

Effects of salinity stress (NaCl) on growth attributes and some nutrient accumulation in cowpea (*Vigna unguiculata*)

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ABSTRACT

This study investigates the impact of salinity (NaCl) on growth and ions accumulation in the leaves of three cowpea genotypes: OU100 and KEB-CP118 from Cameroon and ICV12 from Kenya. Four levels of salinity were used (0 mM, 50 mM, 100 mM, and 150 mM), and the experiment was carried out in the greenhouse. Growth parameters were measured on 8-week-old plants. Leaf ions concentrations (Na⁺, K⁺, and K⁺/Na⁺) were determined. It was observed that increasing salinity induced a significant increase in Na⁺ and substantial reduction in the accumulation of K⁺ in the leaves of all genotypes. Pearson's correlation analysis revealed significant association among most of the growth parameters. Water content in shoots was not affected by salinity for all genotypes; however, salinity induced a reduction of water content in the root for ICV12 and OU100 genotypes. In general, results highlighted that high salt concentrations significantly delayed the growth process. The delay was more pronounced for OU100 genotype. Therefore, genotype effect toward salinity tolerance was noticed. KEB-CP118 was the most tolerant genotype as salinity did not affect negatively growth parameters. Its remarkable behavior under salinity indicates that it should be explored in selection programs, used in the development of tolerant varieties, and promoted for cultivation in tropical zones affected by salinity.

KEY WORDS: Cowpea, salinity tolerance, genotype, growth, ion accumulation

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walps, is an important food legume in developing countries, mostly in sub-Saharan Africa, Asia, and Central and South America (Singh *et al.* 1997). Cowpea has been referred to as "poor man's meat" because of its high protein content (20-25%) (Diouf and Hilu, 2005; with Kareem and Taiwo, 2007; Sharmar *et al.*, 2013). In Cameroon, the Far North Region is the largest contributor to the national production of cowpea, and the crop ranks second after groundnuts in the category of leguminous crop (Dugje *et al.*, 2009). The production in this region ranges from 300 to 500 kg/ha in farmers' fields and 1200-2000 kg/ha in research stations (Dugje *et al.*, 2009). According to Bidima (2012), the Western region also produces significant quantities. Overall, the national production in Cameroon is estimated to about 110,000 tons from a planted area of 105,000 hectares (Bidima, 2012).

Biotic and abiotic factors affect significantly yield and productivity of many crops worldwide. Among the abiotic factors, the salinity of the soil appears as the major factor that severely reduces agricultural productivity throughout the world (Epstein *et al.*, 1980). According to Rengasamy (2010), salinity affects more than 800 million hectares of the cultivable land worldwide. Salinity stress in the soil is induced by a wide range of dissolved salts, but sodium chloride (NaCl) is the most widespread one which explains the intensive investigations carried out (Munns and Tester, 2008). Salinization of soils occurs primarily due to agricultural practices that include poor water management, high evaporation, and exposure to seawater (Pitman and Lauchli, 2002). This salinity is quite widespread in many arid and coastal zones. The important presence of salt in the soil is a problem that restricts yield of several crops on millions hectares worldwide of irrigated lands (Epstein *et al.*, 1980). This is why a large proportion of irrigated lands are being removed every year from crop production (Epstein *et al.*, 1980).

Several studies screening crops for salinity tolerance using different methodologies have been carried out on cowpea and other related crops (Taffouo *et al.*, 2009; Patel *et al.*, 2010; Gogile *et al.*, 2013; Khalid *et al.* 2015; Sakina *et al.* 2016; Hamayun *et al.* 2010; Gulzar *et al.*, 2003; Pandolfi *et al.*, 2012). Screening under controlled conditions has given better results because of reduced environmental effects. In general, seed germination, seedling survival, and plant growth are inhibited by salinity. The level of inhibition may vary with plant species and crop varieties with their tolerance level (Munns and Termaat, 1986). High levels of soil salinity create a combined effect of high osmotic potential and specific ion toxicity on the plant. This high salt concentration reduces the ability of plants to take up water, and this can significantly inhibit seed germination and seedling growth (Grieve and Suarez, 1997).

To overcome the adverse effect of salinity on germination and growth, the best practice would be to identify crop genotypes that are able to grow and develop on saline soils. This follows suggestions of Baccio *et al.* (2004) who propose the introduction of salt tolerant genotypes as one of the ways to utilize saline lands. This research was therefore carried out to evaluate the effects of salinity stress on growth attributes and ions accumulation in the leaves of some cowpea genotypes with that aim of identifying the salt tolerant one that could be proposed in saline zones.

MATERIALS AND METHODS

Study Site and Plant Material

The study was carried out at the Genetics Experimentations Greenhouse of the Research and Teaching Farm of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, located in the West Region of Cameroon at latitude of 5°20' North and longitude of 10°05' East, and 1407 m above the sea level. The annual rainfall of the study site ranges from 1800 to 2000 mm. The average annual temperature and relative humidity are around 20.50°C and 76.8%, respectively. Tree cowpea genotypes were used for the study. These were KEB-CP118 and OU100 from Cameroon and ICV12 from Kenya. The soil used for the experiment was collected from the ploughed field of a nearby university site. Soil's characteristics are presented in Table 1.

Experimental Design, NaCl Treatments, and Parameters Measurements

Eight seeds of each genotype were planted in each plastic pot containing 7 kg of soil. Thinning was carried out

Table 1: Chemical and physical characteristics of the soil used (0-20 cm depth)

Elements	Content
Clay (%)	13.00
Sand (%)	70.00
Silt (%)	17.00
Carbon (%)	3.56
Nitrogen (g/kg)	2.69
Ratio C/N	13.00
Assimilable phosphorus (mg/kg)	9.87
Exchangeable potassium (mEq/100 g)	0.11
Exchangeable magnesium (mEq/100 g)	1.27
Exchangeable calcium (mEq/100 g)	2.70
Exchangeable sodium (mEq/100 g)	0.14
pH-water	5.30
EC ($\mu\text{s}/\text{cm}$)	25.50

2 weeks after planting and leaving four plants in each pot. The pots used had no holes at the bottom. The experimental design was randomized complete block design with three replications. Salinity treatments were applied as NaCl solutions at four levels that were 0, 50, 100, and 150 mM. Pots were irrigated with 200 ml saline solution every 3 days for 6 weeks and from 2 weeks after planting. Plant height (PH) was taken at weekly basis on two plants in each repetition. At 8 weeks, the experiment was concluded, and all other measurements on growth parameters (shoot fresh weight [SFW], shoot dry weight [SDW], root fresh weight [RFW], root dry weight [RDW], RDW-SDW ratio, root length [RL], number of leaves [NL], and RL-PH ratio), ions accumulation in the leaves (Na^+ , K^+ , and K^+/Na^+), water content in the shoot (SWC), and root (RWC) were carried out. Dry weights were measured after drying plants at 70°C for 48 h as suggested by Bohm (1979). To determine ions accumulation, Ash of the different plant samples was obtained by heating dried samples at 450°C for 6 h. The Ash obtained was then dissolved in diluted HCl using few milliliters of nitric acid. This solution was used to determine Na^+ and K^+ by means of flame photometer. SWC and RWC were determined as follows:

$$\text{SWC} = \frac{(\text{SFW} - \text{SDW})}{\text{SFW}} \quad \text{RWC} = \frac{(\text{RFW} - \text{RDW})}{\text{RFW}}$$

Statistical Analysis

Data for each measured parameter were subjected to monofactor analysis of variance (ANOVA) using GraphPad Prism 6.0 statistical software package for Windows. Where ANOVA test showed significant differences among means, Tukey's multiple range test of XLSTAT 2014 software was performed at the 0.05 level of probability to separate means. Pearson correlation coefficients were performed to assess relations between growth parameters.

RESULTS

Growth Parameters

On the basis of ANOVA, there was a significant difference in different NaCl treatments for growth expressed in terms of centimeter per week (cm/week) for genotypes ICV12 and OU100 ($P < 0.05$ significance level). However, growth of genotype KEB-CP118 was statistically similar for the different treatments (Figure 1). For other growth parameters, the ANOVA revealed in general significant difference among genotypes and treatments. Dry weights (SDW and RDW) and SFW were not affected by salinity for ICV12 and KEB-CP118 genotypes. However, SDW, RDW, and SFW of OU100 genotype decreased significantly with salinity ($P < 0.050$, Table 2). The maximum heights of seedling (PH) and RFW were observed in controls for all genotypes. They decreased significantly with increasing salinity for ICV12 and OU100 genotypes. The decrease of PH and RFW was not significant for KEB-CP118. Salinity did not have any effect on RL for ICV12 genotype. However, salinity decreased significantly RL for OU100 genotype and tends to increase the RL of KEB-CP118 genotype. The number of leave per plant (NL) decreased significantly with increasing salt concentration for OU100 genotype. Salinity, however, did not affect the NL of ICV12 and KEB-CP118 plants. In general, growth parameters significantly ($P < 0.050$) decreased (7 out of 8 parameters) with increasing salinity in OU100 genotype. Salinity had no effect on five out of eight growth parameters for ICV12

genotype and on six out of eight growth parameters for KEB-CP118 genotype (Table 2).

Ions Accumulations and Water Content

Ions accumulations in the leaves were affected by salinity in all three cowpea genotypes. K^+ levels and K^+/Na^+ ratios decreased significantly with increasing salinity for all the three genotypes. Na^+ levels increased generally with increasing salt concentration. This increase was significant ($P < 0.050$) for ICV12 and KEB-CP118 only (Table 3). Shoot had significantly more water content compared to root for all three genotypes (Table 4). Salinity had no impact on water content in shoots for all three genotypes. However, salinity significantly decreased water content in the root for ICV12 and OU100 genotypes and increased that of KEB-CP118 genotype.

Correlations Analysis

Pearson's correlation coefficients were determined for any pair of the nine growth parameters used for the study. Among the 36 correlations estimated, twenty associations (55.56%) were significant and positive; six associations (16.67%) were significant and negative. 10 associations (27.78%) had no relationship (Table 5). This study revealed that PH, NL, SFW, SDW, RFW, and RDW had significant associations with most of growth parameters. RL, however, had no relationship with most of growth variables, such as correlation between RL and PH that was not significant ($r = 0.015$, $P > 0.050$, Table 5).

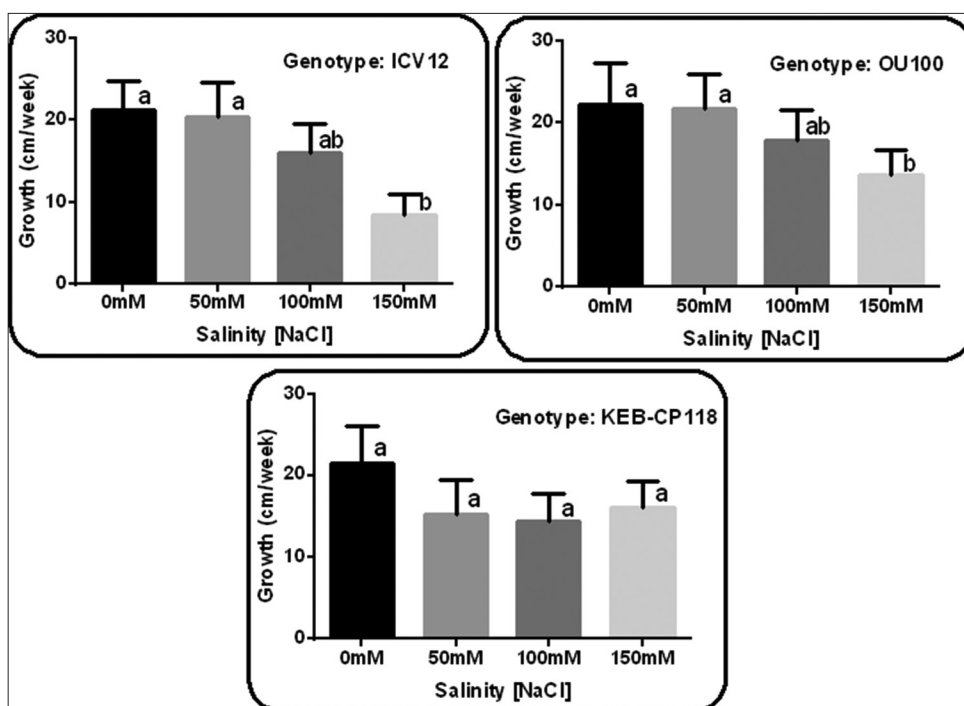


Figure 1: Growth of three genotypes of *Vigna unguiculata* under salinity conditions at 8 weeks

Table 2: Effects of NaCl on growth attributes of cultivated *V. unguiculata* at 8 weeks

Genotype	Growth attributes	Salinity (NaCl)				F
		0 mM	50 mM	100 mM	150 mM	
ICV12	SDW (g)	1.40±0.26 ^a	1.26±0.13 ^a	1.10±0.19 ^a	1.17±0.11 ^a	0.4869 ^{NS}
	RDW (g)	0.47±0.06 ^a	0.44±0.09 ^a	0.36±0.05 ^a	0.40±0.06 ^a	0.3619 ^{NS}
	SFW (g)	12.01±2.64 ^a	10.28±0.82 ^a	9.43±1.72 ^a	10.23±1.13 ^a	0.6713 ^{NS}
	RFW (g)	1.57±0.20 ^a	1.10±0.08 ^{ab}	0.97±0.19 ^b	1.09±0.17 ^{ab}	2.535 [*]
	RL (cm)	20.50±1.50 ^a	25.30±4.29 ^a	19.40±1.37 ^a	19.40±1.74 ^a	1.307 ^{NS}
	PH (cm)	25.60±0.83 ^a	25.00±0.30 ^b	22.31±0.90 ^b	23.70±0.50 ^{ab}	4.853 ^{**}
	NL	5.20±0.42 ^a	5.40±0.36 ^a	4.80±0.45 ^a	5.00±0.29 ^a	0.436 ^{NS}
	RL/PH	0.72±0.07 ^b	0.99±0.16 ^a	0.77±0.05 ^b	0.80±0.06 ^b	2.659 [*]
OU100	SDW (g)	1.62±0.12 ^a	0.89±0.20 ^b	0.87±0.25 ^b	0.51±0.07 ^b	6.35 ^{**}
	RDW (g)	0.28±0.02 ^a	0.18±0.03 ^b	0.19±0.03 ^b	0.15±0.02 ^b	4.006 ^{**}
	SFW (g)	12.77±1.97 ^a	7.37±1.56 ^b	6.59±0.79 ^b	4.03±0.62 ^c	7.531 ^{***}
	RFW (g)	1.12±0.15 ^a	0.61±0.10 ^b	0.69±0.07 ^b	0.39±0.06 ^c	10.51 ^{***}
	RL (cm)	23.70±2.26 ^a	18.25±2.51 ^b	14.20±1.13 ^{bc}	12.75±0.67 ^c	7.73 ^{***}
	PH (cm)	35.50±5.35 ^a	35.13±8.04 ^a	34.40±7.37 ^a	21.00±2.30 ^b	3.991 [*]
	NL	8.00±0.20 ^a	6.25±0.57 ^{ab}	5.80±0.81 ^b	3.50±0.15 ^c	11.74 ^{***}
	RL/PH	0.72±0.15 ^a	0.44±0.10 ^a	0.47±0.07 ^a	0.60±0.02 ^a	2.39 ^{NS}
KEB-CP118	SDW (g)	1.96±0.50 ^a	2.27±0.60 ^a	1.76±0.35 ^a	2.93±0.99 ^a	0.610 ^{NS}
	RDW (g)	0.57±0.11 ^a	0.53±0.09 ^a	0.44±0.03 ^a	0.42±0.06 ^a	0.688 ^{NS}
	SFW (g)	13.87±3.52 ^a	18.45±4.91 ^a	13.73±2.82 ^a	22.65±7.47 ^a	0.722 ^{NS}
	RFW (g)	2.07±0.50 ^a	2.66±0.57 ^a	1.92±0.24 ^a	1.83±0.36 ^a	0.727 ^{NS}
	RL (cm)	31.60±5.06 ^b	48.00±6.49 ^{ab}	53.60±2.68 ^a	40.50±7.05 ^b	2.918 [*]
	PH (cm)	39.50±7.91 ^a	28.80±1.00 ^a	27.60±1.74 ^a	36.20±5.86 ^a	1.310 ^{NS}
	NL	7.80±0.39 ^a	7.00±0.56 ^a	7.40±0.90 ^a	9.20±1.99 ^a	1.506 ^{NS}
	RL/PH	1.04±0.16 ^b	1.65±0.19 ^{ab}	2.07±0.16 ^a	1.44±0.30 ^{ab}	3.936 [*]

Means followed by the same letter in the same row are not significantly different at $P=0.050$ probability level. *V. unguiculata*: *Vigna unguiculata*, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, RL: Root length, NL: Number of leaves, PH: Plant height

Table 3: Accumulation of sodium and potassium contents, potassium/sodium ratio in three genotypes of *V. unguiculata* under salinity for 8 weeks

Genotype	Nutrient uptake	Salinity (NaCl)				F
		0 mM	50 mM	100 mM	150 mM	
ICV12	K ⁺ (mg/g)	14.23±1.42 ^a	13.37±1.78 ^a	13.76±0.20 ^a	10.06±0.35 ^b	2.868 [*]
	Na ⁺ (mg/g)	1.06±0.11 ^c	1.33±0.10 ^{bc}	1.66±0.09 ^{ab}	1.77±0.07 ^a	12.371 ^{***}
	K ⁺ /Na ⁺ ratio	13.42±0.93 ^a	10.01±0.95 ^{ab}	8.29±0.49 ^{ab}	5.76±0.13 ^b	6.342 ^{**}
OU100	K ⁺ (mg/g)	10.22±1.36 ^a	8.53±1.28 ^{ab}	8.13±1.95 ^{ab}	7.44±1.36 ^b	2.611 [*]
	Na ⁺ (mg/g)	0.84±0.15 ^a	0.95±0.16 ^a	1.14±0.18 ^a	1.16±0.21 ^a	0.778 ^{NS}
	K ⁺ /Na ⁺ ratio	12.09±0.24 ^a	9.15±0.26 ^{ab}	7.16±0.35 ^b	6.61±0.26 ^c	25.007 ^{***}
KEB-CP118	K ⁺ (mg/g)	14.04±1.87 ^b	12.24±1.96 ^b	7.49±1.15 ^a	7.09±0.46 ^a	5.376 ^{**}
	Na ⁺ (mg/g)	0.71±0.18 ^b	0.81±0.19 ^b	1.39±0.09 ^a	1.45±0.02 ^a	7.361 ^{**}
	K ⁺ /Na ⁺ ratio	19.63±1.19 ^a	15.14±0.92 ^b	5.69±0.89 ^c	4.98±0.73 ^c	12.997 ^{***}

Means followed by the same letter in the same row are not significantly different at $P=0.050$ probability level. *V. unguiculata*: *Vigna unguiculata*

Table 4: Water content in shoot and root of three genotypes of *V. unguiculata* under salinity at 8 weeks

Treatment	Genotype								
	ICV12			OU100			KEB-CP118		
	Shoot			Shoot			Shoot		
Salinity (NaCl)	SFW (g)	SDW (g)	SWC (%)	SFW (g)	SDW (g)	SWC (%)	SFW (g)	SDW (g)	SWC (%)
0 mM	12.01	1.4	88.34	12.77	1.62	87.31	13.87	1.96	85.87
50 mM	10.28	1.26	87.74	7.37	0.89	87.92	18.45	2.27	87.69
100 mM	9.43	1.1	88.32	6.59	0.87	86.79	13.73	1.76	87.18
150 mM	10.23	1.17	87.58	4.03	0.51	87.34	22.65	2.93	87.06
	Root			Root			Root		
Salinity (NaCl)	RFW (g)	RDW (g)	RWC (%)	RFW (g)	RDW (g)	RWC (%)	RFW (g)	RDW (g)	RWC (%)
0 mM	1.57	0.47	70.06	1.12	0.28	75	2.08	0.57	72.48
50 mM	1.1	0.44	60	0.61	0.18	70.49	2.36	0.53	77.54
100 mM	0.97	0.36	62.88	0.69	0.19	72.46	1.92	0.44	77.08
150 mM	1.09	0.4	63.3	0.39	0.15	61.53	1.83	0.42	77.05

V. unguiculata: *Vigna unguiculata*, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, SWC: Shoot water content

Table 5: Pearson correlation coefficients between growth attributes of *V. unguiculata* under salinity at 8 weeks

	SDW	RDW	RDW/SDW	SFW	RFW	RL	PH	NL	RL/PH
SDW	1.000								
RDW	0.803***	1.000							
RDW/SDW	-0.510***	-0.118 ^{NS}	1.000						
SFW	0.995***	0.788***	-0.507***	1.000					
RFW	0.864***	0.949***	-0.267*	0.869***	1.000				
RL	0.148 ^{NS}	0.394**	0.333*	0.143 ^{NS}	0.321*	1.000			
PH	0.618***	0.557***	-0.411**	0.600***	0.497***	0.015 ^{NS}	1.000		
NL	0.868***	0.557***	-0.474***	0.870***	0.630***	0.110 ^{NS}	0.641***	1.000	
RL/PH	-0.148 ^{NS}	-0.040 ^{NS}	0.417**	0.148 ^{NS}	-0.057 ^{NS}	0.494***	-0.319*	-0.152 ^{NS}	1.000

*P<0.050, **P<0.010, ***P<0.001. *V. unguiculata*: *Vigna unguiculata*, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, RL: Root length, NL: Number of leaves, PH: Plant height, NS: Not significant

DISCUSSION

The level of salinity presents in the soil represents one of the major abiotic stresses that reduce crop yield. Although many surveys related to physiological and growth responses of plants to different levels of salinity have been reported and are flourishing the literature (Sakina *et al.*, 2016; Zhang *et al.*, 2012; Hamayun *et al.*, 2010; Hakim *et al.*, 2010; Yang *et al.*, 2009; Khan *et al.*, 2000), very few studies have been reported on the response of cowpea to salinity (Gogile *et al.*, 2013; Patel *et al.*, 2010; Taffouo *et al.*, 2009). This information is particularly lacking in Africa and Cameroon where arid land represents significant proportion in the country. In this study, shoot and root growth, ions accumulation in the leaves, and water content of *V. unguiculata* plants were investigated under different levels of salinity stress in three cowpea genotypes. The effect of salinity was clearly manifested as the impact was visible in terms of the reduction of PH. Evidently, other growth attributes generally decreased as the concentration of NaCl increased in the soil. Similar observation of reduction of growth under salinity was observed for a number of plant species such as mungbean (Rafiq *et al.*, 2008), eggplant (Shaheen *et al.*, 2012), and rice (Shahbaz and Zia, 2011). Growth reduction due to NaCl treatment illustrates the negative effect of salinity on plants activated by the osmotic potentials of salt on the root that limits the gain of the required water quantity (Mer *et al.*, 2000). The growth and development of plants are therefore inhibited because of defection of metabolism such as change of the membrane permeability (Cramer *et al.*, 1985). Khalid *et al.* (2015) reported also the salinity reduces crop productivity by causing disruption of cell membranes that is the origin of nutrient imbalances and the source of dysfunction of growth regulators. Singularly, shoot and root growths of KEB-CP118 genotype were reduced with increasing salinity, but the reduction was not significant. In general, all three genotypes had lower shoot and root growth at higher levels of salinity. This might likely be attributable to the reduction of water

absorption that happens under increasing crucial osmotic force as reported by Kumar *et al.* (2005).

The variance analysis showed that the nutrient content of all genotypes was affected by increasing salinity. The concentration of ions accumulation in leaves (Na⁺, K⁺, and K⁺/Na⁺ ratio) are presented in Table 3. The tree genotypes had a higher Na⁺ accumulation in the control groups. Epstein (1972) reported that nutrient deficiencies can occur in plant when high concentration of Na⁺ in the soil reduces the amount of available K⁺. As Na⁺ level increased with salinity, it reduces the ability of cowpea plant to take up water and mineral such as K⁺. As K⁺ is known as a cofactor that activates many biochemical enzymes, its limitation in the cell will result of high cytosolic Na⁺ and low value of K⁺/Na⁺ ratio (Munns *et al.*, 2006). Our result indicates that, with the increasing salt concentration, Na⁺ increase in the leaves and the proportion of K⁺ and K⁺/Na⁺ ratio are reduced. The trend of Na⁺ accumulation in the leaves of the studied genotypes was, therefore, the contrary of the trend of K⁺ accumulation. Studies on barley (Wolf *et al.*, 1991) and soybean (Li *et al.*, (2006) reported similar results. These nutrient deficiencies are likely the reason of the delay in growth under salinity observed in the cowpea genotypes studied. Na⁺ likely has a toxic effect as its affect the structural and functional integrity of cell membranes (Kurth *et al.*, 1986). Our result also agrees with the report of Greenway *et al.* (1966) on *Atriplex nummularia* with the leaf sodium content increasing while leaf potassium content decreasing with increasing salt concentration. Increasing salinity results in gradual decrease of water content of cells (Hashem *et al.*, 2015). The water content in the root decreased with increasing salt concentration for ICV12 and OU100 genotypes. This observation agrees with the report of Azza *et al.* (2007) which shows that salinity postpones seedling growth by delaying radicle emergence because of insufficient water absorption. Similar observations of reduction of water content in cells of plants under salinity were also reported with cowpea (Hashem *et al.*, 2015).

CONCLUSION

The reduction of growth attributes by salinity differs between crop species and between genotypes within a crop species. This is due to their different aptitudes to tolerate salinity. Our results indicate that there are significant differences among cowpea genotypes in their responses to salinity. KEB-CP118 appears as a tolerant genotype as its growth was not significantly reduced by the different concentration of the NaCl treatments. Growth of ICV12 and OU100 genotypes was not reduced at low concentration of salt but was significantly inhibited by a higher level of NaCl concentrations.

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