

Growth Morphology and Chlorophyll Synthesis of *Vigna unguiculata* (L). Walp Varieties (White and Brown) Grown Under Salinity Stress

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ABSTRACT

The impact of salt (MgSO_4) on some growth parameters and chlorophyll synthesis of Cowpea (*Vigna unguiculata*) (L). Walp varieties was studied in the greenhouse. Mature dry seeds of *Vigna unguiculata* was planted in sandy loam soil after sterilization. Three (3) groups of experiment were used for each variety: group 1 (control), group 2 (1% MgSO_4) and group 3 (0.1% MgSO_4). The set-up was done in complete randomized block design (CRBD) in triplicates. The morphological parameters studied were: seedling height, leaf area, internode length, number of nodes, root length, moisture content, petiole length and chlorophyll content. The results revealed that salt (MgSO_4) stress had significant ($p=0.05$) reduction effects on growth morphology of *Vigna unguiculata*. It was observed from the results of this research that the relationship between chlorophyll “a” and “b” at the same concentration of MgSO_4 followed a different sequence for the two bean varieties (white and brown). The result represented showed a decrease in chlorophyll “a” and “b” and carotenoids in both varieties. The results of this research also indicates that *V. unguiculata*, which widely used in Nigeria and Africa as a whole suffers morphological and physiological alterations that make them unable to develop and thrive effectively, thus reducing productivity of this very important crop. Thus, this crops should not be planted on saline soils as this will adversely affect its growth and productivity.

Keywords: Chlorophyll, Cowpea, Magnesium Sulphate, *Vigna unguiculata* and Salinity Stress.

I. INTRODUCTION

Soil salinity is a term used to describe the amount of mineral salts present in soil (Richard, 1954; Shan, 2009). The mineral salts constitute a mixture of electrolytes. The major cations in saline soils include Na^+ , Ca^{2+} , Mg^{2+} , and K^+ ; the major anions include Cl^- , SO_4^{2-} , HCO_3^- , CO_3^{2-} , and NO_3^- . High concentrations of soluble salts occur in terrestrial environments or in aquatic environments, which may happen naturally or anthropogenically (Larcher, 1995; Maria *et al.*, 2011). The naturally occurring salinization is recognized as primary and the anthropogenic form as secondary (Williams, 1999; Maria *et al.*, 2011). The primary salinization is a natural process which occurs in regions where there is water deficit, in other words, low rainfall and high evaporation potential, which leads to a progressive increase in the concentration of salts

released by weathering or by sea spray that may reach the water bodies as consequence of storms and winds (Suzuki *et al.*, 2002; Roache *et al.*, 2006; Maria *et al.*, 2011). Unlike primary salinization, secondary salinization results from human activities (Neumann, 1997). The secondary salinization of water bodies may occur through irrigation of agricultural crops which may leach the accumulated salt in irrigated soils into river and lake waters downstream (Rengasamy, 2006) and, in the specific case of coastal lagoons, through the process of opening sand fences between the sea and the coastal lagoons (Suzuki *et al.*, 1998; 2002; Maria *et al.*, 2011).

Saline solutions impose both ionic and osmotic stresses on plants. These stresses can be distinguished at several levels. In salt sensitive plants, shoot and to a lesser extent root growth is permanently reduced within hours of salt stress and this effect does not appear to depend on

Na⁺ concentration in the growing tissues, but rather a response to the osmolality of the external solution (Munns, 2002). Na⁺ specific damage is associated with the accumulation of Na⁺ in leaf Tissues and results in necrosis of older leaves; starting at the tips and margins and working back through the leaf. Seven percent of the land surface and five percent of cultivated lands are affected by salinity, with salt stress being one of the most serious environmental factors limiting the productivity of crop plants (Munns *et al.*, 2000). The electrolytes sodium (a cation) and chloride (an anion) are extremely toxic to most plants at relatively low soil water concentrations, due to deleterious effects on cellular metabolism and ultra-structure (Erhenhi *et al.*, 2008).

Nutrients, in general, have several functions in plant structure, metabolism and osmoregulation of plant cells (Taiz and Zeiger, 2009; Maria *et al.*, 2011). However, one of the most important salt stress effects on plants is induced by nutritional disorders, which result from salinity effect on availability, absorption and transport of nutrients within the plant (Munns and Tester, 2008). Nutrient deficiency as well as ion toxicity and osmotic stress are factors attributed to the deleterious effect of salinity on plant growth and productivity (Nublat *et al.*, 2001; Maria *et al.*, 2011).

Chlorophyll is the principal agent responsible for photosynthesis and, under adverse conditions; chlorophyll level is a good indicator of photosynthetic activity (XinWen *et al.*, 2008). Thus, we quantified the content of photosynthetic pigments in order to infer the effect of salt concentrations on the photosynthetic activity of *Vigna unguiculata*. Many studies confirm the inhibitory effect of salinity on biochemical processes, of which photosynthesis is the most important. The effect on photosynthesis can be gauged from the effect on the photosynthetic pigments. The results of specific studies clearly indicate that salinity reduces the content of photosynthetic pigments in treated plants.

Vigna unguiculata is a warm-season, annual legume belonging to the family Fabaceae, commonly known to the Ibibio people of South South Nigeria as Akoti. It exhibits a wide range of growth habits. Varieties may be short and bushy, prostrate, or tall and vine-like. Canopy heights can be 2–3 feet, depending on the variety

(Albala, 2007). The common bean is a highly variable species with a long history. Bush varieties form erect bushes 20–60 cm (8–20 in) tall, while pole or running varieties form vines 2–3 m (7–10 ft) long. All varieties bear alternate, green or purple leaves, which are divided into three oval, smooth-edged leaflets, each 6–15 cm (2–6 in) long and 3–11 cm (1–4 in) wide. The white, pink, or purple flowers are about 1 cm long, and they give way to pods 8–20 cm (3–8 in) long and 1–1.5 cm wide. These may be green, yellow, black, or purple in color, each containing 4–6 beans. The beans are smooth, plump, kidney-shaped, up to 1.5 cm long, range widely in colour, and are often mottled in two or more colours.

The leaves and seeds of *Vigna unguiculata* are used in Africa as an important food source for over 6000 years and its use has spread around the world. In rural Africa, the young fresh leaves and pods are eaten as a green vegetable. The leaves and beans are also dried, stored and used for food during winter month. The young leaves have up to fifteen times more protein than the dried seeds and are a valuable food source. Apart from being abundant with proteins, these types of legumes may also be loaded important vitamins and minerals which are important for great health.



Plate 1.1: *Vigna unguiculata* seeds (White)



Plate 1.2: *Vigna unguiculata* seed pods

Beans are a rich source of protein and carbohydrates, as well as being a good source of vitamin B complex such as niacin, riboflavin, folic acid and thiamine. It also provides iron, copper, zinc, phosphorus, potassium, magnesium and calcium, furthermore, has high fiber content. It is also an excellent source of polyunsaturated fatty acids. Currently, it is accepted that the common bean has medicinal use based on indigenous customs and practices.



Plate 1.3: *Vigna unguiculata* seeds (brown)



Plate 1.4: *Vigna unguiculata* whole plant

Agriculture plays a pioneering role in economic development in many countries. However, salinity, which affects most areas of Nigeria, represents one of the main obstacles that limit the expansion of the agricultural area or the increase in agricultural production for many crops. High salinity is due to the high concentration of soluble salts in irrigation waters and the high rate of evaporation caused by the high temperatures, inefficient drainage, or soil type. Bean is one of the important economic cereal crops, used as food for both people and animals, besides its capacity to tolerate salinity. It is based on the importance of these crops and the rapid increasing salinity problems

necessitate this study to find-out the effect on the salt stress on the morphological parameters and chlorophyll content of *Vigna unguiculata* varieties.

II. METHODS AND MATERIAL

A. Plant Materials

The experiment was carried-out in the greenhouse of Botany and Ecological Studies Department, University of Uyo, Akwa Ibom State. Viable seeds were obtained from the local market and are one of the types grown in Nigeria.

B. Preparing and Sowing of Seeds

Seeds of white and brown varieties of *Vigna unguiculata* free from wrinkles were chosen for sowing. Ninety seeds of white and brown beans were selected and five seeds were grown in each polybag (4 kg of soil). The seeds were left to grow inside the greenhouse under natural lighting, (day/night). The polybags were distributed uniformly in rows and columns and only homogenous seedlings showing the strongest growth, were selected and left to grow until 4 weeks (from the un-set of germination).

C. Salt Preparation/Procedures

Sterile distilled water was used as control (Group 1). 100 ml of distilled water was added to 1g of MgSO_4 to give 1% solution of MgSO_4 (Group 2). 1ml of 1% MgSO_4 was added to 9 ml of distilled water to form 0.1% solution of MgSO_4 (Group 3).

D. Salinity Treatments

The Polybags were irrigated once every day, then once in every 3 days and once in a week until 4 weeks old. Then plants were irrigated with distilled water for group 1. Each group were replicated three times and chosen for each morphological and physiological measurement (at an average of three plants per replica).

E. Growth Measurements

Growth parameter measurements for the plants exposed to saline treatments were taken at times mentioned

previously according to the methods of by Hoyt and Brafield (1962), Umoh and Esenowo (1996). The three replicates taken for each treatment were used to calculate the mean of each measurement. The measurements taken include: Number of Nodes, Leaf area, Root length, Seedling height, Petiole length, Internode length and Moisture content.

F. Determination of Chlorophyll Contents

Chlorophyll contents of the experimental plants were determined using AtLeaf Chlorophyll Meter.

G. Statistical Analysis

Results are expressed as mean \pm Standard Error (M.S.E) of three replicates. Statistical significance between the different groups was determined by two-way Analysis of Variance (ANOVA). $P=0.05$ was considered as statistically significant (Ubom, 2004).

III. RESULTS AND DISCUSSION

The effect salinity stress on growth parameters of *Vigna unguiculata* (white and brown variety) was studied by measuring its parameters viz: seedling height, leaf area, root length, petiole length, internode length, number of

nodes, percentage germination and moisture content during the weeks of study.

The different effect of salt stress on *Vigna unguiculata* (white variety) was observed; seedling height was recorded: 44.93 ± 1.94 , 24.80 ± 8.10 and 44.17 ± 18.88 in the control, 1% of $MgSO_4$ and 0.1% of $MgSO_4$ respectively. Leaf Area also recorded the following; control (19.40 ± 3.00), 1% of $MgSO_4$ (17.4 ± 3.99) and 0.1% of $MgSO_4$ was (35.22 ± 15.68). Root Length was also observed control, 1% of $MgSO_4$ and 0.1% of $MgSO_4$ with the following data 5.90 ± 1.42 , 6.74 ± 0.28 and 10.28 ± 9.03 respectively. Petiole Length also recorded thus; control (2.83 ± 1.83) 1% of $MgSO_4$ (2.96 ± 0.26) and 0.1% of $MgSO_4$ (3.97 ± 0.44). Internode length was calculated to be 2.89 ± 0.81 , 4.21 ± 2.3 and 1.7 ± 0.50 for control, 1% of $MgSO_4$ and 0.1% of $MgSO_4$ respectively. However, the numbers of nodes were accurately recorded. For the control (3.78 ± 0.31) 1% of $MgSO_4$ (3.17 ± 0.16) and 0.1% of $MgSO_4$ was 4.0 ± 0.81 . Thus the percentage germination for control was recorded 73.3 ± 9.43 while 1% of $MgSO_4$ 70.00 ± 10.00 and 0.1% of $MgSO_4$ 20.00 ± 0.00 . Moisture content was also observed and recoded as follows: for control (45.7 ± 6.96), 1% of $MgSO_4$ (44.6 ± 2.20) and 0.1% of $MgSO_4$ (55.6 ± 6.76) (Table 1).

Table 1: Effect of different Salt Stress on growth morphology of *Vigna unguiculata* (white variety) for 4 weeks after germination

Conc./ Para.	Seedling Height (cm)	Leaf Area (cm ²)	Root Length (cm)	Petiole Length (cm)	Internode Length (cm)	Number of Nodes	Percentage Germination (%)	Moisture Content (%)
Group 1 (Control)	44.93 ± 1.94	19.40 ± 3.00	5.90 ± 1.42	2.83 ± 1.83	2.89 ± 0.81	3.78 ± 0.31	73.3 ± 9.43	45.7 ± 6.96
Group 2 (1%)	24.80 ± 8.10	17.4 ± 3.99	6.74 ± 0.28	2.96 ± 0.26	4.21 ± 2.3	3.17 ± 0.16	70.0 ± 10.0	44.6 ± 2.20
Group 3 (0.1%)	44.17 ± 18.88	35.22 ± 15.68	10.28 ± 9.03	3.97 ± 0.44	1.7 ± 0.50	4.00 ± 0.81	20.0 ± 0.00	55.6 ± 6.76

Data are processed and expressed as mean \pm Standard Error of three replicates

Table 2: Effect of different Salt Stress on growth morphology of *Vigna unguiculata* (brown variety) for 4 weeks after germination

Conc. / Para.	Seedling Height (cm)	Leaf Area (cm ²)	Root Length (cm)	Petiole Length (cm)	Internode Length (cm)	Number Of Nodes	Percentage Germination n (%)	Moisture Content (%)
Group 1 (Control)	17.67±0.1 0	14.09±1.68	7.92±1.24	0.58±0.08	2.24±1.10	2.70±0.27	60.0 ± 16.30	45.02±7.49
Group 2 (1%)	18.59±0.9 1	16.38±2.74	5.17±0.05	0.39±0.19	3.46±1.07	1.33±0.00	70.0 ± 10.0	39.34±2.05
Group 3 (0.1%)	19.70±1.4 1	14.60±2.55	13.42±2.68	0.41±0.05	3.34±0.94	2.15±0.09	73.33±24.94	41.45±6.01

Data are processed and expressed as mean ± Standard Error of three replicates

Table 3: Effect of different Salt Stress on chlorophyll content of *Vigna unguiculata* (white variety) for 4 weeks after germination

Conc. Para.	CCI	Chlorophyll "a"	Chlorophyll "b"	Carotenoids
Group 1 (Control)	46.20 ± 7.2	32.32 ± 5.20	9.70 ± 1.54	3.23±0.5
Group 2 (1%)	26.2 ± 18.90	18.40 ± 13.20	5.51 ± 3.96	1.84 ± 1.32
Group 3 (0.1%)	40.70 ± 9.02	28.50 ± 6.31	8.54 ± 1.89	2.85 ± 0.63

Data are processed and expressed as mean ± Standard Error of three replicates

CCI = Chlorophyll Concentration Index

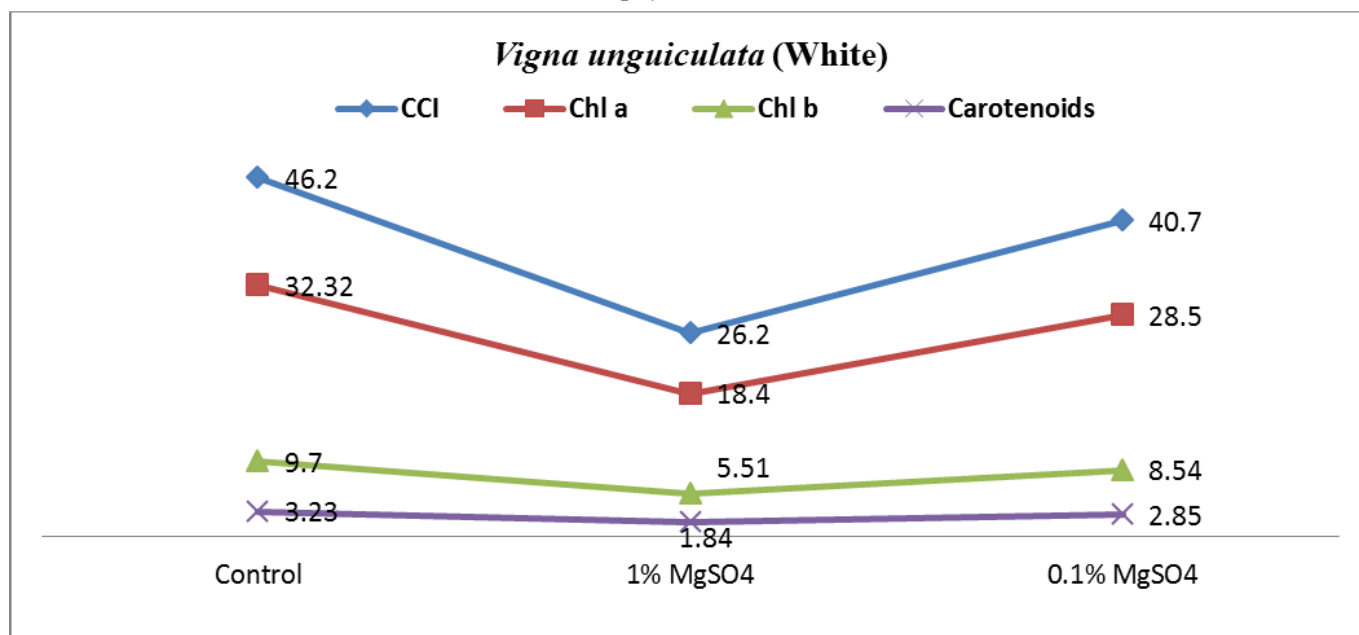


Figure 1: Effect of different Salt Stress on chlorophyll content of *Vigna unguiculata* (white variety) for 4 weeks after germination

Table 4: Effect of different Salt Stress on chlorophyll content of *Vigna unguiculata* (brown variety) for 4 weeks after germination

Conc.	Para.	CCI	Chlorophyll "a"	Chlorophyll "b"	Carotenoids
Group 1 (Control)		41.74 ± 9.14	29.22 ± 6.39	8.80 ± 1.91	2.92 ± 0.63
Group 2 (1%)		21.33 ± 18.30	18.04 ± 12.80	5.41 ± 3.84	2.36 ± 2.30
Group 3 (0.1%)		43.3 ± 9.30	30.31 ± 6.51	9.09 ± 1.95	3.03 ± 0.65

Data are processed and expressed as mean ± Standard Error of three replicates
CCI = Chlorophyll Concentration Index

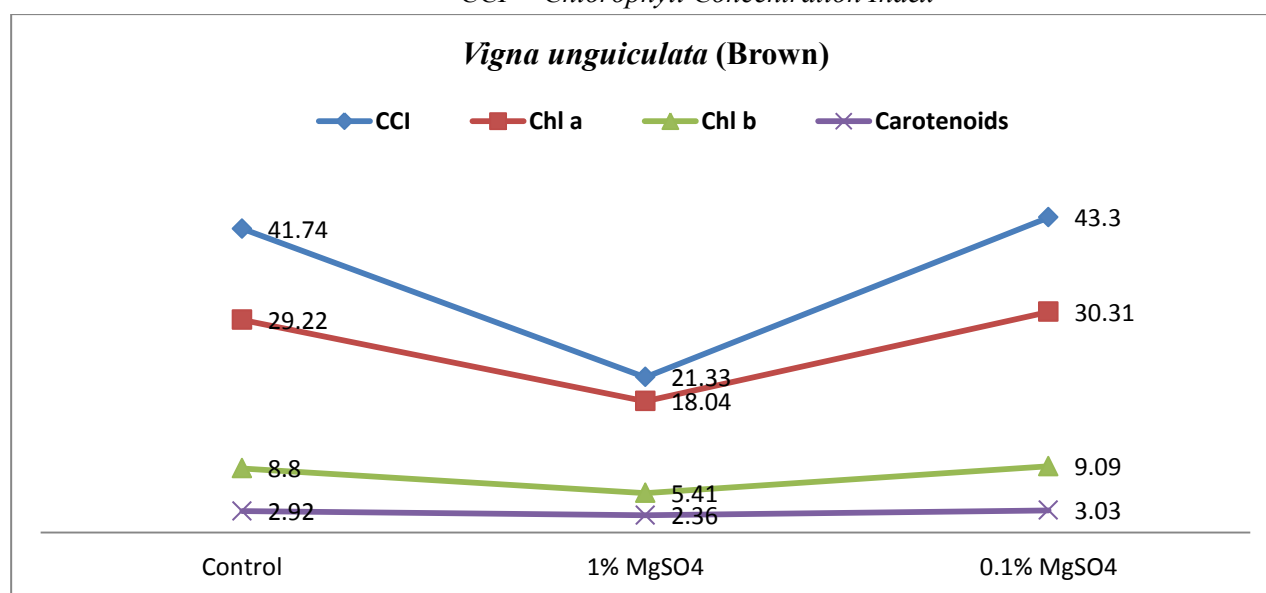


Figure 2: Effect of different Salt Stress on chlorophyll content of *Vigna unguiculata* (brown variety) for 4 weeks after germination

The same effect of salt stress was duly carried out during the four weeks study with *Vigna unguiculata* using the brown variety. The same parameters were also considered. However, seedling height was recorded: for control (17.67 ± 0.10), 1% of MgSO_4 (18.59 ± 0.91) and 0.1% of MgSO_4 (17.70 ± 1.14). Leaf area was recorded thus: control (14.09 ± 1.69), 1% of MgSO_4 (16.38 ± 2.74) and 0.1% of MgSO_4 (14.60 ± 2.55). Also root length recorded the following; control (7.92 ± 1.24), 1% of MgSO_4 and 0.1% of MgSO_4 was 28.9 ± 2.68 . Petiole Length was also observed; control, 1% of MgSO_4 and 0.1% of MgSO_4 with the following data 0.58 ± 0.08 , 0.39 ± 0.19 and 0.41 ± 0.05 respectively. Similarly, internode length was observed to be 2.24 ± 1.10 for control, 3.46 ± 1.07 for 1% of MgSO_4 and 3.34 ± 0.94 for 0.1% of MgSO_4 . The number of nodes were carefully observed and recorded using the three replicates; control (2.70 ± 0.27), 1% of MgSO_4 ($1.33 \pm$

0.00) and 0.1% of MgSO_4 to be 2.5 ± 0.10 . The percentage germination was also found to be 60.0 ± 16.30 for control, 70.0 ± 10.10 (1% of MgSO_4) and 73.33 ± 24.94 for (0.1% of MgSO_4). Moisture content was carefully weighed and data for control was 46.8 ± 7.49 and 1% of MgSO_4 was recorded at 39.9 ± 2.05 while 0.1% of MgSO_4 was 39.7 ± 6.01 (Table 2).

The impact of different salt stress on the chlorophyll content of *Vigna unguiculata* (white variety) was also analyzed and the data was recorded for each group. The chlorophyll concentration index of group one was 46.20 ± 7.2 and group two (2.6 ± 18.9) while group three recorded 40.70 ± 9.02 . Chlorophyll 'a' for group one recorded 32.32 ± 5.20 , group two (18.40 ± 13.20) and group three (28.50 ± 6.31). Also, chlorophyll 'b' 9.70 ± 1.54 was recorded for group one and 5.5 ± 3.96 was recorded for group two while 8.54 ± 1.89 represented

group three. The carotenoids in group one was observed to be 3.23 ± 0.5 , group two (1.84 ± 1.32) and group three recorded 2.85 ± 0.63 (Table 3, Figure 1).

The impact of different salt stress on the chlorophyll content of *Vigna unguiculata* (brown variety) was presented below: The following data represent the chlorophyll concentration index of group one (41.74 ± 9.14), group two (21.33 ± 18.30) and group three (43.3 ± 9.30). For chlorophyll 'a' 29.22 ± 6.39 for group one and 18.04 ± 12.80 for group two while 30.31 ± 6.51 was recorded for group three. Meanwhile, chlorophyll 'b' was observed and recorded for group one to be 8.80 ± 1.91 , group two (5.41 ± 3.84) and group three (9.09 ± 1.95). Similarly, carotenoids in group one represented the mean value and standard error of 2.92 ± 0.63 , for group two (2.36 ± 2.30) while group three recorded 3.03 ± 0.65 (Table 4, Figure 2).

The analysis of variance showed a significant ($p=0.05$) reduction on growth morphology. Similar decrease in plant height, number of leaves, and leaf area in stressed plants were observed in asparagus bean when exposed to the same saline medium (Chen *et al.*, 2007). *Vigna unguiculata* acted as an absorber of the salt ($MgSO_4$) used, and was unable to cope with it leading to reduction in morphological features of the plant due to the toxic effects of the salt, thus resulting in reduced internode length, petiole length, number of nodes and leaf area. As observed in this study, abiotic stress from the salt inhibits the plant growth, thus leading to a decrease in leaf area and seedling height.

High $MgSO_4$ concentration in the growth medium of the plant affected the plant growth and development adversely and negatively. The initial effect of salt stress on the plant growth was ionic toxicity and osmotic stress. Ionic toxicity occurs because high concentration of salt in the plant cells and interrupt several biochemical, physiological and morphological processes and osmotic induced by lowering the water potential causing turgor reduction and cellular loss and decrease in productivity (Chen *et al.*, 2007).

Secondary effects of salt stress are inhibition of mineral uptake, membrane dysfunction and generation of reactive oxygen species in the cells (Ghoulam *et al.*, 2002; Agarwal and Pandey, 2004; Upadhyay and Panda, 2005). Increasing salinity levels caused a significant

($p=0.05$) decrease in vegetative growth of *Vigna unguiculata* (both varieties), in which the morphological changes are probably caused by the reduction in turgor pressure within the cells that restricted cell expansion (Jampeetong and Brix, 2006). Consequently, the expansion of leaf was reduced. The reduction in plant growth has also led to a reduction in water absorption due to osmotic effect, nutritional deficiency in plants because of ionic imbalance and so much decrease in many metabolic activities according Kumar *et al.* (2005). Despite results of many researchers indicating a positive effect for salt stress on protein content, they are people who presented contrary result as well, indicating a negative effect for salt stress, Chen *et al.*, 2007 found that exposing *Vigna unguiculata* (L.) plant, of fourteen days, in salt treatment using 75mM of NaCl, reduced soluble protein content in the plant.

However, it was observed from the results of this research that the relationship between chlorophyll "a" and "b" at the same concentration of $MgSO_4$ followed a different sequence for the two bean varieties (white and brown). The result represented showed a decrease in chlorophyll "a" and "b" and carotenoids in both varieties this is in agreement with the findings of Schreiber and Stanbery (1965) that the exposure of Barley (*Hordeum vulgare* L.) to 0, 120 and 240 mM of $MgSO_4$ leads to the decrease in chlorophyll 'a', and chlorophyll 'b' and carotenoids.

Also, carotene content during exposure of the bean plants to salt stress appear in both pest crop that salt stress was an inhibiting factor for the formation of carotenes inside the stressed plants after days of treatment, where the carotene content was found decreased.

IV. CONCLUSION

From the result of this investigation, it is obvious that salt stress caused significant ($p=0.05$) growth stress on *V. unguiculata* and these two varieties (white and brown) exhibited significant and high variations for adaptation to salt stress. The results of this research also indicates that *V. unguiculata*, which widely used in Nigeria and Africa as a whole suffers morphological and physiological alterations under salinity stress that make them unable to develop and thrive effectively, thus reducing productivity of this very important crop.

V. REFERENCES

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