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Full Length Research Article

Effects of Salinity on the Growth and Visocosity of Fruits of Okra (*Abelmoschus esculentus* L.)

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A local okra variety (Ishiagu early) of *Abelmoschus esculentus L.* seeds were collected from Ishiagu in Ebonyi State in the southeastern region of Nigeria and were grown in plastic horticultural bags filled with a mixture of top garden soil, poultry manure and river sand as growth media. Seven days after germination, the plants were subjected to different levels of salt stress (control, 20mM, 50 mM, 100 mM and 200 mM Nacl). The growth parameters were measured at 2, 4, 6, 8, 10 weeks after planting. The growth parameters considered were germination percentage, shoot height stem girth, leaf length and width, fresh and dry weight of the plants, fresh pod yield and viscosity of the mucilage extracted from the fruits. Increasing salinity caused a reduction in plant growth. Germination was 100% in the control, however there was a sharp reduction as the level of salinity increased with 30% germination seen in 200nM treated soil. Shoot height decreased continuously with increment in the salinity level with a minimum of 38.54 cm in control and a maximum of 24.30cm in 200 nm treated saline soil. The shoot thickness also decreased considerably as the salinity level increased (control – 1.88 cm, 30 mM – 1.4 cm, and 200 mM – 1.30 cm). The fresh weights of the shoots of the plants were drastically affected and this is evident from the wide margin seen in 30 nm and 200 nm saline soil (16.76g and 4.12 respectively). A similar trend was observed in the dry weight. The pod weight was also affected negatively (25.94 g and 20.93 g for 30 nM and 200 nM treated soil respectively). The viscosity of the mucilage of the fruits was affected by the salinity of the soil. There was a reduction in the viscosity of mucilage as the salinity level increased. The highest was observed in control soil (198 mPa.s) and the lowest was in 200 nM treated soil (191 mPa.s). Visible physiological changes were also observed on the leaves, such as yellowing of older leaves, reduction in thickness and senescence of the older leaves with increase in salinity. The salt stress generally affected the okra germination rate, plant growth, development and yield as well as the viscosity of mucilage of the fruit of okra.

Key words: Okra, Salt stress, NaCl, Viscosity, Mucilage.

INTRODUCTION

Salinity is an environmental stress that limits growth and development in plants (Flowers *et al.,* 2010). Salinity is an expanding problem. More than 800 million hectares of land is salt affected which is over 60% of the world's land area (Flowers and Yeo, 1995). Salt affected land is increasing worldwide through vegetation clearance and irrigation both of which raises the water tables bringing dissolved salts to the surface. It is estimated that up to half of irrigation schemes worldwide are salt affected (Flowers and Yeo, 1995). Salt stress affects plants at both whole plant and cellular levels through osmotic and ionic stress (Murphy and Durako, 2003). This was also noted by Munns (2002), that soil salinity stresses plants in two ways, first the high concentrations of salt in soil make it harder for shoots to extract water (osmotic stress) and secondly '|high concentrations of salt within the plant can be toxic (ionic stress). Meanwhile, high salt content reduces growth and production by affecting physiological process including modification of ion balance, water status, mineral nutrition, stomatal conductance behavior and photosynthetic efficiency (Munns, 1993). The lack of absorption of water by plant growing in saline water results in physiological drought or dryness within the plant (Pooja, 2010). Okra (*Abelmoschus esculentus L.*) belongs to the family Malvaceae. It originated in tropical Africa and was grown in Mediterranean region (Abid *et al.,* 2002). Okra is an important vegetable crop in much of the tropics including Nigeria. Young fruits are consumed fresh or cooked (Fajinmi and Fajinmi, 2010).

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In West Africa, okra is utilized essentially because of its high mucilage content which is used in thickening of soup (Fatokun *et al.,* 1979). Mucilages and gums are water soluble polysaccharides found in widespread number of plants and also in some microorganisms (Hurst and Jones, 1978). Okra mucilage refers to the thick and slimy substance found in fresh as well as dried pods. Okra products are mucilaginous resulting in the characteristic slime when the seeded pods are cooked, mucilage contain a stable form of soluble fibre. The mucilage substances are usually concentrated in the fruit walls (not in seeds) and are chemically acidic polysaccharides associated with proteins and minerals (Kumar *et al.,* 2010). Viscosity is described as fluids internal resistance to flow and may be thought of as a measure of fluid friction. A liquid with the viscosity less than water is known as a mobile liquid, while a substance with a viscosity greater than water is simply called a viscous liquid (Edgeworth *et al.,* 1984). Okra's mucilage physical and chemical properties include high water solubility, plasticity, elasticity and viscosity (BeMiller *et al.,* 1993). Most of its physical and chemical properties are influenced by factors such as temperature, pH, sugar, salt content and storage time (Woolfe *et al.,* 1977; Baht and Tharanatha, 1987). This study is therefore aimed at investigating the effects of salt stress on the growth, yield and viscosity of fruits of okra grown under saline condition.

MATERIALS AND METHODS

Study site

The experiment was conducted in the green house of Michael Okpara University of Agriculture, Umudike, Nigeria. Umudike is located at Longitude 07°34" E, Latitude 05°29" N and an elevation of 122 m above sea level.

Plant material

Okra (*Abelmoschus esculentus L.*) cultivars used for this study were Ishiagu early and a dwarf type. It is popular among local farmers and it shows good adaptation to the local environment.

Horticultural bag preparations

Medium sized black horticultural polythene bags measuring 20 cm in diameter and 25 cm deep were filled with a mixture of top garden soil, poultry manure and river sand in the ratio of (3:1:1). These bags were placed on the concrete benches in the green house. The bags were fully irrigated with water in preparation for the seed planting.

Experimental design and treatments

The experiment was designed in a Completely Randomized Design (CRD) with three replicates. This was used in each case and all treatments were replicated for shoot height, shoot girth, fresh shoot weight, dry shoot weight, fresh pod yield. Five levels of solutions of NaCl were used as salt treatments. They were, control (00mM), 30 mM, 50 mM, 100 mM and 200 mM. The seedlings were irrigated with water for seven days after germination. At the end of the seventh day after germination, the different levels of NaCl solutions were used in irrigating the plants until the emergence of buds in preparation for flowering of the plants, they were twenty five horticultural bags altogether. The different levels of NaCl solutions were labeled according to treatment levels starting from 0 mM as the control, 30 mM as Treatment 1 (T_1), 50 mM as Treatment 2 (T_2), 100 mM as Treatment 3 (T_3) and 200 mM as Treatment 4 (T_4). Five horticultural bags with 3 plants in each one of them were assigned to the different levels of treatment.

Planting

Seeds of one local cultivar namely; Ishiagu early was planted in the prepared black horticultural polythene bags. Seed germination was calculated separately in Petri dishes by applying the different levels of NaCl solution on the filter papers. The seeds were washed with distilled water and then sown in Petri dishes with the filter papers moistened with the different levels of salt treatment solution in three replicates (10 seeds in each Petri dish).

Crop attributes measured

Records were taken every two weeks starting from 2 weeks after planting on the following crop attributes;

- Germination percentage (%) taken 5 days after planting.
- Shoot height (cm) taken 2 weeks after planting.
- Shoot girth (cm) taken 2 weeks after planting.
- Leaf length and width (cm) taken 10 weeks after planting.
- Fresh weight of the whole plants (g) taken 10 weeks after planting.
- Dry weight of the whole plants (g) taken 10 weeks after planting.
- Fresh pod weight (g) taken 10 weeks after planting.
- Viscosity of the fruits (mPa.s) taken in 10 weeks after planting.

Germination Percentage (%) – After five days, number of seeds germinated in each Petri dish was counted. The germination percentage was calculated. The shoot height was obtained by measuring the distance from the ground to the visible overlap of the plant shoot with a measuring tape and getting the average of each bag; while the shoot girth was measured using calipers and the average of each bag were calculated. The length and width of the fully matured leaves of three plants each from different treatment levels were plucked, measured with a measuring tape and recorded.
The plants were harvested at 10 weeks after planting. At each harvest, the plants with the roots and leaves were uprooted. The roots were washed with water to remove sands attached to them. Then fresh weights of the whole plants were obtained using an electronic weighing balance. Later, they were dried in an oven after which the dry weights of the whole plant were obtained using an electronic weighing balance. The weight of the fresh pod were also taken with an electronic weighing balance and recorded.

Extraction of the mucilages

Fresh and matured fruits of okra cultivar being studied were obtained from the experimental site immediately after harvesting of the whole plants. Fresh pod of the okra of the different treatment levels were harvested 10 weeks after planting. The fresh pods were labelled according to their different treatment levels as they were harvested. The fresh pods after weighing was taken to the laboratory on the same day for the extraction of the mucilages and measurement of the viscosities of the fruits from different treatment levels. The okra was sliced with a knife, blended for one minute. After blending, it was homogenised with ten times its weight with water (1:10). The viscous solution was separated from the debris using fine cloth (Thanatcha and Pranee, 2011).

Viscosity measurement of the mucilages extracted

Viscosity of various solutions of the different levels of treatment was measured using Torsion Viscometer (mPa.s). The readings were taken at 25 °C within 1 minute. It was measured in triplicate and the average taken. The pH of the mucilage was pH 6.0.

Statistical analysis

The data collected were subjected to one way analysis of variance. Mean separation was done using the Duncan Multiple Range Test. Consequently, the error bar was presented to facilitate interpretation and comparison of results.

RESULTS AND DISCUSSION

Visible changes on the leaves

The results of the effects of salinity on the visible changes on the leaves of the okra studied are presented in Plates 1 – 5. Minimum effects were shown by control (00 mM) followed by 30 mM, 50 mM, 100 mM and 200 mM. The observed changes in colour of mature leaves from dark green in colour in control to yellowish green in treatment level 200 mM could be due to the effects of salinity on plants which may become obvious over weeks especially in more sensitive species (that is those with high rates of salt uptake or inability to compartmentalize salt once it builds up in leaves). The injury may be visible as yellowing or death of older leaves (Munns, 2002). The progressive loss in leaf number of the plants of treatment levels 100 mM and 200 mM as against the control in which the leaf loss was reduced may be due to in salt-sensitive species, in which salt is not effectively excluded from the transpiration stream, salt will build up to toxjic levels in the leaves that has been transpiring the longest. This results in a progressive loss of the older leaves with time (Munns, 2002). The significant reduction in leaf size observed in the leaves of different treatment levels of 100 mM and 200 mM might be due to the decreased rate of leaf growth after increase in soil salinity which is primarily due to the osmotic effect of salt around the roots. A sudden increase in soil salinity causes leaf cell to lose water. Over days reduction in cell elongation and cell division will lead to slower leaf appearance and smaller final size (Munns and Tester, 2008). The premature deaths of some plants at the treatment level 200 mM were also observed in this study. This might be due to changes in cell elongation and cell division which lead to slower leaf appearance and smaller final size, and leaf growth is usually more affected than root. So plants with high salt uptake rates, the oldest leaf may start to show symptoms of injury. After weeks, it is clear that lateral shoots have been inhibited and a number of leaves may be dead. After months, the difference between plants with high and low salt uptake rates becomes very apparent, with a large amount of leaf injury and complete death in some cases if the salinity level is high enough (Munns, 2002). Similar results were found in wheat (Munns, 1993).

Germination Percentage (%)

Influence of sodium chloride on the seedling performance is given in Table 1 and Fig. 1. The maximum reduction in germination was observed at the treatment level 200 mM as compared to control which has 100% maximum percentage, 30 mM had 90% followed by 50 mM which had 80%, 100 mM which had 60% while 200 mM had 30%. The germination of the okra seeds were affected because high salt concentration solution sprayed on the filter paper in the petri dishes increased the osmotic potential which caused the seeds to use more energy to absorb water from the filter papers hence effects germination (Shahid *et al.,* 2011).

Shoot height

The data in Table 1 and Fig. 2 show that increasing concentration of NaCl had significant ($P<0.05$) adverse effect on the shoot height. It decreased continuously with the increment in salinity level from control to 200 mM. The control showed the highest shoot height of 38.54 cm, followed by the treatment levels 30 mM which had 30.52 cm, 50 mM had 28.41 cm, 100 mM had 25.889 cm and 200 mM with the lowest value 24.31 cm. The okra plants under control may have adjusted osmotically to the growing conditions as a result of which they were successful in maintaining required cell enlargement so they showed maximum shoot height (Shahid *et al.,* 2011; Kaya *et al.,* 2000) recorded that the visible symptoms of salinity on plant were stunting shoot and root growth, and smaller leaves in size.

Shoot girth

Sodium chloride also exerted a significant ($P<0.05$) effect on the shoot girth development by affecting its thickness (Table I and Fig. 3). All the four different treatment levels: 30 mM – 1.47, 50 mM – 1.42 cm, 100 mM – 1.31 cm and 200 mM – 1.30 cm caused reduction in the thickness of the shoot as compared to the control which had 1.88 cm. This may be due to the effects of high salt concentration on the elongation process, because the excess salt modifies the metabolic activities of the cell wall leading to the deposition of various materials which causes a reduction in the cell wall elasticity. Secondary cell wall form sooner as a result of which cell wall becomes rigid hence causing a decrease in turgor pressure efficiency in cell wall enlargement (Aslam *et al.,* 2011).

Fresh shoot weight and Dry shoot weight

The salt treatment significantly ($P<0.05$) affected the fresh shoot weight of the plants (Table 1 and Fig. 4). The maximum fresh weight value was 26.8 g in control, 30 mM had 16.7 g, 50 mM had 10.99 g and 100 mM had 7.8 g. The minimum was 200 mM which had 4.21 g. In addition, dry shoot weight were also significantly ($P<0.05$) affected by NaCl applications (Table 1 and Fig. 2). The maximum dry weight 5.22 g was obtained from the control, while minimum, 2.83 g, was got from 200 mM. Other different treatment levels gave 30 mM – 3.06 g, 50 mM – 2.23 g and 100 mM gave 1.5 g. It was evident that at 100 mM and 200 mM that reduction in fresh shoot weight and dry shoot weight of these plants were more. These plants failed to activate the dehydration avoidance mechanism like making the root membranes impermeable for toxic ions of Na^+ and Cl^- and did not maintain stomatal conductance up to desired rate (Abbruzzese *et al.,* 2009), thus could not with stand high salt stress and experienced the reduction in growth of fresh and dry shoot weight (Shahid *et al.,* 2011).

Fresh Pod Yield

The salt stress also showed significant ($P<0.05$) influence on the yield of okra (Table 1 and Fig. 5). Maximum decrease was recorded at 200 mM which had 20.39 g, 100 mM had 22.5 g, 50 mM had 23.77 g and 30 mM had 25.94 g as compared to the non-saline control with the highest pod weight of 57.67 g. The reduction shown by the okra plants of treatment level 100 mM and 200 mM may be due to the high salt concentration of the soil which caused shrinkage of the cell contents as well as reductions in the development and differentiation of the tissues unbalanced nutrition, damage of membrane and disturbed avoidance mechanism. These entire factors contribute towards the reduction in plant yield (Aslam *et al.,* 2011).

Viscosity measurement on the mucilage of the fruits of the okra

The result of the effects of salinity on the viscosity of the mucilages of the fruit is presented in Fig. 7. The maximum viscosity 198 mPa.s was given by control. The different treatment levels 30 mM gave 196 mPa.s, 50 mM gave 195 mPa.s, 100 mM gave 193 mPa.s and minimum was at 200 mM which gave 191 mPa.s. Okra grown under salinity showed rapid decrease in salt concentrations. This may be as a result of the interactions of the ions with the acid groups of the polysaccharides casuing a change in molecular shape and hence viscosity (Woolfe *et al.,* 1977). Pour value of 0.1 percent okra solution were measured before and after addition of Na2SO4, NaCl and $CaCl_2$. The results indicated that the pour value (ropiness) decreases in the following ions Ca^{++}, Na^+, $SO_4^{--}$and Cl^- and the tolerance of okra for ions decreased from monovalent to trivalent ions. In addition to support this, Woolfe *et al.,* (1977) showed that the addition of calcium ions purified okra and purified baobab mucilage brought about decrease in the viscosity of both mucilages. This behaviours is again similar in gum Arabic.

High concentrations of salts in the soil make it harder for shoots to extract water from the soil. Sonneveld and Voogt (1990) reported that water in tomatoes was reduced with application of high sodium chloride. Kaya *et al.,* (2001) showed that high salinity in root medium reduced water use in tomatoes, cucumber and pepper. Also plants at high salinity had very leaky root systems as evidence by high K^+ efflux – high NaCl in nutrient solution has a detrimental effect on root membrane integrity. High K^+ efflux in root is evidence that high salt causes damage to the root medium. Root permeability of plants decreased under salt stress (Kaya *et al.,* 2011) and may have led to reduction in water absorption rate, resulting in dryness within the plnat. This condition may affect growth, flowing, and fruiting of plants (Aslam *et al.,* 2011; Adetuyi *et al.,* 2011) worked on the viscosities of different selected cultivars of okra and they showed that the viscosity range of the different cultivars to be 56.42 – 68.12 cp with the Ikaro local okra having the highest viscosity of 68.12 cp, which was significantly (P<0.05) higher than other varieties observed. They concluded that it is expected that the higher the moisture content, the higher the viscosity which could have been contributed to Ikara local okra having the highest viscosity. This may possibly be part of an explanation for the decrease in viscosity of the mucilage of okra as the NaCl treatment increased. The water content of the fruit of okra plants must have lowered due to the dryness within the plant caused by lack of absorption of water from the soil by the okra plants. This confirms the work of Kaya *et al.,* (2011) that reported lower leaf relative water content in spinach when subjected to salt stress.

Conclusion

The salt stress affected the okra germination rate, plant growth, development and yield as well as the viscosity of mucilage of the fruit of okra. The study assumes from the results obtained that the local okra cultivars can perform well at control and 30 mM of NaCl treatment levels and the viscosities of these treatment level were not so much affected. The results of the salt stress on the growth of okra have the potential to benefit farmers, vegetable growers as well as researchers. With rapid urbanization and population growth, market-oriented okra production is increasing in pre-urban zones. Okra is now cultivated as irrigated crop during dry season. One of the effective ways to overcome salinity problem in the soil is the introduction of salt tolerance crops by plant breeders.

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