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Full Length Research Paper

Growth and physiological response of African nightshades (Solanum Scabrum Mill.) to Sodium chloride salinity stress

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African nightshades (Solanum scabrum Mill.) are one of the African indigenous vegetables widely consumed in Kenyan diet as a cooked vegetable. They are known as a good source of vitamin c and they help in boosting body immune system. Salinity is one of the most serious threats to agriculture all over the world and one major environmental factor that limit crop growth and performance. To elucidate possible adaptive strategies that enable this species to survive areas subjected to salinity, growth and physiological characters such as shoot height, stem diameter, leaf area, leaf number, biomass, chlorophyll concentration and fluorescence were studied in controls and plants subjected to various NaCl salinity levels for four weeks. The study was conducted at Maseno University, Kenya under Glasshouse conditions. The plants were subjected to five different levels of NaCl salinity (0, 25, 50, 75 and 100mM). High salinity levels (75 and 100mM) significantly (p < 0.05) reduced shoot height, number of leaves, leaf area, fresh as well as dry weight of shoots and roots, stem diameter, chlorophyll concentration and fluorescence. Injuries such as leaf senescence and abscission were not observed but some salinised plants showed signs of leaf chlorosis. The causes of the reduction in growth of African nightshades could be as a result of decreased water uptake, toxicity due to sodium and chloride ions as well as reduced photosynthesis which may partly be attributed to decrease in chlorophyll concentration and loss of chloroplast activity. Based on the results we recommend this species of African nightshades to be grown in areas with salinity levels of not more than 75 mM NaCl.

Keywords: African nightshade; chlorophyll content; chlorophyll fluorescence; growth; Sodium chloride salinity

INTRODUCTION

Solanum scabrum is one of the commonly consumed African indigenous vegetable in Kenya (Edmond and Chweya, 1997; Muthomi and Musyimi, 2008). African nightshades are amongst the common and popular leafy vegetables found in the warmer and humid zones of Africa (Edmond and Chweya, 1997). In several places, it is the most important leaf crop surpassing cabbage and kales. It is highly nutritious as well as a medicinal plant. It reduces malnutrition in young children and women of child bearing age. African nightshades provide vitamin C riboflavin, folic acid, carotenes, protein, iron, vitamin A, lodine, Zinc and Selenium (www.new.ag.infor/focusItem.php?a=422). The high levels of vitamin C and macro nutrients are especially

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important to people at risk of malnutrition and HIV/AIDS (www.new.ag.infor/focusItem.php?a=422). Solanum scabrum leaves extract have been used to treat diarrhoea and jaundice in children. Raw fruits are also used to treat stomach ulcers when chewed and swallowed (http://www//database.prota.org/PROTAhtml/). It is also used as fodder for livestock.

Salinity refers to the occurrence of high concentrations of dissolved major inorganic ions in solution; including Na⁺, Mg²⁺, Ca²⁺, K⁺, HCO₃, SO₄²⁻ and Cl⁻ (Musyimi *et al.*, 2007). The total area of saline soils is 397 million ha (FAO, 2004). Of the current 230 million ha of irrigated land, 45 million ha are salt-affected soils (19.5 percent) and of the almost 1 500 million ha of dryland agriculture, 32 million are salt-affected soils (2.1 percent) to varying degrees the result of human-induced processes (FAO, 2004). Salt affected soils (EC > 4 dS m⁻¹) can be characterized by the excess levels of water-soluble salts, including sodium chloride (NaCl), sodium sulfate (Na₂SO₄), calcium chloride (CaCl₂) and magnesium chloride (MgCl₂), among others (Cha-um, and Kirdmanee, 2010). Salt is a natural element of soils and water soil salinity is the salt content in the soil. The ions responsible for salinization are Ca^{2+} , Na^+ , Mg^{2+} and Cl. As the Na⁺ (sodium) predominates soils can become sodic and tend to have very poor structure which limits or prevents water infiltration and drainage over a long period of time

(http://www.fao.org/docrop/x50871e/x587ieoo.htm). Land salinization is among the environmental constraints that affect crop agricultural production production of marginal agricultural soils in many parts of the world (Pandey and Thakares, 1997; Munns, 2002). It leads to perturbation of all or some of physiological and biochemical processes in plants (Sidari et al., 2007). It is a common problem in many parts of Kenya (Musyimi et al., 2007). The area affected by salinity is increasing steadily worldwide, as a result of mismanaged irrigation (Almodares et al., 2007). High concentrations of salts in soils account for large decreases in yield of a wide variety of crops all over the world (Levitt, 1980; Yildrim et al., 2006). Most crops tolerate salinity up to a threshold level above which growth or yield decreases as salinity increases (Maas, 1986). This threshold varies with species (Pasternak and Malach, 1994). Salinity affects plant growth by ion toxicity, osmotic stress, mineral deficiencies or combination of any of these factors (Hasegawa et al., 2000; Musyimi et al., 2007). Plants must increase the energy to obtain water from the soil and the energy used would otherwise be useful for anabolic activities of the plants (Munns, 2002).

Saline conditions disrupt several physiological processes in plants leading to reduction in growth and yield (Saranga *et al.*, 1993; Ashraf and Shahbaz, 2003; Zadeh and Naeini, 2007). Salt limits the normal plant metabolism, water quality and nutrient uptake of plants.

Because many salts are also plant nutrients, high salt levels in the soil can upset the nutrient balance in the plant or interfere with the uptake of some nutrients (Ali et al., 2001). Reductions in chlorophyll concentration under elevated salinity conditions have been reported in some salt-sensitive plant species (Ashraf et al., 2000; Jungklang et al., 2003; Lee et al., 2004). Chlorophyll concentration in salt-tolerant plants either does not decline or else rises with increasing salinity (Qui and Lu, 2003). According to Yeo and Flowers (1986), chlorophyll concentration can be used as a sensitive indicator of the cellular metabolic state since its decrease signifies toxicity in tissues due to accumulation of ions. Salt stress causes a substantial damage to photosystem II (PS II) and of the two photosystems, PS II is found to be more prone to the hazardous effects of salinity (Abdeshahian et al., 2010). Measurement of chlorophyll fluorescence has been used to evaluate the integrity of photosystem II upon exposure to stress (Shabala, 2002). The functionality of PS II can be assessed by measuring different attributes of chlorophyll fluorescence. A number of studies on photosystem II performance have been conducted on different plant species in the field (Bacelar et al., 2009), on cultivated plants under salt stress (Sofo et al., 2009), and on detached leaves (Catala et al., 2007). These studies have reported a good correlation between plant growth and changes in the chlorophyll fluorescence parameters in response to salt stress conditions. Of different chlorophyll fluorescence parameters the most important one is the leaf maximum quantum yield of PSII (Fv/Fm) that results in a severe reduction under stress conditions.

The problems of salinity are becoming more severe worldwide, there is need to grow alternative plants and crops that are suited to moderately saline conditions. The option of introducing under-exploited and salt-tolerant minor crops should also be considered. Due to the growing interest in improving food security in Kenya research is required to evaluate the tolerance of African nightshades (*Solanum scabrum*) to NaCl salinity which is a possible candidate crop to be introduced in areas faced with salinity problem in Kenya. The future of growing salt tolerant genotypes of African nightshades is bright. However, despite large amounts of literature on responses of plants to salinity stress, no information is available on African nightshades.

The main aim of the present study was to determine growth and physiology parameters of African nightshade plants when subjected to various NaCl salinity levels.

MATERIALS AND METHODS

Experimental Materials and growth conditions

Seeds of *Solanum scabrum* were obtained from Botanic

garden of Maseno University, Kenya. Ten viable seeds were sown in 4.5 litre plastic pots containing soil collected from Maseno University, botanic garden. Maseno soils are classifield as acrisol deep reddish brown clay and well drained with a pH range of 4.5-5.4 (Mwai, 2001). After germination, seedlings were thinned to two seedlings per pot. Plants were subjected to five levels of sodium chloride solutions whose concentrations were: 0 (control), 25, 50, 75 and 100mM NaCl with five replications. The pots containing the plants were arranged in a completely randomized design inside a glass house. 300 ml of tap water and NaCl solution was added to every pot on daily basis in the morning upto the end of the experiment. To reduce osmotic shock, saline treatment was imposed incrementally, increasing the concentration by 25 mM NaCl, every second day until the final 100mM NaCl was reached. Granule fertilizer (Foliar Nitro-Phoska) containing nitrogen, phosphorus, potassium, magnesium (20-19-19-0.5) was applied at a rate of 2g per pot after 2 weeks. Plants were grown under naturally illuminated glasshouse conditions of 30/25 ^UC day/night temperature, 70% relative air humidity, and a $230 \mu mol m^2 s^{-1}$ photon flux density of of photosynthetically active radiation at plant height. Data collection commenced immediately after the initiation of the treatments and continued for four weeks.

Determination of growth parameters

At the end of experiment, shoot height was measured from the base of the stem, just above the soil surface, to its apex using a millimeter rule. The total number of leaves on both the stem and branches were also counted and recorded at the end of the study. Leaf area was obtained by measuring the length of the leaf and width of the leaf and calculated following the formula of Otusanya *et al.* (2007) as shown below:

 $LA=0.5(L_I \times W_I)$, where L_I is the leaf length and WI is the maximum width measured for each leaf on each plant.

At the end of the experiment, plants were harvested carefully from the pots. Roots were separated from the shoots and then weighed immediately using an electronic weighing balance. The roots and shoots were packaged separately in envelopes and dried in an oven at 80°C to constant weight for 48 hours. The weights were then determined using an electronic weighing balance. Stem diameter was measured 5cm above soil level of each plant using a vernier caliper.

Chlorophyll concentration determination

Chlorophyll concentration was determined at the end of the experiment by sampling the fourth fully expanded leaf from the shoot apex from all the treatments. 0.3g of leaves from Solanum scabrum were soaked overnight in 5ml 80% (v/v) acetone. The resulting supernatant was read at 664nm and 647nm using uv-visible

spectrophotometer. Total chlorophyll content was determined using the following equation according to Adelusi *et al.* (2006).

Total chlorophyll = $7.93 \ A664 + 19.53 \ A647 \ (mg g^{-1} fresh weight).$ Where,

A664 is the absorbance at 664 nm

A 647 is the absorbance at 647 nm.

Determination of chlorophyll fluorescence

Chlorophyll fluorescence measurements were taken at the end of the experiment using a portable nonmodulated fluorometer, (Hansatech, U.K.). PEA, Measurements were done on leaves (second fully expanded leaves from the top of the plants), which had been dark -adapted using leaf clips for 15 minutes. An actinic light pulse was used to saturate the photosystems. The parameters of fast chlorophyll fluorescence: maximum fluorescence yield from PSII following a saturating pulse of photons in a light-adapted plant (F_{M}), steady state yield of PSII fluorescence in the light (Fs), electron transport rate through PSII (ETR), actual efficiency of PSII [quantum yield of PSII electron transport, (OPSII); Belkhodja et al. 1999], were determined during the day between 1100 and 1400 hours. Quantum yield of PSII electron transport, (**PSII**) was calculated as [Fm'-Fs]/Fm' (Maricle et al., 2007). Efficiency of excitation capture of open photosystem II (Fv/Fm) was also measured following the guidelines of Maxwell and Johnson (2000).

Statistical analyses

Data collected was subjected to analysis of variance (ANOVA) using SAS statistical computer package and least significance difference (L.S.D) test at 5% level of significance was used to separate and to compare the means.

RESULTS

Shoot height was significantly (p < 0.05) reduced by NaCl saline water treatment (Table 1). There were significant (p < 0.05) differences in shoot height reductions among the treatments compared to the control. The reduction in shoot height was 70% and 64% of control plants at 75 and 100 NaCl mM. Low levels of salinity (0, 25 and 50 NaCl mM) had no significant (p > 0.05) impacts on leaf number increase (Table 1). Saline water treatments 75 and 100 NaCl mM significantly (p < 0.05) reduced the

NaCI Mm	Shoot height (cm)	Leaf number per plant
0	13.0500a	10.1600a
25	12.1312ab	9. 7600a
50	11.2156b	9.5600a
75	9.1720c	8.9200 b
100	8.3240c	8.4000b

Table 1. Effect of NaCl salinity on shoot height and leaf number of

 Solanum scabrum after four weeks of saline water treatments.

Means in each column followed by the same letter are not significantly different at p<0.05.

 Table 2. Effect of five levels of NaCl salinity on shoot and root fresh and dry weight of Solanum scabrum after four weeks of saline water treatments.

NaCI Mm	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
0 (control)	10.760a	3.8720a	0.9200a	0.50000a
25	9.478ab	3. 1500a	0.9200a	0.44800a
50	9.120bc	2.7500b	0.7300b	0.36000b
75	6.924c	1.5180bc	0.5500b	0.22000b
100	6. 422c	1. 3900c	0.5000b	0.2000b

Means in each column followed by the same letter are not significantly different at p<0.05.

Table 3. Effect of NaCl salinity on stem diameter, leaf area and chlorophyll concentration of Solanum scabrum after four weeks of saline water treatments.

NaCI Mm	Stem diameter (mm)	Leaf area (cm ²)	Total chlorophyll (mg/g)
0	0.48400a	15.370a	59.546a
25	0.45200a	14. 202ba	46.124ba
50	0.43600a	12.426bc	43.838bc.
75	0.36000b	10.078c	38.930bc
100	0.35400b	9. 310c	31.220c

Means in each column followed by the same letter are not significantly different at p<0.05

number of leaves per plant, which was 87.8 % and 82.7% of control plants respectively.

The shoot and root fresh and dry weights were significantly (p < 0.05) reduced as the salinity level increased (Table 2). There were significant (p < 0.05) differences in shoot fresh weight among treatments. Root fresh weight for control treatment and 25 mM NaCl treatment were not significantly different from each other, but there were significant differences between treatments 50, 75 and 100 mM. There were no significant (p < 0.05) differences in shoot and root dry weights between control and 25 Mm NaCl level, however there were significant (p < 0.05) differences in shoot and root dry weights between NaCl treatments 50, 75 and 100mM. At 100mM NaCl, shoots fresh and dry weights showed about 59.7% and 54.3 % reduction than roots compared to control.

Salinity treatments significantly (p < 0.05) reduced the stem diameter of the plants (Table 3). Stem diameter reduced at about 73% of control plants at 100 mM NaCl treatment. Compared to control plants, leaf area of NaCl

treated plants were significantly(p < 0.05) different, and decreased with increase in NaCl salinity level (Table 3).The decrease was about 61% of control plants at 100 mM NaCl. Chlorophyll concentration of *Solanum scabrum* leaves reduced significantly (p < 0.05) with increase in NaCl salinity (Table 3). Chlorophyll concentration at higher salinity level, 100 mM NaCl was more reduced than in the other salinity levels. The decrease in chlorophyll concentration was 52.4% of control plants at 100 mM NaCl.

Maximum quantum yield of primary photochemical reaction (Fv/Fm) decreased significantly (p < 0.05) with increase in salinity of irrigation water (Table 4). The reduction was 96.3% and 93.5% of control plants at 75 and 100mM NaCl respectively. Quantum yield of PSII electron transport (Φ PSII) decreased significantly (p < 0.05) between treatments even though there was no significant difference between control and 25 mM NaCl treatments (Table 4).The decrease in Φ PSII at 75 mM NaCl was 94.9% of control plants and at 100 mM NaCl,

Table 4. Effect of five levels of NaCl salinity on maximum quantum yield of primary photochemical reaction (Fv/Fm), quantum yield of PSII electron transport (Φ PSII) and electron transport rate (ETR) of *Solanum scabrum* after four weeks saline water treatments.

NaCl Mm	Fv/Fm	ΦPS II	ETR
0 (control)	0.979a	0.862a	46.21a
25	0.967ab	0.852a	39.8a
50	0.954bc	0.824b	38.6b
75	0.943c	0.818bc	36.33b
100	0.915d	0.785c	31.2c

Means in each column followed by the same letter are not significantly different at p<0.05.

the decrease was 91.1% of control plants. Electron transport rate (ETR) also decreased significantly (p < 0.05) as the salinity level of irrigation water increased (Table 4). The decrease was 83.5% and 78.6% of control plants at 75 and 100mM NaCl respectively.

DISCUSSION

Saline environment can cause a wide number of responses in plants leading to growth inhibition (Jaleel et al., 2008). Reduction of plant growth under saline conditions is a common phenomenon (Ashraf and Harris, 2004) but such reduction occurs differently in different plant organs. High salinity levels significantly reduced plant height, stem diameter, fresh as well as dry weight of shoots and roots, the number of leaves, and leaf area of solanum scabrum. For example, in the present study, shoot dry weight was reduced more than root dry weight by salt stress. Similar kinds of results have been reported by Jamil et al., (2005). The results reported here are in agreement with those reported by Essa and Al-Ani (2001). Similar decreases in growth parameters were found in Withania somnifera under salt stress and in Salvodora persica under saline conditions (Jaleel et al., 2008). The present results confirmed the earlier studies that indicated roots to be more resistant to salinity compared to shoots (Essa , 2002; Al-Shoaib and Al-Sobhi, 2004). Roots might seem the part of the plant most vulnerable to salinity, as they are directly exposed to salt or to drying soil but their growth rate is not affected as that of shoots (Munns, 2002). Rapid root growth inhibition by high NaCl levels has been observed for most crops (Reinhardt and Rost, 1995). Salt stress inhibited root growth in cleome gynandra (Mwai, 2001), however root growth was less pronounced and consistent compared to the shoot responses. Reinhardt and Rost (1995) have noted that the most common salt stress effect is a general stunting of plant growth.

The reduction in shoot dry weight could be associated with lower leaf production and development of smaller leaves. Salt stress alters cellular metabolic activities such as normal protein synthesis (Lutts *et al.*, 1996; Sharma and Hall, 1992), which could be one of the reasons for the decrease in fresh and dry weights at higher salt concentrations in this study. Salinity may decrease biomass production because it causes a lowering of plant water potentials, specific ion toxicities, or ionic imbalances (Neumann, 1997). Plants typically respond to salinity stress by reduced shoot and root growth with shoot growth reduction occurring earlier (Munns, 2002).

Leaf production and expansion may be the processes particularly sensitive to salinity (Lutts et al., 1996). Reduction in leaf growth of plants exposed to salinity has been attributed to reduced turgor (Neumann et al., 1988) or reduction in extensibility of expanding cell walls (Neumann, 1993). The inhibition of leaf growth in the short term may be due to water stress while on long term scale, leaf growth is affected by ion toxicity when the ions move through the transpiration stream and accumulate in the leaves (Yeo et al., 1991; Munns et al., 2006). The reduced leaf area is an adaptation to reduced ion uptake by roots (Neumann et al., 1988). Reduction in leaf area contributes to less photosynthesis and hence less dry matter accumulation. Salt stress reduces leaf growth rate by shortening the length of the leaf elongating zone (Bernstein et al., 1993). Cell expansion in leaves can be inhibited by salt stress and this may lead to reduction in leaf area and decline in shoot dry weight (Atlassipak et al., 2009). Greenway and Munns (1980) reported that the effect of salinity on leaf area was greater than on dry weight, as salt accumulation in the shoot occurs via transpiration stream. The reduction in growth of Solanum scabrum could be due to decreased water uptake, toxicity of sodium and chloride ions as well as reduced photosynthetic rates. Effect of salinity on plant growth may result from impairment of supply of photosynthetic assimilates (Atlassipak et al., 2009).

Reduction in chlorophyll concentration in this study could be attributed to the inhibitory effect of accumulated ions of salts on the biosynthesis of the different chlorophyll fractions. This reduction has also been linked to the destruction of chlorophyll pigments and the instability of the pigment protein complex (Jaleel *et al.*, 2008). Similarly, a decrease in leaf chlorophyll content has been described in maize irrigated with water high concentration NaCl (Demir containing and Kocacaliskan, 2008). These results are not in agreement with those of Jamil et al., (2007) who found that chlorophyll content increased significantly with increasing salinity in sugar beet and cabbage. Decreases in photosynthetic pigment concentration have been reported in salt stressed plants before (Mickelbart and Arpaia, 2002; Musyimi et al., 2007). Results reported by Araus et al. (1997) do not agree with our findings because salt stress had no significant effect on chlorophyll content in their study. Leaf senescence is most often quantified by decreases in protein or chlorophyll concentration (Lutts et al., 1996). The concentrations of chlorophyll components of the photosynthetic apparatus are normally well correlated with photosynthetic capacity under non-stress conditions. The decrease in chlorophyll content under salt stress as observed in this study could be attributed to the adverse effects of the salts on membrane stability (Ashraf and Bhatti, 2000). Similar findings have been reported in spider plant (Mwai, 2001). Salinity reduces the chlorophyll content in salt susceptible plants and increases it in salt tolerant plants (Heidari, 2012).

Fv/Fm is almost constant for different plant species measured under non-stressed conditions, with 0.80≤ Fv/Fm ≤0.86. For the most severe salt stress, Fv/Fm decreases to 0.588±0.019 (Abdeshahian et al., 2010). The decline in Fv/ Fm, ΦPS II, and ETR with increase in NaCl treatments in our study may be attributed to the effects of the salts on the reaction centers of PS II system directly such as losses in chloroplast activity, inhibition of electron transport at both acceptor and donor sides of PSII (Rao et al., 1987; Gong et al., 2008) and partly to decrease in Rubisco activity and content (Lobban and Harrison, 1994). Salt-induced decline in Fv/Fm is a promising indicator of the functionality of photosynthetic apparatus of plants (Gomathi and Rakkiyapan, 2011). Previously, Jiang et al. (2006) reported a significant reduction in *electron* transport rate (ETR), and maximum quantum efficiency of PSII in different cultivars of barley, which is in agreement with the current results. Reduction in plant chlorophyll fluorescence parameters has been positively correlated with a decrease in different photosynthetic parameters and biomass production. Reduction in chlorophyll concentration could partially explain the decreases in chlorophyll fluorescence parameters in salt-treated plants. Some studies have shown that salt stress inhibits PSII activity (Gong et al., 2008) whereas others have demonstrated that salt stress has no effect on PSII (Morales et al., 1992; Abadía et al., 1999; Netondo et al., 2004). This shows that the sensitivity of PSII to salt stress is different in different plant species. The major process involved in the protection against photo damage is probably the photochemical quenching energy dissipation, which

reduces the relative quantum yield of PSII in order to maintain an adequate balance between photosynthetic electron transport and carbon metabolism (Abdeshahian *et al.*, 2010).

CONCLUSION AND RECOMMENDATIONS

The results indicated that NaCl salinity reduced plant height, stem diameter, leaf number, leaf area, chlorophyll concentration and photosynthetic performance of African nightshades. The results indicate that *Solanum scabrum* is sensitive to high salinity stress above 75mM NaCl and therefore relatively low to moderate salinity must be maintained to achieve high growth rate of African nightshades. Further research is needed to evaluate other varieties of African nightshades to salinity tolerance in order to understand their physiology in relation to salt stress..

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