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RESEARCH ARTICLE

# EFFECT OF SALINITY STRESS ON MORPHO-PHYSIOLOGICAL ATTRIBUTES OF KODOMILLET HYBRIDS DURING PRE-ANTHESIS

**Prasanthi Kumari, R. and *Vishnuvardhan, Z**

Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjunanagar – 522 510, Guntur, Andhra Pradesh, India

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**ABSTRACT**

Kodomillet *(Paspalum scrobiculatum* L.) is one of the hardiest crop among all the small millets. It has a capability to stand for the biotic and a biotic stresses. But increased soil salinity majorly limits the plant productivity and quality. Present work was focused on evaluation of Kodomillet genotypes such as IPS 145, IPS 610, IPS 351, IC 382888, IPS 583 and IC 426676 for their salt tolerance efficiency subjected to different salinity levels i.e. 0, 200, 400 and 600 mM NaCl at 45 DAS. The results obtained were revealed that root length, shoot length, number of leaves and root length density found to be insignificant whereas root shoot ratio, root and shoot relative water content and total chlorophyll content were correlated significantly. Among all the test types IC 426676 and IPS 583 showed more salt tolerance efficiency and IPS 145 reported less salt tolerance efficiency.



## INTRODUCTION

Agricultural productivity is severely affected by soil salinity and the damaging effects of salt accumulation in agricultural soils have influenced both ancient and modern civilizations (Munns, 2002; Chinnuswamy *et al.,* 2005). Salinity stress is a major environmental constraint to crop productivity in the arid and semiarid regions of the World. High concentrations of salts cause ion imbalance and hyper osmotic stress in plants. Saline environments affect the plant growth in different ways such as a decrease in water uptake, an accumulation of ions to toxic levels, and a reduction of nutrient availability. In some extensive reviews concerning strategies of overcoming the salinity problem, two primary lines of action were emphasized one is reclamation of salt-affected soils by chemical amendments and second one is using of saline soils to grow salt-tolerant plants (Ashraf and McNeilly, 2004). High salt concentration in the soil solution is bound to create high osmotic pressure in the root zone and reduce availability of water and nutrients to plants. Such conditions are known to affect plant physiological activities, which ultimately determines crop yield (Munns *et al*., 2006). NaCl being the dominant salt in nature, $Na^+$ and $Cl^-$ ions can enter into the cells because of their prevalence and have their direct toxic effects on cell membranes, as well as on metabolic activities in the cytosol (Hasegawa *et al*., 2000). Kodomillet germplasm lines were not substitute for the damaging effects of NaCl even though they are having the ability to with stand to the abiotic stress conditions. Moreover there should be varietal differences between inbreds in defending the abnormal changes due to salt stress. This situation demands the identification of salt tolerable lines from the available Kodomillet seed bank.

***Corresponding author: Vishnuvardhan,** *Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjunanagar – 522 510, Guntur, Andhra Pradesh, India.*

## MATERIALS AND METHODS

Six Kodomillet varieties such as IPS 145, IPS 610, IPS 351, IC 382888, IPS 583 and IC 426676 were obtained from ICRISAT and NBPGR Hyderabad, Andhra Pradesh, India. The experiment was carried out in the Botanical garden of Acharya Nagarjuna University. Forest bags (10.0'' diameter and 15'' height) were filled with 5.0 kg red loamy soil along with vermi compost in 4:1 ratio. A total of five sterilized seeds per pot were sown, after germination, plants were thinned to one in a pot, and grown for 45 days under rainout shelters. All the pots were irrigated everyday to maintain the sufficient soil water status. All the plants were treated with different salt solutions of NaCl viz., 0, 200, 400 and 600 mM at 45 DAS for about 6 days. On final day of the treatment the responses of the following traits such as root length, shoot length, whole plant fresh and dry weights, root shoot ratio, root and shoot relative water content (RWC), root length density and total chlorophyll content was recorded both in control and treated plants.

**Shoot and root length**

Plant height was recorded from the ground level to the growing tip of the main shoot. Measurements were taken from five randomly selected plants in each treatment and the average height was calculated using scale and expressed in centimetres. The length of all the roots in a clump up to the tip was measured with the help of a measuring scale and thread expressed as average root length in centimetres.

**Number of Tillers**

Number of tillers for plant was measured by carefully plucking out the plant without disturbing root system and leaves using a small plucker.

**Root shoot ratio**

Root shoot ratio of all the test types before and after treatment was calculated according to Anbumalarmathi *et al.* (2008).

$$\text{Root shoot ratio} = \frac{\text{Root dry weight (g)}}{\text{Shoot dry weight (g)}}$$

**Root length density**

Root length density was measured basing on water replacement method (Anbumalarmathi *et al.,* 2008). Root length density was measured using the following formula.

$$\text{Root length density} = \frac{\text{Root length (cm)}}{\text{Root volume (cc)}}$$

**Relative Water Content (RWC)**

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

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

**Chlorophyll content**

Total chlorophyll content was measured using dimethyl sulfoxide (DMSO) method (Hisscox and Israelstam 1979). Thirty milligrams of fresh leaf material was taken in dried test tube and to which 10 ml of DMSO was added. Then test tubes were kept in hot water bath for about one hour at 60°C (until the leaf samples become colorless). Amount of chlorophyll was estimated using the following formula and expressed in mg/g fr.wt.

$$\text{Chl} = (20.2 \times \text{O.D at 645 nm} + 8.02 \times \text{O.D at 663 nm}) \times V \times \frac{W}{10}$$

**Whole plant fresh and dry weight**

Fresh weights of plant material were determined on the final day of the treatment. Dry weights of plant material were measured with the help of an electric balance after drying at 70°C for about 72 hours (Afzal *et al.,* 2005).

## RESULTS AND DISCUSSION

Shoot length of all the cultivars under salinity stress during preanthesis was measured (Fig. 1). All cultivars showed reduced shoot growth with increased salinity level when compared with the controls. During preflowering stage shoot lengths of IPS 610 (17.33 cm) and IC 426676 (32.20 cm) are reported to be significant upto 200 mM NaCl stress. This decrease in shoot growth is may be due to salinization effect of NaCl on both cell division and cell expansion in rapidly growing tissues like maristamatic tissue (Zidan *et al.,* 1990). Similar type of Reduction in shoot length was observed by Arshi *et al.* (2002) in *Cassia angustifolia* plants growing under salt stress. All these results were in coincide with the present study where the cultivars IPS 145 and IPS 610 found to be more sensitive to the increased sodium chloride with high reduction in shoot length. The root length ranges from 11.43 - 24.40 cm among the controls.

The significant highest root length was observed in IC 426676 (C: 21.83 cm; 200 mM: 21.77 cm) and IPS 583 (C: 17.33 cm; 200 mM: 17.00 cm) at 0 mM and 200 mM concentration of NaCl (Fig. 2) whereas the lowest root length was reported in IPS 145 (C: 13.6 cm; 200 mM: 9.8 cm) at 0 mM and 200 mM concentration of salinity stress. In remaining treatments such as 400 mM and 600 mM the root length was found to be non significant. The reduction in root length was due to altered external water potential, increase in ion toxicity or because of an ion imbalance (Jaleel *et al.,* 2007a; Jaleel *et al.,* 2007b). This condition brings biochemical restraints on cell wall expansion, which in turn can inhibit root growth (Hernandez and Almansa, 2002). Moreover accumulation of inorganic ions, predominantly of $Na^+$ has a role in the process of osmotic adjustment (Gzik, 1996; Arshi *et al.,* 2005). In the present study the varieties IC 426676 and IPS 583 found to be managed the root growth up to some extent this may be because of having potent mechanism to stop the excess input of $Na^+$ into the external medium of their cells. The effect of different saline concentrations on growth of tillers was tested. The tiller number ranges from 16.33 (IPS 145) to 24.67 (IC 76) (Fig. 3). The highest number of tillers was maintained by IC 76 and IPS 583 both in controls and 200 mM (C : 24.67; 200 mM: 21.67 and C : 24.33; 200 mM : 16.67). At 400 and 600 mM concentration of NaCl no variety found to be significant. Similar results were reported by Natrajan (1992) in Chilli and Varghese *et al.* (1979) in cabbage. The root shoot ratio varied significantly among all the treatments in all the test varieties. The cultivars IPS 145 and IPS 610 didn't exhibit significant root shoot ratio among all the treatments at preanthesis stage under salinity stress. The accessions IPS 351 (0.414 g) and IC 426676 (0.988 g) varied significantly in controls only. But the genotypes IC 382888 and IPS 583 recorded significant root shoot ratio at flowering stress up to 200 mM concentration respectively (Fig. 4).

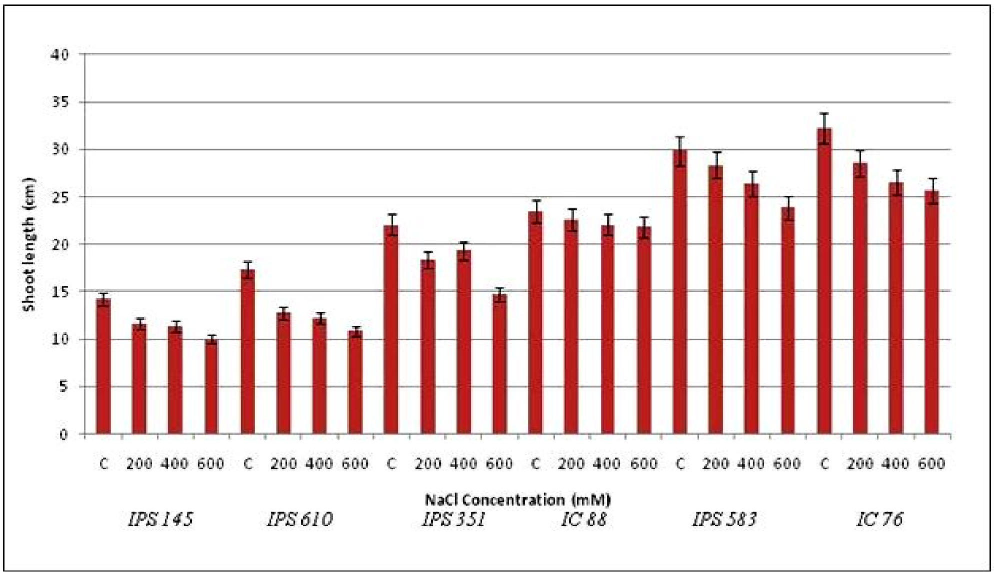


**Fig. 1. Effect of NaCl on shoot length at flowering stress**

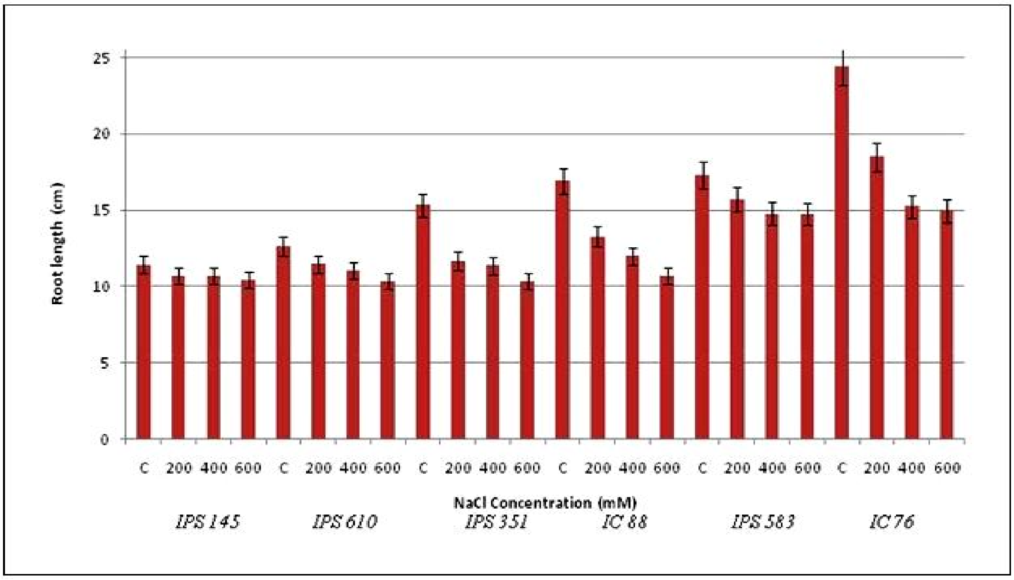


**Fig. 2. Effect of NaCl on root length**

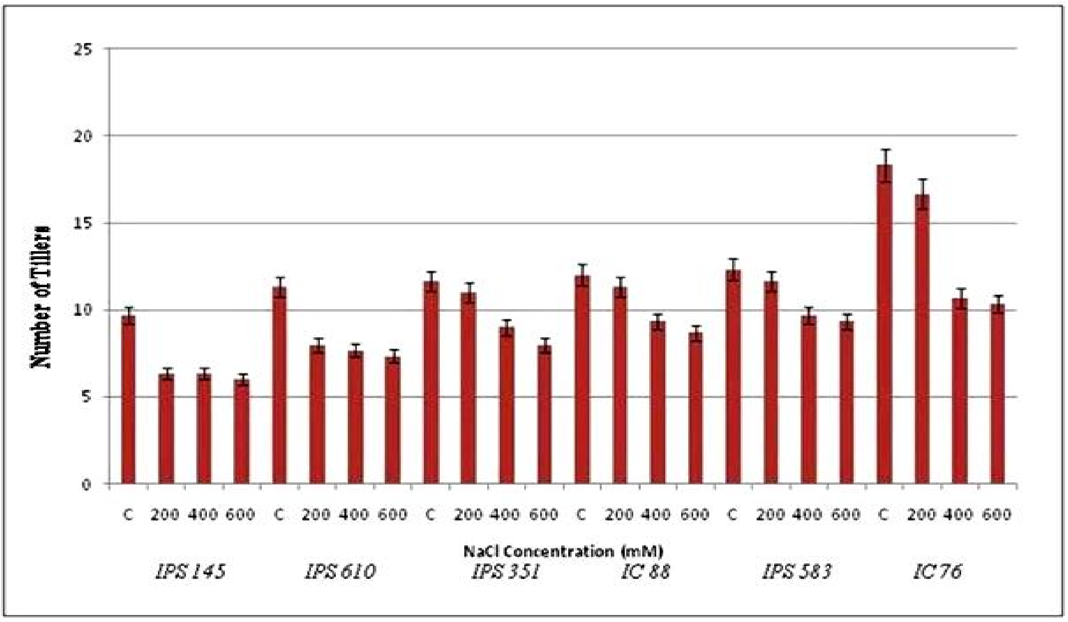


**Fig. 3. Effect of NaCl on growth of Tillers**

Similar type of reduction in root shoot ratio was observed in wheat (Afzal *et al.,* 2007) and pearl millet (Yakubu *et al.,* 2010 and Bukhari *et al.,* 2012) and foxtail millet, proso millet (Islam *et al.,* 2011). Under salinity stress during preanthesis highest RLD was observed in controls of IC 426676 (0.508 $cc^3$) whereas the lowest value was observed in IPS 145 at 200 mM concentration (Fig. 5).

both in root and shoot. The root relative water content was reported to be significant in the controls of genotypes IPS 610, IPS 351, IC 382888, IPS 583 and IC 426676 (Fig. 6) whereas the accession IPS 145 found to be significant up to 200 mM. The highest shoot RWC during flowering stress was observed in controls of IC 426676 (85.51%) followed by IPS 583 (84.72%).

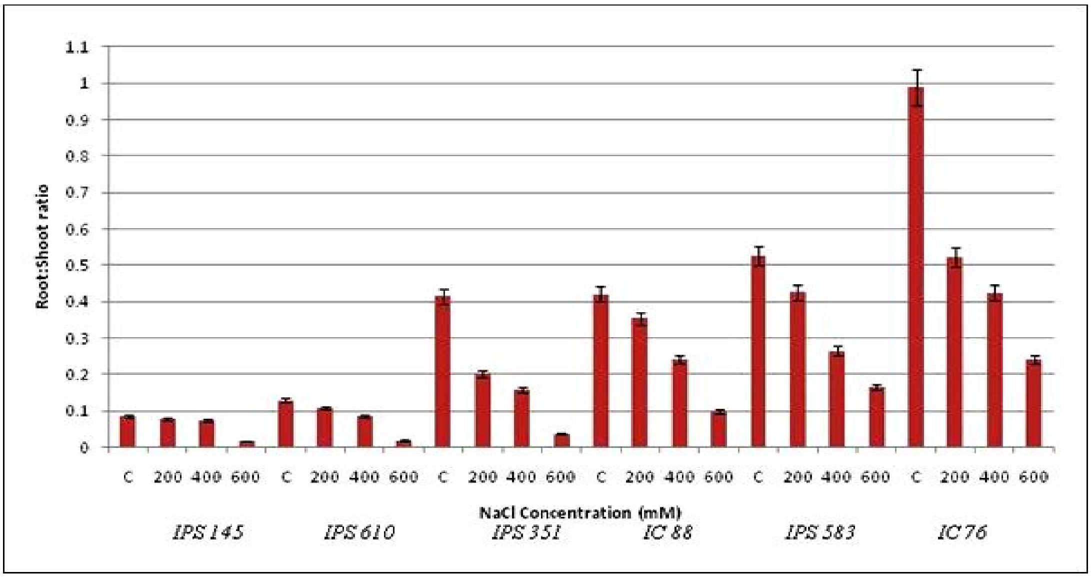


**Fig. 4. Variation in root to shoot ratio against NaCl stress**

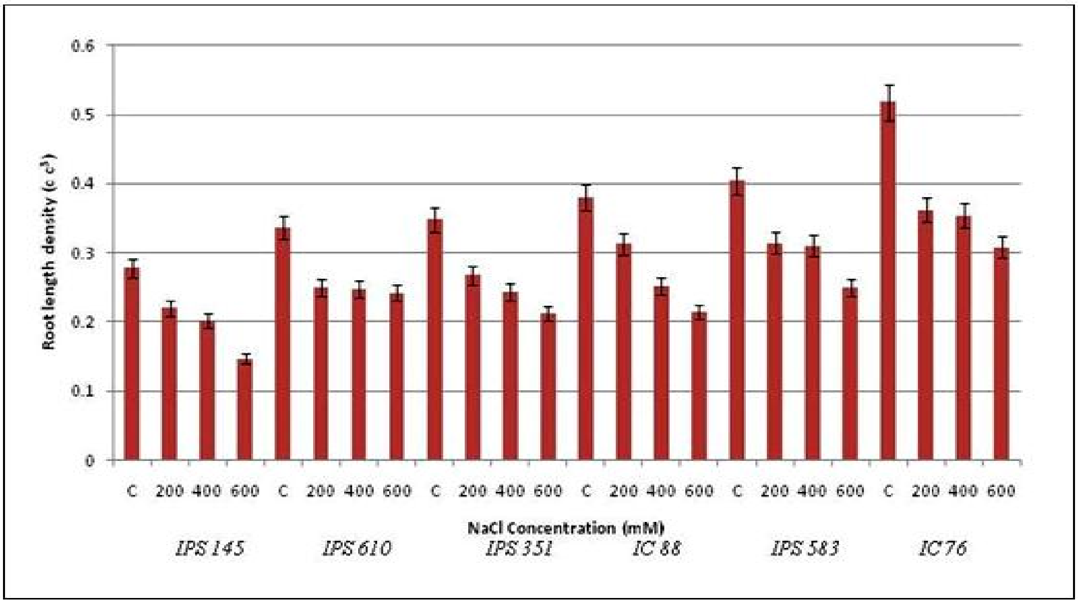


**Fig. 5. Differences in RLD against NaCl stress at anthesis**

Under salt stress there is a decrease in the root length along with a decrease in the total root length density. Among all the tested varieties the highest root length density was maintained by the cultivars IC426676 and IPS 583 in NaCl stress whereas lowest RLD was fed with IPS 145 and IPS 610. This may be due to the decrease in cell division and cell expansion. These reports are in agreement with Magdi *et al.* (2010) in *Vicia faba* and Beemarao *et al.* (2007) in *Abelmoschus esculentus* (L.) Moench. The relative water content of all the varieties tested

These two varieties recorded high shoot RWC in 200 mM concentration (77.20%; 58.32%) (Fig. 7). The genotype IC382888 recorded high RWC in 200 mM (66.91%) followed by IPS 351 (66.88%). The decrease in RWC indicated a loss of turgor that resulted in limited water availability for the cell extension process (Katerji *et al.,* 1997). This might be the reason for the decrease in root growth and shoot growth in all the genotypes with increase in the saline stress. Even though there is a increase in the salinity stress which imposes high effect on root RWC and shoot RWC the cultivars IC 426676 and IPS 583 recorded high RWC indicated that these accessions maintained their turgor at limited water supply and sustain their lives in all the NaCl stress levels. The amount chlorophyll present in the salt effected leaves found to be varied significantly during pre-flowering stress. Among the controls IPS 145 (3.28 mg/g), IPS 610 (6.38 mg/g), IPS 351 (6.45 mg/g) and IPS 583 (9.80 mg/g) found to be significant. The accessions IC 382888 (C : 7.49 mg/g; 200 mM : 4.15 mg/g) and IC 426676 (C : 9.89 mg/g; 200 mM : 9.89 mg/g) recorded significant up to 200 mM (Fig. 8). Chlorophyll contents have been suggested as one of the parameters of salt tolerance in crop plants (Hernandez *et al.,* 1995). The reduction in leaf chlorophyll under salinity has been attributed to the destruction of the chlorophyll pigments and the instability of the pigment protein complex (Levitt 1980). It is also attributed to the interference of salt ions with the denovo synthesis of proteins, the structural component of chloroplast rather than the breakdown of chlorophyll (Megdiche *et al.,* 2008). Similar results were reported by Nieman *et al*. (1988) in pepper, Bethke and Drew (1992) in capsicum.

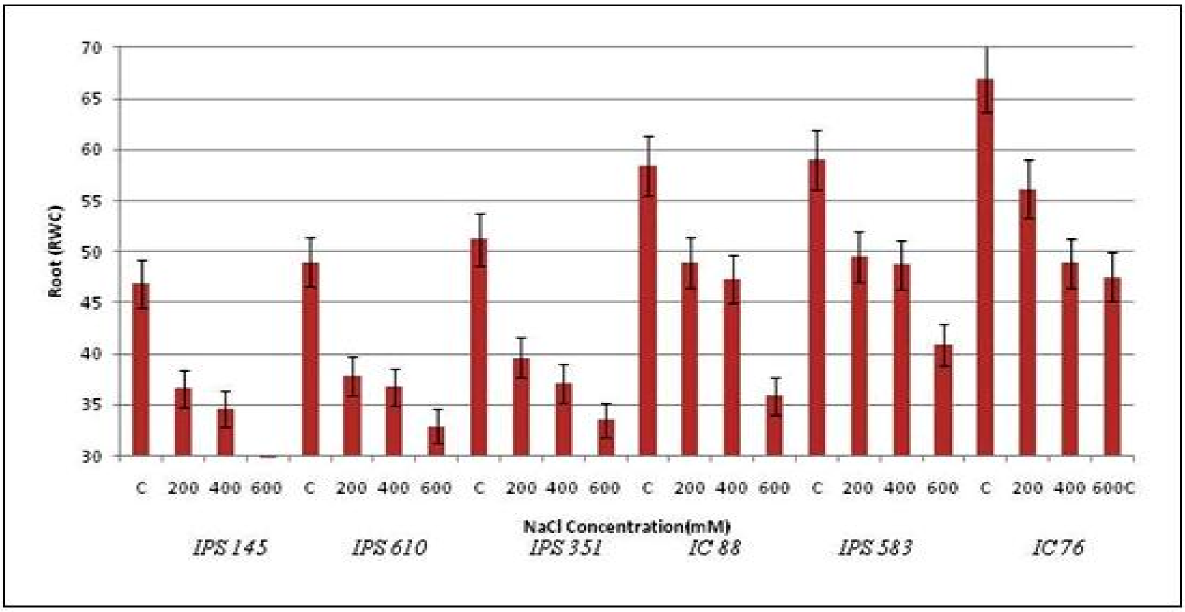


**Fig. 6. Root RWC against NaCl stress at anthesis**

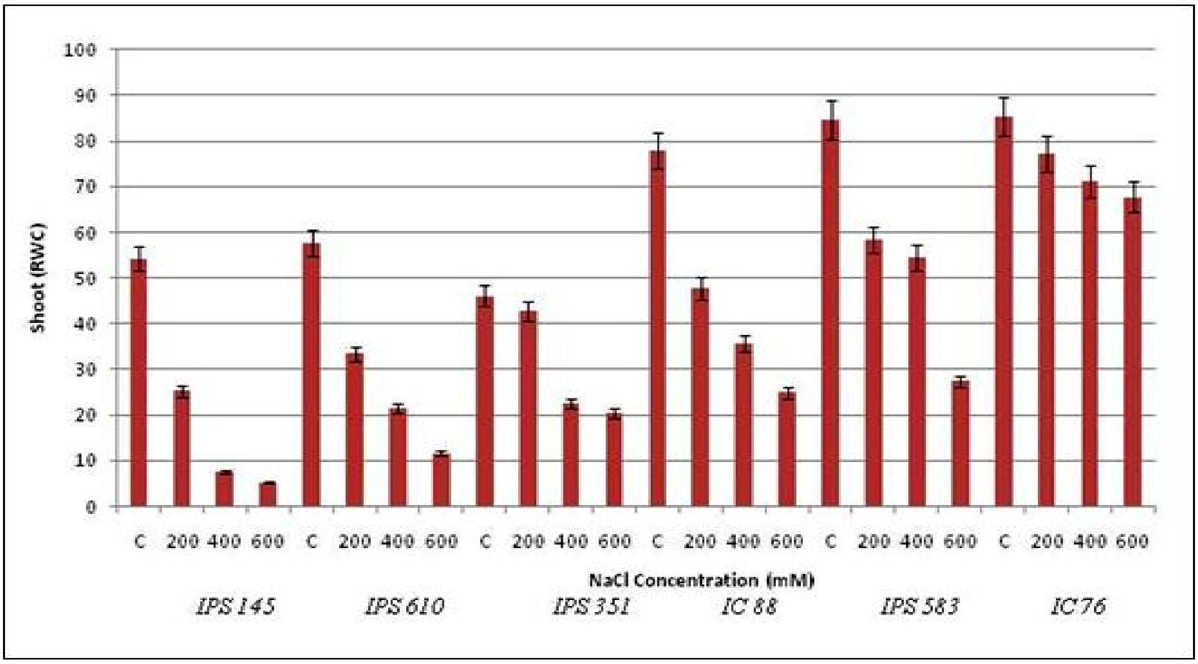


**Fig. 7. Shoot RWC against NaCl stress**

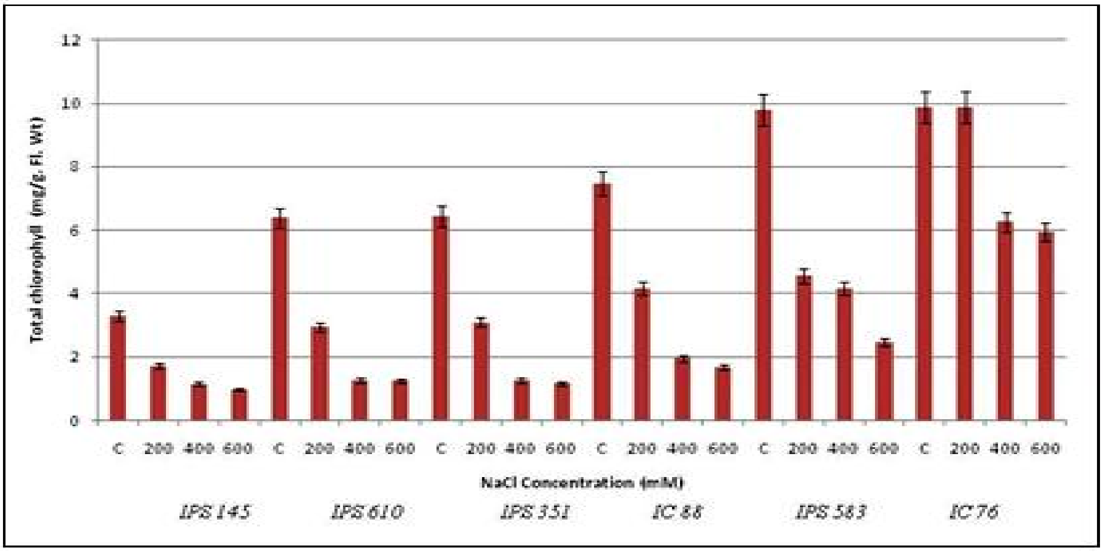


**Fig. 8. Total chlorophyll against NaCl stress**

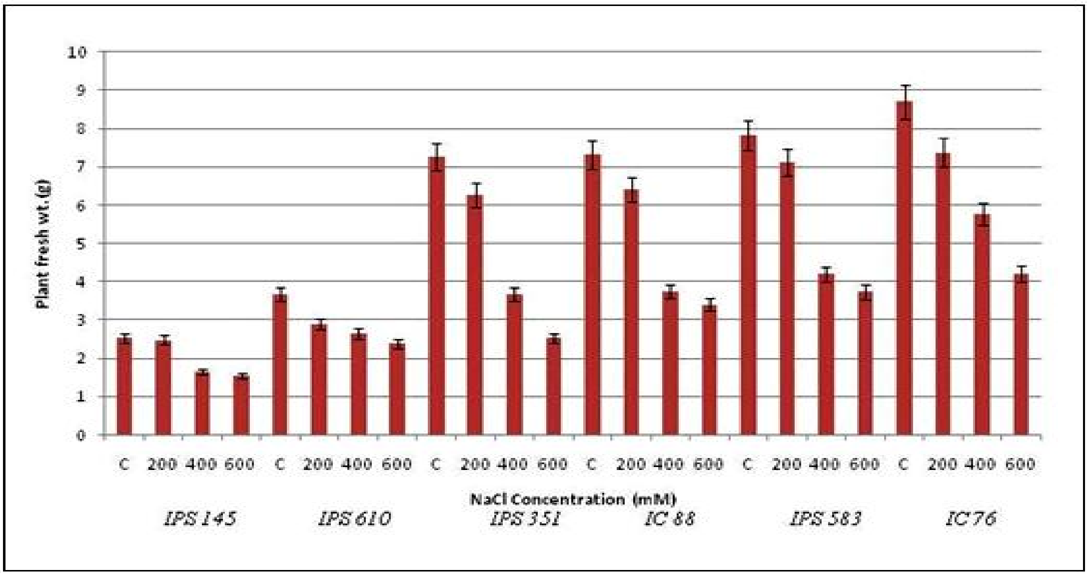


**Fig. 9. Reduction in fresh weight with increase in NaCl stress at anthesis**

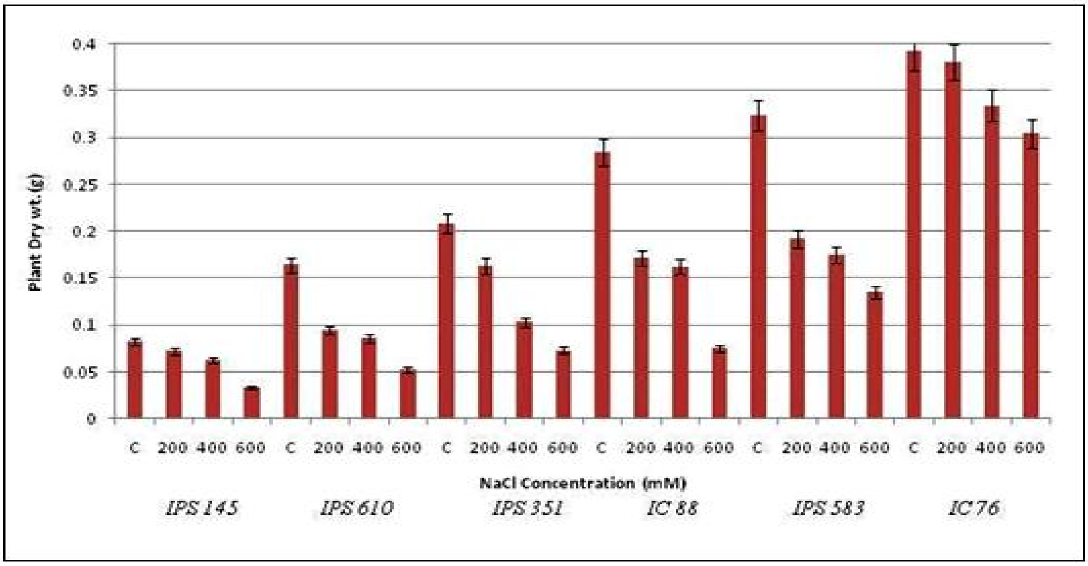


**Fig. 10. Reduction in Dry weight with increase in NaCl stress**

The total biomass of plants has been affected by salt stress. Both fresh and dry weights were reduced with increasing salinity (Fig. 9 and Fig. 10). Among all the test types the highest fresh and dry weights were observed at 0 mM NaCl, whereas in case of treatments the varieties IPS 583 and IC 426676 recorded significant fresh and dry weights upto 400 mM later on the weights were decreased with enhanced NaCl stress. The decrease in fresh and dry weights may be due to altered osmotic adjustments (Wilson *et al.,* 1989) which in turn reflects the activity of photosynthesis by provoking stomatal closure (Hernandez and Almansa, 2002). Similar reports were also found in pearl millet (Bukhari *et al.,* 2012) finger millet (Pradhan *et al.,* 2014).

**Conclusion**

Kodomillet varieties IPS 583 and IC 426676 showed tolerance even under high salinity (600 mM) than other test lines by having adequate relative water content, root length density and chlorophyll content which in turn helps to overcome the stress effects and leads better growth and biomass.

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