity at $P < 0.05$. Shoot dry weight at the highest salinity (150 mM) had the lowest values compared to non-stressed (0 mM) plants in both accessions (Table 1).

Salinity affected ions uptakes of the two cowpea accessions (Tables 2 and 3). shoot nutrient analysis of the two cowpea accessions indicated that Na⁺, Mg²⁺, Na⁺, Cl⁻, SO₄²⁻, PO₄³⁻ and Na⁺: K⁺ ratio significantly increased under saline condition ($p \leq 0.05$) with increasing NaCl and Na₂SO₄ concentrations except K⁺, and Ca²⁺ (Tables 2 and 3). K⁺ uptake of salt stressed cowpea plants appeared to be differentially influenced by NaCl and

Table 2. Effect of varying concentrations of NaCl and Na₂SO₄ on inorganic ionic contents of leaves of two accessions of *V. unguiculata* (L.) Walp.

Salt	Conc. (mM)	K ⁺		Na ⁺		Ca ²⁺		Na ⁺ : K ⁺		Mg ²⁺		PO ₄ ³⁻		SO ₄ ²⁻		Cl ⁻	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
NaCl	0	1.00 ^a	1.80 ^a	1.99 ^c	2.17 ^d	6.26 ^a	5.82 ^a	2.06 ^c	1.21 ^d	0.95 ^a	0.91 ^a	1.93 ^d	1.31 ^d	2.00 ^d	2.01 ^d	1.69 ^d	1.78 ^d
	50	0.64 ^b	0.84 ^b	2.27 ^b	3.07 ^c	5.88 ^b	5.29 ^b	3.58 ^c	3.69 ^c	0.77 ^b	0.88 ^a	3.16 ^c	3.02 ^c	3.02 ^c	2.64 ^c	2.70 ^c	2.99 ^c
	100	0.42 ^c	0.64 ^c	2.41 ^b	4.95 ^b	5.30 ^c	4.77 ^c	4.28 ^b	7.82 ^b	0.75 ^b	0.82 ^b	4.29 ^b	3.79 ^b	3.84 ^b	3.68 ^b	3.35 ^b	3.71 ^b
	150	0.21 ^d	0.44 ^d	2.91 ^a	6.11 ^a	4.95 ^d	4.33 ^d	5.86 ^a	3.97 ^a	0.68 ^c	0.79 ^b	4.83 ^a	4.18 ^a	4.12 ^a	4.13 ^a	4.06 ^a	4.18 ^a
Na ₂ SO ₄	0	5.00 ^a	2.00 ^a	1.80 ^d	1.99 ^d	5.00 ^a	4.72 ^a	0.36 ^d	0.99 ^d	1.21 ^a	0.92 ^a	1.15 ^d	1.86 ^a	1.90 ^d	1.83 ^a	1.60 ^a	1.62 ^d
	50	1.55 ^b	0.76 ^b	2.01 ^c	2.38 ^c	4.15 ^b	4.08 ^b	1.31 ^c	3.13 ^c	1.13 ^a	0.86 ^b	1.60 ^c	2.19 ^b	2.08 ^c	2.20 ^b	2.63 ^b	2.91 ^c
	100	0.94 ^c	0.61 ^b	2.24 ^b	2.85 ^b	4.04 ^b	3.36 ^c	2.40 ^b	4.71 ^b	1.08 ^a	0.83 ^c	1.89 ^b	2.86 ^c	2.78 ^b	2.91 ^c	3.15 ^c	3.84 ^b
	150	0.74 ^c	0.80 ^c	2.60 ^a	3.22 ^a	3.76 ^c	2.91 ^d	3.55 ^a	4.02 ^a	0.89 ^b	0.80 ^c	0.19 ^a	3.54 ^d	3.10 ^a	3.30 ^d	3.91 ^a	4.28 ^a

Each value is a mean of three replicates. Values in the same column with the same letter(s) were not significantly different at P≥0.05 using Duncan's Multiple Range Test (DMRT). A, TVu 11711; B, TVu 15245

Table 3. Effect of varying concentrations of NaCl and Na₂SO₄ applications on inorganic ionic contents of stems of two accessions *V. unguiculata* (L.) Walp.

Salt	Conc. (mM)	K ⁺		Na ⁺		Ca ²⁺		Na ⁺ : K ⁺		Mg ²⁺		PO ₄ ³⁻		SO ₄ ²⁻		Cl ⁻	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
NaCl	0	0.81 ^a	0.89 ^a	1.04 ^d	1.81 ^d	4.48 ^a	2.28 ^a	1.29 ^c	2.07 ^c	0.83 ^a	0.195 ^c	1.41 ^d	1.01 ^d	1.60 ^d	1.03 ^a	1.50 ^d	1.62 ^d
	50	0.51 ^b	0.62 ^b	1.40 ^c	2.08 ^c	4.02 ^b	1.30 ^b	2.75 ^{bc}	3.36 ^c	0.80 ^a	0.51 ^a	2.20 ^c	1.99 ^c	1.91 ^c	0.79 ^b	2.60 ^c	3.23 ^c
	100	0.29 ^c	0.58 ^b	1.84 ^b	3.83 ^b	3.30 ^c	0.93 ^c	6.48 ^b	6.73 ^b	0.54 ^b	0.35 ^b	2.98 ^b	2.98 ^b	2.08 ^b	0.63 ^c	3.23 ^b	4.67 ^b
	150	0.15 ^d	0.34 ^c	2.14 ^a	5.38 ^a	3.02 ^d	0.66 ^d	15.43 ^a	5.90 ^a	0.39 ^c	0.255 ^c	3.84 ^a	3.79 ^a	2.48 ^a	0.40 ^d	4.01 ^a	5.19 ^a
Na ₂ SO ₄	0	2.85 ^a	1.65 ^a	1.11 ^d	1.35 ^d	3.35 ^a	3.93 ^a	0.39 ^c	0.82 ^c	0.61 ^a	0.70 ^a	0.89 ^d	1.52 ^d	1.33 ^d	1.18 ^d	1.27 ^d	1.50 ^d
	50	0.82 ^b	0.56 ^c	1.56 ^c	2.13 ^c	2.50 ^b	3.08 ^b	1.91 ^b	3.81 ^b	0.42 ^b	0.55 ^b	1.12 ^c	1.70 ^c	1.63 ^c	1.49 ^c	2.11 ^c	2.07 ^c
	100	0.83 ^b	0.43 ^d	1.81 ^b	2.47 ^b	2.09 ^b	2.61 ^c	2.18 ^b	5.79 ^a	0.26 ^c	0.43 ^c	1.28 ^b	1.90 ^b	1.89 ^b	1.79 ^b	3.18 ^b	3.15 ^b
	150	0.62 ^c	0.73 ^b	2.14 ^a	3.11 ^a	1.75 ^c	2.33 ^d	3.46 ^a	4.31 ^b	0.15 ^d	0.31 ^d	1.53 ^a	2.07 ^a	2.02 ^a	1.99 ^a	3.67 ^a	4.05 ^a

Each value is a mean of three replicates. Values in the same column with the same letter(s) were not significantly different at P≥0.05 using Duncan's Multiple Range Test (DMRT). A, TVu 11711; B, TVu 15245.

Na₂SO₄. NaCl application reduced the uptake of K⁺ in both leaf and stem. Similarly, the uptake of K⁺ content in the accessions treated with Na₂SO₄ was significantly reduced with increased in concentration. The amount of Na⁺ uptake increased markedly in response to increasing salt

levels. Besides, increased amounts of Na⁺, the Na⁺/K⁺ ratio rose significantly by increasing salts concentrations in both accessions. Calcium uptake in leaf was also reduced by both salts in the accessions (Tables 2 and 3), to a great extent by Na₂SO₄, NaCl also followed the same trend

but the rate of absorption was significantly lower than in Na₂SO₄ in the accessions with increased in concentrations. The uptake of magnesium was also affected by increasing concentration of salinity in the accessions in both NaCl and Na₂SO₄ (Tables 2 and 3). There was a

decreased in the content of magnesium from 0 - 150 mM of salt treatment and this decreased were reflected in the chlorophyll contents of both accessions in both treatments since magnesium is majorly responsible for chlorophyll formation in leaf. Leaf and stem phosphate and sulfate ion concentrations from control plants did not differ significantly among the different accessions, in both salt types (Tables 2 and 3). However, the uptake of SO_4^{2-} and PO_4^{3-} concentrations in the shoot from salt-stressed plants increased with increase in the salts concentrations from 0 - 150 mM of Na_2SO_4 and NaCl

DISCUSSION

In the present study, salt stress caused a great reduction in fresh and dry weights of shoot and root of both cowpea accessions with the increase in saline contents. Reduction in plant growth as a result of salt stress had also been reported in several other plant species (Ashraf and O'leary, 1997).

The increase in Na^+ content and decrease in K^+ uptake disturbs ionic imbalance as observed in cowpea plant exposed to salt stress. The diminution of K^+ concentration in tissue may also be due to direct competition between K^+ and Na^+ at plasma membrane, inhibition of Na^+ on K^+ transport process in xylem tissues and/or Na^+ induced K^+ efflux from the roots. K^+ and Ca^{2+} have been reported to be the major cations in cell organization as well as the major contributors to osmotic adjustment under stress conditions in several plant species (Santos-Diaz and Alejo-Ochoa, 1994; Hirschi, 2004). In the present study, the level of K^+ , Mg^{2+} and Ca^{2+} in the salt-stressed cowpea plants gradually decreased while that of Na^+ was dramatically increased. Increasing levels of NaCl induced a progressive absorption of Na and Cl in plant, agreeing with Taban et al. (1999) and Turan et al. (2007a, 2007b). Excessive Na^+ concentration in the plant tissue hinders nutrient balance, osmotic regulation and causes toxicity (Amdouni et al., 2014).

Accumulation of Cl^- in the root tissue is disruptive to membrane uptake mechanisms, and these results in increased translocation of Cl^- to the shoots (Turan et al., 2009). When NaCl was applied to the soil, the levels of K in plant were reduced in accordance with the antagonism between Na^+ and K^+ (Alberico and Cramer, 1993; Azevedo and Tabosa, 2000). Mansour (1997) showed that excess NaCl leads to the loss of potassium due to membrane depolarization by sodium ions. The decrease in K^+ and Ca^{2+} content under stress condition had been previously reported in other species particularly in the salt-sensitive lines (Lutts et al., 2004). According to Weimberg (1987) cited in Summart et al. (2010) high levels of Na^+ inhibit the K^+ uptake and as a result of this it causes an increase in the Na^+/K^+ ratio. According to Ahmed El Sayed (2011), Na^+ and Ca^{2+} ions probably compete much more for common uptake sites. From the

result obtained, it appears that Na^+ in combination with SO_4^{2-} is more toxic than with Cl^- . Phosphate content was increased due to salt stress in both leaf and stem. The influence of salinity on phosphorus in phosphate uptake is controversial. A suppression of P uptake due to salt stress was been reported by Ahmed El Sayed (2011) whereas increased PO_4 content due to salt stress was reported by Garg et al. (2005). Indeed symptoms of PO_4^- toxicity induced by salinity have been recognized by Nieman and Shannon (1976). According Ahmed El Sayed (2011) resistance to secondary salt induced stress in *Glycine falcata* Benth. was due to its ability to maintain a high P content in the presence of salt stress. It is possible that a high phosphate content in stem and leaf of salinized cowpea plant played similar role which corroborated with Silva et al. (2003) who reported an increase in leaf P concentration resulting from salt stress in mature and immature cowpea leaves. On the other hand, P accumulation in salt-stressed plants could be a consequence of reduced translocation associated with a decreased demand for growth.

Addition of NaCl and Na_2SO_4 had an adverse effect on the growth of cowpea plant. Salinity caused a significant effect on shoot fresh and dry weights. The reason for growth reduction in cowpea plant could be due to water shortage and ionic toxicity caused by salinity. Assessment of pattern of accumulation of toxic ions in a species is vital importance to understand, whether the species uses partial exclusion or inclusion mechanism for tolerating toxic ions present in its growth medium (Khalid et al., 2009).

Conclusion

It is concluded that salinity had adverse effect on growth of cowpea plant. Growth was reduced with the increase in salinity levels. Its ionic contents also varied significantly under salt stress. So, salinity had adverse effects on plant life cycle. However, further research should be conducted in field in other to authenticate these findings.

Conflict of Interest

The authors have not declared any conflict of interest.

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