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Changes in growth and nutrient uptake in response to foliar application of sodium and calcium chloride in cowpea cultivars (*Vigna unguiculata* L. Walp)

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In this study, the effects of NaCl and $CaCl_2$ on growth and nutrients uptake of cowpea (*Vigna unguiculata* L. Walp cvs. Mouola GG; Mouola PG, Garoua GG, Garoua PG and Tsacre) were investigated. Three treatments [0 (unsprayed), 50 mM NaCl or 50 mM $CaCl_2$] were adopted, with five replications. NaCl and $CaCl_2$ were applied as foliar spray twice a week during 30 days after sowing. Application of NaCl resulted in significant decreases in plant height, dry weights of root and shoot of Garoua GG, Mouola PG and Tsacre compared to Garoua PG and Mouola GG cultivars, while those growth parameters were significantly reduced under $CaCl_2$ treatment in all cowpea cultivars except Mouola PG. K^+, Ca^{2+} and Mg^{2+} contents decreased under NaCl stress in leaves of Garoua PG and Mouola GG, but no significant changes were observed in those of Garoua GG, Mouola PG and Tsacre. The main strategy of salt tolerance in Mouola GG and Garoua PG seems to be as a result of increased osmotic adjustment through the accumulation of Na^+ in leaves while in salt sensitive Mouola PG, Garoua GG and Tsacre, the osmotic adjustment may be due to the accumulation of inorganic ions (K^+, Ca^{2+} and Mg^{2+}) in leaves, contributing to the maintenance of water uptake and cell turgor, allowing for physiological processes. In the analysis of growth parameters measured, the results suggested that Mouola GG and Garoua PG cultivars were relatively more tolerant to salinity than others, suggesting that those cultivars could be cultivated in the environment with varying salinity. $CaCl_2$ treatment significantly increased growth parameters and nutrients uptake specially Ca^{2+} in Mouola PG cultivar, suggesting that it could grow and develop on calcareous soils.

Key word: Foliar spray, growth, nutrients uptake, saline and calcareous soils, *Vigna unguiculata*.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is one of the most important food legumes grown in the semi-arid tropical regions (Singh, 2003). 14.5 million hectares of land is planted to cowpea each year worldwide. Global production of dried cowpeas in 2010 was 5.5 million metric tons (Abate et al., 2011). The top producers are the West and Central African sub regions which contribute to about 64% of the global production and an estimated production of 3 million tons of cowpea seeds produced annually (Fery, 2002). The protein content in cowpea leaves annually consumed by Africans and Asians is equivalent to 5 million tons of dry cowpea seeds, representing as much as 30% of the total food legume production in the lowland tropics (Steele et al., 1985). The economic importance of cowpea species show a number of advantages that make them particularly valuable for inclusion in many types of cropping systems (Fery, 2002). Salinity is one of the most serious abiotic stresses that lead to the deterioration of agricultural lands and reduction in crop productivity in many parts of the world especially in arid and semi-arid regions (Munns, 2002; Taffouo et al., 2010a).

The ability of vegetation to survive under higher salinity conditions is essential for the distribution of plants and agricultural grounds around the world (Yousif et al., 2010). Excessive salinity in marginal soils results from natural processes, whereas in arable land it is mostly anthropically generated, due to the progressive accumulation of the ions dissolved in irrigation water (Neumann, 1997). This later salinisation results to an alarming situation for agriculture especially in arid and semi-arid regions which about half of their surface is already affected by salt stress, to a higher or lower degree, and more than 10 million ha of agricultural land are lost every year due to salinisation (Munns and Tester, 2008). Maintaining adequate nutrient elements in the growth media under salinity is a common goal in grain legume production. Soil salinity can inhibit plant growth by a number of mechanisms such as low external water potential, toxicity of absorbed Na^+ and Cl^- ions, due to the inhibition of many enzymatic activities and different cellular processes (protein synthesis or generation of reactive oxygen species) and interference with the uptake of essential nutrients, such as K^+ and Ca^{2+} (Munns, 2002; Grigore et al., 2011). The severity of each of these factors to plant growth depends on the plant genotype and environmental conditions (Zadeh and Naeini, 2007). The nutritional imbalance in plants caused by salt stress may result from the effect of salinity on nutrient availability, competitive uptake and transport or partitioning within the plant (Munns and Tester, 2008).

NaCl toxicity which is the major form of salt in most saline soils enhances the Na^+ and Cl^- contents and consequently affects the absorption of other mineral elements (Greenway and Munns, 1980). It is stated that high levels of Na^+ inhibits Ca^{2+} and K^+ absorption, which results in a Na^+/K^+ antagonism (Turan et al., 2007). The fundamental mechanisms of salt tolerance in salt tolerant plants seem to be mostly dependent on their capacities to sequestrate toxic ions (Na^+, Cl^-) in the vacuoles and to accumulate compatible osmotica in the cytoplasm (Munns, 2002). Ca^{2+} plays a vital role in many physiological processes such as membrane structure and stomatal functioning cell division, cell wall synthesis and osmoregulation, which influence growth and responses towards environmental stresses (Kusvuran, 2012). The maintenance of an adequate supply of Ca^{2+} in saline soil solutions is an important factor in controlling the severity of specific ion toxicities, particularly in crops which are susceptible to Na^+ and Cl^- injuries (Grattan and Grieve, 1999). Under this condition, the ameliorative action of supplemental Ca^{2+} is crucial to alleviate high salinity stress. Ca^{2+} has a role in building salt tolerance in plants (Amuthavalli et al., 2012). Externally supplied Ca^{2+} reduces the toxic effects of NaCl, presumably by facilitating a high K^+/Na^+ selectivity (Liu and Zhu, 1998). Other beneficial effects that $CaCl_2$ has on plant physiology include membrane permeability and reduction in Na^+ concentration (Amuthavalli et al., 2012). Studies about the use of foliar organic or inorganic substances application on vegetable species are scarcely reported in the literature, however, some investigations have been conducted in groundnut (Lee, 1990), cucumber (Ozdamar Unlu et al., 2011), cowpea (Khalil and Mandurah, 1989), watermelon (Silva-Matos et al., 2012), pepper (Karakurt et al., 2009), rice (Kaur and Singh, 1987), white gourd (Ali et al., 2010) and common bean (Rahman et al., 2014), with promising results. However, the effect of foliar spray of NaCl and $CaCl_2$ on the growth and nutrient uptake of cowpea seedlings has been poorly quantified.

The objective of this study, therefore, was to investigate the effect of foliar application of NaCl and $CaCl_2$ on growth and nutrients accumulation and determine the main strategy of cowpea salt tolerance and identification of salt tolerant cultivars which could be grown in saline or calcareous soils.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of cowpea (*V. unguiculata* L. Walp) cvs. Mouola GG; Mouola PG, Garoua GG, Garoua PG and Tsacre provided by the breeding program of the Agronomic Institute for Research and Development of Cameroon were used in this study. The experiment was conducted from July 2011 to December 2011 at the Faculty of Science, University of Douala, Cameroon. Seeds with similar sizes and weights were washed with distilled water. The seeds were then sterilized for 20 min using sodium hypochlorite 3% (w/v) and rinsed 5 times using distilled water. Three days after germination, the primordial leaves were established. The seedlings were transferred

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to pots with 750 cm^3 capacity filled with 0.7 kg of sterilized quartz sand as substrate. The pots were kept in the laboratory (temperature: 26°C/20°C, light: 5000 lux for 12 h alternating periods and relative humidity of 61%) and supplied every three days with modified Wacquant (1974) nutrient solution containing 0.4 mM KNO_3, 0.2 mM KH_2PO_4 and 0.4 mM $MgSO_4$.

Treatments

A completely randomized block design with three treatments [0 (unsprayed), 50 mM NaCl or 50 mM $CaCl_2$] was adopted, with five replications of five seedlings each, with a total of 75 seedlings of all cultivars. The exogenous substances (NaCl and $CaCl_2$) were diluted using distilled water to get two different treatment solutions. Cowpea seedlings were sprayed with 30 ml NaCl or $CaCl_2$ twice a week until the end of the experiment (30 days) with a hand sprayer in the evening late hours to avoid dehydration at midday. Five randomly chosen plants from each cultivar and treatment were harvested after 30 days and used for physiological analysis.

Plant height and dry weights

The plant heights were measured every two days in each group of seedlings. Plant samples were harvested after 30 days and the harvested leaves, stems and roots were weighed to determine their fresh weights (FW). Dry weights (DW) were determined after the leaves, stems and roots were dried in an oven at 70°C for 72 h.

Nutrient analysis

For chemical analysis, the plant samples were dried in an oven for 72 h at 70°C. The Na^+ and K^+ contents in plants were determined according to Savouré (1980) methodology, while Mg^{2+}, Ca^{2+}, P and N contents were determined using the method described by Taffouo (1994).

Statistical analysis

All analyses were carried out in accordance with completely randomized design. Data were statistically analysed by analysis of variance using the SPSS software package (SPSS 10.0 for Windows 2001). The statistical differences between the experimental and control groups were established by the ANOVA. Each data point was the mean of five replicates (n = 5) and comparison between means was done using the Duncan's multiple range tests at 5% probability level.

RESULTS

Effect of foliar application of NaCl and $CaCl_2$ on dry weights of cowpea organs

The growth parameters such as shoot and root dry weights were measured after spraying exogenous substances (NaCl or $CaCl_2$) on leaves of cowpea cultivars (Figure 1A and B). The present study shows that NaCl treatment significantly inhibited the shoot dry weights of Garoua GG, Mouola PG and Tsacre cultivars while there were no significant differences in those of Garoua PG and Mouola GG, as compared with the control groups (Figure 1A). The application of NaCl did not cause significant changes in root dry weights of all cowpea cultivars, except in Tsacre (Figure 1B). The application of $CaCl_2$ significantly reduced the shoot dry weights of Garoua GG, Garoua PG, Mouola GG and Tsacre but no significant changes were observed in Mouola PG cultivars (Figure 1A), as compared with the control groups, while the root dry weights were not significantly reduced for these cultivars except in Tsacre (Figure 1B).

Effect of foliar application of NaCl and $CaCl_2$ on plant height

The present study shows that the foliar spray of NaCl and $CaCl_2$ caused a significant reduction in the plant height of the cowpea cultivars as compared to the control, except for Mouola GG (Figure 2). The magnitude of plant height reduction was highly dependent upon cowpea cultivars. The plant heights of Tsacre and Garoua GG cultivars showed higher decreases (44.87 and 39.28% respectively, compared to the control) than those of Mouola PG, Garoua PG and Mouola GG (21.94, 19.83 and 0.06%, respectively) under NaCl application (Figure 2).

The plant heights of all the cowpea cultivars decreased under $CaCl_2$ application. Cultivars Mouola PG, Garoua GG and Mouola GG were the least affected, with decreases of 17.98, 19.42 and 24.47%, respectively, compared to the control. Tsacre and Garoua PG cultivars showed the highest reductions in plant heights, with decreases of 44.00 and 33.45%, respectively (Figure 2).

Effect of foliar application of NaCl on nutrient uptake

The effects of foliar spray of NaCl on nutrient uptake were examined in the leaves of cowpea cultivars (Table 1). All cowpea cultivars accumulated Na^+ ($P<0.05$) in leaves compared to the control. The concentrations of Na^+ in leaves of Mouola GG and Garoua PG were higher (17.8 and 12.4 mg g^{-1} DW) than those of Mouola PG, Garoua GG and Tsacre (11.5, 10.5 and 10.1 mg g^{-1} DW), respectively. The Na^+/K^+ ratio in the leaves of Mouola GG and Garoua PG were slightly higher (0.70 and 0.59, respectively, compared to the control) than those of Mouola PG, Garoua GG and Tsacre (0.40, 0.30 and 0.37 respectively). The K^+, Ca^{2+} and Mg^{2+} concentrations in leaves of Mouola GG and Garoua PG cultivars were markedly decreased on salt treatment; however, those elements in leaves of Garoua GG, Mouola PG and Tsacre were not changed by NaCl application, as compared to the control (Table 1). On the other hand, N and P uptake of Garoua GG, Mouola PG and Tsacre cultivars were significantly decreased on salt treatment; however, those elements in leaves of Mouola GG and Garoua PG cultivars were not affected by NaCl application as compared to the control (Table 1).

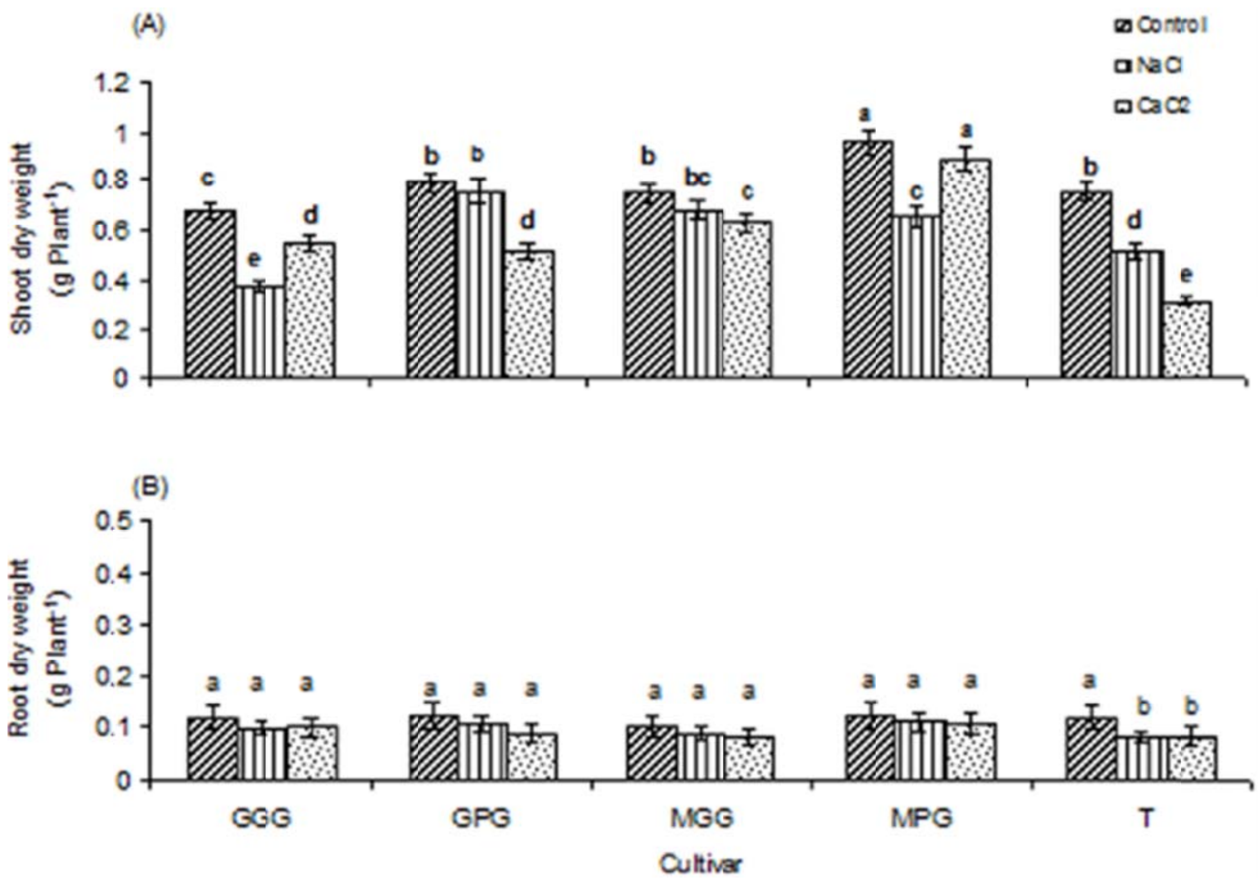

Figure 1. Shoot dry weight (A), root dry weight (B) of cowpea cultivars as affected by foliar application of NaCl and $CaCl_2$ during vegetative stage. Values are given as mean±SD, n = 5 for each cultivar; Garoua GG (GGG), Garoua PG (GPG), Mouola GG (MGG), Mouola PG (MPG) and Tsacre (T). Values of each bar followed by the same letter indicate no significant difference (P < 0.05) according to Duncan's test.

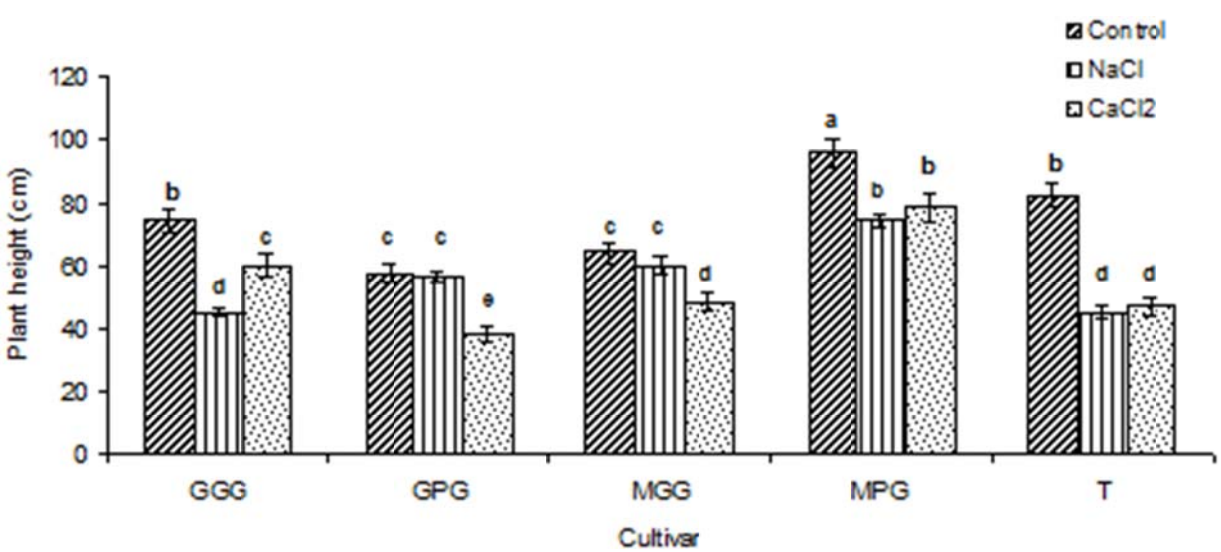

Figure 2. Plant height of cowpea cultivars as affected by foliar application of NaCl and $CaCl_2$ during vegetative stage. Values are given as mean±SD, n = 5 for each cultivar; Garoua GG (GGG), Garoua PG (GPG), Mouola GG (MGG), Mouola PG (MPG) and Tsacre (T). Values of each bar followed by the same letter indicate no significant difference (P < 0.05) according to Duncan's test.

Table 1. Effect of foliar application of NaCl on nutrient contents in leaves of cowpea cultivars during vegetative stage.

Cultivar	NaCl concentration (mM)	Nutrient concentrations (mg g^{-1} DW)						Na^+/K^+ ratio
		Na^+	K^+	Ca^{2+}	Mg^{2+}	N	P	
Garoua GG	0	4.1±0.2^d	38.7±1.3^a	16.7±1.7^{ab}	6.5±0.3^a	40.6±1.1^a	6.1±0.4^a	0.11
	50	10.9±0.4^c	36.5±1.4^a	15.3±0.6^b	5.1±0.1^a	26.4±0.4^c	4.2±0.3^b	0.30
Garoua PG	0	3.2±0.1^d	30.3±0.8^{bc}	19.1±1.1^a	7.4±0.5^a	42.2±2.3^a	6.3±0.4^a	0.11
	50	12.4±0.2^b	20.9±0.2^d	11.2±0.6^c	2.2±0.2^b	41.5±1.3^a	5.8±0.6^a	0.59
Mouola GG	0	2.9±0.1^d	33.8±1.6^b	22.8±1.3^a	6.4±0.7^a	40.7±0.7^a	6.1±0.7^a	0.09
	50	17.8±0.5^a	25.5±1.9^c	13.5±1.2^{bc}	2.1±0.3^b	38.6±1.3^a	5.3±0.4^a	0.70
Mouola PG	0	3.5±0.3^d	32.4±1.2^b	18.5±1.8^a	5.8±0.2^a	41.2±3.0^a	6.6±0.2^a	0.11
	50	11.5±0.4^{bc}	29.0±0.5^b	16.9±0.2^{ab}	4.2±0.7^a	33.6±2.1^b	4.6±0.1^b	0.40
Tsacre	0	2.9±0.7^d	29.5±1.9^b	21.7±2.1^a	7.5±0.8^a	43.9±1.1^a	6.7±0.6^a	0.10
	50	10.1±0.2^c	27.2±1.4^{bc}	19.6±1.6^a	6.3±0.4^a	32.8±2.0^b	4.1±0.2^b	0.37

Data represent mean ± SE (n = 5); within columns, means followed by the same letter are not significantly different (p<0.05) by Duncan test.

Table 2. Effect of foliar application of $CaCl_2$ on nutrient contents in leaves of cowpea cultivars during vegetative stage.

Cultivar	$CaCl_2$ concentration (mM)	Nutrient concentrations (mg g^{-1} DW)						Na^+/Ca^{2+} ratio
		Na^+	K^+	Ca^{2+}	Mg^{2+}	N	P	
Garoua GG	0	8.7±0.2^b	28.7±1.3^b	20.9±1.7^b	6.5±0.3^a	40.6±1.1^b	6.1±0.4^a	0.41
	50	4.1±0.7^c	26.3±2.9^c	19.1±0.2^b	4.6±0.2^b	39.1±0.4^b	5.6±0.3^a	0.21
Garoua PG	0	7.1±0.1^b	30.3±0.8^b	19.1±1.1^b	7.4±0.5^a	41.2±2.3^b	6.3±0.4^a	0.37
	50	3.2±0.4^c	33.0±1.9^a	17.8±1.5^{bc}	6.6±0.9^a	40.8±3.1^b	5.3±0.3^a	0.18
Mouola GG	0	13.1±0.1^a	25.8±1.6^c	22.8±1.3^b	6.4±0.7^a	40.7±0.7^b	6.1±0.7^a	0.57
	50	2.9±0.5^c	24.9±0.3^c	24.1±0.7^b	7.1±0.8^a	41.9±1.6^b	5.2±0.7^a	0.12
Mouola PG	0	13.5±0.3^{ab}	25.4±1.2^c	18.5±1.8^c	5.8±0.2^a	41.2±3.0^b	6.6±0.2^a	0.73
	50	8.5±0.3^c	26.9±2.2^c	26.2±1.6^a	6.0±1.4^a	40.4±2.1^b	5.1±0.4^a	0.32
Tsacre	0	6.2±0.7^b	26.5±1.9^c	21.7±2.1^b	7.5±0.8^a	43.9±1.1^a	6.7±0.6^a	0.29
	50	2.9±0.3^c	24.1±1.6^d	23.2±0.2^b	5.2±1.1^b	41.3±1.1^b	4.4±0.5^b	0.13

Data represent mean ± SE (n = 5); within columns, means followed by the same letter are not significantly different (p<0.05) by Duncan test.

Effect of foliar application of $CaCl_2$ on nutrient uptake

Changes in nutrient contents in the leaves of cowpea cultivars subjected to foliar application of $CaCl_2$ during seedling stages are presented in Table 2. As can be shown by statistical analyses (P<0.05), Na^+ content was significantly decreased by $CaCl_2$ application in the leaves of all cowpea cultivars. In this study, $CaCl_2$ treatment decreased significantly K^+ and Mg^{2+} contents in the leaves of Garoua GG and Tsacre cultivars while those of Mouola GG and Mouola PG did not significantly affected. On the other hand, Ca^{2+} content was significantly increased in the leaves of Mouola PG by the application of Ca^{2+}, as compared to the control and the other cultivars studied (Table 2). The Na^+/Ca^{2+} ratio in the leaves of Mouola PG was slightly higher (0.32) than those of Garoua GG, Garoua PG, Mouola GG and Tsacre cultivars (0.21, 0.18, 0.12 and 0.13), respectively as compared to the control. $CaCl_2$ application decreased significantly N and P contents in the leaves of Tsacre cultivar (Table 2).

DISCUSSION

In the present study, NaCl treatment inhibited significantly the shoot dry weights of Garoua GG, Mouola PG and Tsacre cultivars while there were no significant differences in those of Garoua PG and Mouola GG as compared to the control (Figure 1A). Crop responses to foliar application have been mixed either positive or negative responses depending on crop species and nutrients applied. Numerous studies conducted on foliar fertilization with N, P, K and S during early vegetative growth stages or late reproductive growth stages showed inconsistent growth and grain yields increases (Parker and Boswel, 1980; Haq and Mallarino, 2000; Nelson et al., 2005). These authors reported that leaf damage due to foliar fertilization sometimes were severe enough to cause growth and yield reductions. Reduced seedling growth under saline conditions has also been reported by Huang and Redmann (1995) on barley, Taffouo et al. (2010a) on bambara groundnut and Erum Mukhtar et al. (2013) on canola, respectively. The effect of salinity application on seedling growth found a reduction in shoot dry weight that may be due to toxic effects of NaCl used as well as unbalanced nutrient uptake by the seedlings (Grigore et al., 2011). According to Jaleel et al. (2009), the reduction of plant growth under salinity is the result of the alteration of many physiological activities in the plant, such as photosynthetic activity, mineral uptake and antioxidant activity. Rahman et al. (2008) reported that salinity depressed shoot than root dry weights and increased root/shoot ratio. Shoot dry weight of all cowpea cultivars (Figure 1A) was significantly reduced with $CaCl_2$ treatment except in Mouola PG compared to control, while the root dry weight was not significantly reduced for these cultivars except in Tsacre (Figure 2A). According to Levitt (1980), Mouola PG cultivar can be considered as calciophile plants that are able to grow and develop on calcareous soils at high Ca^{2+} levels. In those obligate calciophile plants, the Ca^{2+} plays an essential role in processes that preserve the structural and functional integrity of plant cell membranes, stabilize cell wall structures, regulate ion transport and selectivity, and control ion-exchange behaviour as well as cell wall enzyme activities (Marschner, 1995). In this study, the shoot dry weights of Garoua GG, Garoua PG, Mouola GG and Tsacre cultivars were significantly ($P<0.05$) reduced with $CaCl_2$ application; those cultivars appear to be typical calciophobe plants. Similar reductions in the dry weights were observed in *Gossypium hirsutum* plants growing under $CaCl_2$ conditions (Amuthavalli et al., 2012). In the presence of excess Ca^{2+}, the calciophobe plants would absorb considerable Ca^{2+} at the expensive of other ions, and would therefore suffer from a deficiency. This implies that the Ca^{2+} resistance of the calciophile plants is due to avoidance of a secondary deficiency stress by Ca^{2+} exclusion (Levitt, 1980).

The present study shows that the foliar application of $NaCl_2$ caused a significant reduction in the plant heights of the cowpea cultivars as compared to the control, except for Mouola GG (Figure 2). Numerous studies have reported the reduction of plant heights stimulated by NaCl salinity (Khan et al., 2000; Zadeh and Naeini, 2007). In this work, $CaCl_2$ application caused a significant reduc-tion in the plant heights of all the cowpea cultivars as compared to the control (Figure 2). Similar observa-tions for plant height reductions by $CaCl_2$ application were reported in *G. hirsutum* (Amuthavalli et al., 2012). For these cultivars, the $CaCl_2$ may reduce the growth by upsetting water and nutritional balance of the plant (Al-Khateeb, 2006).

In this research, the plant height, the dry weight of roots and shoot of the Garoua GG, Mouola PG and Tsacre showed higher decreases compared to those of Mouola GG and Garoua PG cultivars after 4 weeks of NaCl application (Figures 1 and 2). These results demonstrate that Garoua GG, Mouola PG and Tsacre cultivars, in common with certain other leguminous plant (e.g. beans), are highly sensitive to salt with severe effects even at 50 mM NaCl (Levitt, 1980). As stated by Munns (2002), the reduction of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of $NaCl_2$. It can inhibit plant growth by a range of mechanisms, including low external water potential, ion toxicity and interference with the uptake of nutrients (Salam et al., 2011). According to Yousif et al. (2010) the growth of glycophytes decreases with salinity, while that of halophytes improves. In this work, the growth of Garoua PG and Mouola GG cultivars were less affected under NaCl application, agreeing with previous data reported in *Ceriops roxburghiana*, *Phaseolus adenanthus*, *Lagenaria siceraria, Vigna subterranea*, *Tetragonia tetragonioides* and *Oryza sativa* genotypes described as salt-tolerant (Rajest et al., 1998; Taffouo et al., 2008; 2010b; Yousif et al., 2010; Mehede et al., 2014). There are a great number of plant species which are regarded as salt tolerant, the most competitive being those that are able to become established, grow to maturity and survive until they are able to reproduce (Turan et al., 2007).

In this study, NaCl application increased significantly Na^+ concentration and reduced K^+, Ca^{2+} and Mg^{2+} contents in the leaves of Mouola GG and Garoua PG cultivars, however, those elements in the leaves of Garoua GG, Mouola PG and Tsacre were not changed by NaCl Treatment (Table 1). The increase of Na^+ accumulation in Mouola GG and Garoua PG cultivars was associated with reduced K^+, Mg^{2+} and Ca^{2+}, indicating a restriction in the uptake of these nutrients, as noted in other salt tolerant plants (Sagir et al., 2002; Yousif et al., 2010). Under saline conditions, tolerant plants tend to take up and accumulate Na^+ in their vacuoles and use it as an osmoticum (Glenn and Brown, 1999). Similar outcome were obtained earlier by Al-Khateeb (2006) and Turan et al. (2007). Salinity is known to significantly reduce K^+ uptake related with reduce intracellular K^+ concentration especially in the vacuolar pool (Cuin et al., 2003). The decrease in K^+ concentration under NaCl application may be due to the competition of Na^+ with uptake of K^+, resulting in Na^+/K^+ antagonism (Carvajal et al., 2000; Turan et al., 2007). High Na^+ levels in the external medium greatly reduce the physiochemical activity of the dissolved Ca^{2+} and may thus displace Ca^{2+} from the plasma membrane of the root cells (Cramer et al., 1985). In this study, the uptake of N by Garoua GG, Mouola PG and Tsacre cultivars was decreased and that of Garoua PG and Mouola GG was not affected by NaCl application (Table 1). According to Parida and Das (2004), salinity reduces N uptake in many plants and this is attributed to antagonism between NO_3^- and Cl^-. In this work, the N uptake of Garoua PG and Mouola GG cultivars was not affected by NaCl application. According to Hu and Schmidhalter (2005), N is an essential nutrient element that plants require in the largest amounts for the biosynthesis of nitrogenous organic solutes in plants; therefore N deficiency inhibits plant growth. Adequate supply of N is beneficial for carbohydrate and protein metabolism that promotes cell division and enlargement resulting in higher yield (Shehu et al., 2010). The results showed that NaCl application decreased P concentration in leaves of all cowpea cultivar (Table 1). Grattan and Grieve (1999) reported that P concentration in plants depended on the species, growth conditions and cultivar.

Changes in nutrient contents in leaves of cowpea cultivars subjected to foliar applied $CaCl_2$ during seedling stage are presented in Table 2. As can be shown by statistical analyses (P<0.05), Na^+ content was significantly decreased by $CaCl_2$ application in leaves of all cowpea cultivars. Externally supplied Ca^{2+} reduces the toxic effects of NaCl, presumably by facilitating a high K^+/Na^+ selectivity (Liu and Zhu, 1998). Other beneficial effects that $CaCl_2$ has on plant physiology include membrane permeability and reduction in Na^+ concentration (Amuthavalli et al., 2012). According to Arshi et al. (2010) the Ca^{2+} ameliorated the deleterious effects of NaCl stress and stimulated the plant metabolism and growth when application of NaCl and $CaCl_2$ was combined than with the NaCl treatment alone. In this study, $CaCl_2$ treatment showed significant increase of Ca^{2+} in the leaves of Mouola PG (Table 2). Arshi et al. (2010) found significant increase of Ca^{2+} concentration in plant parts of *Cichorium intybus* under $CaCl_2$ application. $CaCl_{2,}$ as compared to $NaCl_2$ treatment, which may be necessary for the osmotic adjustment by accumulation of inorganic ions (K^+, Ca^{2+} and Mg^{2+}) in cowpea leaves, contributing to the maintenance of water uptake and cell turgor, allowing for physiological processes like stomatal conductance, photosynthesis and cell expansion (Munns, 2002).

In conclusion, the growth of Mouola GG and Garoua PG cultivars were less affected under NaCl application but those of Mouola PG, Garoua GG and Tsacre were markedly decreased, indicating that Mouola GG and Garoua PG cultivars are salt tolerant. The main strategy of salt tolerance in Mouola GG and Garoua PG seems to be as a result of increased osmotic adjustment through the accumulation of Na^+ in leaves while in salt sensitive Mouola PG, Garoua GG and Tsacre, the osmotic adjustment may be due to the accumulation of inorganic ions (K^+, Ca^{2+} and Mg^{2+}) in leaves, contributing to the maintenance of water uptake and cell turgor, allowing for physiological processes. The application of $CaCl_2$ ameliorated the deleterious effects of NaCl stress and stimulated the plant growth by reducing significantly Na^+ content in the leaves of all cowpea cultivars. $CaCl_2$ treatment significantly increased growth parameters and nutrients uptake especially Ca^{2+} in Mouola PG. This cultivar may be a calciophile plant that is able to grow and develop on calcareous soils. In the analysis of growth parameters measured, the results suggested that Mouola GG and Garoua PG cultivars were relatively more tolerant to salinity than others, suggesting that those cultivars could be cultivated in the environment with varying salinity.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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