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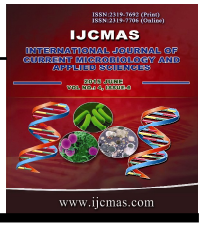

Original Research Article

Effect of Salinity on Growth, Protein and Antioxidant Enzymes in Three Kodo Millet (*Paspalum scrobiculatum*) Germplasm

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Keywords

Salinity stress, Kodo millet, Germination percentage, RGR, RWC, RSIR

A B S T R A C T

Salinity stress is a major adverse factor that limits agricultural productivity. It is one of the abiotic stresses that affect plant growth, physiological activities and developmental processes. The effect of salinity on growth, protein content and antioxidant enzymes was studied in three kodomillet germplasms (IC 426676, IC 382888 and IPS 145). Seedlings were subjected to NaCl stress (50, 100, 150 and 200 mM) for 6 days and caused a reduction in germination percentage, relative germination rate (RGR), relative salt injury rate (RSIR) and relative water content (RWC). Seedlings of IPS 145 were greatly affected compared to control plants. However, salinity stress caused only slight decrease in relative germination rate and relative water content of IC 382888 and IC 426676. All the plants under NaCl stress exhibited reduction in total protein content. The ROS activity was increased in all the tested accessions. Among the three, IC 426676 was exhibited highest enzyme activity followed by IC 382888 and IPS 145. The results indicated that under salinity stress the Kodo millet seedlings were responded more with reduced protein content. Of the three accessions, IC 426676 appeared to be stress tolerant and should be used as a promising variety to cultivate under saline conditions.

Introduction

One of the unique properties of living organisms is growth. Growth is the final morphological expression of various metabolic activities taking place in the plants. Exposure of plants to the abiotic stresses such as salinity results in the adverse effects on plant morphology, anatomy and physiology (Hussain *et al.*, 2010)

Plants growing in saline environments have developed a number of adaptive mechanisms, which enable them to survive and grow in the presence of toxic salts. Several reports revealed that salinity stress causes many adverse effects on the growth and development of millets (Hussain *et al.*, 2010)

Previously researchers depend upon simple primary data in the form of weights, areas, volumes and contents of plant components to evaluate the plant performance under stressful conditions (Causton and Venus, 1981; Hunt, 1990).

But now the use of physiological data along with the primary data found to be promising in screening of stress tolerant genotypes (Garnier *et al*., 1999).

The objective of the present study is to know the salinity effect on growth, protein and antioxidant enzymes activity of three Kodo millet varieties during germination and seedling emergence.

Materials and Methods

The seeds of accessions IC 426676, IC 382888 and IPS 145 of kodo millet (*Paspalum scrobiculatum* L.) were obtained from ICRISAT and NBPGR, Hyderabad for the present study and were surface sterilized with 2.0% aqueous sodium hypochlorite for 15 minutes at room temperature and then rinsed thoroughly with distilled water and germinated on moistened Whatmann No-1 filter paper in petri dishes of 12 cm in diameter.

Salt stress

The effect of salt stress on plant growth was studied using different concentrations of NaCl solutions *viz.*, 50 mM, 100 mM, 150 mM and 200 mM.

Experimental design

The experiment was designed in RBD with five replications and the entire setup was kept in an incubator and was maintained at 25°C.

Germination per cent

Five seedlings from each treatment were taken to measure seed germination. Percentage germination was measured using the following formula (Abdul Kabir, 2009).

$$\text{Germination per cent} = \frac{\text{No. of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Relative Water Content (RWC)

Relative water content was estimated according Fletcher *et al.* (1988) on the final day of the experiment and was calculated by the formula given by Kramer (1983).

$$\text{RWC} = \frac{\text{Fresh weight - dry weight}}{\text{Turgid weight - dry weight}} \times 100$$

Relative germination rate

The germinating frequency of seeds against different salt treatments was measured according to Yan Li (2008).

$$\text{RGR} = \frac{\text{Germination \% in NaCl concentration}}{\text{Germination \% in control}}$$

Relative salt injury rate

Relative salt injury rate indicates the effect of salt concentration on the rate of germination and it was determined using the formula of Yan Li (2008).

$$\text{RSIR} = \frac{\text{Germination \% of control} - \text{Germination\% of NaCl}}{\text{Germination \% of Control}}$$

Catalase activity (CAT)

Activity of catalase was performed according Prathibha Devi (2006). One gram of plant material was taken and macerated into thin paste using pH 7 phosphate buffer and the enzyme extract was filtered through muslin cloth. Two milliliter of the enzyme extract was taken into 50 ml clear conical flask and to this 1 ml of 0.45 molar H_2O_2 was added and the set up was kept for 5 min incubation and enzyme activity was stopped by adding 1 ml of 12% H_2SO_4. This extract was titrated against 0.05 N of $KMnO_4$ taken in a burette, appearance of pink color which remains constant for about 30 seconds was considered as the end point. The amount of H_2O_2 destroyed by catalase is calculated by the formula. The enzyme activity was expressed as enzyme units per gram leaf material. One unit of catalase is defined as that amount of enzyme, which breaks down / µmol / of H_2O_2 / min.

$$\text{Catalase activity} = \frac{25 \times 0.85}{2} \times \frac{V}{W}$$

Where W= Wt. of material used

V= Vol. of KMNO4 utilized (Blank sample value)

Superoxide dismutase (SOD)

Seed sample of 500 mg was homogenized in an ice cold 50 mM potassium phosphate buffer (pH=7.8) with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in cooling micro centrifuge (Eppendorf – 5415 R) at 10, 000 rpm. The supernatant was used for enzyme activity assay (Esfandiari *et al.,* 2007) within 12h of extraction.

SOD activity was estimated by recording the decrease in absorbance of superoxide nitro blue tetrazolium complex by the enzyme (Sen Gupta *et al.,* 1993). A reaction cocktail of 33 ml was prepared by mixing the reagents in the following ratio *viz.,* 50 mM Phosphate buffer - 60 µl; 13 mM Methionine - 390 µl; 02 µM Riboflavin - 0.6 µl; 0.1 mM EDTA - 60 µl; 75 mM NBT - 300 µl and Enzyme extract - 50 µl.

A blank was set without enzyme and NBT to calibrate the spectrophotometer. Another control was set having NBT but no enzyme as reference control. All the tubes were exposed to 400 W bulb (4* 100W bulbs) for 15 min and the absorbance was measured at 560 nm and percentage inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity. The enzyme activity is expressed as units / mg of protein.

Estimation of protein

Protein was estimated by micro-Kjeldhal's method (Bremner, 1975). The powdered samples of 100 mg were taken in a Kjeldhal's tubes and to this 1:4 ratio of $CuSO_4$ and K_2SO_4 was added along with 4 ml of concentrated H_2SO_4. Then the contents of the tubes were kept for digestion. The digested material was taken in Kjeldhal's tubes. The tubes were placed on the Kjeldhal's ammonia distillation unit and 10 ml of 40% NaOH were added to each tube.

Boric acid (3%) solution (10 ml) was taken in a conical flask with a few drops of methyl red indicator. When the distillate was approximately double the solution of conical flask, the distillation was stopped. The distillate was cooled for a few minutes and titrated against 0.01 N standard H_2SO_4 till the solution turned pink which is considered

as the end point. A blank was run for the complete procedure.

Protein content of seed = % of N_2 × 6.25

$$\% \text{ of Nitrogen} = \frac{X \times \text{Normality of acid} \times 14 \times 1000}{\text{Sample weight} \times 1000}$$

Where
X = Amount of HCl run down
Normality of acid = 0.02

Results and Discussion

Germination percentage

Germination was greatly reduced at the highest level of salt treatment. The per cent germination recorded above 90% in controls of all the varieties except IPS 145 and no variety recorded 100% germination (Fig. 1). Among all the treatments highest % germination was observed in 50 mM (98.80%) and the lowest was reported in 200 mM (8.80%) in IC 76 and IPS 145 respectively. The germination decreased by 2.57% , 37.7%, 63.63% and 92% in seeds germinated at 50, 100, 150, 200 mM NaCl salinity levels, respectively over the control. The data indicated that the percentage of germination was decreased with each increasing level of salinity from 50 mM to 200 mM over the control.

Analysis of variance has also revealed that, all the accessions differed significantly between the varieties and among the treatments and $p< 0.05$. This decrease in germination may be due to increased osmotic pressure and reduced protein hydration which induces changes in the activities of many enzymes in germinating seeds (Waisel, 1972). These results were in agreement with Guan *et al.*, (2009) in *Medicago ruthenica* and Basalah (1991) in *Cucurbita pepo*.

RGR

At 50 mM NaCl treatment relatively highest RGR was found in IC 76. It was varied from 0.94% to 1.01% and the lowest RGR was observed in IPS 145 (0.06%) (Fig. 2). At 100 mM IC 76 showed higher RGR (0.77%), whereas it was lower in IPS 145. The 200 mM concentration of NaCl has greater effect on RGR and all the tested genotypes were recorded low relative growth rate (Fig. 2). This change in RGR between the genotypes is may be due to their genetic potential to cope with the change in environmental conditions and their quick adaptation to the change in saline levels. These results were similar to Jamil *et al.* (2005) cabbage and Al–Harbi *et al.* (2008) in tomato.

RSIR

As the concentration increases the relative salt injury rate was increased in all the varieties (Fig. 3). In 50 mM NaCl treatment highest RSIR was noticed in IPS 145 (0.06) and very less effect was observed in IC 76 (0.01). Significant damage due to NaCl was observed in IPS 145 in 100, 150 and 200 mM concentrations whereas in IC 88 this damage was found to be significant in 150 and 200 mM concentration NaCl. In case of IC 76 the damage was significant at 200 mM only. This increased damage is may be due to change in cell expansion process which is controlled by processes related to cellular water uptake and cell wall extension (Cramer and Bowman, 1993).

RWC

Relative water content was determined in all the test varieties at 144 hrs of experiment and the highest relative water content was maintained by the genotype IC 76 in all the treatments. The lowest readings were reported in IPS 145 (Fig. 4).

Fig.1 Effect of NaCl on germination percentage

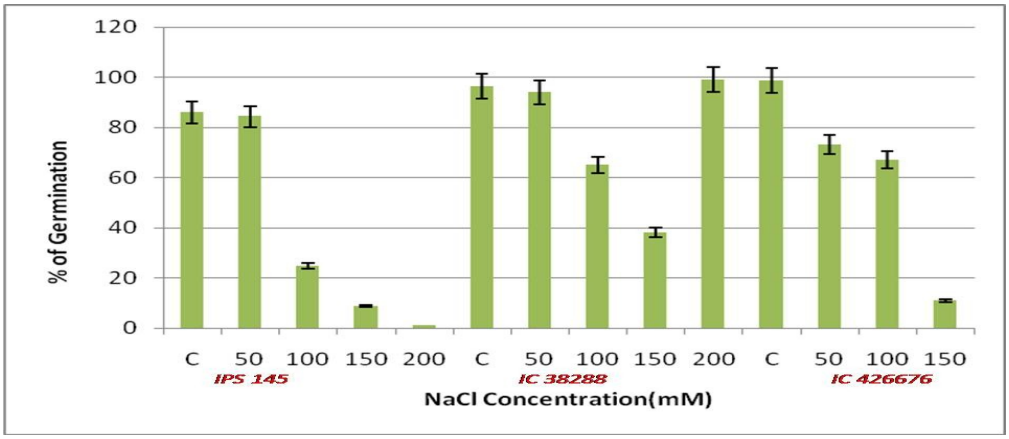


Fig.2 Effect of NaCl stress on relative germination rate

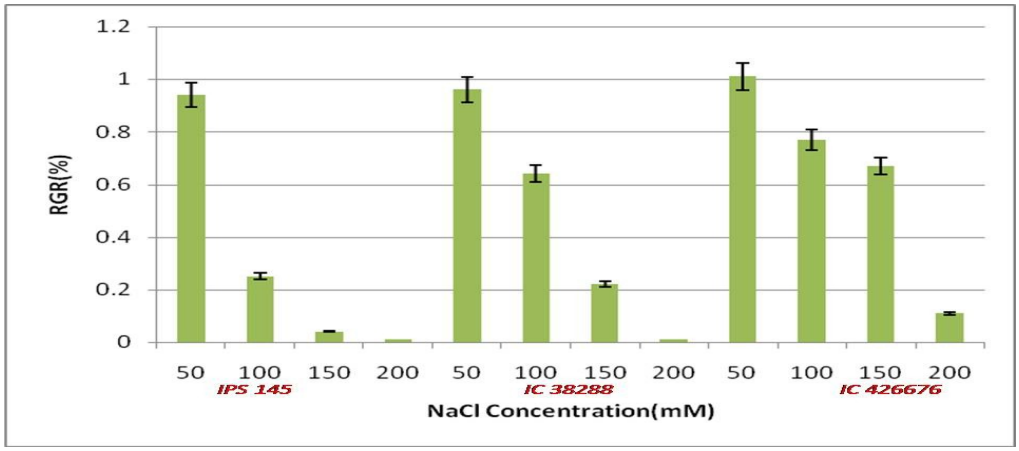


Fig.3 Relative salt injury rate in tested genotypes under NaCl stress

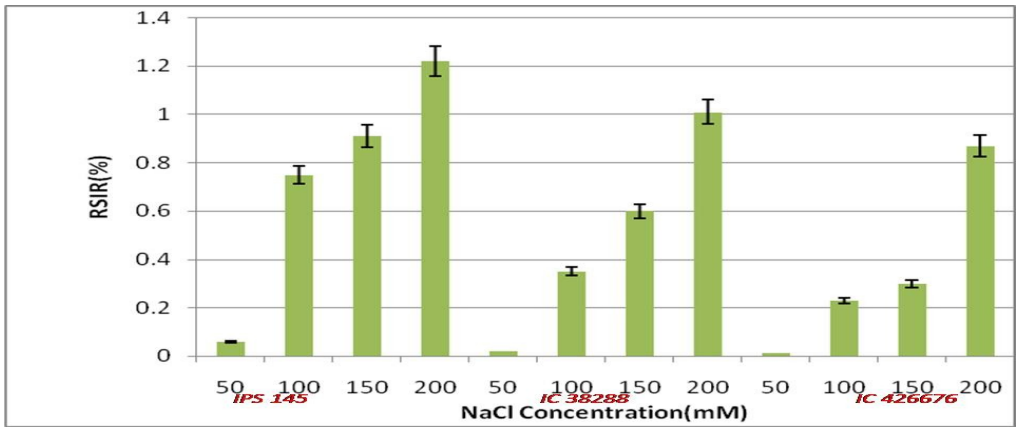


Fig.4 Effect of NaCl on relative water content

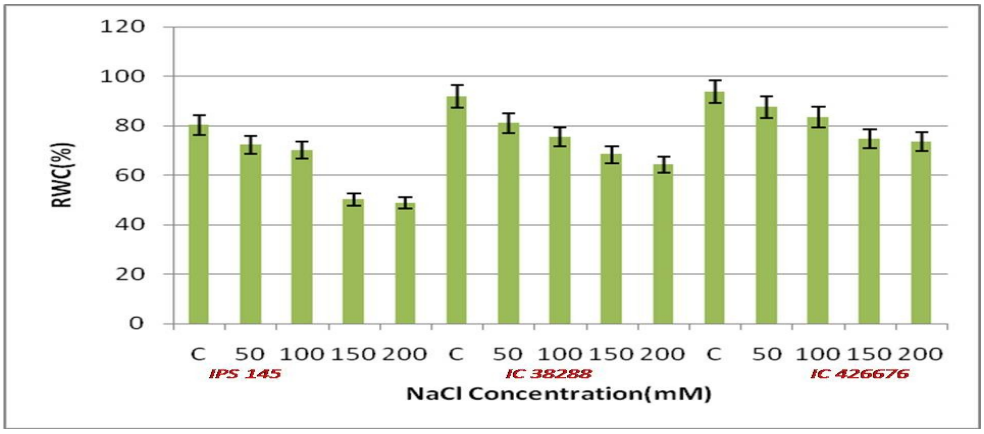


Fig.5 Decrease in protein levels with increase in NaCl concentration

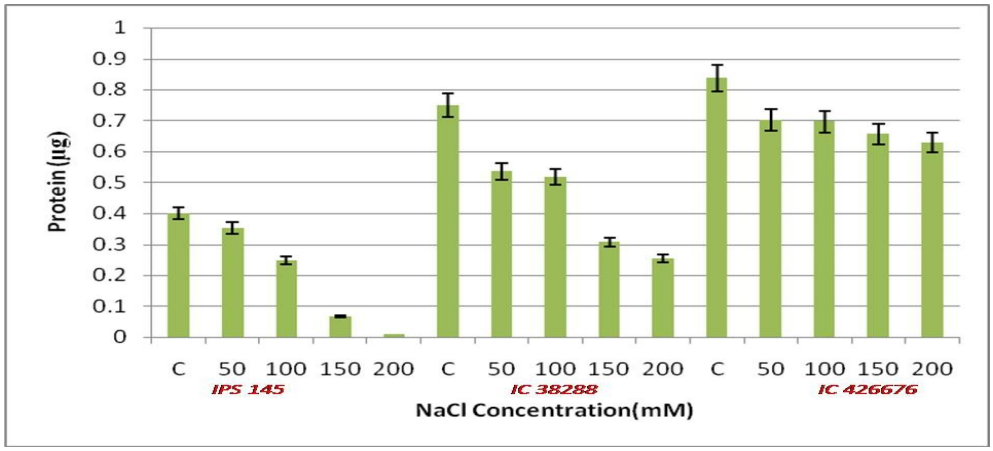


Fig.6 Reduced Catalase activity at different NaCl stress levels

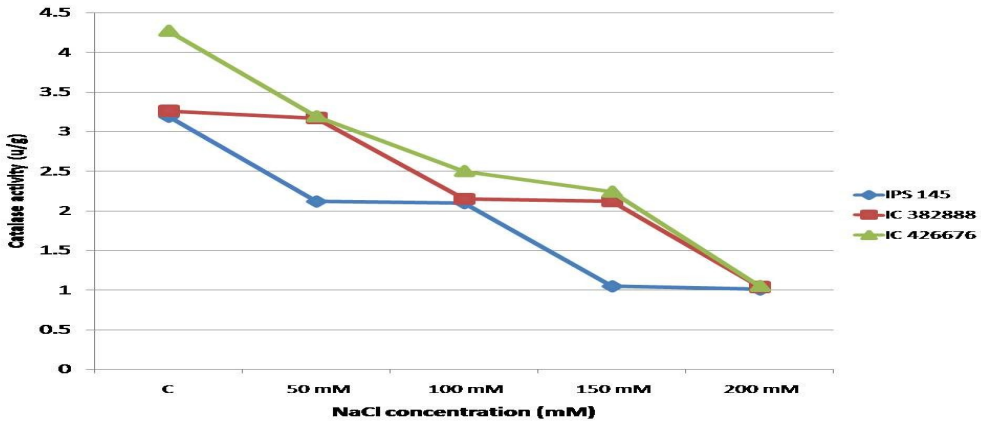
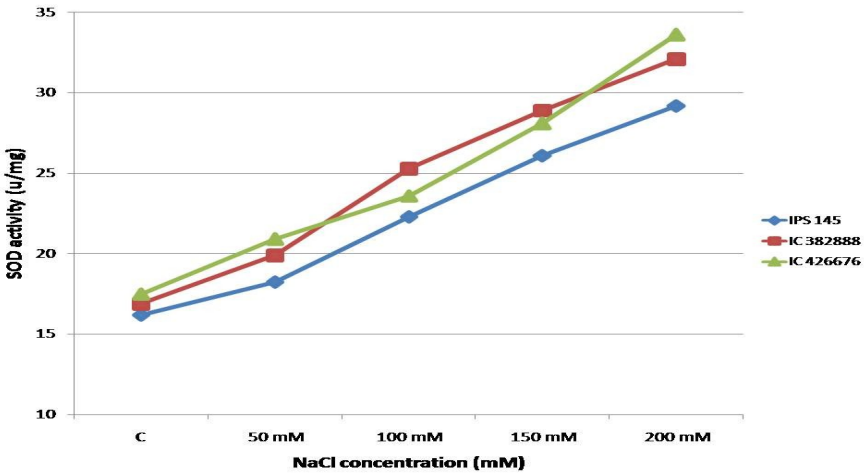


Fig.7 Increased Superoxide dismutase activity under different NaCl concentrations



It is because of reduction in water supply to the cells by increasing in Na^+ ions in cytoplasm which compete with K^+ ions resulted in decrease in osmotic potential in cell cytoplasm of all the cultivars (Murillo *et al.*, 2002 and Szigeti, 1991).

Protein

As the concentration of NaCl increases, the availability of the protein seems to be decreases in all the accessions. Even though at high salt treatments the variety IC 76 maintained its available protein and the variety IPS 145 was failed to keep up its protein levels with increased salinity stress. IC 88 tried to manage their protein levels in spite of increase in the salinity levels (Fig. 5). This decrease was may be due to the increasing activity of acid and alkaline proteases in order to keep osmotic stress during NaCl stress (Parida *et al.*, 2002). Our results are in agreement with Kennedy and De Fillippis (1999) in *Grevillea ilicifolia* and *G. arenaria* under saline stress.

Catalase

The activity of catalase was found to be less and its activity was goes on decreased with increased in salinity stress. The variety IC 76 maintained its catalase activity in all the stress levels with a very less decrease in catalase levels (C: 4.27; 50 mM : 3.19; 100 mM : 2.50; 150 mM : 2.24 and 200 mM : 1.05) when compared to other varieties (Fig. 6). The decline in CAT activity is considered as a general response to many stresses (Gunes *et al.*, 2008). The reduction of CAT activity is supposedly due to the inhibition of enzyme synthesis or change in the assembly of enzyme subunits under stress conditions.

Superoxide dismutase (SOD)

The activity of SOD was found to be increased with increased NaCl stress levels. In all the tested varieties maximum SOD activity was observed at 200 mM NaCl concentration. Among the three tested genotypes IC 76 showed high SOD activity in all the treatments (C – 17.5; 50 mM – 20.9; 100 mm – 23.6; 150 mM – 28.1 and 200 mM – 33.6) (Fig. 7). The increased SOD activity under salinity stress is may be due to its critical role in the survival of the plant, when SOD activity was high, ROS, especially superoxide radical, scavenging was done properly and thus, damage to membranes and oxidative stress decreased,

leading to the increase of tolerance to oxidative stress (Esfandiari *et al.*, 2007).

Acknowledgements

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