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GROWTH AND BIOMASS ACCUMULATION OF SEEDLINGS OF COWPEA (*Vigna unguiculata* L. WALP) IN RESPONSE TO SOIL SALINITY

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Abstract: This study was carried out during the early planting season at the mini Research Farm of the Department of Botany, Delta State University, site 2 Abraka, Delta State, Nigeria to ascertain the effect of various levels of soil salinity (0.00, 90, 180, 360 Mm NaCl treatment) on the growth, plant height, number of leaves, leaf area and biomass accumulation (fresh and dry weight) of cowpea (*Vigna unguiculata* L. Walp) seedling (cultivar IT 870–9411). The result showed that the saline soil significantly ($P \geq 0.05$) affected the growth performance of cowpea negatively as the control (0.00% w/w) showed the best morphological record. However, flowering and pod production occurred earlier in the 90Mm NaCl treatment. The results indicated that high salinity level (16.20% w/w) was detrimental to the growth of the plant. These variations in growth and biomass accumulation of this plant species in response to salinity were discussed.

Keywords: Biomass accumulation, *Vigna uguiculata,* soil salinity

Introduction

Cowpea (*Vigna unguiculata* L. Walp), also known as southern pea, black eye pea, crowder pea or lubiais a legume belonging to the Fabiacea family and a native of Africa and Latin America. It is widely grown in Africa, Latin America, South Asia and in the Southern United States. Cowpea is a warm season, annual herbaceous legume plant which varies in habit from indeterminate to fairly determinate (Silva *et al.,* 2003; Sousa *et al.,* 2003). Cowpea performs well on a variety of soil and soil condition but performs on well drained sandy loam or sandy soils where soil pH is in the range of 5.8 – 6.5. Cowpea seed is a nutritious component in the human and live stock diet. It contains about 24.8% protein, 1.9% fat, 63.6% carbohydrates, 6.3% fiber, and few traces of vitamins and mineral (Achuba, 2006). The proteinous seed is rich in the amino-acid, lysine and tryptophan compared to cereal grains; however, it is deficient in methionine and cysteine when compared to animal protein (Achuba, 2006). Therefore, cowpea is valued as a nutritional supplement to cereals and as an extender of animal protein (Agbogidi and Egbo, 2012). In Africa, cowpea is the most popular legume and the largest part of world production originates from this continent (Agbogidi and Egbo, 2012). It is a food security crop in the semi and zone of West and Central Africa which ensures farm household subsistence food supply even in dry years (Silva *et al.,* 2003). Being a legume, cowpea serves as a cover crop and adds about 40-80 kg nitrogen per hectare, and are grown as a sole crop, as inter crop or in alley planting (Awe, 2008).

Salinity is one of the serious environmental problems that cause osmotic stress and reduction in plant growth and crop productivity (Flower, 2004). According to FAO (2002), a saline soil is that which has an electrical conductivity of the saturation extract (ECo) of 4 dsm^{-1} or more, and soils with ECo's exceeding 15 dsm^{-1} are considered strongly saline. The cations associated with salinity include Na^+ Ca^{2+} and Mg^{2+}, while the common anions are Cl^-, SO_4^{2-} and HCO_3. However, Na^+ and Cl^- are considered the most important, since Na^+ in particular causes deterioration of the physical structure of the soil and both Na^+ and Cl^- are toxic to plants (Patel *et al*., 2010).

The Niger Delta in the southern region of Nigeria falls within the tropical rainforest zone. It is highly diverse and supportive of numerous species of terrestrial, aquatic flora and fauna (Vincent and Paul, 2012). These areas are however, exposed to salinity. In the tropics saline soils are typified by lagoons and mangrove swamps and in some rainforest near the sea. Toxic concentrations often occur in such soil because the soluble constituents are carried away in drainage water to the table below the root range. Saline soil affects plant growth in various ways, it increases water deficits in soils, increase the concentration of certain anions that have inhibitory effect in plant metabolism, deteriorates the physical structure of the soil, and consequently, affected plant become stunted with dark green leaves, sometimes, leaf burn, and defoliation, and in severe cases, death occur (Silva *et al.*, 2003; Ashishi *et al.*, 2010; Leyla, 2013; Qu *et al.*, 2008). High salt salinity also cause nutrient imbalance, result in the accumulation of one element toxic to plants and reduces water infiltration if the level of one salt element-sodium is high. Soil salinity is regarded as being responsible for reduction in plant growth and yield in arid and semi-arid regions of the world (Gheyi, 2000; Munns, 2002).

The salt-water balance is also important for electrochemical functions within the plant. Osmotic or salt-water imbalance of plants under dehydration and increased concentration of salts in the plant sap and tissues would lead to the disturbance in the metabolic integrity of the plant system such as photosynthesis, respiration, nutrient availability; hence plant productivity is adversely altered (Patel and Pandey, 2007; Costa *et al*., 2003). Some responses of seedling grown under salt stress have been observed and it is reported that there is a delay in germination and seedling establishment (Xiao-Xia *et al*., 2008; Ramiliya *et al.*, 2004; Raptan *et al.*, 2001). Silva *et al.* (2003) reported that plant acclimation to salt stress is considered an integrated responses at different organs, especially roots and leaves. Some results have shown that most of the physiological and biology changes in leaves as related to stress intensity and duration (Slater *et al*., 2003; Singh *et al*., 2011; Yakit and Tuna, 2006; Shouping *et al.*, 2011). Soil with high salt concentrations affects plant growth and production (Leyla, 2013; Ramiliya *et al*., 2006). Due to multidimensional uses of cowpea, a lot of researches have been conducted on cowpea and as its importance increases, up to date researches are necessary to facilitate innovative technique. There is however paucity of information regarding the effect of different salt concentration on the growth of cowpea. It is therefore against this backdrop that this study was conducted with a view of evaluating the effects of different levels of saline soil on the morpho-physiological changes on the seedlings of cowpea.

Materials and Methods

Study area

The experiment was conducted at the nursery site of the Department of Botany, Delta State University, Abraka Delta State, Nigeria. Abraka is in Ethiope East Local Government

Area of Delta State, Nigeria. Abraka lies within the tropical rain forest zone at approximately latitude 5^0 47'N and longitude 6^0 47'E of the equator (Vincent and Paul, 2012). The area is characterized by a mean annual rainfall of 3.097 mm, annual temperature of 30.6^0C and relative humidity of 68% per annum. The mean monthly soil temperature at 100 cm depth is 29.7ºC; soil pH ranges from 4.5 to 8 with mean monthly sunshine of 4.8 bars (Efe and Aruegodore, 2003).

Source of soil sample

Soil sample was collected at surface depth (0-15 cm) from a loamy soil land at site 2, Delta State University, Abraka, having no pollution history. The soil was air dried and then sieved to remove stones, roots and other materials that may be detrimental to emergence of plumule upon germination.

Pre-planting soil analysis

Prior to experimentation, air dried, crushed and sieved soil samples were taken to analytical unit, Department of Chemistry Delta State University Abraka, Delta State for physio-chemical analysis using standard methods.

Source of cowpea seed

Young healthy seedlings of *Vigna unguiculata* (IT 870-9411) were obtained from the Agricultural Development Programme (ADP) office in Warri, Delta State. Seeds were subjected to viability test using floatation method- soaking of seeds in a container of water for five minutes and the seeds that sank are physically termed viable.

Source of salt

The salts were obtained at Abraka market, Abraka, Delta State. Saline solution was prepared artificially by dissolving calculated and weighed amount of commercially available table salt (NACL) dissolved in water to make the various concentrations (0, 90, 180 and 260 MM) following the procedure of Tozlu *et al.* (2000).

Procedure and experimental design

Three seeds were planted per hole in a depth of 5 cm in polypots (25.5 cm wide and 20.9 cm deep), filled with 2000 g of soil. Poly pots were perforated at the bottom to facilitate drainage and the experiment was laid out in a Randomized Complete Block Design (RCBD) with three replicates. There were a total of 12 polypots in each replicate and a total of 48 polypots in the experimental field. The plants were water regularly to filled capacity for three weeks to allow seedling stabilize in the soil. Treatment of NaCl solution of concentration of 0, 90, 180 and 360 mM were applied using the 10 ml beaker at two days interval. Plant height (cm) was determined using a meter rule from the top of the soil to the terminal bud; number of leaves was obtained by visual counting the number of leaves. The leaf area (cm^2) was determined by measuring, using a meter rule, the maximum length and breadth of the leaf multiplied by the correction factor (0.75) following the formula of (Agbogidi and Ofoku, 2005). The fresh weight (g) was determined using a triple beam balance while the dry weight was obtained using an electronic weighing balance after oven drying the plant for 72 h at 65^0C until a constant weight was achieved following the procedure of Agbogidi and Egbo (2012).

Statistical analysis

The experiment was laid out in a randomized complete block design with three replicates. Data collected were subjected to Analysis of variance (ANOVA) and the significant means were separated with the DUNCAN'S multiple range tests using the statistical analytical system (SAS, 2005).

Result and Discussion

The initial pre-planting properties of the soil (Table 5) showed that the soil is marginally fertile. The observed 6.46 value of pH of the soil indicates that the soil is slightly acidic and this can be attributed to high rainfall prevalent in the area leading to leaching of the basic cations from the surface area of the soil. The soil is sandy loam in texture with characteristics of 70.3% sand, 26.9% clay and 2.8% silt. The highest mean plant height (75.13 cm) was recorded at 0 mM NaCl treatment and the lowest (8.96 cm) at 360Mm NaCl treatment and there was a significant difference between them ($P \geq 0.05$) (Table 1). This gave credence to earlier reports of Lacerda *et al.* (2003) who observed a marked reduction in plant height of sorghum under salt stress.

Table 1: Effects of various levels of salinity on the plant height (cm) of cowpea (*Vigna unguiculata*)

NaCl (Mm)	Weeks after planting/plant height							Means
	1	2	3	4	5	6	7	
0	25.0	56.1	80.06	84.78	86.0	95.1	98.9	75.13^a
90	23.4	36.8	40.2	57.0	60.0	59.8	60.1	48.19^b
180	23.6	29.1	25.2	30.0	31.1	0.0	0.0	19.86^c
360	21.2	21.3	20	9.2	0.0	0.0	0.0	8.96^d

Means with different alphabets are significantly different at $P \geq 0.05$ using the Duncan's multiple range tests

Table 2: Effect of various levels of salinity on the number of leaves of cowpea (*Vigna unguiculata*)

NaCl (Mm)	Number of leaves/weeks after planting							Means
	1	2	3	4	5	6	7	
0.0	11.2	13.9	17.5	28.0	32.8	33.1	35.0	24.5^a
90	10	13.2	16.0	17.0	17.3	14.2	0.5	10.3^b
180	9.1	9.4	9.5	2.5	0.0	0.0	0.0	4.4^c
360	10.2	10.0	1.9	0.4	0.0	0.0	0.0	3.2^d

Means with different alphabets are significantly different at $P>0.05$ using the Duncan's multiple range tests

Table 3: Effect of various levels of salinity on the leaf area of cowpea (*V. unguiculata*)

NaCl (Mm)	Leaf area (cm^2)/weeks after planting							Means
	1	2	3	4	5	6	7	
0.0	36.5	49.0	46.1	46.8	50.6	52	52.9	47.7^a
90	31.0	47.8	52.3	51.3	48.1	0.8	0.0	39.9^b
180	80.0	38.6	36.0	15.1	0.0	0.0	0.0	17.1^c
360	35.1	42.0	43.0	0.2	0.0	0.0	0.0	17.2^d

Means with different alphabets are significantly different at $P>0.05$ using the Duncan's multiple range tests

Salinity inhibits shoot growth by suppressing leaf initiation and leaf expansion at internodes growth of leaves leading to reduction in plant growth (Qu *et al*., 2008; Essa, 2002; Besma and Mounir, 2010). This could be attributed to decreasing osmotic potential of the soil solution. The leaf area investigated showed that, leaf area decreased considerably as salinity level increases (Table 3), which is in corroboration with Munns (2002), who observed that salt stress inhibits leaf growth with exception of primary leaves, such an inhibition was related to developmental stages, that is, younger leaves showed higher reduction in the final size, which may be mainly associated with osmotic component of salt stress. This change in morphology (Plates 1 and 2) can contribute to plant acclimation to salt stress through the adjustments of leaf area to water available. The reduction of leaf number was also recorded as the level of salinity increased. This is in accordance with Sousa *et al.* (2003), who reported that the reduction in the number of leaves may be attributed to the accumulation of Na^+ and Cl^- ion which may have become toxic to leaves, turning them chlorotic and leading to loss of leaves. Salinity may directly or indirectly affect cell division and enlargement in the growing points resulting in stunted leaves and stem (Munns, 2005). Fresh and dry weight (g) of the cowpea seedling in Table 4 showed that saline soil significantly affect ($P \geq 0.05$) the biomass accumulation of cowpea. The control (0 mM) recorded the highest amount of fresh and dry weight. This is in line with earlier reports of Raptan *et al*. (2001) who reported that saline soil inhibit growth and total dry matter production of mung bean. Under NaCl stress, the roots and stem seems to absorb NaCl than leaves and fruit (Xiao-Xia *et al.*, 2008).

Table 4: Effects of various levels of salinity on fresh and dry weight (g) of cowpea (*V. unguiculata*)

NaCl (Mm)	Fresh weight	Dry weight
0.0	22.0^{a}	6.2^{a}
90	13.9^{b}	1.7^{b}
180	0.0^{c}	0.0^{c}
360	0.0^{d}	0.0^{d}

Means with different alphabets are significantly different at P>0.05 using the Duncan's multiple range tests

Table 5: Physio-chemical properties of soil before experimentation

S/N	Parameters	Values
1	Soil pH	6.46
2	Electrical conductivity (µs/cm)	34.6
3	Soil temperature (°C)	29.6
4	Moisture content	1.86
5	Total hydrocarbon content (mg/kg)	0.39
6	Total organic carbon (%)	1.53
7	Particulate size distribution	
	% sand	70.3
	% clay	26.9
8	% silt	2.8
9	Calcium (mg/kg)	19.23
10	Magnesium (mg/kg)	13.54
11	Lead (mg/kg)	1.75
12	Iron (mg/kg)	397.29
13	Manganese (mg/kg)	23.83
14	Zinc (mg/kg)	44.22
15	Nickel (mg/kg)	1.09
16	Potassium (mg/kg)	2.32

°C = Degree centigrade; mg/kg = Milligram per kilogram; % = Percentage; µs/cm = Micro Siemens per centimeter

Plate 1: Performance of cowpea before the commencement of treatment

Plate 2: Morphological difference in cowpea in response to various salinity levels

Biomass takes an important part of NaCl accumulation so application of NaCl (180 and 360 Mm) significantly increased the amount of NaCl accumulation in the plant as shown in drastically reduced biomass. At 360 mM of NaCl treatment, death was recorded (Plate 2) Although the effects of high salt content on metabolic processes are yet to be fully elucidated, it has been reported that salinity reduces protein hydration (Camara *et al*., 2000; Slater *et al*., 2003; Achuba, 2006) and induces changes in the activities of many enzymes involved in plant growth and development (Ramiliya *et al*., 2006).

The result of the present study confirmed that the difference in growth and biomass accumulation of cowpea in response to salinity is dose dependent suggesting that *Vigna unguiculata* is relatively salt tolerant. This slight salt tolerance of cowpea at low NaCl concentration is dependent on integration of different genetic attributes that may help alleviate NaCl stress.

Conclusion

In conclusion, the result of this experiment indicates that NaCl disturbed the mechanism of growth and biomass accumulation in cowpea. It is suggested that for future research, more cultivars should be selected for field trial and the range of NaCl increased. The study has a great implication in environmental and food security especially in Niger Delta where salinity level of most soil is high.

Conflict of Interest

Author declares that there is no conflict of interest reported on this work.

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