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Variation of some growth, agronomical and biochemical parameters of *Vigna unguiculata* (L. Walp) under salinity stress

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The variation of some growth, agronomical and biochemical parameters of *Vigna unguiculata* (L. Walp) under salinity stress were investigated in the greenhouse and farm conditions. Plants were subjected to 0, 50, 100 and 200 mM NaCl. The increases of NaCl in the culture medium significantly (P< 0.001) decreased the growth parameters (number of leaves, noose diameter, leaf area and stem height), dry biomass (roots, shoots and total), chlorophyll contents and micronutrients (Zn, Fe and Mn) from 100 mM NaCl. Macronutrients (K, Ca and Mg) and K/Na ratio significantly (P < 0.001) decreased in plant partitioning with increasing salinity. The metabolites (proline, total soluble carbohydrates, soluble proteins, total free amino acids, total phenolic and flavonoids contents) significantly (P < 0.001) increased from 50 mM NaCl in variety Ekomcalle compared to Mouala GG and could be considered as biochemical indicators of early selection and osmotic adjustment ability for salt tolerant plants. At 50 mM NaCl, a relative tolerance of variety Ekomcalle compared to Mouala GG for all agronomic parameters (the flowering time, number of flowers per plant, number of pods/plant, number of seeds/pod, pod yield, seed yield and harvest index) was observed suggesting that Ekomcalle could increase cowpeas production on salty soils.

Key words: *Vigna unguiculata*, salinity, growth, metabolites, mineral uptake, agronomic parameters.

INTRODUCTION

In the coastal, arid and semi-arid regions, the high soil salinity severely impacts crop production by limiting plant growth and development (Ashraf and Ali, 2008; Santhi et al., 2013; Gouveitcha et al., 2021). In the other words, salinity negatively influences the homeostatic balance of water potential and ion uptake within a plant. Under high salinity, sodium toxicity may cause a range of disorders affecting germination, development, photosynthesis,

protein synthesis, lipid metabolism, leaf chlorosis, and senescence (Rahneshana et al., 2018). The responses to the changes carried out by salt stress are often accompanied by a decrease in leaf area, increase of leaf thickness and succulence, abscission of leaves, necrosis of plant, and decrease of internode lengths (Parida and Das, 2005; Rahneshana et al., 2018). Salinization of soil is caused by two main factors: high evaporation relative to precipitation in association with weak leaching in soils, and salt accumulation as a result of the use of saline water (Singh, 2015; Hand et al., 2021). Previous workers like Rhoades and Loveday (1990), Santhi et al. (2013), and Hand et al. (2017) estimated that, nowadays 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity.

Facing the detrimental effects of salinity stress in many species, compatible osmoprotectants, such as proline, total soluble carbohydrates, proteins content and total free amino acids are produced to protect the cells against the adverse effects from salt stress (Meguekam et al., 2014; Nouck et al., 2016; Hand et al., 2021). Plants develop several mechanisms that induce tolerance to overcome salinity effects like the osmotic adjustment mechanism which consists of the sequestration of large amounts of salt ions in the vacuole and/or synthesis of organic osmolytes (Munns, 2002; Rais et al., 2013). The impact of salinity stress and mineral nutrient solution on growth and productivity has been largely studies on different crop plants (Li et al., 2008; Taffouo et al., 2010; Hand et al., 2021). It has been observed that high levels of sodium (Na) inhibit potassium (K), calcium (Ca) and magnesium (Mg) uptake in leaves, which results in a K/Na antagonism. Net photosynthesis is affected strongly by NaCl conditions as it is related directly to the closure of stomata due to low levels of intercellular CO_2 (Turan et al., 2007). Plants cultivated in saline areas often showed microelements such as zinc (Zn), ion (Fe) and manganese (Mn) deficiency symptoms due to their low solubility (Hand et al., 2021). NaCl may change the solubility of these micronutrients and their available concentration in the soil (Sharply et al., 1992).

Cowpea is an economically important crop with nutritional and medicinal values. It can consist of 25% protein and is low in anti-nutritional factors, provides a rich source of proteins and calories, as well as minerals and vitamins (Sreerama et al., 2012). Their cultivation enables farmers and traders to generate more income, provide employment opportunities and so improve the economy of the nation. With the rising trend of global population, the demand for food is also on the increase. Cowpeas are grown for dry seeds and as leafy vegetables in different parts of the world (Summerfield et al., 1974; Singh et al., 2003). They resist drought stress and can recover rapidly during vegetative growth stage by re-watering because of their efficiency in using soil water (French, 1998). The main challenge in salty areas therefore is how to enhance plant growth to improve crop production under saline conditions. In order to address these challenges to the world's food security, the identification of salt tolerant crop is considered as a valuable tool to enhance productivity and develop sustainable agriculture in salt affected lands. The main objective of this work was to compare the responses of two cowpeas cultivars exhibiting differences in salt tolerance on the growth, chlorophyll content, macro and micronutrient uptake, metabolites and agronomic parameters under saline stress in order to identify a potential biochemical indicator for early selection of salt-tolerant plants and discuss the physiological responses and adaptive strategies.

MATERIALS AND METHODS

Study area and plant

The study was carried out in a greenhouse of Faculty of Science, The University of Bamenda located in Bambili-Cameroon, with elevation of 1444 m, found in Mezam division of the NorthWest region of Cameroon. The work was carried out from December 2020 to April 2022. Average rain fall, and temperatures are 854 mm/year and 30°C and relative humidity is nearest to 84%. Prevailing winds carry the tropical monsoon. The seeds of two varieties of cowpeas (Ekomcalle and Mouala GG) used for the experiment were obtained from the breeding program of Agronomic Institute Research and Development (IRAD) Nkolbisson, Yaounde-Cameroon. The two varieties were chosen for their resistance to pathogens and socio-economic rank.

Plant growth conditions and salt treatments

Cowpeas seeds were sterilized after a viability test with 3% of sodium hypochlorite for 10 min, washed eight times with demineralized water and planted into 2 L polythene bags previously filled with 2 kg of sterilized sand, with one plant each and five replications per treatment. The plants were arranged in a complete randomized block design and daily supplied with a modified nutrient solution (in g L^{-1}): of 150 g $Ca(NO_3)_2$, 70 g KNO_3, 15 g Fe–EDTA, 0.14 g KH_2PO_4, 1.60 g K_2SO_4, 11 g $MgSO_4$, 2.5 g $CaSO_4$, 1.18 g $MnSO_4$, 0.16 g $ZnSO_4$, 3.10 g H_3BO_4, 0.17 g $CuSO_4$ and 0.08 g MOO_3 (Hoagland and Arnon, 1950). The pH of the nutrient solution was adjusted to 7.0 by adding HNO_3 0.1 mM. Plants were subjected to different salt concentrations (0, 50, 100 and 200 mM NaCl) with 0 mM NaCl as a control in the culture medium for a period of six weeks to determine the physiological and biochemical responses of cultivars to salt stress. The average day and night temperatures in the greenhouse were between 25 and 18°C, respectively during the growth period with average relative air humidity of 75%. Parameters evaluated under greenhouse conditions: number of leaves, noose diameter, stem height, leaf area, dry biomass (roots and shoots) and ratio (roots/shoots), chlorophyll (a+b), total soluble proteins, total free amino acids, proline, total soluble carbohydrates, total phenol and flavonoids content and mineral (Na, K, Ca, Mg, Fe, Mn and Zn contents) of roots and shoots.

Plant growth

The leaf area, stem height, number of leaves, noose diameter, and dry weights were recorded after six weeks. The leaf area was calculated using the formula, surface area (cm^2) = 1/3 (length × width). The parts (Roots and shoots) of the plant were dried

separately (roots and shoots) at 65°C for 72 h in an oven and their dry biomasses were determined (Ndouma et al., 2020). The stem height was determined by measuring with a ruler.

Mineral uptake

In order to extract Na, K, Ca, Mg, Fe, Zn and Mn, 2 g of dried plant organs were separately added to 20 mL of Hcl for 24 h. The filtrate was analysed with an atomic absorption spectrophotometer (Rayleigh WFX-100) using the Pauwels et al. (1992) method.

Chlorophyll content

The Arnon (1949) method was used to determine chlorophyll (a+b) content. 1 g of fresh cowpeas leaves were crushed, and their contents extracted with 80% of alkaline acetone (v/v). The filtrate was analyzed using a spectrophotometer (Pharmaspec model UV-1700) at 645 and 663 nm wavelengths.

Biochemical parameters

Primary metabolites

Soluble protein content: PR content was determined by Bradford (1976) method. An appropriate volume (0 - 100 µl) of sample was put into a test tube and the total volume was augmented to 100 µl with distilled water. 1 mL of Bradford working solution was added to the sample. Then the mixture was thoroughly mixed with a vortex mixer. The absorbance
was read at 595 nm with a spectrophotometer UV (PG instruments T60) after 2 min. The standard curve was used to determine PR content.

Proline content: PRO was estimated using Bates et al. (1973) method. 0.5 g of fresh leaves were weighed, crushed and put inside a flask. 10 mL of 3% aqueous sulphosalicylic acid was poured in the same flask. The mixture was homogenized, and then filtered with a Whatman No. 1 filter paper. 2 mL of filtered solution was poured into a test tube, and then 2 mL of glacial acetic acid and ninhydrin acid were respectively added into the same tube. The test tube was heated in a warm water bath for 1 h. The reaction was stopped by placing the test tube in an ice bath. 4 mL of toluene was added to the test tube and stirred. A purple-coloured mixture was obtained and its absorbance was read at 520 nm by spectrophotometer UV (Pharmaspec model UV-1700). The concentration of PRO was determined using the standard curve (µg/g FW).

Soluble carbohydrate content: CH was obtained using phenol-sulphuric acid (Dubois et al., 1956). The fresh leaves (1 g) were ground in 5 mL of 80% ethanol and filtered with the Whatman No. 1 filter paper. The extract was diluted with deionized water to make up 50 mL. 1 mL of sample was poured in test tube, followed by the addition of 1 mL of phenol solution and 5 mL of sulphuric acid. The mixture was then swirled. The absorbance was read at 490 nm using a spectrophotometer (Pharmaspec UV-1700 model). The quantity of CH was deduced from the glucose standard curve.

Total free amino acids content: FAA content was determined by the ninhydrin method (Yemm and Cocking, 1955). Fresh leaves (1 g) were ground in 5 mL of ethanol 80%, amino acids were then extracted using reflux technique in boiling ethanol for 30 min. After decanting, the supernatant was filtered using Whatman No. 1 filter paper. The filtrate was collected, and the residue used to repeat the extraction. The two filtrates were mixed, and the raw extract of amino acid content was measured using ninhydrine method. The absorbance of purplish-blue complex was read at 570 nm wavelength. The standard curve was established using 0.1 mg/mL of glycine.

Secondary metabolites

Flavonoids content: FLA of crude extract was determined by using the aluminium chloride colorimetric method (Chang et al., 2002). 50 µL of crude extract (1 mg/mL ethanol) was made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution was added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was recorded on spectrophotometer (Pharmaspec UV-1700 model) at 510 nm wavelength. FLA content was calculated from a grutin calibration curve, and the result was expressed as grutin equivalent per g dry weight.

Total phenolic content: TP content of the extract was determined by the Folin Ciocalteu method (Marigo, 1973). Subsamples (1 g) of fresh leaves were ground at 4°C in 3 mL of 0.1 N HCl. After incubation at 4°C for 20 min, the homogenate was centrifuged at 6000 g for 40 min. The supernatant was collected, the pellet re-suspended in 3 mL of 0.1 N HCl and centrifuged as previously. The two supernatants were mixed. 15 µL of the mixture was extracted and 100 µL Folin-Ciocalteu reagents, 0.5 mL of 20% Na_2CO_3 added before incubating at 40°C for 20 min and. the absorbance was read at 720 nm using a spectrophotometer (Pharmaspec UV-1700 model). A standard curve was established using chlorogenic acid. TP content was expressed as mg g^{-1} fresh weight.

Agronomic parameters

The farm experiment was done at the University of Bamenda agricultural research farm located in Bambili-Cameroon. Bambili, elevation 1444 m, found in Mezam division of the NorthWest region of Cameroon. The work was carried out from March 2020 to April 2022. Average rain fall, and temperatures are 854 mm/year and 31°C and relative humidity is nearest to 84%. Prevailing winds carry the tropical monsoon. Table 1 shows the physico-chemical properties of the soil taken from 0 to 20 cm depth of the experimental site in Bambili. The plots were arranged in a randomized complete block design within a split-plot layout with two main treatments 0 and 50 mM NaCl, three replications and 0 as a control. The surface area of the plots was 5×1 m surface with intra spacing of 1.5 m. The cultivars were planted at 0.50 m spacing. Data from crop yield were collected from fifteen plants per repetition for each variant of the experiment. The agronomic parameters assessed were the flowering time, number of flowers per plant, number of pods/plant, number of seeds/pod, pod yield, seed yield and harvest index. The number of flowers was determined by counting flowers every week for each treatment until the emergence of the first pods. The number of pods per plant was determined every week for each treatment until harvest time. The flowering time was gotten by noting the date of first appearance of flower for each treatment. The yield was obtained:

Yield (t/h) = Total production (tonne)/surface (hectare).

The harvest index (HI) was calculated (Bijalwan and Manmohan, 2014):

HI = WP/(WP+ Biomass (shoot and root)) × 100 [30]

Table 1. Physico-chemical properties of the soil (Bambili, Cameroon). Source: Experimental data analysis.

Property	Values	Property	Values
Fine sand (%)	17.66±1.52	Ratio C/N	2.68±0.03
Coarse sand (%)	16.33±0.57	Exchangeable cations (cmol + kg^{-1})	0.2±0.04
Fine silt (%)	15.16±0.76	Cation Exchange capacity (cmol + kg^{-1})	11.23±0.25
Coarse silt (%)	15.66±1.15	Phosphoros (ppm)	63.73±0.25
Clay (%)	36.66±1.52	Potassium (g kg^{-1})	0.01±0.01
Moisture content (%)	14.37±2.37	Calcium (g kg^{-1})	2.22±0.07
porosity (%)	38.33±1.52	Magnesium (g kg^{-1})	1.35±0.14
pH water	5.45±0.2	Sodium (g kg^{-1})	0.04±0.01
pH kcl	4.93±0.05	Sulfur (g kg^{-1})	3.56±0.03
Organic carbon (%)	5.04±0.05	Iron (g kg^{-1})	117.97±6.90
Organic mater (%)	8.69±0.20	Conductivity (mS/cm)	0.08±0.01
Nitrogen (%)	1.88±0.07		

Source: Experimental data analysis.

where HI = Harvest Index and WP = Weight of pods.

Statistical analysis

The experiment was performed using a completely randomized design. All data were presented in terms of mean (± standard deviation), statistically analysed using Graph pad Prism version 5.01 and subjected to analysis of variance (ANOVA). Statistical differences between treatment means were established using the Fisher Least Significant Difference (LSD) at $P < 0.05$. Probability level was calculated using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Plant growth

Growth parameters

The inhibition effect of salinity on the growth of *V. unguiculata* was significantly (P<0.01) noted from 100 mM NaCl for both study varieties (Table 2). The cultivar Ekomcalle showed greater tolerance to NaCl treatment than cultivar Mouala GG in all growth parameters. The interactions cultivar × salinity was significant (P<0.05) for stem height and leaf area total dry weight (Table 1). These results are in line with the findings of Rais et al. (2013), Hand et al. (2017), and Ndouma et al. (2020). These authors explained that salinity stress negatively affects plant growth and photosynthetic functions of crop plants due to the presence of excessive amounts of Na$^+$ and Cl$^-$ ions which cause osmotic effects, nutrients imbalance, and inhibition of enzymes activities, cell division and affect plants metabolism.

The number of leaves decreased significantly ($p < 0.05$) with increased salt concentration from (100 mM NaCl) (Table 2). These results are consistent with the reports of Shrivastava and Kumar (2015) on soil salinity and Kamran et al. (2020) on hazardous impacts on soil salinity. These authors reported that soil salinity leads to a reduction in the amount of water taken up by plants and the accumulation of Na$^+$ in the leaves, eventually injuring the cells. The noose diameter significantly ($p < 0.05$) reduced with increasing salinity (100 mM NaCl) (Table 2). These results are substantiated by those of Nassar et al. (2020) on *Triticum aestivum* L. and Santhi et al. (2013) on *Solanum nigrum* L. According to their findings, the reduction of noose diameter with increasing salinity was due to physiological responses from the plant due to modifications of the ionic balance, mineral nutrition and photosynthetic efficiency which affect the formation of both phloem and cells. Similar observations were made for leaf area and stem height. Both parameters also decreased with increasing soil salinity. A significant decrease ($p < 0.05$) of leaf area and stem height was observed from 100 mM NaCl (Table 2). Heidari et al. (2014) affirmed this pattern in his work, stating that the loss of water by plant cells caused the loss of cell turgor and shrinkage, reducing the rate of cell elongation. This contributed to the formation of shorter plant stems and smaller leaves with reduced leaf area. Karam et al. (2020) in conformity with this trend reported that photosynthetic rates were retarded under high salinity due to decreased efficiency of growth hormones, resulting in decreased stem height and low water uptake, leading to lesser leaf area development. In this study, the improvement observed in the Ekomcalle variety was much more for all growth parameters as compared to the Mouala GG variety. This is because, effect of salinity on photosynthetic activities, ions imbalance, cell division and growth of Ekomcalle crop plant are less than in the Mouala GG variety (Rais et al., 2013; Heidari et al., 2014).

Dry weight partitioning

Generally, salinity has a negative impact on plant

Table 2. Effects of salinity stress on plant growth of cowpeas cultivars at different salt concentrations.

Cultivar	Treatment (mM NaCl)	Roots dry weight	Plant dry weight (g plant-1) Shoots dry weight	Total plant dry weight	Roots/Shoots	Stem height (cm)	Leaf area (cm^2)	Number of leaves	Noose diameter (mm)
Mouala GG	0	2.26±0.08[a]	4.68±0.07[a]	6.94±0.15[a]	0.48±0.01[a]	28.34±0.85[a]	8.28±0.4[a]	17.6±0.54[a]	1.63±0.01[a]
	50	2.14±0.05[a]	4.38±0.09[a]	6.52±0.08[a]	0.48±0.02[a]	26.06±0.60[a]	7.85±0.92[a]	14.8±0.83[b]	1.54±0.02[a]
	100	1.30±0.06[c]	3.43±0.07[c]	4.74±0.09[c]	0.37±0.02[c]	21.96±1.11[c]	5.96±0.21[bc]	11.8±0.83[c]	1.24±0.08[c]
	200	0.70±0.05[d]	1.92±0.03[d]	2.63±0.07[d]	0.37±0.16[c]	16.04±1.05[d]	4.72±0.44[c]	9.6±0.55[d]	1.07±0.07[d]
Ekomcalle	0	2.68±0.05[a]	5,84±0.02[e]	7.60±2.05[e]	0.45±0.01[d]	37.18±1.34[e]	10.5±0.78[e]	19.6±0.54[e]	2.03±0.08[e]
	50	2.41±0.03[a]	5.40±0.04[e]	7.82±0.04[e]	0.44±0.01[d]	35.14±1.12[e]	10.2±0.17[e]	18.4±0.53[e]	1.85±0.04[f]
	100	1.71±0.05[g]	4.27±0.08[e]	5.98±0.09[g]	0.39±0.01[g]	29.3±0.54[a]	7.38±0.40[a]	15.6±0.89[b]	1.46±0.01[a]
	200	1.04±0.10[h]	2.81±0.06[h]	3.86±0.13[h]	0.37±0.03[g]	23.58±1.15[h]	6.06±0.67[b]	10.4±0.54[d]	1.22±0.01[c]
Two way ANOVA Result									
Cultivar (C)		*	*	*	*	*	*	*	*
Salt treatments (S)		*	*	*	*	*	*	*	*
Interactions C×S		ns	*	*	ns	*	*	ns	ns

Mean results of five replications ± SD; within each column, mean followed by the same letter are not significantly different (p < 0.05). The asterisk indicates the interactions between cultivars and salt treatment. *significant (p < 0.05) and ns= Not significant.
Source: Experimental data analysis.

dry biomass. The dry biomass of *V. unguiculata* significantly decreased (p < 0.001) in plant partitions with increased salinity (Table 2). These results are in conformity with those of Heidari (2012). He explained that the reduced ability of plants to take in water under saline conditions caused slower growth and also that excessive salts from the transpiration stream injured the cells of transpiring leaves and finally contribute to reduced plant biomass. The results also showed that the dry biomass of the roots decreased significantly (p < 0.01) from 100 mM NaCl for variety Mouala GG while those of the Ekomcalle variety decreased significantly (p < 0.05) at the same concentration. The same observations were seen in the shoots and total dry biomass of both varieties. The dry biomass of variety Ekomcalle improved more compared to variety Mouala GG in

all concentrations and plant parts (Table 2). This can be explained by the inhibition of minerals uptake in the tissues and decreased in the photosynthetic activities of Mouala GG variety which caused the decrease in dry biomass as compared to that of the Ekomcalle variety (Hand et al., 2017; Ndouma et al., 2020). It was also observed that, the reduction in water uptake and the hydrolysis of food reserves from storage tissue to the developing embryo (Menguekam et al., 2014) were less for the Ekomcalle variety. This is proof that this variety is more tolerant compared to Mouala GG. The ratio root/shoot dry biomass decreases significantly (p < 0.05) in the culture medium from 100 mM NaCl for both varieties (Table 2). These results corroborate those of Assimakoupoulou et al. (2015) on three green bean cultivars. He explained that the

decreased of root/shoot ratio may improve salinity tolerance by restricting the flux of toxic ions to the shoot, delaying the onset of the tolerance threshold.

Mineral distribution

The Na$^+$ concentration significantly increased (p < 0.001) with increased salinity in both shoots and roots while K$^+$, Ca^{2+} and Mg^{2+} decreased significantly (p < 0.001) (Table 3). According to Assaha et al. (2017), Na$^+$ in plants increased with increased salinity due to imbalance in the Na$^+$/K$^+$ transporters. Similarly, Greenway and Munns (1980) and Taffouo et al. (2010) showed that, the ratio is distorted by the high salt concentration in the soil, which enhanced the uptake of Na$^+$ and

Table 3. Effects of salinity stress on mineral uptake (µg g^{-1} DW) of cowpeas after six weeks at different salt concentrations.

Cultivar		Treatment	Mineral uptake (µg g^{-1}DW)							
		mM NaCl	Na	K	Ca	Mg	Zn	Mn	Fe	K/Na
Mouala GG	Roots	0	1.41±0.06[a]	8.6±0.72[a]	14.71±1.35[a]	17.51±1.23[a]	233.34±22.14[a]	205.33±26.14[a]	394.74±37.18[a]	6.09[a]
		50	2.13±0.04[b]	4.34±0.66[b]	8.31±1.01[b]	14.73±1.05[b]	201.11±26.53[b]	184.64±20.19[b]	378.64±30.14[b]	2.03[b]
		100	3.85±0.08[c]	2.56±0.07[c]	5.02±0.03[c]	11.22±0.72[c]	163.23±10.28[c]	133,73±28.77[c]	329.35±33.82[c]	0.66[c]
		200	5.96±0.55[d]	1.51±0.04[d]	2.33±0.05[d]	5.22±0.06[d]	138.29±17.19[d]	126.39±19.46[d]	301.17±43.89[d]	0.25[d]
	Shoots	0	2.71±0.04[b]	25.81±1.32[e]	31.22±2.6[e]	43.12±4.03[e]	341.55±22.14[e]	315.86±25.81[e]	554.93±55.61[e]	9.52[e]
		50	4.83±0.25[f]	20.11±2.64[f]	26.55±3.12[f]	35.23±2.88[f]	317.24±17.32[f]	286.12±37.94[f]	521.58±34.19[f]	4.16[f]
		100	10.31±0.64[g]	15.73±2.13[g]	20.74±1.83[g]	26.11±1.36[g]	271.43±33.56[g]	257.37±30.17[g]	491.33±29.72[g]	1.52[g]
		200	14.44±1.13[h]	11.71±1.22[h]	14.25±1.33[h]	21.93±3.24[h]	209.64±27.23[h]	232.67±19.41[h]	417.94±27.66[h]	0.81[c]
Ekomcalle	Roots	0	1.69±0.09[a]	10.11±1.03[i]	13.22±0.99[i]	20.11±1.53[h]	251.61±22.13[i]	221.64±23.52[d]	422.88±34.79[h]	5.98[a]
		50	2.94±0.07[b]	6.67±0.53[j]	12.33±1.28[j]	17.88±1.52[a]	229.89±17.68[j]	201.28±19.58[a]	401.54±29.71[j]	2.26[b]
		100	5.22±0.06[d]	3.01±0.07[c]	8.89±0.25[b]	12.42±0.94[c]	178.45±20.34[k]	147.88±14.73[c]	379.89±22.84[b]	0.57[c]
		200	7.62±0.41[l]	1.45±0.08[d]	5.31±0.07[c]	8.08±0,41[l]	154.33±22.76[l]	139.47±18.35[c]	319.73±28.64[l]	0.19[l]
	Shoots	0	2.34±0.04[b]	29.73±2.9[m]	43.66±5.23[m]	51.29±5.44[m]	366.24±33.26[m]	331.28±24.81[m]	601.63±42.49[m]	12.70[m]
		50	6.87±0.09[n]	23.88±3.32[n]	32.67±2.44[e]	42.33±4.91[e]	349.69±44.87[e]	306.73±31.84[n]	587.96±37.12[n]	3.47[n]
		100	11.86±0.88[o]	18.22±1.66[o]	24.06±1.23[o]	31.74±3.28[o]	294.71±26.19[h]	279.54±22.19[o]	514.46±19.12[f]	1.53[g]
		200	17.23±1.73[p]	14.24±1.04[g]	19.92±1.77[p]	25.74±1.85[g]	238.92±17.15[j]	236.39±22.86[p]	453.48±23.98[p]	0.82[c]
Two way ANOVA Result										
Cultivar (C)			*	*	*	*	*	*	*	ns
Salt treatments (S)			*	*	*	*	*	*	*	*
Interactions C×S			*	*	ns	ns	*	*	*	ns

Mean results of five replications ± SD; within each column, mean followed by the same letter are not significantly different ($p < 0.05$). The asterisk indicates the interactions between cultivars and salt treatment *significant ($p < 0.05$) and ns= Not significant.
Source: Experimental data analysis.

Cl^- and affects the uptake of other minerals. Previous authors like Munns and Tester (2008) and Rais et al. (2013) explained that the competition of Na^+ and K^+ for aerial plants resulted in greater accumulation of Na^+ in the shoots than in the roots. It can be also caused by the loss of osmotic potential of root medium, specific ion toxicity and the lack of nutritional ions. In the same line, Marschner (1995) and Rahneshan et al. (2018) showed that K^+ plays a crucial role in the regulation of stomata movement, activation of the enzymes needed in the metabolism, proteins and carbohydrates synthesis, osmotic adjustment and cell turgor. In this study, Ekomcalle variety accumulated more Na^+ in the shoots and roots

compared to Mouala GG variety and higher accumulation is observed in the shoots compared to the roots in both varieties. This can be explained by the fact that Ekomcalle variety easily translocates the Na^+ from the roots to the shoots causing the leaves to get dry and fall rapidly. This can be considered as a tolerant mechanism for the tolerant glycophytes under salinity.

Ca^{2+} decreased significantly ($p < 0.001$) in the culture medium with increased salinity (Table 3). According to Rahneshan et al. (2018), Ca^{2+} ameliorates salt stress through osmotic adjustments by enhancing the selective absorption of potassium and other minerals in plants (Schachtman and Liu, 1999). In saline medium, Hosseni and Thengane (2007) and Hand et al. (2017) also indicated that the uptake of Na^+ inhibits the uptake and transportation of other mineral elements to the leaves. Na^+ replaces Ca^{2+} resulting in the disintegration of cell membranes and cell walls and acts as a secondary messenger in the regulation of signal transduction pathways for the response to abiotic stress and in the promotion of K^+/Na^+ selectivity (Shabala et al., 2006). In the present work, the decrease of calcium from membranes and cell walls by sodium of Ekomcalle variety can be suggested to be one of the responses to salinity stress tolerance compared to Mouala GG variety.

The decrease of Mg^{2+} with increased salinity as reported by Shabala et al. (2006) and Hand et al. (2017) was attributed to the Na^+/K^+ antagonism which favoured the uptake of Na^+ over other minerals (Table 3). Rahneshan et al. (2018) also reported that many key chloroplast enzymes are strongly influenced by slight variations in levels in the cytosol and chloroplast. Our results revealed a decreased Mg^{2+} content in both cultivars partitioning. This decrease had less influence Ekomcalle and Mouala GG growth due to Mg^{2+} deficiency, and the activity of some enzymes, which requires Mg^{2+} for catalysis as well as chlorophyll synthesis (Khan et al., 2000; Rahneshan et al., 2018).

The increased intake doses of NaCl in the culture medium generally influenced the Fe, Mn and Zn. These minerals decreased significantly ($p < 0.05$) in plant partitioning (Table 3). This can be explained by the mineral disorder, the competition for mineral uptake, transport or mineral distribution within the crop plant (Munns and Tester, 2008). The presence of Nacl in the soil leads to change and increased the solubility of study micronutrients and their availabilities (Sharply et al., 1992). In this work, Fe, Mn and Zn contents were higher in the shoots as compared to the roots for both varieties. These results are in consonance with those of Marschner (1995). He explained that the ability of genotypes of plants to metabolize micromineral vary under salinity stress and depend on the plant crop. The mineral contents were higher in Ekomcalle variety compared to Mouala GG in all concentrations and plant parts. We can say that the variety Ekomcalle is more tolerant than Mouala GG.

Chlorophyll (a+b) content

In general, salinity has detrimental effects on chlorophyll (a+b). The effect of increased soil salinity was observed at 100 mM NaCl, with a significantly reduction ($p < 0.05$) in Mouala GG as compared to Ekomcalle at the same concentration (Figure 1). These results are in consonance with those of Santhi et al. (2013) on *S. nigrum*; Najar et al. (2019) on *Medicago truncatula.* They explained that salinity contributes to stomatal closure which reduces CO_2 assimilation in the leaves and conduct to the reduction of photosynthetic activities. In other words, the accumulation of salts in older leaves leads them to eventually die and drop off. In this study, the photoinhibitory damages caused by chlorophyllase or reactive oxygen species (ROS) formation under salt stress explained by Heidari et al. (2014) and Najar et al. (2019) had less effect on variety Ekomcalle as compared to Mouala GG.
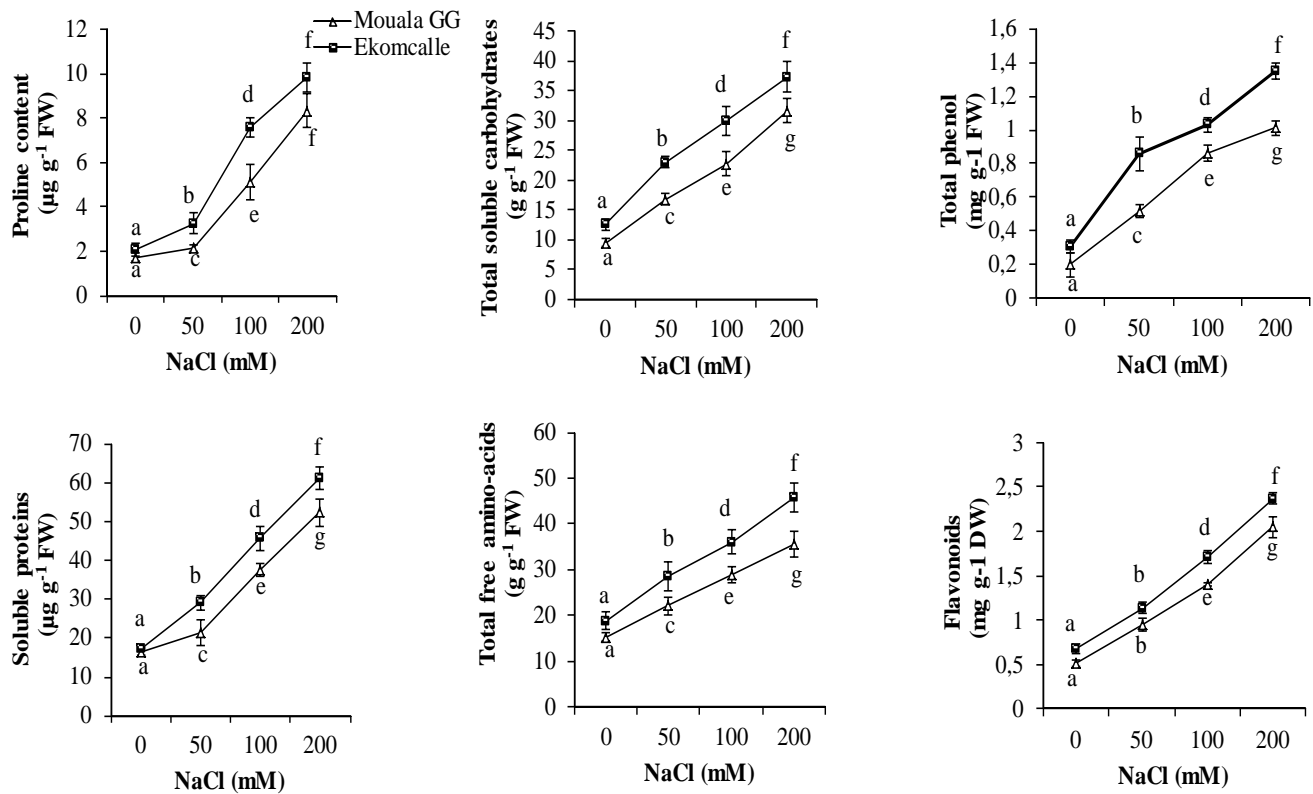
Metabolites

Primary metabolites

The increased concentrations of NaCl in the culture medium significantly increased ($p < 0.001$) the total free amino acids, total soluble carbohydrates, soluble proteins and proline in all cultivars (Figure 2). These results are in consonance with the findings of Hasegawa et al. (2000), Munns and Tester (2008), Chelli-Chaabouni et al. (2010) and Gouveitcha et al. (2021). They said that metabolites like FAA, CH, PR and PRO are salt-tolerance indicators and possess osmoprotective qualities which are one of the common responses of plants to changes in the external osmotic potential. In this work, the PRO content of variety Ekomcalle and Mouala GG increased with the intake doses of salt from 50 mM NaCl. The PRO accumulation was much more marked in variety Ekomcalle compared to Mouala GG. This can be explained that Ekomcalle variety is more tolerant than Mouala GG. It is considered to be compatible regulator due to its accumulation under salinity stress. According to previous workers, Solomon et al. (1994), Smirnoff and Cumbes (1989), Venekamp (1989) and Gouveitcha et al. (2021), PRO plays the role of protective and stabilizing agent for enzymes and membranes; a free radical scavenger, a carbon and nitrogen storage compound and the regulation of cytosolic pH, respectively.

The total free amino acids increased with increased intake doses of NaCl during the experimentation (Figure 2). The findings of Cusido et al. (1987), Ndouma et al. (2020), Hand et al. (2017) and Negrao et al. (2017) showed that salinity increased the levels of total free amino-acids due to the reduction of osmotic potential to maintain the turgid potential and may be a good indicator for screening salt-tolerant genotypes. These results are in
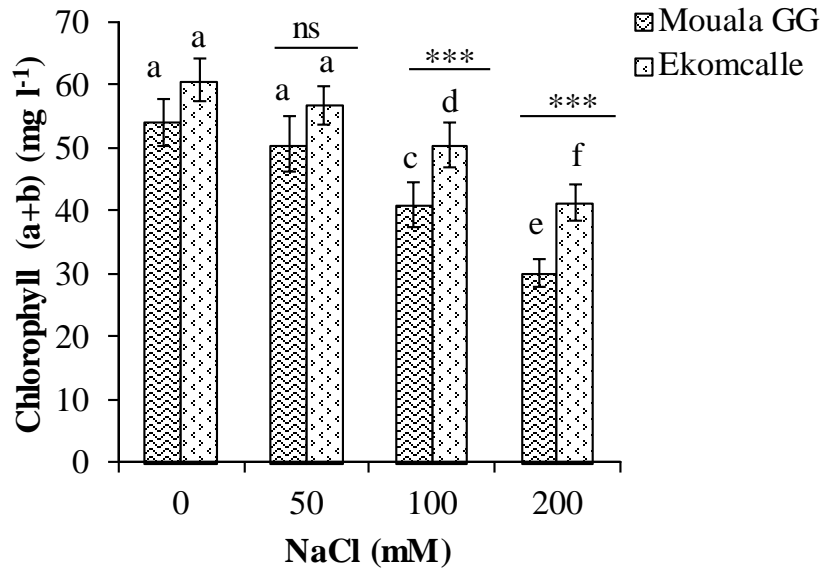
Figure 1. Effects of salinity stress on chlorophyll (a+b) of cowpeas cultivars after six weeks at different salt concentrations. Mean results of five replications ± SD. Mean followed by the same letter are not significantly different (p < 0.05). The asterisk indicates the difference between the control vs treatment, ***significant (p < 0.001) and ns= Not significant.
Source: Experimental data analysis.

Figure 2. Effects of salinity stress on metabolites of cowpeas after six weeks at different salt concentrations. Mean results of five replications ± SD. Mean followed by the same letter are not significantly different (p < 0.05).
Source: Experimental data analysis.

Table 4. Effects of salinity stress on some agronomical parameters of cowpeas at the mature stage.

Cultivar	Treatment mM NaCl	Flowering time (Week)	Number of flower per plant	Number of pod per plant	Number of seed per pod	Pods yield (t/ha)	Seed yield (t/ha)	Harvest index (%)
Mouala GG	0	4.1 ± 0.22^a	24.20 ± 1.44^a	20.80 ± 1.83^a	9.40 ± 0.54^a	2.91 ± 0.11^a	1.27 ± 0.07^a	0.74 ± 0.03^a
	50	6.04 ± 0.30^b	17.80 ± 1.83^b	13.60 ± 0.89^b	6.80 ± 0.44^b	2.34 ± 0.05^b	1.11 ± 0.01^b	0.62 ± 0.02^b
Ekomcalle	0	4.55 ± 0.31^a	29.4 ± 1.54^c	25.20 ± 2.83^c	12.60 ± 0.89^c	3.36 ± 0.08^c	1.63 ± 0.06^c	0.82 ± 0.06^c
	50	7.15 ± 0.08^d	22.20 ± 1.80^d	18.40 ± 1.14^d	8.20 ± 0.44^a	3.05 ± 0.10^d	1.52 ± 0.01^d	0.73 ± 0.01^a
Two way ANOVA Result								
Cultivar (C)		*	*	*	*	**	**	*
Salt treatments (S)		*	*	*	*	*	*	*
Interactions C×S		ns	ns	ns	ns	*	*	ns

Mean results of five replications ± SD; within each column, mean followed by the same letter are not significantly different ($p < 0.05$). *Significant ($p < 0.05$). The asterisk indicates the interactions between cultivars and salt treatment ** significant ($p < 0.01$) and ns= Not significant.
Source: Experimental data analysis.

consonance with the findings of Kosová et al. (2013) and Hand et al. (2017). They reported that soluble proteins increased due to regulatory adjustments to stress resulting in its active synthesis. This is because soluble proteins enhance plant salt tolerance. Its production is considered as an adaptive mechanism of plants to salinity.

From the results obtained, the total soluble carbohydrates increased significantly with salinity in the leaves of two cowpeas varieties. According to Movafegh et al. (2012), the accumulation of total soluble carbohydrates in plant tissues under salinity conditions stress was due to regulatory osmotic adjustment in current stress. The osmotic balance of the cytoplasm relies on an active synthesis of organic compounds such as soluble carbohydrates which enhances the plant salt tolerance through osmotic adjustment (Hajiboland et al., 2014).

Secondary metabolites

The results of non-enzymatic antioxidants (total phenolic (TP) and flavonoid (FLA)) accumulated under stress condition as shown in Figure 2. This is corroborated by the findings of Menguekam et al. (2014) and Ndouma et al. (2020). These authors maintain that, the accumulation of TP and FLA are physiological responses to plant stress. Their accumulation is a cellular adaptive mechanism for scavenging oxygen free radicals, while maintaining chlorophyll levels and cell turgor to protect photosynthetic activities. These results agree with those of Radi et al. (2013) who reported that salinity stress affects the phenolic compounds content by the induced disturbance of the metabolic processes leading to an increase in phenolic compounds. This study showed that an increase in salt concentrations led to a significant increase in total phenol contents. According to

Hichem et al. (2009), the high accumulation of phenolics compound in plants physiologically is important in overcoming the salinity-induced oxidative stress.

Agronomic parameters

The yield components were generally influenced by salinity in the field (Table 4). The number of flowers per plant, number of pods/plant, number of seeds/pod, pod yield, seed yield and harvest index were significantly ($p < 0.05$) decreased in the presence of NaCl for both varieties (Table 4). According to Alam et al. (2004), salinity might have reduced the production of crop by overturning water and nutritional balance of plant and loss of photosynthetic capacity. The latter is a limiting factor to the supply of carbohydrates for plant grow. Our findings showed that the variety

Ekomcalle improves all agronomic parameters compared to Mouala GG variety. Munns (2002) explained that salinity reduced plant parts development by reducing turgor in growing plant roots and shoots due to limited water potential in roots growth medium, consequently Ekomcalle is more tolerant than Mouala GG. In addition, Villora et al. (2000) showed that the yield of tolerant crops could not be affected by a low level of salinity, even though the leaf area and the shoot biomass are reduced.

Conclusion

The conducted study confirmed that the two cowpeas varieties were generally affected by NaCl stress throughout the entire experiment. The results revealed that, the growth parameters (number of leaves, stem height, leaf area and noose diameter), the dry biomass (roots, shoots, total and roots/shoots), the mineral uptake (K, Ca, Mg, Zn, Fe Mn and K/Na) and Chlorophyll (a+b) decreased significantly with increasing intake doses of NaCl from 100 mM NaCl while Na^+ and metabolites (PRO, PR, CH, FAA, TP and FLA) increased from 50 mM NaCl. In the field experiment, agronomic parameters (flowering time, number of flowers per plant, number of pods/plant, number of seeds/pod, pod yield, seed yield and harvest index) were improved at 50 mM NaCl in the variety Ekomcalle compared to Mouala GG at the same concentration. The variety Ekomcalle was less affected by the detrimental impacts of salinity resulting to significant improvements in all the studied parameters compared to variety Mouala GG. The high accumulation of metabolites with increased salinity doses, the dry biomass and chlorophyll content could be added as indicators of early identification and of osmotic adjustment ability for salt-tolerant plants in salt stress conditions. The varieties Ekomcalle and Mouala GG could be cultivated in areas with moderate salinity with preference given to the Ekomcalle variety.

COMPETING INTERESTS

The authors have declared any conflict of interests.

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