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A Hydroponic Approach to Evaluate Morpho-Physiological and Biochemical Responses of Kodo Millet (Paspalum Scrobiculatum) genotypes Against Calcium Chloride Stress

Prasanthi Kumari.R[1], Vishnuvardhan.Z[2], Babu.K[3]

[1]Department of Botany, Andhra Christian College, Guntur
[2,3]Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar - Guntur, Andhra Pradesh, India.

Abstract
Kodo millet (*Paspalum scrobiculatum)* belongs to the family Poaceae, and is locally known as rice grass, ditch millet and cow grass. Kodo millet is one of the small grain cereals with an ability to tolerate the biotic and abiotic stresses. Soil salinity is one of the major stresses especially in arid and semi-arid regions leads to reduced yields. Some of the millet species have developed adaptive mechanism to overcome the salinity stress and hence, thorough screening of the germplasm through hydroponics or field testing is essential to identify the potential genotypes. Plant response of salinity stress towards morphological, physiological, biochemical features have been delineated. In our work we studied the morphological, physiological and biochemical responses of six genotypes of kodo millet (IPS 145, IPS 610, IPS 351, IC 382888, IPS 583 and IC 426676) against $CaCl_2$ salinity through hydroponic experiment. Germinated seeds were grown in beakers supplemented with Hoagland nutrient solution containing $CaCl_2$ (0, 50, 100, 150 and 200 mM) for about 120 hours and the data was collected for every 18 hours interval on root length, shoot length, Germination Index (GI), Germination Energy (GE), Seedling Vigour Index (SVI), Polyphenol Oxidase (PPO), Peroxidase (POD) and Superoxide Dismutase (SOD) . Among all the varieties IC 426676 and IPS 583 showed highest $CaCl_2$ concentration when compared to other genotypes.

Keywords: Kodo millet,Hydroponic, Salinity Stress, $CaCl_2$ CAT, SOD, POX

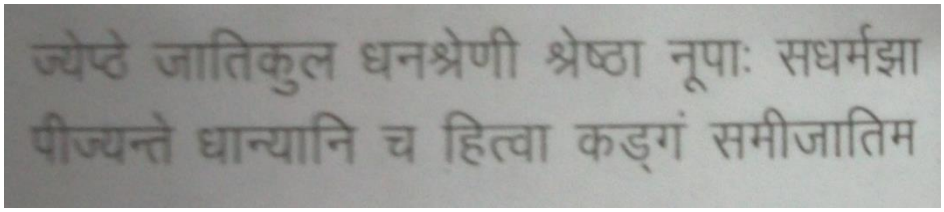
1. Introduction

Salinity stress can be referred to the accumulation of soluble salts like Cl, SO_4, HCO_3, Na +, Ca $^{2+}$, and Mg^{2+}. These ions chiefly come from the irrigation and poor soil drainage (Acosta Motos et al. 2017). Salinity reduces plant growth chiefly in three mechanism- osmotically induced water stress, specific ion toxicity due to high concentration of sodium and chloride ion and nutrient imbalances which hampers the uptake of water by plants (Greenway and Munns 1980). The hydroponic system of screening is more reliable, less time consuming and can be used to diminish the environmental variation commonly encountered in the field trials (Chen et al. 2008).

Calcium is known to increase salinity tolerance in many crop plants. Calcium is a divalent cation that is extremely important in maintaining the strength of stems and stalks of plants. Under saline conditions

root growth has been found to be regulated by calcium and found to mitigate the adverse effects (Cramer et al). Calcium is well-known as a secondary messenger involved in various physiological and biochemical processes in osmotic stress in plants (Ranty et al., 2016; Tuteja and Mahajan, 2007)Exogenous application of calcium has shown to ameliorate the adverse effects of salt, cold, heat, and water deficit by modulating antioxidant, growth performance, photosynthetic efficiency, and osmolytes production (Shoresh et al., 2011; Tan et al., 2011; Upadhyaya et al., 2011; Xu et al., 2013).

Kodomillet (*Paspalum scrobiculatum)* is one of the small grain cereals native of India with cultivation history of more than 3,000 years in Indian sub-continent and is mentioned in ***BRIHAD SANHITA***.



According to it, when Jupiter is leader in the year of Jyeshtha, majority of the population will enter in trouble due to famine and only the grain like kodo (*Kodra*) and kutki (*Samai*) will survive. It grows on a fairly large scale for food and livestock feed in India and Africa. Kodo millet is a balanced and staple food of tribal and economically poor section of the population. It provides low priced proteins; minerals and vitamins in form of sustainable food. The fiber content of the whole grain is very high. Kodo millet contains 11% protein and nutritional value of protein has been found to be slightly better than that of other minor millets.

2.Materials and Methods

2.1 Plant material

The experimental plant materials (genotypes IPS 145, IPS 351, IPS 583, IPS 610, IC 426676 and IC 382888) were collected from International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) and National Bureau of Plant Genetic Resources (NBPGR), Hyderabad, India. The research was carried out at cytogenetics lab, department of Botany and Microbiology, ANU.

2.2 Hydroponic system setup and salt treatment

Twenty-five seeds per each variety were taken and were placed on a moist filter paper that was kept over 250 ml beakers containing half strength modified Hoagland solution (Hershey and Merritt, 1986). Seeds were allowed to germinate up to 2 mm, and after 4 days seedlings were treated with $CaCl_2$ solutions of 0 mM, 50 mM, 100 mM, 150 mM and 200 mM concentrations up to 120 hrs. Solutions were aerated with an aerator for 5 minutes both at dusk and dawn. After 120 hrs of treatment final data was recorded. The following morphological, physiological and biochemical characters were studied i.e. root length, shoot length, germination energy, germination index, seedling vigor index, polyphenol oxidase, peroxidase, catalase and superoxide dismutase. The data were analyzed statistically by using ANOVA.

2.3 Analysis of Morphological Properties

2.3.1 Root length

The length of all the roots in a clump up to the tip was measured with the help of a measuring scale thread and expressed in centimeters as average root length.

2.3.2 Shoot length

Plant height was recorded from the ground level to the growing tip of the main shoot and calculated using

scale and expressed in centimeters.

2.4 Analysis of Physiological Properties

2.4.1Germination Index (GI)

Czabater (1962) defined the germination index (GI) as a measure of the ability of a seed to germinate and develop into a seedling. GI can be expressed as a function of total germination and mean germination rate.

GI = Total germination × Mean germination rate

2.4.2 Germination Energy (GE)

It is expressed as the percentage of seeds germinating within a given time under defined conditions or the time (days) required for a given percentage of seeds to germinate. Accordingly, it reflects germination rate, uniformity, vigour and viability. Germination energy was calculated by the following formula given by Yan Li (2008).

$$GE = \frac{\text{Germinated seeds in NaCl conc. in 3 days}}{\text{Number of seeds for germination}}$$

2.4.3 Seedling Vigour Index (SVI)

Seedling vigour index (SVI) was calculated by using modified formula of Abdul-Baki and Anderson (1973).

SVI = [seedling length (cm) × germination percentage]

2.5 Analysis of Biochemical Properties

The activity of catalase, peroxidase and polyphenol oxidase was assayed by the method of Prathiba Devi (2006).

2.5.1 Polyphenol Oxidase (PPO)

Enzyme Extraction

Five hundred milligrams of plant material was grinded using pre chilled pestle and mortar by adding 30 - 40 ml phosphate buffer (0.02 M). The contents were filtered through cheese cloth and centrifuged at 2000 rpm for 10 minutes. The extract was made 100 ml with the phosphate buffer and preserved for the analysis of PPO.

The reaction mix was prepared by adding 2 ml of buffer and 1 ml of enzyme extract. The mixture was incubated for 5 minutes and the reaction was stopped by adding 1 ml 2.5 N H_2SO_4. Optical density was measured at 420 nm against a blank containing 1 ml of H_2SO_4, 2 ml of buffer, 1ml of pyrogallol and 1 ml of boiled enzyme extract. Enzyme activity was calculated by subtracting the absorbance value of blank from the sample and expressed the enzyme activity as absorbing units per 1 g fresh weight per 5 min.

2.5.2 Peroxidase activity (POX)

Enzyme Extraction

Five hundred milligrams of plant material was grinded using pre chilled pestle and mortar by adding 30 - 40 ml phosphate buffer (0.02 M). The contents were filtered through cheese cloth and centrifuged at 2000 rpm for 10 minutes. The extract was made 100 ml with the phosphate buffer and preserved for the analysis of POX.

Reaction mixture was prepared by adding 3ml of pyrogallol phosphate buffer and 0.1 ml of enzyme extract into a cuvette 0.5 ml of H_2O_2 was added to the reaction mixture and gently shaken. The absorbance was measured after 3min at 420 nm. The same procedure was continued to know the control value by using boiled enzyme extract. The enzyme activity was measured by subtracting the absorbance value of the

blank from the sample and expressed the enzyme activity as absorbing units per 1 g fresh weight per 3 minutes.

2.5.3 Catalase activity (CAT)

Germinating seeds were taken and seeds coat was removed. One gram of this material was macerated into thin paste by using pH 7 Phosphate buffer and the enzyme extract was filtered through muslin cloth.The filtrate was used for enzyme assay.

Two milliliter of the enzyme extract was taken into 50 ml clear conical flask and to this 1ml of 0.45 molar H_2O_2 was added. The set up was incubated for 5 minutes incubation and enzyme activity was stopped by adding 1ml of 12% H_2SO_4. This extract was titrated against 0.05 N of $KMnO_4$ taken in a burette. Appearance of pink color that remains for about 30 seconds was considered as the end point.

The amount of H_2O_2 destroyed by catalase is calculated and the enzyme activity was expressed in enzyme units per gm leaf material. One unit of catalase is defined as that amount of enzyme, which breaks down /µmol/ of H_2O_2 / min.

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

Where W= weight. of material used

V= volume of $KMNO_4$ utilized (Blank sample value)

2.5.4 Superoxide dismutase (SOD)

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

SOD activity was estimated by recording the decrease in absorbance of superoxide nitro blue tetrazolium complex by the enzyme (Sen Gupta *et al.*, 1993).

Estimation

A reaction cocktail of 33 ml was prepared by mixing the reagents in the following ratio i.e 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, 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 that were with reaction mixture were exposed to 400 W bulb (4 * 100W bulbs) for 15 min.The absorbance was measured at 560 nm immediately and calculated the percentage inhibition of the reaction between riboflavin and NBT in the presence of methionine is taken as one unit of SOD activity. The enzyme activity is expressed as units/mg of protein.

Preparation of Reagents

Potassium Phosphate buffer 250 mM pH 7.8

Solution A was prepared by using potassium mono hydrogen phosphate 250 mM and solution B was prepared by taking potassium dimono hydrogen phosphate 250 mM. Both the solutions were mixed and pH was set at 7.8.

Methionine was prepared by taking 100 mM in 10 ml distilled water. Riboflavin was prepared by taking 10 mM in 10ml distilled water, EDTA was made by taking 5 mM in 10 ml distilled water and nitroblue

tetrazolium (NBT) was prepared by taking 750 µM in 300 µl distilled water.

3.Results

3.1 Growth performance of kodo millet seedlings in response to saline ($CaCl_2$)

The efficiency of the selected varieties against different levels of salt stress during the juvenile stages of plant was observed through Hydroponics experiment. The obtained results were analyzed here under.

3.2 Effect of salinity on morphological properties

Root length and shoot length were significantly changed when plants were exposed to the salt stress conditions (Table 1). All the varieties tried to maintain their root length with change in $CaCl_2$ concentration. The variety IC426676 succeeded well in this issue by having the root lengths i.e. Control: 2.34 cm; 50 mM : 2.19 cm; 100 mM : 2.18 cm; 150 mM : 1.45 cm and 200 mM : 1.21 cm. This is followed by IPS 583 and IC3828 88. The highest effect $CaCl_2$ was seen in IPS 145 at all the concentrations. The genotype IC 426676 was recorded the significant values in control, 50 mM and 100 mM concentration of $CaCl_2$ (Table 1)(Figure 1).Highest shoot length was maintained by IC426676 and IPS 583 among all the varieties in all levels of $CaCl_2$ stress. The least shoot length was observed in IPS 145 (Table 1). The genotypes IPS 610, IC 38288, IPS 583 and IC 426676 were recorded significant and maintained the shoot length up to 150 mM concentrations of $CaCl_2$ (Table 1) (Figure 2).

3.3 Effect of salinity on physiological properties

In $CaCl_2$ treated seeds the variety IC 426676 was showed the highest germination energy in control (0.99), followed by the varieties IPS 583 (0.90) and IC 382888, IPS 351 (0.80). Among all the genotype IC 426676 maintained its germination energy well at all treatments of $CaCl_2$ (Table 1). The varieties IPS 610, IPS 351 and IC 382888 were found to be significant in controls and at 50 mM concentration of $CaCl_2$, and the remaining varieties were found to be significant in controls only. The mean germination index was ranged from 4.00% to 0.33% in $CaCl_2$. The highest germination index was reported in IC 426676 in all the treatments and the variety IPS 145 recorded minimum germination index. The remaining varieties fall in moderate germination index. All the genotypes recorded significant in controls and at 50 mM concentration of $CaCl_2$, but the accession IPS 583 reported significant in control only (Table 1). The seeds of IC426676 and IPS 583 keep up their SVI even at high levels of $CaCl_2$ concentration whereas the germplasm IPS 145 was recorded less SVI with increased concentration of $CaCl_2$. All the varieties were maintained significant levels of SVI at 50 mM concentration of $CaCl_2$ (Table 1) (Figure 3).

3.4 Effect of salinity on biochemical properties

The highest peroxidase values were maintained with IC426676 and IPS 583 in $CaCl_2$ treated samples in all the levels of $CaCl_2$ stress. These results were quiet opposite in case of IPS 145 and IPS 610 where these cultivars were recorded lower peroxidase activity. All the genotypes were reported significant peroxidase activity in 150 mM and 200 mM concentrations of $CaCl_2$ (Table 1) (Figure 4). In $CaCl_2$ treated genotypes the highest activity of PPO was observed in IC426676 (0.61 ug^{-1}) and IPS 583 (0.59 ug^{-1}) at 200 mM concentration of $CaCl_2$, and 0.54 ug^{-1} and 0.52 ug^{-1} at 150 mM concentration in the same varieties i.e IC426676 and IPS 583 respectively. The polyphenol oxidase activity was found to be significant in all the varieties at 150 mM and 200 mM concentration of $CaCl_2$ (Table 1) (Figure 5).

The activity of SOD was increased with increased $CaCl_2$ concentration in all the studied varieties (Table 1) (Figure 6). The increase in SOD activity was ranged from 18.52 umg^{-1} to 27.89 umg^{-1}. The cultivars IPS 145 and IPS 610 were recorded the lowest SOD activity in all the levels of $CaCl_2$ stress but the genotypes like IPS 583 and IC 426676 were reported the highest SOD activities in all the levels of $CaCl_2$ stress. The highest concentration of $CaCl_2$ stress results in the depletion of available catalase among all the treatments. In spite of increased $CaCl_2$ stress the genotypes IC426676 and IPS 583 were performed well in keep up their catalase contents in all the treatments (Table 1) (Figure 7).

4. Discussion

Growing plants in hydroponic solution with an addition of salinizing salts is an easy technique that rigorously controls the root environment for evaluation of the response of the plants to salinity (Feigin et al., 1987; 1992 and Shibli, 1993). With this technique most of the complexities and interferences induced by soil and environmental factors are avoided and better control of the experiment is achieved (Meyer et al., 1989). Basing on the above conclusions the present study was taken up with an aim to evaluate morphological and biochemical responses of kodo millet to different salt stress levels in selected germplasm of Kodo millet through hydroponic.

Rasool *et al.,* (2010) have suggested that salinity decrease radicle/root and plumule/shoot growth and root length density. It is reported that soil salinity suppresses shoot growth more than the root growth (Ramoliya and Pandey, 2003) which are in confirmation with the present results. This is may be due to change in the external water potential, increasing ion toxicity and ion imbalance (Jaleel *et al.,* 2007a and Jaleel *et al.,* 2007b), and this situation brings biochemical restraints on cell wall expansion, which in turn can inhibit plant growth (Hernandez and Almansa, 2002). Moreover, accumulation of inorganic ions, predominantly of Na^+ has a role in the process of osmotic adjustment (Gzik, 1996 and Arshi *et al.,* 2005). High Na^+ levels in the external medium greatly reduce the physiochemical activity of the dissolved calcium (Cramer *et al.,* 1986) and may thus displace Ca^{2+} from the plasma membrane of the root cells (Cramer *et al.,* 1985) which in turn inhibit the root and shoot growth. Since plant growth is a result of massive and irreversible expansion of young cells produced by ongoing meristematic divisions, salinization can inhibit both cell division and cell expansion in growing tissues of roots, stems, and leaves (Zidan *et al.,* 1990). But the varieties IC 426676 and IPS 583 found to be showed increased radicle/root growth and plumule/shoot growth up to some extent this may be because of having potent mechanism stopping the excess input into their cells. The similar results were found in *Vicia faba* (Magdi *et al.,* 2010) in *Abelmoschus esculentus* (L.) Moench (Beema rao *et al.,* 2007).

The reduction in germination energy was observed in all the genotypes in all treatments. It is may be due to less availability of simple sugars which can directly useful in respiration and immediate energy releasing process (Dendy, 1995) and inability to uptake water (Daiber, 1975). Further highest percent loss of germination energy was reported in $CaCl_2$ treated plants in all the genotypes in all the treatments, which is opposite to the ameliorative effect of $CaCl_2$. But in present study the cultivars IC 426676 and IPS 583 were maintained their osmotic potentiality which help them to overcome the harmful effects of these salts. Germination index can be considered as a perfect measure of plant response to salinity because the formula takes into consideration the speed and total germinated seeds. This situation is may be due to accumulation of Na^+ and Ca^+ ions into the actively growing tissues especially meristematic cells (Li *et al.,* 2007). Moreover, to regulating the K^+/Na^+ value requires a certain time for the adaptation of this process of the salt environment. Among all the studied genotypes IC 426676 and IPS 583 proved to be maintained high

GI in $CaCl_2$ stress with better adaptation. These reports are in agreement with Karagüzel (2003) Lipunus various and Khan *et al.* (2009) hot pepper.

In present study genotypes IC 426676 and IPS 583 were showed high SVI by maintaining effective alternate pathways so as to produce reactive oxygen species in order to cope up the increasing salinity stress. These results are in agreement with Kader and Jutzi (2004) in sorghum.

In present study salt tolerant genotypes showed increased peroxidase activity $CaCl_2$ stress. Significant roles of POX have been suggested in plant development processes (Gaspar *et al*., 1985), which was involved in scavenging of H_2O_2 produced in chloroplasts (Asish and Anath, 2005). Aghaleh and Niknam (2009) found that salinity increased total POX activity in explants of soybean under salinity stress. The enhancement of POX activity by salinity has also been observed in rice (Lee *et al*., 2001), pea (Shahid *et al*., 2011).

The PPO activity was enhanced with increase in $CaCl_2$ concentration. High polyphenol oxidase activity under stress suggests that its ability to oxidize and to reduce the toxic substances such as phenolic compounds which are generally described to be accumulated during salt stress (Ashish and Anath, 2005). In present study highest PPO activity was observed in IC 426676 and IPS 583 at 200 mM indicating that they are having high protective mechanism against oxygen free radicles. The results obtained in this study were in accordance with those found in the roots of rice seedlings (Lin and Kao, 1999).

Total superoxide dismutase activity is enhanced at 200 mM $CaCl_2$ salinity stress especially in genotypes IC 426676 and IPS 583. Superoxide dismutase (SOD) catalysis the conversions of superoxide anions to hydrogen peroxide and water (Pratap and Sharma, 2010). Several reports have stated that over-expression of superoxide dismutase leads to increased tolerance to abiotic stress (Bohnert and Sheveleva, 1998). Martinez *et al.* (2001) in potato, Zhao *et al.* (2006) in rice, Esfandiari *et al.* (2007) in wheat also reported over expression of SOD activity and associated with decrease in oxidative damage.

A decline in catalase activity under stress conditions were observed in the present study. The decline in CAT activity is regarded as a general response to many stresses (Pan *et al.*, 2006 and 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. It may also be associated with degradation caused by induced peroxisomal proteases. But the cultivars IC 426676 and IPS 583 tend to maintain the catalase activity Because of which these cultivars managed high germination process. The same results were obtained in *Catharanthus roseus* (Jaleel *et al.*, 2007a; Hamed *et al.*, 2013).

5. Conclusion

Use of hydroponic methods to screen the salt tolerant cultivars found to be successful and dependable especially in case of minor millet such as Kodomillet As a result, we studied the effects of calcium chloride on the morphological, physiological and biochemical response through hydroponic method under saline conditions. we analyzed seedling growth parameters and activity of antioxidant enzymes. In conclusion, IC 426676 and IPS 583 were performed well against different $CaCl_2$ stress levels and can be used further to examine their tolerance through pot culture and field trials in order to suggest them as potent lines to with stand to the high saline soils predominantly calcium chloride stress.

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Declaration of Interest Statement

The authors declare no conflict of interest.

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Table 1: Effect of different concentrations of $CaCl_2$ on seedling characters of kodo millet in Hydroponic Experiment

Variety →	Effect of $CaCl_2$ on IPS 145					Effect of $CaCl_2$ on IPS 610					Effect of $CaCl_2$ on IPS 351				
Parameter ↓	Control	50 mM	100 mM	150 mM	200 mM	Control	50 mM	100 mM	150 mM	200 mM	Control	50 mM	100 mM	150 mM	200 mM
Germination Index (%)	0.66*	0.65*	0.51	0.34	0.33	1.52*	0.31*	0.66	0.66	0.33	2.65*	2.14*	1.65	1.32	1.16
Germination Energy (%)	0.20	0.20	0.14	0.10	0.09	0.45*	0.40*	0.30	0.20	0.10	0.80*	0.62*	0.50	0.49	0.34
WSeedling Vigou	35.60*		4.40	2.00	1.40			46.60	28.20	13.40			151.60	146.80	132.40

r Index		28.20*				141.60*	129.20*				309.00*	171.20			
Root length (cm)	0.57*	0.20	0.13	0.07	0.05	0.96*	0.84*	0.52	0.49	0.37	1.52*	1.06*	1.00*	0.65	0.38
Shoot length (cm)	1.65*	0.92*	0.77	0.13	0.07	2.26*	2.14*	1.99*	0.83	0.67	3.91*	2.82*	2.43	1.98	1.48
Peroxidase (mg g-1 F wt)	0.33	0.37	0.41	0.47*	0.52*	0.35	0.39	0.42	0.48*	0.53*	0.35	0.44	0.46	0.49*	0.56*
Polyphenol oxidase (mg g-1 F wt)	0.33	0.37	0.41	0.43*	0.48*	0.35	0.38	0.42	0.48*	0.55*	0.36	0.38	0.44	0.53*	0.56*
Catalase (µg-1)	0.039*	0.037*	0.36*	0.019	0.017	0.058*	0.040*	0.038*	0.020	0.018	0.059*	0.041*	0.038*	0.020	0.019
Superoxide Dismutase (µ mg-1)	18.52	19.52	21.54	23.20*	24.84*	17.20	18.54	19.63	20.35*	22.36*	20.51	22.68*	23.61	24.89*	26.87*

Variety→	**Effect of $CaCl_2$ on IC 382888**					**Effect of $CaCl_2$ on IPS 583**					**Effect of $CaCl_2$ on IC 426676**					**CD at 5%**	**F-value**
Parameters↓	**Control**	**50 mM**	**100 mM**	**150 mM**	**200 mM**	**Control**	**50 mM**	**100 mM**	**150 mM**	**200 mM**	**Control**	**50 mM**	**100 mM**	**150 mM**	**200 mM**		
Germination Index (%)	0.31*	2.16*	2.09	1.66	1.32	3.30*	2.17	2.16	1.99	1.55	4.00*	3.11*	2.83	2.60	1.99	0.10	SIG

Germination Energy (%)	0.80*	0.65*	0.50	0.50	0.41	0.90*	0.65	0.60	0.50	0.44	0.99*	0.65	0.62	0.60	0.50	0.07	SIG
Seedling Vigour Index	337.00	316.20*	215.00	195.80	114.00	645.00*	316.20	268.20	133.40	115.00	675.40*	463.20*	350.80	146.80	132.40	2.66	SIG
Root length(cm)	1.34*	1.28*	0.90	0.56	0.54	2.32*	1.63*	1.53*	0.97	0.79	2.34*	2.19*	2.18*	1.45	1.21	0.01	SIG
Shoot length(cm)	5.81*	3.87*	3.63*	2.12	2.09	6.27*	3.96*	3.74	2.64	2.32	8.34*	6.25	5.80*	3.38	2.38	0.07	SIG
Peroxidase (mg g^{-1} Fwt)	0.36	0.44	0.47	0.50*	0.58*	0.40	0.44	0.48	0.52*	0.59*	0.44	0.45	0.58	0.54*	0.61*	0.01	SIG
Polyphenol oxidase (mg g^{-1} Fwt)	0.36	0.42	0.45	0.53*	0.57*	0.38	0.43	0.46	0.53*	0.58*	0.44	0.47	0.50	0.55*	0.61*	0.01	SIG
Catalase (µg^{-1})	0.060*	0.045*	0.038	0.020	0.019	0.061*	0.056	0.041*	0.020	0.019	0.062*	0.059*	0.042*	0.023	0.023	0.001	SIG
Superoxide	20.36	21.54	22.36*	23.45*	24.58*	22.01	23.51	24.67*	25.89*	27.61*	23.22	24.56	25.36*	26.54*	27.89*	2.1	SIG

Dismutase (μ mg^{-1})																01	

Figure 1. Effect of $CaCl_2$ salinity on root length of kodomillet

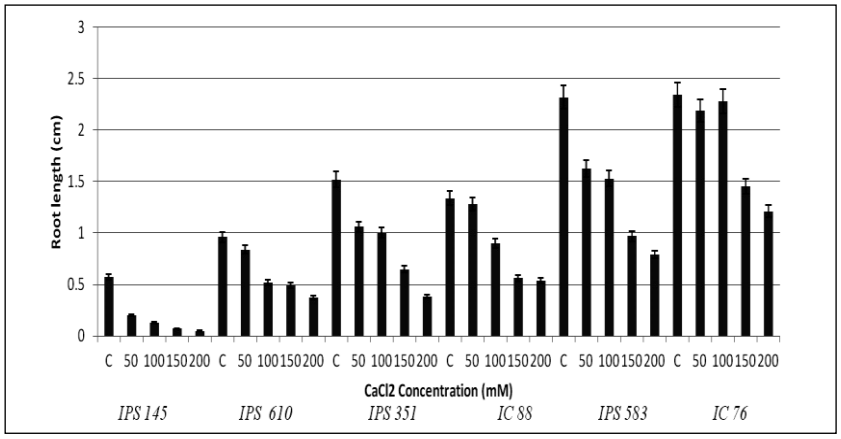

Figure 2. Effect of $CaCl_2$ salinity on shoot length

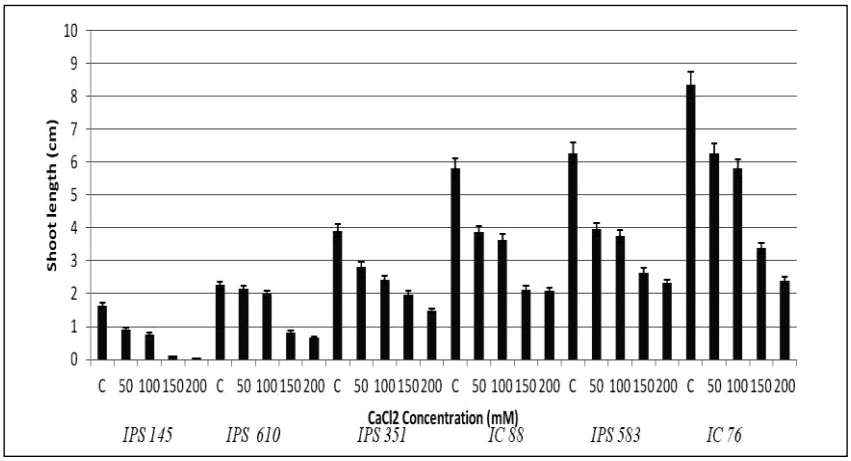

Figure 3. Seedling vigour index under $CaCl_2$ stress

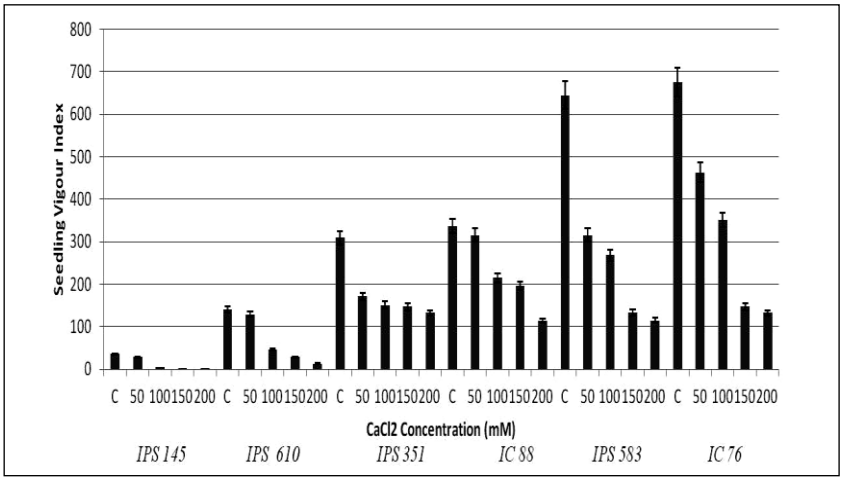

Figure 4. Peroxidase activity on treatment with $CaCl_2$

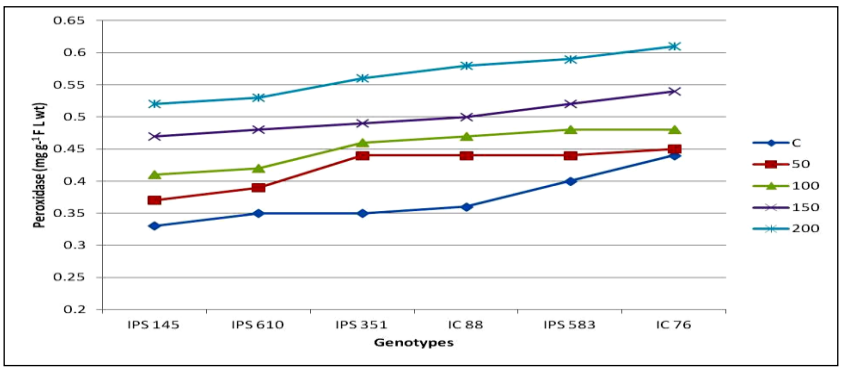

Figure 5. Hike in polyphenol oxidase activity under $CaCl_2$ stress

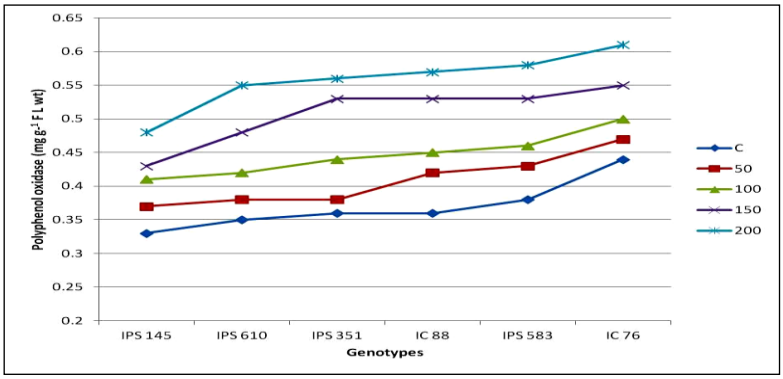

Figure 6. Superoxide dismutase activity under $CaCl_2$ stress

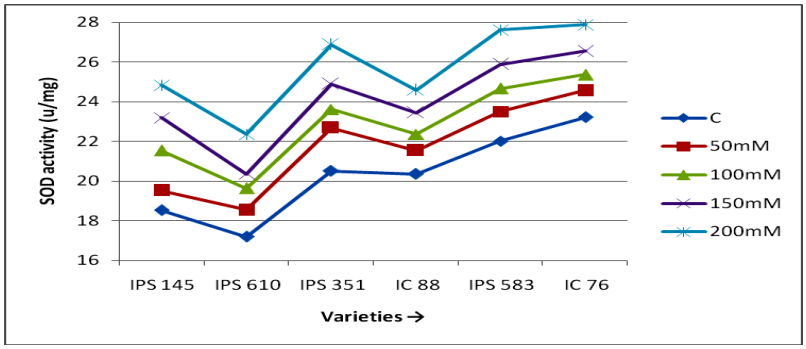

Figure 7. Catalase activity under $CaCl_2$ stress