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Kikuyu Grass: A Valuable Salt-Tolerant Fodder Grass

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Abstract: The turf grass *Pennisetum clandestinum* Hochst. (kikuyu grass) is one of the candidate plants for utilization and reclamation of salinized areas. The capability of kikuyu grass to grow under saline conditions was tested during 6 months, under various irrigation treatments (tap water control, 80-mM, 150-mM, 200-mM, 240-mM NaCl). Plant biomass production was visibly affected only at NaCl concentrations greater than 150-mM NaCl. Plant growth and plant regeneration capability in the 200- and 240-mM NaCl treatments gradually decreased as the experiment progressed in time. The photosynthetic potential of the plants remained unchanged and was neither affected by the treatment nor with time. Proline content of leaves as well as the content of Na^+ and Cl^- increased with increasing salinity stress. Apparently, kikuyu grass can withstand moderate concentrations of NaCl for prolonged periods and under repeated mowing. Thus, this grass can be used as a potential ground cover and as fodder grass in saline habitats.

Keywords: *Pennisetum clandestinum*, proline, salinity, sodium chloride

INTRODUCTION

Salt stress is one of the most serious environmental factors limiting the productivity of crop plants (Ashraf 1999). Saline soils present special challenges for plant subsistence and growth. Moreover, salinity plays a major role in natural ecosystem functioning, limiting plant development, particularly in arid and semiarid regions. In such ecosystems, reduced precipitation leads

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to reduced leaching of salt from the soil and consequently to salinization of the upper soil horizon. Such a process leads to the alteration of the soil structure, causing severe soil erosion, plant cover reduction, and a deterioration of the natural vegetation. Amelioration of saline soils can be achieved by the introduction of salt-resistant ground cover species (Chapman 1960). Certain varieties of kikuyu grass (*Pennisetum clandestinum* Hochst.) are known to be tolerant to salinity (Russell 1976; Skerman and Riveros 1990), tolerant to drought (Whiteman 1990), and tolerant to water logging (Dale and Read 1975). Thus, this species seems to be a good candidate for planting and use in such habitats.

Kikuyu grass is a perennial grass native to East and Central Africa (Skerman and Riveros 1990) that was introduced to many parts of the world (Rumball 1991; Herreroborgonon, Cristobal, and Crespo 1995). Considering its fast growth, dense ground cover, and well-developed root system, kikuyu grass can be an exceptional species for erosion control on desert edges and salinized soils. Kikuyu grass can also be used as a pasture plant, because of its good nutritive properties (Butler and Bailey 1973; Marais, Figenschou, and de Figueredo 1992).

Moreover, its capability to regenerate rapidly following repeated mowing is a highly important trait of fodder plants, especially for those that are grown under saline conditions (Gugenheim and Waisel 1977).

Salt tolerance of plants involves the preservation of a basic ionic balance in their cells and certain metabolic changes that decrease salt injury. Accumulation of Cl^- was reported as one of the means that enables salt tolerance in some plants (Azmi and Alam 1990; Ashraf and O'Leary 1995). Increased Na^+ content generally disturbs the nutrient balance and osmotic regulation of sensitive plants but is controlled in salt-tolerant plants, where it plays an important role in their adaptation (Waisel 1989).

The decline in productivity of many salt-affected plant species that are subjected to excess salinity is often associated with a reduction in photosynthetic capacity (Long and Baker 1986). One of the common adaptive responses of plants to salt stress is expressed by their proline metabolism. Proline is an important factor in establishment of an osmotic equilibrium in salt-affected plants, and thus is a good method for monitoring of stress tolerance of such plants (Delauney and Verma 1993; Sidari, Panuccio, and Muscolo 2004). The fast and aggressive reproduction and spread of kikuyu grass made it a weed in numerous agricultural and recreational areas (Wilen et al. 1995; Wilen and Holt 1996). However, such characteristics could be an advantage for reclamation of salt-affected sites. Thus, the present study has tackled the following questions:

a. How do increasing concentrations of NaCl affect above- and belowground biomass production of kikuyu grass?
b. Does salinity affect the photosynthetic efficiency of this plant species?

c. How much is the accumulation of ions in leaves of kikuyu grass affected by the increasing salt concentrations of the irrigation water?
d. What is the role of proline in osmotic adaptation of this species?

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Cuttings and runners of *P. clandestinum* were grown in pots filled with 10 kg of sand. The pots were watered with a nutrient solution equivalent to half of Hoagland's nutrient solution. The plants were grown in a ventilated greenhouse (~25°C) under long-day conditions (16/8-h photoperiod).

Experimental Treatments

The plants were irrigated daily with different NaCl treatments (80-mM, 150-mM, 200-mM, and 240-mM of NaCl, supplemented with the nutrient solution), with 12 replicates per each treatment. Control pots were treated with nutrient solution only. The treatments were continued for 6 months.

Plant Biomass

Plants were pruned regularly every month at the height of 4 cm above the ground. The clipped shoots were oven dried at 80°C for 2 days and then weighed. At the end of the experiment, 80 days after transplantation, the plants were removed from the pots. Shoots and roots were separated, washed, and dried at 80°C for 2 days, and the dry weight was then determined.

Photosynthetic Yield Measurements

Pulse amplitude modulated fluorometry (PAM, Walz Gmbh, Effeltrich, Germany) was used to measure the photosynthetic quantum yield of dark adapted fully developed leaves of the treated plants after 6 months of treatment. Leaf clips (Dark Leaf Clip DLC-8) were placed on different leaves, taken randomly from the treated plants, to have them completely dark adapted for a period of 30 min. Maximal quantum yields were evaluated using the equation

$$\frac{F_v}{F_m} = \frac{(F_m - F_o)}{F_m}$$

where F_v is the variable fluorescence, F_o is the fluorescence measured after dark adaptation, and F_m is the maximal fluorescence of the dark-adapted leaves after the application of a 0.8-s pulse of light that is saturating for photosynthesis

(∼6000 μmol photons $m^{-2} s^{-1}$) (Beer et al. 1998; Beer et al. 2000; Kitajima and Butler 1975).

Proline Estimation

Fresh leaf segments, taken from plants of the different treatments, were cut and ground in liquid nitrogen. Approximately 2 mL of 3% sulfasalicylic acid were added, and the test tube was then centrifuged at 14 k rpm for 5 min. Proline was determined using ninhydrin acid reagent according to Bates, Waldren, and Teare (1973) and L-proline as standard.

Determination of Na^+ and Cl^-

Dried leaves, taken from each of the different treatments, were weighed and ashed in the furnace at 550°C for 6 h. The ash was then dissolved in 5 mL of 2 M nitric acid, diluted, and measured by flame spectrophotometry. Chloride was determined with a chloride analyzer (MKII Chloride Analyzer 926, Sherwood Scientific Ltd., Cambridge, England).

Statistical Analysis

Data was analyzed using one- and two-way analyses of variance (ANOVA), and treatment means of the significant differences were separated using the Tukey test ($p < 0.05$).

RESULTS

Effects of Salinity on Biomass Production

Exposure of kikuyu grass to all the salinity treatments for 6 months reduced the aboveground shoot biomass as well as the root growth (Figure 1). Highest inhibition was noted at the 200-mM NaCl treatment. Root biomass was strongly affected by the salt treatment, and differences in root growth were found (cf. Shalhevet, Huck, and Schroeder 1995). The root–shoot ratio dropped down in the salt-exposed plants and was lowest in the 240-mM NaCl treatment (Figure 2).

Effect of Shoot Pruning at Definite Intervals

Shoot regeneration was affected by all irrigation treatments with saline water. Inhibition was positively correlated with the salinity treatments. For example, shoot production for the 200-mM treatment at the first clipping was only 47%

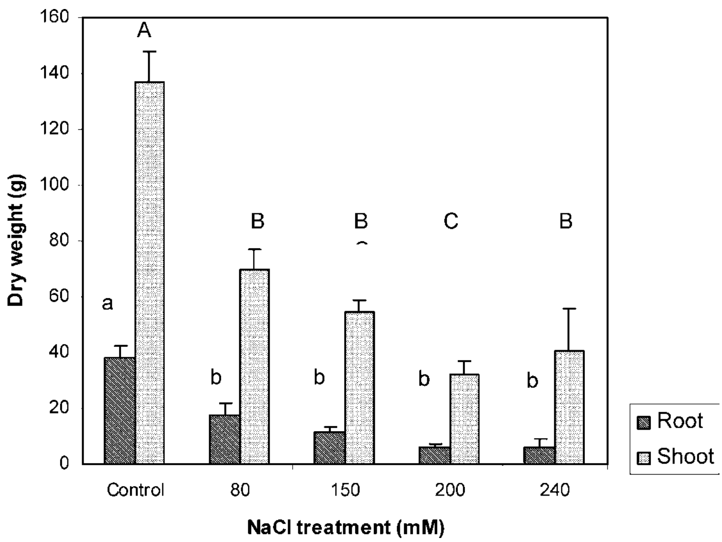

Figure 1. Effects of increasing NaCl concentrations on dry root and shoot biomass of kikuyu grass (mean $\pm$ SE). Means with common letter do not differ from each other (Tukey HSD, $p < 0.001$). Capital letters refer to shoot grouping and lowercase letters indicate root grouping.

of the production of the control plants but 74% for the plants of the 240-mM treatment.

After 6 months, the regrowth had dropped down to 88% of the initial values at 200 mM of NaCl and was practically abolished at the 240-mM treatment, leading eventually to the death of many of the plants of this treatment (Figure 3).

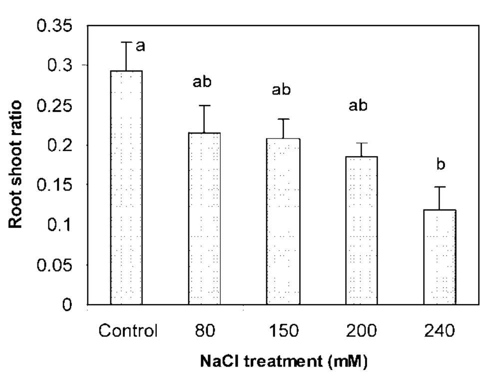

Figure 2. Effect of increasing NaCl concentration on root–shoot ratio of kikuyu grass (mean $\pm$ SE). Means with common letters, do not differ from each other (Tukey HSD, $p < 0.05$).

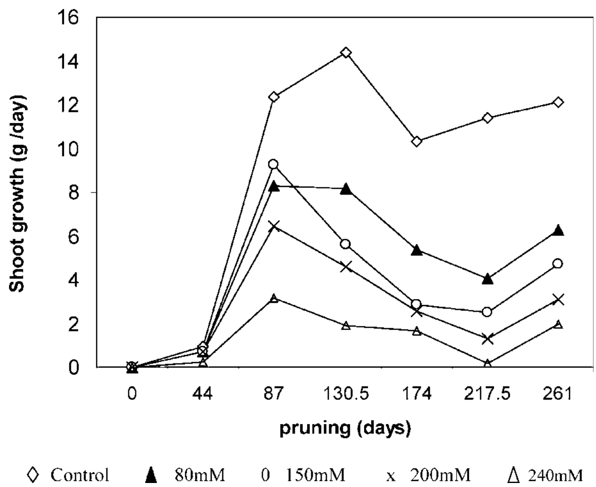

Figure 3. Effect of salinity on dry weight production: pruning at different times.

Proline Accumulation

To clarify the mechanisms of osmotic adaptation by kikuyu grass plants, proline content of the leaves of the treated plant was monitored (Figure 4). Up to a four-fold increase in proline content (15.14 μmol/g fresh weight) was measured in salt-stressed shoots of the 240-mM NaCl treatment as compared to 3.5 μmol/g fresh weight in the 80-mM NaCl treatment.

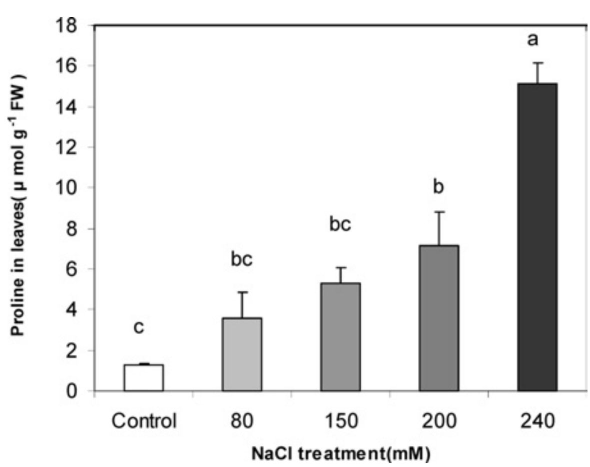

Figure 4. Effects of salt stress on proline accumulation (μmol g^{-1} FW) of leaves in kikuyu grass exposed to different salt concentrations (mean ± SE). Means with common letters do not differ from each other (Tukey HSD, $p < 0.001$).

Photosynthetic Response of Chlorophyll Fluorescence to Salt Stress

The photosynthetic quantum yield of leaves of the treated plants was measured. The photosynthetic yield obtained was similar for all treatments, except for the 200-mM NaCl treatment (−70%). Apparently the applied salt stress had no substantial effect on PSII photochemistry (Figure 5).

Ion Content in Leaves

Sodium and chloride concentrations of the leaves of the treated kikuyu grass plants increased with the salinity of the medium. Sodium and chloride content in the control were 9.5 mg g^{-1} and 21.5 mg g^{-1} respectively and reached 17.7 mg g^{-1} and 30.72 mg g^{-1} respectively in the 200-mM treatment. Leaves of plants with high ion accumulation exhibited bleaching and scorched leaves that eventually lead to death. In some plants of the 240-mM NaCl treatment, where leaf turnover was fast, sodium and chloride content in the leaves was rather low (Figure 6), apparently representing the ion content only of the young regenerating leaves.

DISCUSSION

Growth of kikuyu grass plants was little affected by low salt concentrations of the irrigation water. The plants showed a gradual decrease in leaf production that was distinct from the 150-mM treatment and up. This confirmed the results that were previously reported (Skerman and Riveros 1990; Muscolo, Panuccio, and Sidari 2003) that marked the salt tolerance of this grass at the 200-mM NaCl concentration.

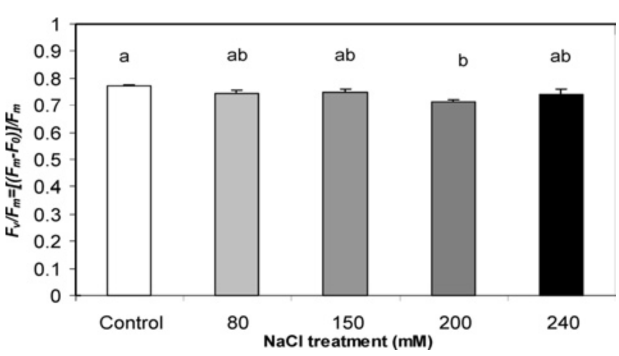

Figure 5. Maximal photosynthetic quantum yield (F_v/F_m) in leaves of kikuyu grass exposed to different concentrations of salt (mean ± SE). Means with common letters do not differ from each other (Tukey HSD, $p < 0.05$).

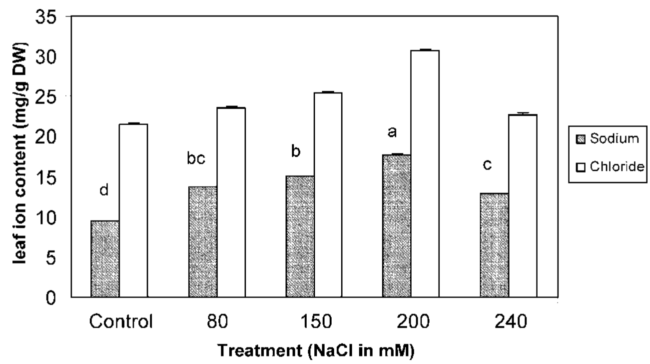

Figure 6. Effect of salinity on ion content in leaves of kikuyu grass exposed to different concentrations of salt stress (mean ± SE). Means with common letters do not differ from each other (Tukey HSD, $p < 0.001$).

Growth inhibition under saline conditions might be caused either by the lack of osmotic adaptation or by specific poisoning. The lack of osmotic adjustment reduces water uptake and causes physiological drought. This has long been considered the major cause of salinity injury to plants (Waisel 1972; Levitt 1980). Adaptation is reached either by accumulation of inorganic ions or by accumulation of compatible solutes. Some grasses are capable of reaching a fast and complete adjustment under salinity stress (Marcum and Murdoch 1990). Judging from the changes in proline content of the salt-exposed kikuyu plants, such a trait also applies to them.

In the present study, we also observed the inhibitory effects of repetitive leaf pruning. Such a trait lowers the value of plants planned to be used as turf grasses or grown for grazing. However, at moderate salinities, even a reduced production is of value.

Could it be that reduced growth resulted from a reduction in photosynthesis? Several reports have mentioned that salt stress is enhancing photoinhibition (Sharma and Hall 1991) and by that affect net photosynthesis. However, the presented results imply that the photosynthetic potential of kikuyu grass was not really lowered by the salinity treatments. Because the stolons and rhizomes were not damaged by the repeated leaf clippings, we have to conclude that the regeneration potential of the plants was lowered by the removal of photosynthesizing leaves and not by reduction of PSII yield. Concomitantly, adaptation of the PSII system in salt-stressed leaves can be explained as an important strategy of plant adaptation particularly in arid and warm regions (Lu and Zhang 1998). Another cause for the decline in growth under saline conditions appears to be related to an excessive buildup of ions in leaf tissues (Muscolo, Panuccio, and Sidari 2003) and to the inability of plants to produce new leaves to replace the senescent ones (cf. Guggenheim and Waisel 1977). This was reconfirmed in the present study.

Accumulation of sodium and chloride ions is instrumental for leaf osmotic adjustment in halophytes (Waisel 1989; Marcum 1999) and is enhanced by salinity (Rawson, Long, and Munns 1988; Ghoulam, Foursy, and Fares 2002). Such adaptation is limited to a certain concentration range. In the present study, it has peaked in plants of the 200-mM treatment. The decline in the average leaf content of sodium and chloride, at high concentrations of external NaCl (cf. Hussain, Caemerer, and Munns 2004) is not because of the reduction in ion accumulation by the individual leaves but because of an unbalanced leaf turnover, a fast shedding of the salt-loaded old leaves concomitantly with some production of low-salt young leaves.

Proline accumulation is often considered to be a major factor involved in osmotic adaptation and is used as a measure for stress tolerance (Delauney and Verma 1993). Accumulated proline in plants acts not only as an osmolyte. It involves an improved NADPH supply and is an "easy to handle" energy reservoir as well as an energy shuttle between plastids and mitochondria (Hare and Cress 1997). Thus, accumulation of proline serves a multitude of adaptation systems. Nevertheless, its role in plant adaptation to salinity remains controversial. Thus, the osmotic adaptation of kikuyu grass plants is achieved by two means: sodium and chloride accumulation on one hand and proline accumulation on the other. **Q1**

Findings of this study present kikuyu grass as a reasonably good turf and forage grass, as well as a cover grass that can endure relatively high soil salinity. Kikuyu grass can be a good candidate to combat the ongoing land degradation and can play an important role in saline land reclamation, combating the spread of salinization in arid and semiarid regions.

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