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Physiology of salt tolerance in proso millet under *in-vitro* screening

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Abstract
Millets are small grained cereals, have the tradition and heritage in India, the smallest of which include kodo, foxtail, proso, little and barnyard millets. They are the staple food of the millions inhabiting the arid and semi-arid tropics of the world, and are distributed in most of the Asian and African countries and parts of Europe. Soil salinity is one of the major abiotic stress, which play a detrimental role in crop production and productivity in semi-arid regions and tropics. Soil salinity is the salt content in the soil; the process of increasing the salt content is known as salinization. Salts occur naturally within soils and water. Salination can be caused by natural processes such as mineral weathering or by the gradual withdrawn of an ocean. The ions responsible for salinization are Na^+, K^+,Ca^{2+},Mg^{2+} and Cl^-. Sodium is predominant in saline soil. Proso millet has been reported to have comparable tolerant level to drought and salinity. The tolerance of the Proso millet, ATL 1 towards drought and salinity have been studied by many researchers. The tolerance towards proso millet under salinity is still unknown. In this *in-vitro* study was carried out at Agricultural College and Research Institute, Vazhavachanur. We have used salts such as NaCl and $CaCl_2$ at the concentrations of 50 mM, 75 mM and 150 mM. The seeds are sown in the petriplates containing the solutions of NaCl and $CaCl_2$ at the concentrations of 50 mM, 75 mM and 150 mM respectively. After 15 days of salinity stress treatments, the seeds sowed in 50 mM NaCl showed better germination percentage, root length, shoot length, leaf length, seedling length, fresh & dry weight of seedling, promptness index and seedling vigour index followed by 75 mM NaCl. The results revealed that the seeds sowed in 75 mM and 150 mM $CaCl_2$ was proves to be very lethal.

Keywords: Climate change, shoot length, root length, promptness index and seedling vigour index

Introduction
Proso millet is one of the important nuti-cereal grain crop and is grown in many parts of the world known by different names; such as broom corn millet, hog millet, Hershey millet, proso millet or common millet, etc. It is grown extensively in India, Japan, China, Egypt, Arabia and Western Europe. In India proso millet is largely grown in Madhya Pradesh, eastern Uttar Pradesh, Bihar, Tamil Nadu, Maharashtra, Andhra Pradesh and Karnataka. Qadir (2014) [9] reported that, soil salinity reduce the crop yield significantly. Proso millet have gluten-free grain has protein and fatty acids. Besides it also contains polyphenols and essential minerals like phosphorus, manganese, and magnesium. Moreover, proso millet bran is an excellent source of fiber. Salt induction reduced the seed germination could be induced the osmotic stress or iron toxicity of the plants (Hung, 1995) [11].
Salt affected soils are designated as problem soils. Salt affected soils are unproductive unless excess salts are reduced or removed. These soils occur most extensively in arid climates but these soils are also found in coastal sea areas, where soils are inundated by ocean or sea water. Soils are saline if they contain an excess soluble salts such as Na^+,K^+,Ca_2^+,Mg^{2+} and Cl^-.Saline soil contains high contents of soluble salt. Accumulation of soluble salts in soils lead to essential criteria for assessing the salt tolerance in plants under unfavourable soil-plant-water-air relationships (Brady, 2002) [10].

Materials and Methods
The *in-vitro* experiment was carried out at Agricultural College & Research Institute, Vazhavachanur and the seeds were procured from Centre of Excellence in Millets, Athiyandal. The experiment was conducted in Completely Randomised Design (CRD). The salinity tolerance was studied using NaCl and $CaCl_2$ in various concentrations *viz*., 50 mM, 75 mM, 150 mM and control.

After five days of germination, observation on seedling growth parameters *viz*., germination percentage, shoot length, root length, leaf length, total seedling length, fresh weight of seedlings, dry weight of seedlings were recorded. Based on the above parameters, the promptness index, shoot length stress tolerance index, root length stress tolerance index and seedling Vigor index were calculated. Observation was also done on 5th, 9th and 15th days after sowing and the data were recorded. The pooled data was subjected to statistical analysis.

Results and Discussion

During this study, all the observations for salinity stress treatments were recorded at 5th, 9th and 15th days after sowing. The data of the parameters *viz*., germination percentage, shoot length, root length, leaf length, total seedling length, fresh weight of seedlings, dry weight of seedling, promptness index, shoot length stress tolerance index, root length stress tolerance index and seedling Vigor index were recorded on 15th DAS for the full and complete germination. The data of the parameters is given in the table 1.

Table 1: Effect of salt responses of proso millet on germination percentage (%), shoot length (cm), root length (cm), dry weight of seedlings, promptness index and seeding vigour index

Treatments	Germination Percentage (%)	Shoot length (cm)	Root length (cm)	Dry weight of seedlings (g)	Promptness index	Seedling vigour index
T_1 - Control	75	8.31	8.60	0.005	13.7575	1220
T_2 -50 mM NaCl	100	9.37	8.81	0.006	17.5	1360
T_3- 75 mM NaCl	100	7.43	5.17	0.003	11.75	966
T_4 -150 mM NaCl	100	7.23	3.80	0.003	10.00	643
T_5 -50 mM $CaCl_2$	83.3	6.60	4.97	0.004	13.00	905
T_6 -75 mM $CaCl_2$	100	4.83	3.17	0.005	9.27	783
T_7 -150 mM $CaCl_2$	50	2.03	1.23	0.002	7.25	330
MEAN	86.9	6.69	4.86	0.0037	11.73	886
SE(d)	19.15	0.28	0.18	0.17	3.36	13.78
CD	23.53	0.86	0.56	0.38	12.64	42.46

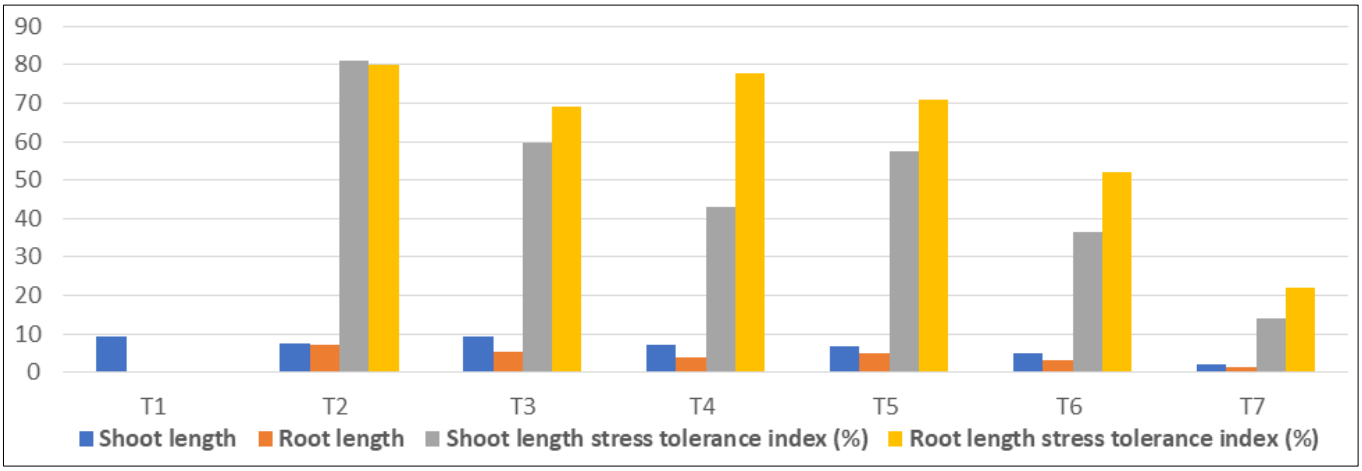

Fig 1: Effect of salt salinity response in proso millet

During this study the salinity stress caused with NaCl treatments were revealed great inhibitory effects in comparison to $CaCl_2$ treatments. Moreover the increase in the concentrations of NaCl (150 mM) and $CaCl_2$ (75 mM, 150 mM) reflected less inhibition to salt stress. With the increase in the concentrations of both salts (NaCl and $CaCl_2$), the shoot formation was more suppressed than root formation. The lowest concentartions of NaCl (50 mM) showed better root and shoot formation when compared to control. The hundred percentage of germination was observed in treatments containing 50 mM NaCl and 75 mM NaCl. The shoot (9.37 cm) and root length (8.81 cm) (Table. 1) were found to be highest in the salt solutions containing 50 mM NaCl. Based on the above parameters the salt tolerance indices like promptness index, shoot length stress tolerance index, root length stress tolerance index and seedling vigor index were calculated. The treatments containing 50 mM NaCl showed better results in all parameters when compared to control. Germination was greatly reduced at the highest level of salt especially at high $CaCl_2$ levels. In our study, it is found that the germination percentage was reduced when the salinity level increases. Reported that pearl millet varieties ICMB 90111 are sensitivity to salinity stress during germination and Tift 23D2B1 are tolerant to NaCl in 75 mM concentration during germination. Sintho Wahyuning Ardie *et al*. (2016) [4] have assessed the shoot length of Foxtail millet under salinity condition with different NaCl salt concentrations. He recorded that the genotype ICERI 5 shown the shoot length of 2.54 cm over than the control. Subramanian *et al*. (2015) [2] had conducted the study on *In vitro* and *In vivo* screening of Barnyard millet. During his study, He recorded the root length of barnyard millet variety MDU^{-1} was 6.6 cm at control and it gradually decreased with increasing level of NaCl salt concentrations. He recorded the Promptness index of barnyard millet variety MDU^{-1} was 9.9 at control and it gradually decreased with increasing level of NaCl salt concentrations. Prabhu *et al*. (2014) [5] have assessed the total seedling length under *In-vitro* screening of finger millet. In that study they considered the two salt concentrations 3000 ppm and 6000 ppm together, the seedling length was least affected in the genotype AF 459 at 20 and 30 days of culture. Francis *et al*. (2021) [3] have assessed seed vigour index in small millets

under drought stress. During this study, He observed the seed vigour index as percentage over control values of the respective genotypes gives as fairer comparison of vigour of the seedling parameters under various level of stresses. Under high stress level maximum seedling vigour was recorded for foxtail millet variety CO 7 (69%) and minimum seedling vigour was for little millet variety ATL 1 (4%). Yakubu *et al*. (2006) [12] reported up to 85 and 70% reduction in shoot and root dry weights, respectively, in Amaranthus grown high salt 13.54dSm of salt.

Conclusion

The growth of the proso millet on comparing to the control, was found to be prominent and relatively steady in T_2 (NaCl 50 mM) with the higher germination percentage, shoot length, root length, leaf length, total seedling length, fresh weight, dry weight, promptness index, plant shoot length stress tolerance index, plant root length stress tolerance index and seed vigour index.

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