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The negative effects of Calcium Chloride on Kodo millet (*Paspalum scrobiculatum*) germplasm during germination

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Abstract: *Calcium is one of the important components in the growth of plants and their metabolic activities. It is found to increase the growth rate especially during the times of abiotic stresses like drought and salinity stress such as NaCl and other metal toxicity. But when there is hike in $CaCl_2$ individually the scenario may changes completely and reversed some times. The same thing was happened in case of Kodomillet (Paspalum scrobiculatum) also at times of germination when treated with different concentrations of $CaCl_2$. Different germination parameters such as % germination, germination energy, germination index, reduction percentage of germination were tested besides physiological and biochemical factors like protein percentage, RWC, PRO, POX, PPO, CAT and SOD. Significant changes were observed in the activity of these reactive oxygen species and were statistically correlated. The cultivars IC 76 and IPS 583 defend best when compare with the other genotypes IPS 351, IC 88 and least tolerance was observed in IPS 145 and IPS 610.*
Keywords: *$CaCl_2$, Kodomillet, % germination, relative water content and superoxide dismutase.*

I. Introduction

Plant growth and productivity is adversely affected by various abiotic and biotic stress factors. Plants are frequently exposed to many stress conditions such as low temperature, salt, drought, flooding, heat, oxidative stress and heavy metal toxicity. Salt stress is certainly one of the most serious environmental factor limiting the productivity of crop plants [1]. It is due to the fact that salinity affects most aspects of plant physiology, growth and development [2].

Salt stress induces various biochemical and physiological responses in plants and affects almost all plant processes [3]. An important consequence of salinity stress in plants is the excessive generation of ROS such as the superoxide anion (O^{2-}), H_2O_2 and the hydroxyl radicals [4]. In order to survive under stress conditions, plants are equipped with oxygen radical detoxifying enzymes such as superoxide dismutase, peroxidase, polyphenol oxidase and catalase [5]. Antioxidant mechanisms may provide a strategy to enhance salt tolerance in plants.

One possible approach to reducing the effect of salinity on plant productivity is through the addition of calcium supplements to irrigation water [6], [7]. Calcium is a divalent cation that is extremely important in maintaining the growth of plants. Calcium functions in plant cell elongation and division, structure and permeability of cell membranes, nitrogen metabolism and carbohydrate translocation. Calcium is known to increase salinity tolerance in many crop plants. Under saline conditions root growth has been found to be regulated by calcium and found to mitigate the adverse effects [8].

Kodo millet (*Paspalum scrobiculatum*) is a minor grain crop in India and in the Deccan plateau. The fiber content of the whole grain is very high. Kodo millet has around 11% protein and the nutritional value of the protein has been found to be slightly better than that of other small millets [9].

We all know about the ameliorative nature of the Ca^+. It is found to be true in most of the plant species. But the effect of $CaCl_2$ stress on Kodomillet was not clearly understood and moreover there is no earlier reports regarding the influence of Ca^+ on kodomillet at germination and seedling emergence stage. And it is very significant to study the changes in plant metabolism against $CaCl_2$ stress.

II. Materials And Methods

Six genotypes of Kodo millet seeds were surface sterilized by soaking them in a solution of 2.0% aqueous sodium hypochlorite for 15 minutes at room temperature and then rinsed thoroughly with distilled water. Before germination the seeds were imbibed for 24 h in distilled water at room temperature for quick germination. A total of 25 seeds per plate were taken and were spread evenly on moist two fold Whatmann No. 1 filter paper kept in petri plates of 12 cm in diameter. Seeds were treated with 50, 100, 150 and 200 mM concentrations of $CaCl_2$ solutions and distilled water was used to treat the controls. In each petri plate 5 ml of the appropriate solutions were added on alternate days or when ever required, the entire setup was kept in an incubator and maintained at 25°C.

The experiment was harvested after 144 hrs of setup and the seeds were examined for the following

factors to know their responses at different concentrations of $CaCl_2$. The following parameters were assayed by using standard methods.

2.1 Radicle length

Radicle length from the tip of root to the collar region of the seed was recorded using scale and thread and expressed in centimetre [10].

2.2 Plumule length

Plumule length from the tip of shoot to the collar region of the seed was recorded with the help of thread and scale and expressed in centimetre [10].

2.3 Germination percent (%)

Percent germination was recorded 24 h after the treatment up to 144 h with a gap period of 24 h. The seeds with a radical length of more than 2 mm were considered as germinated [11]. Percentage of germination was measured using the following formula given by Abdul Kabir [12] and it was expressed in terms of percentage (%).

$$\text{Germination per cent} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

2.4 Germination Index (GI)

The germination index (GI) has been defined by Czabator [13] as a measure of the ability of a seed to germinate and develop into a seedling. The germination can be influenced by a number of exogenous and endogenous factors. Czabator [13] also described GI as a measure of seed vigour and defined it as a function of total germination and mean germination rate as shown in the equation below.

$$\text{GI} = \text{Total germination} \times \text{Mean germination rate}$$

2.5 Germination Energy (GE)

A measure of the rapidity of germination expressed as the percentage of seeds germinating within a given time under defined conditions or as the time (days) required for a given percentage to germinate. Accordingly it reflects germination rate, uniformity, vigour and viability. Germination energy was measured by modifying the formula of Yan Li [14].

$$\text{GE} = \frac{\text{Number of seeds germinated in } CaCl_2 \text{ concentration in 3 days}}{\text{Total number of seeds}}$$

2.6 Reduction % of Germination (RPG)

The reduction % of germination was calculated according Said El Madidi *et al.,* [15].

$$\text{RPG} = \{1 - (N_X / N_C)\} \times 100$$

N_X is the number of germinated seedling under stress treatment

N_C is the number of germinated seedling in control

2.7 Relative Water Content (RWC)

Relative water content was estimated according Fletcher *et al*., [16] on the final day of the experiment. Fresh seedlings or leaf material was collected and weighed immediately up to three decimals. This was taken as fresh weight (FW). Then the seedlings or leaf material was dipped in petri dishes containing distilled water and the entire setup was left undisturbedely for four hours. After four hours, seedlings or leaf material was blotted gently and weighed. This was referred to as the turgid weight (TW). After recording turgid weight, the seedling or leaf material was dried in an oven at 96°C for four days and the dry weight (DW) was recorded. RWC (%) was calculated by the formula given by Kramer [17] and was expressed in terms of percentage (%).

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

2.8 Estimation of Protein

Amount of protein present in the seed sample was estimated by micro-Kjeldhal's method [18]. One 100 mg of powdered seed sample was taken in a Kjeldhal's flasks and to this 1:4 ratio of $CuSO_4$ and K_2SO_4 was added, followed by the addition of 4 ml of Concentrated H_2SO_4. Then the contents of the flasks were kept

undisturbedely overnight, later on the flasks were digested by placing them on electrical heater until the contents in the flask became colourless. Now the flasks were ready for distillation. The contents in the flasks were poured into the Kjeldhal's ammonia distillation unit and 10 - 15 ml of 40 % NaOH was added to distillation unit for each flask.

Boric acid (4 %) 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 it with 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

$$\% \text{ of Nitrogen} = \frac{X \text{. Normality of acid}}{\text{Sample weight}} \times \frac{14}{1000} \times 100$$

Where

X = Amount of HCl run down

Normality of acid = 0.02

2.9 Proline

The anti oxidative enzyme proline was extracted and estimated according to Bates *et al.,* [19]. Fresh plant material of 500 mg was homogenized by using 3% of aqueous Sulfosalicylic acid. The homogenate was filtered through four layered muslin cloth and the filtrate was collected. The extraction was repeated twice and all the filtrates were pooled and made up to known volume.

2 ml of filtrate was taken into a test tube and to this 2 ml of Acid Ninhydrin and 2 ml of Glacial acetic acid was added. The tubes were incubated at 100°C for 1 hour in a boiling water bath. After incubation the tubes were transferred to an ice bath to terminate the reaction. To these contents 4 ml toluene was added and the tubes were mixed thoroughly using a test tube stirrer for 15 sec, chromophore containing toluene was aspirated from aqueous phase. Then the absorbance of the solution was measured at 520 nm in a UV-Vis spectrophotometer and pure toluene was used as a blank. The amount of free proline released was measured from the standard curve prepared with authentic proline and its amount was calculated on dry weight basis and expressed as µg/g fresh weight of the leaf material.

Acid Ninhydrin was prepared by warming 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M Phosphoric acid and agitated until it dissolved and kept in a refrigerator at 4°C. As the reagent was remains stable up to 24 hrs only, the reagent was prepared freshly at every time.

Enzyme extraction for determining antioxidant enzymes

The activity of catalase as well as peroxidase and polyphenol oxidase was assayed according to Prathibha devi [20].

500 mg of plant material was grounded 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 min. The extract was made up to 100 ml by adding phosphate buffer and preserved for further biochemical analysis.

2.10 Peroxidase (POX)

Reaction mixture was prepared by adding 3 ml of pyrogallol phosphate buffer and 0.1 ml of enzyme extract into a cuvette. To the reaction mixture 0.5 ml of H_2O_2 was added and gently shaken. The absorbance was measured after 3 min 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.

2.11 Polyphenol oxidase (PPO)

The reaction mix was prepared by adding 2 ml of buffer, 1ml of enzyme extract. The mixture was incubated for 5 min and the reaction was stopped by adding 1 ml of 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 1 ml 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 gm fresh weight per 5 min.

2.12 Catalase (CAT)

Germinating seeds were taken and seed coat was removed. 1 g of this material was macerated into thin paste using pH 7 Phosphate buffer and the enzyme extract was filtered through muslin cloth.

2 ml 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 later 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 below formula. The enzyme activity was expressed as enzyme units per g fresh leaf material. One unit of catalase is defined as that amount of enzyme, which breaks down /μmol/ of H_2O_2 / min.

$$\textbf{Catalase activity} = \frac{25 \times 0.85}{2} \times \frac{V}{W} \quad \textbf{[alignment]}$$

Where W = Weight of material used
V = Volume of $KMnO_4$ utilized (Blank sample value)

2.13 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 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 [21] within 12 h of extraction.

SOD activity was estimated by recording the decrease in absorbance of superoxide nitro blue tetrazolium complex by the enzyme [22].

A reaction cocktail of 33 ml was prepared by mixing the reagents in the following ratio (60 µl-50 mM Phosphate buffer; 390 µl-13 mM Methionine; 0.6 µl- 02 µM Riboflavin; 60 µl-0.1 mM EDTA; 300 µl-75 mM NBT; 50 µl-Enzyme extract).

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 bulbs (4 * 100 W 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 which is taken as 1 unit of SOD activity. The enzyme activity was expressed as units/mg of protein.

Potassium phosphate buffer was prepared by mixing solution A and Solution B. Solution A was prepared by using potassium monohydrogen phosphate 250 mM and Solution B was prepared by taking potassium dimonohydrogen phosphate 250 mM. Both the solutions were added 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 10 ml 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.

2.14 Seedling fresh weight

Seedlings were collected immediately after completion of the experiment and were blotted gently on blotting paper to remove the excess water or polyethylene glycol attached to the seedling. Then the seeds were weighed by using digital weighing balance to record their fresh weight [10].

2.15 Seedling dry weight

After taking the fresh weight of the seedling, they were kept in hot air oven for about three days at 70°C until the seedling maintains the constant weight, these weights were considered as dry weights of the seedlings [10].

III. Results

The per cent germination in control varied from 86% to 96% among the accessions in $CaCl_2$ treatment. In control, highest germination per cent was observed in IC 76 (99.40%) and lowest was observed in IPS 145 (86.40%) respectively. Except IPS 145 all the varieties found to be germinated above 95%. Treatment with 50 mM, 100 mM, 150 mM and 200 mM $CaCl_2$ caused highest % G in IC 76 (85.40%) and lowest was reported in IPS 145 (1.00%). The mean germination percentage of six accessions was 95.66% in control, 68.76% in 50mM, 30.3% in 100 mM, 10.3% in 150 mM and 1.86% in 200 mM $CaCl_2$ salinity levels respectively (Fig 1).

The germination energy among the accessions between the controls varied from 0.45% to 1.01%. Highest germination energy showed in IC 76 (1.01%) and lowest showed in IPS 145 and IPS 610 (0.55%). At 50, 100, 150, and 200 mM $CaCl_2$ the germination energy was less than control. Very poor germination energy

(0.04 to 0.01) was occurred in 100 mM, 150 mM and 200 mM $CaCl_2$ treatment, among all the genotypes (Table 1).

The germination index in controls showed highest in IPS 351(4.17%) and IPS 610 (3.50%) respectively. In treatment 50,100,150, 200 mM $CaCl_2$ the highest germination index was recorded in 50 mM (IC 76: 3.52%) and lowest germination index (0.01%) was recorded in 200 mM $CaCl_2$ concentration among the all accessions. At 200 mM $CaCl_2$ treatment germination index was very less compared to 50,100,150 mM respectively (Fig 2).

The highest RPG was observed in IPS145 followed by IPS 610, whereas lowest RPG observed in IC 76 respectively. IPS 145 reported highest RPG in all treatments 50 mM to 200 mM. RPG was gradually increases as the concentration of $CaCl_2$ increases and it was found to be less in IC 76 and IPS 58 (Fig 3).

Among the accessions, the radicle length in control seedlings was found to be 1.21 cm (IPS 145) to 2.54 cm (IC 76). Accessions IC 76 and IPS 145, showed a maximum (2.54 cm) and minimum (1.21 cm) root length, respectively. At 150 mM, 200 mM $CaCl_2$ treatment very poor root length observed (Table 1).

The relative water content was found to be maintained above 70% in all controls in $CaCl_2$ experiment, except IPS 145 and IPS 610. At 50 mM concentration all varieties marked above 40% of RWC, but it goes on decreasing when the concentration was increases and the highest RWC was reported by IC 76 and lowest was recorded in IPS 145 at 200 mM concentration (Table 1).

The shoot length of control seedlings varied from 4.51 to 7.56 cm. Accessions IC 76, IPS 583 and IPS 145 showed maximum (7.56 cm, 7.43) and minimum (4.51 cm) shoot length respectively. Shoot length of more than 7.0 cm was observed in three accessions (control) IC 76, IPS 583 and IC 88. At 50 mM $CaCl_2$ treatment six accessions showed more (or) less 1 cm and treatment with 100 mM, 150 mM and 200 mM $CaCl_2$ treatment showed less than 1 cm shoot length respectively. In 150 mM and 200 mM $CaCl_2$ treatment the shoot length was very poor (Table 1).

Free proline content was estimated in control and salinity stressed seedlings of kodo millet. The proline content in control was maximum in IC 76 (57.20) and the minimum was showed in IPS 145 (47.40). Seedlings treated with 50 mM, 100 mM, 150 mM and 200 mM $CaCl_2$ salinity, shows the higher proline level in IC 76 and lower proline level recorded in IPS 145, IPS 610 presented in Table. The level of peroxidase activity was estimated in seedlings of control and treated samples. In control the enzyme activity was higher in IC 76 and the enzyme activity was lowest IPS 145. In treatments like 50 mM, 100 mM, 150 mM, 200 mM $CaCl_2$, the enzyme activity was higher in IC 76 and the lower was recorded in IPS 145 (Fig 4).

In control the POX activity was higher in IC 76 and was lowest IPS 145. In treatments like 50 mM, 100 mM, 150 mM, 200 mM $CaCl_2$, the enzyme activity was higher in IC 76 and the lower was recorded in IPS 145 (Fig 5).

When the seeds were treated with concentrations of 50 mM, 100 mM, 150 mM 200 mM $CaCl_{2,}$ the highest activity of PPO was recorded in IC 76 and lower activity was recorded IPS 145. IC 76 maintained maximum enzyme activity in control and in all treatments (Fig 6).

The concentration of the catalase decreased in six varieties as there is increase in the salinity levels. IC 76 was found to be maintaining high catalase even at high calcium levels. And there is no much deviation between all the varieties even at high levels of salinity stress. At 50 mM showed significant changes in all the test samples (Fig 7).

Superoxide dismutase activity observed to be increased among all the accessions with an increase in $CaCl_2$ concentration. The accession s IC 76 and IPS 583 found to be recorded highest SOD activity when compared with other cultivars in all the treatments at 150 mM and 200 mM concentrations of $CaCl_2$ (Fig 8).

The effect of $CaCl_2$ was more on the fresh and dry weights of the test samples. The range of fresh and dry weights is between 0.029 to 0.081 and 0.045 to 0.007 respectively. The highest fresh weight was reported in IC 76, at all the concentrations of the $CaCl_2$ and the lowest was observed in IPS 145 at all the concentrations. Moderate weights were maintained by remaining varieties IPS 583, IC 88, IPS 351 and IPS 610 (Table 1)

IV. Discussion

Germination was greatly reduced at the highest level of salt especially at high $CaCl_2$ levels. In our present study it is found that the percent germination was reduced as the saline level increases. These results were in agreement with Guan et al., [23], Basalah [24], founded that high levels of soil salinity can significantly inhibit seed germination Seed germination is a major factor limiting the establishment of plants under saline conditions, since the germination process represents the first stage of plant growth in addition to its high sensitivity to nutritional and environmental conditions.

The rate of germination energy was decreased in all the test varieties as the concentration increases. It is may be due to less availability of simple sugars which can directly useful in respiration and immediate energy releasing process which in turn sometimes linked with chemical treatments like $CaCl_2$ [25] and inability to

uptake water [26], [27]. This situation results in the lengthening the time needed to complete germination [28]. Further highest percent loss of germination energy was reported in $CaCl_2$ treated plants in all the genotypes at all treatments. Basically Ca^+ is having an ameliorative effect but in case of kodomillet when used individually it showed a negative effect on plant species.

The germination index decreased in all the varieties it is may be due to increase in salinity. The rate of overall germination process was depleted in all the genotypes as the treatment level increases this is in coupled with speed of germination. Both the process were lagging behind and this situation is may be due to high accumulation of Na^+ and Ca^+ ions into the actively growing tissues especially meristamatic cells [29].Which results in the alteration of regular mechanism and cells tend to protect themselves from the change in environmental conditions by triggering the alternate pathways ultimately leads to decreased germination index. These reports are in agreement with Karagüzel [30] in *Lupinus varius* and Khan *et al.* [31] in hot pepper.

The reduction percentage of germination was decreased with the increase in the salt concentration $CaCl_2$ in all treated cultivars. On the other hand, it is known that the inhibition of the germination and the emergence by salinity is mainly due to an osmotic effect [32], [33], while during the growth, the salinity inhibits especially the absorption and the transport of the major elements, which limits the supply of the plant in essential elements for its growth [34] and [35].

Munns and Termaat [36] have suggested that salinity decrease radicle and plumule growth and if we increase salinity level, the amount of reduction will increase. This reduction is due to osmotic pressure which leads to inability in water absorbance. Hence cell division and differentiation reduce and reduction of plumule and radicle length have takes place. It is reported that soil salinity suppresses shoot growth more than the root growth [37], [38] and [39] which are in confirmation with the present results.

In present study it is reported that the RWC decrease in all the accessions in all treatments. The present results were in line with Murillo *et al.* [40] and Szigeti [41] regarding to depletion in relative water content due to hike in salt concentration.

The total protein content of leaf gradually decreased with increasing concentration of $CaCl_2$. This decrease in protein content might be due to the increasing activity of acid and alkaline proteases [42]. Our results are similar with Kennedy and De Fillippis [43], who reported increased activities of both acid and neutral proteases in *Grevillea ilicifolia* and *G. arenaria* under saline stress which are quite similar incase of IC 76 and IPS 583 of present test types. Proteins are hydrolyzed by proteases to release amino acids for storage and/or transport and for osmotic adjustment during NaCl stress and $CaCl_2$ stress in Kodomillet. Osmotic adjustment, protection of cellular macromolecules, storage form of nitrogen, maintaining cellular pH, detoxification of the cells, and scavenging of free radicals are proposed functions of free amino acid accumulation.

Increased proline accumulation under $CaCl_2$ stress may be due to breakdown of proline-rich protein or de novo synthesis of proline. It may also be due to prevention of feedback inhibition of the biosynthetic enzyme caused by sequestering proline away from its site of synthesis or by relaxed feedback inhibition of the regulatory step or by decreased activity of proline oxidase involved in degradation of proline. Therefore, accumulation of proline oxidation or diminished incorporation of proline into protein due to impaired protein synthesis and reduced growth. Proline accumulation under salt stress can be explained by the higher inhibitory rate of proline dehydrogenase and proline oxidase and it protect some enzymes by acting as effective osmoregulator [44], [45]. It has been reported that proline accumulation was not related to salt tolerance or to salt stress acclimation, but probably is just on expression of the seedling reaction to the stress damaging [46].

In contrast, salt tolerant accessions showed increase in the peroxidase activity. Significant roles of POD have been suggested in plant development processes [47], which was involved in scavenging of H_2O_2 produced in chloroplasts [48]. Aghaleh and Niknam [49] found that salinity increased total POD activity in explants of soybean under salinity stress. The enhancement of POD activity by salinity has also been observed in pea [50] and mulberry [51]. In tolerant plants, POD activity was found to be higher to protect plants against the oxidative stresses [44].

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 [52], [47] and [48]. The same increase in PPO against salt stress was reported in large number of plant species [53].

A decline in Catalase activity under stress conditions were observed in the present study which suggests a possible delay in removal of H_2O_2 and toxic peroxides mediated by catalases and in turn as enhancement in the free radical mediated lipid peroxidation under abiotic stress conditions. CAT activity decreased in all experimental plant cultivars. The decline in CAT activity is regarded as a general response to many stresses [54], [55] and [56]. 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 or may be due to the photo-inactivation of the enzyme.

Superoxide dismutase would play a major protective role under anoxic conditions; it may have a critical role in the survival of the plant, when oxygen levels increase as the flooding stress abates. Anaerobic tissue has a very high redox potential and the soil environment surrounding the roots contains highly reduced forms of metal ions such as iron, which could readily reduce atmospheric oxygen to superoxide.

The reduction in biomass production that is shown in this study could be due to the high concentration of salt [57]. These results are supported by [58], [46], [59] and [60]. Reduction in shoot and root dry weights could perhaps be used as one of the good parameters to characterize salinity tolerance in kodomillet.

V. Conclusion

The present study concludes that, even though Ca^+ has an ameliorative effect on the growth of plant species, it may effects adversely on some other plant species, which alters the growth rate and changes the metabolic activities along with an increase in reactive oxygen species and the kodomillet was found to be good example. The cultivars IC 76 and IPS 583 were found to be more tolerant to the $CaCl_2$ stress followed by IPS 351, IC 88 and least tolerance was reported in IPS 145 and IPS 610.

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Table 1. Effect of different concentrations of $CaCl_2$ in petriplate experiment

Variety	IPS 145					IPS 610					IPS 351				
Character	**C**	**50 mM**	**100 mM**	**150 mM**	**200 mM**	**C**	**50 mM**	**100 mM**	**150 mM**	**200 mM**	**C**	**50 mM**	**100 mM**	**150 mM**	**200 Mm**
Germination Energy (%)	0.45*	0.04	0.01	0.01	0.01	0.55*	0.11	0.01	0.01	0.01	0.76*	0.18	0.01	0.01	0.01
Radicle length(cm)	1.21*	0.42*	0.09	0.01	0.01	1.46*	0.43*	0.15	0.01	0.01	1.77*	0.47	0.18	0.02	0.01
Plumule length(cm)	4.51*	1.01	0.01	0.01	0.01	4.86*	0.81	0.09	0.01	0.01	5.97*	1.10	0.10	0.01	0.01
RWC (%)	47.00*	42.10*	40.60*	24.20	2.50	67.20*	55.10*	42.30	37.20	20.70	71.60*	64.00*	47.40	39.80	24.20
Protein	0.537	0.080	0.070	0.023	0.010	0.557	0.213	0.043	0.040	0.030	0.590	0.221	0.200	0.0.150	0.053
Seedling Fresh Weight	0.044*	0.045*	0.036	0.031	0.029	0.053*	0.060*	0.042	0.031	0.034	0.065*	0.061*	0.043	0.036	0.035
Seedling Dry weight	0.018*	0.018*	0.016	0.015	0.007	0.024*	0.022*	0.019	0.019	0.011	0.025*	0.024*	0.023	0.021	0.013

Variety	IC 88					IPS 583					IC 76					CD at 5 %
Character	**C**	**50 mM**	**100 mM**	**150 mM**	**200 mM**	**C**	**50 mM**	**100 mM**	**150 mM**	**200 mM**	**C**	**50 mM**	**100 mM**	**150 mM**	**200 mM**	
Germination Energy (%)	0.85*	0.23	0.02	0.01	0.01	0.92*	0.31*	0.04	0.01	0.01	1.01*	0.34*	0.04	0.02	0.01	0.01
Radicle length (cm)	1.89*	0.59*	0.20	0.03	0.01	2.03*	0.66*	0.22	0.03	0.01	2.54*	0.73*	0.24	0.11	0.01	0.01
Plumule length(cm)	7.20*	1.25	0.10	0.01	0.01	7.43*	1.28	0.16	0.01	0.01	7.56*	1.41	0.18	0.02	0.01	0.03
RWC (%)	74.50*	65.50*	54.20	43.10	34.60	74.80*	67.20*	55.50	44.20	42.24	87.00*	67.60*	57.50	50.60	42.40	0.78
Protein	0.630	0.257	0.243	0.180	0.083	0.710	0.413	0.457	0.203	0.143	0.750	0.633	0.507	0.227	0.203	NS
Seedling Fresh Weight	0.074*	0.062*	0.044	0.039	0.038	0.077*	0.063*	0.045	0.044	0.041	0.081*	0.071*	0.051	0.051	0.045	0.002
Seedling Dry weight	0.026*	0.024*	0.023	0.022	0.013	0.025*	0.024*	0.023	0.021	0.013	0.065*	0.034	0.032	0.028	0.027	0.003

*Significant at 5% level.

Fig 1. Effect of $CaCl_2$ on germination percentage

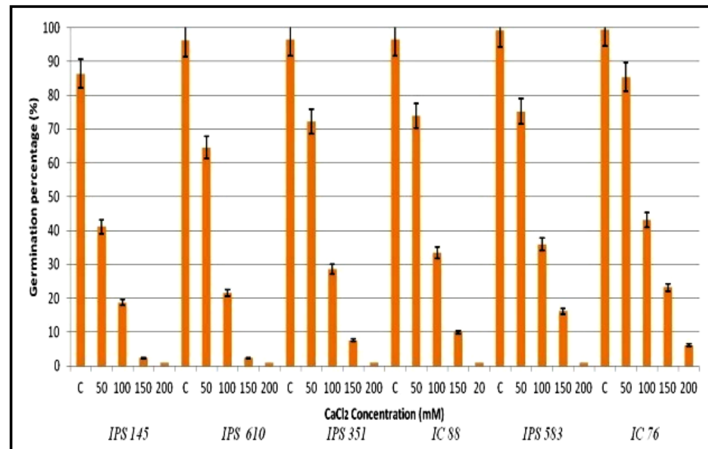

Fig 2. Decrease in germination index with increase in $CaCl_2$

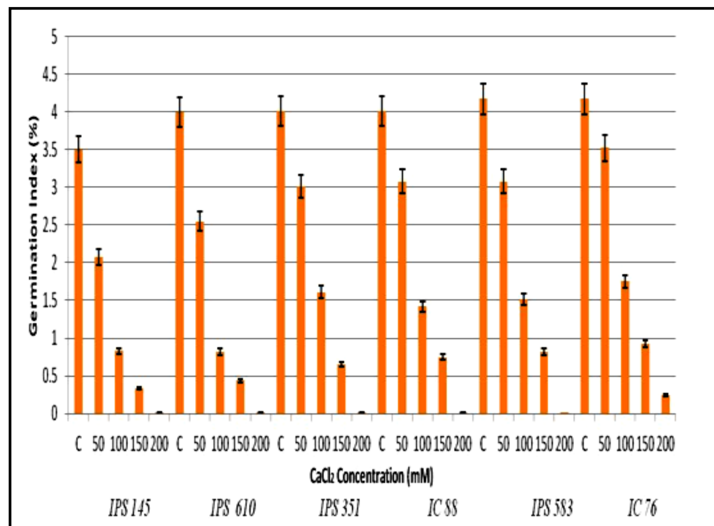

Fig 3. Effect of different $CaCl_2$ concentrations on RPG

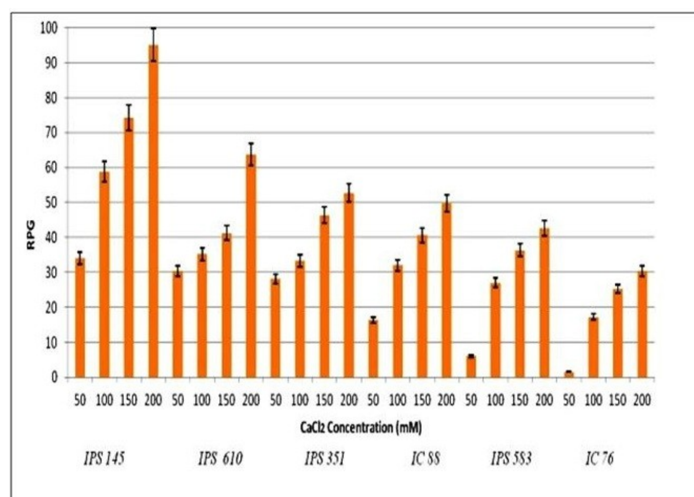

Fig 4. Activity of proline on increase in $CaCl_2$ concentration

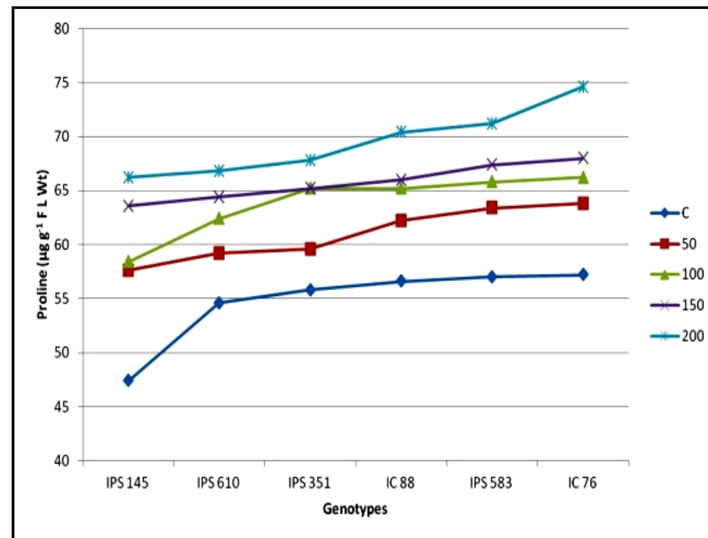

Fig 5. Activity of Peroxidase in $CaCl_2$

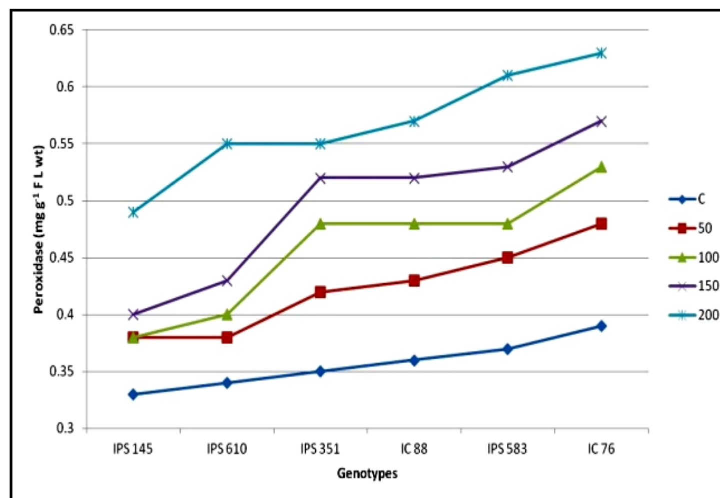

Fig 6. Activity of Polyphenol oxidase in $CaCl_2$

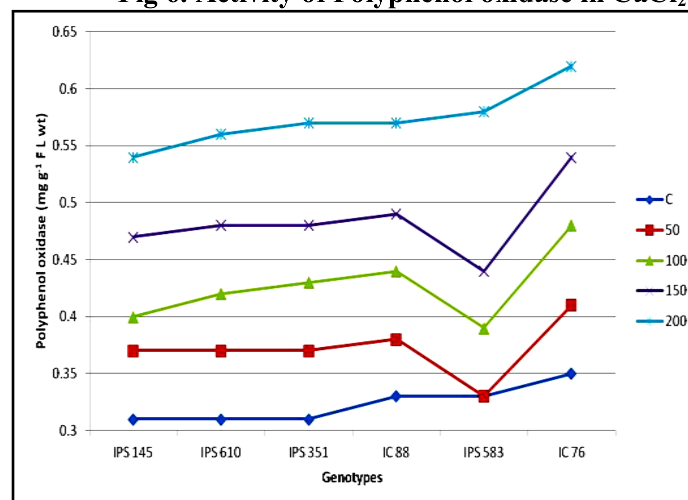

Fig 7. Activity of Catalase in $CaCl_2$

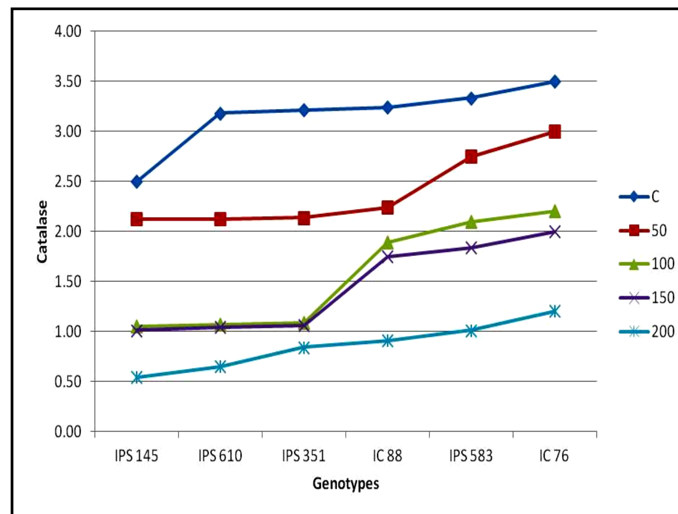

Fig 8. Activity of SOD in $CaCl_2$