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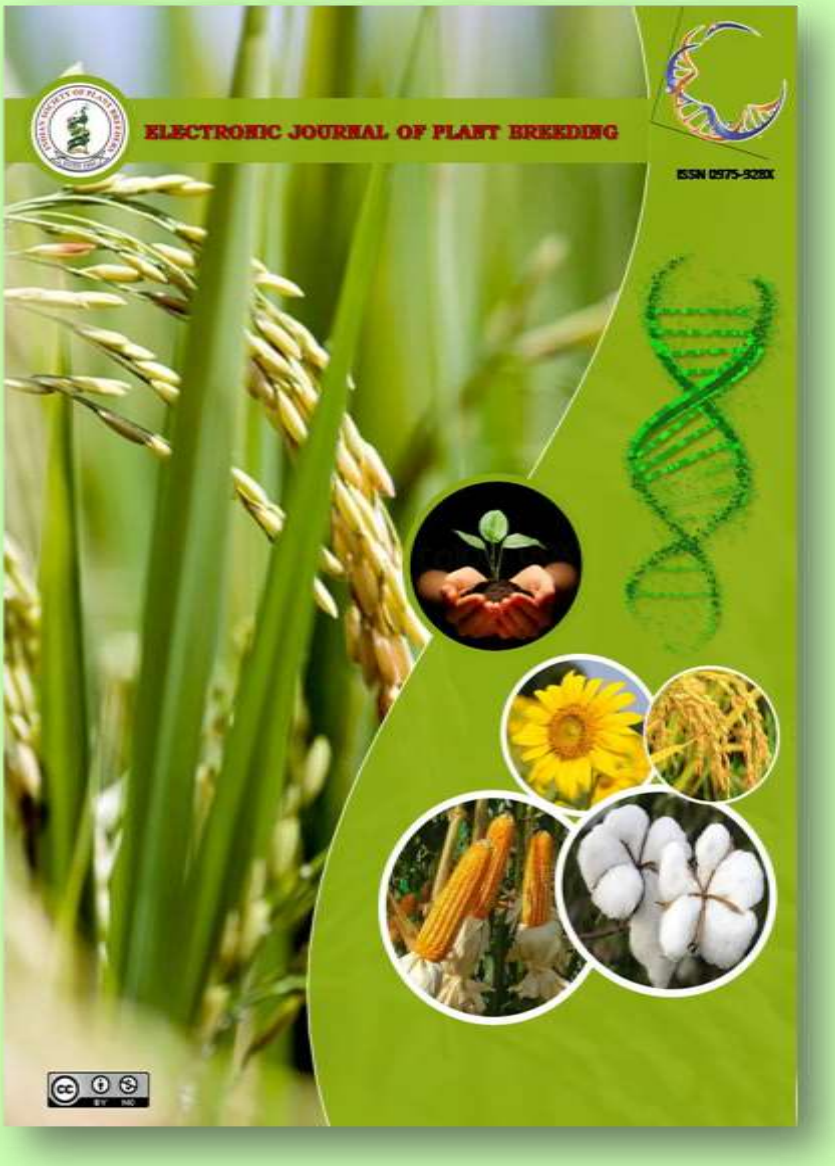

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Research Article

Genetic variability for seedling stage salinity tolerance in barnyard millet [*Echinochloa frumentaceae* (Roxb.) Link]

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Abstract
Barnyard millet (*Echinochloa frumentaceae*) has a potential to withstand salinity. The existing genetic variability for salinity tolerance in 32 barnyard millet accessions was assessed based on the morpho-physiological parameters governing salt tolerance *viz.*, germination percentage, relative germination rate, root length, shoot length, seedling length, vigour index, relative salt injury rate and relative water content. Under gradual increase in the intensity of salt stress, decrease in germination percentage, relative germination rate, root length, shoot length, seedling length, vigour index, relative water content and increase in relative salt injury rate was observed. The antioxidant assay also revealed that catalase and peroxidase activity increased with rise in salt level in tolerant genotypes (ACM161, ACM295, ACM335, GECH10, IEc167) but the enzyme activity in the salt sensitive genotypes (IEc134, IEc348, IEc607) declined with increase in salt concentration, when compared to control. The salt tolerant genotypes maintained higher relative water content and enzyme activity under salt stress. Hence, this may be the underlying mechanism for salt tolerance.

Key words
Barnyard millet, salinity, antioxidant response

Introduction

The genus *Echinochloa* includes nearly 35 species distributed worldwide out of this two species were domesticated and grown as cereals (*Echinochloa esculenta* – Japanese Barnyard millet and *Echinochloa frumentaceae* – Indian Barnyard millet)(Yabuno, 1987). Barnyard millet is a climate smart and nutrient rich crop. It has wider adaptability, short duration, stress tolerance, and superior nutritional qualities (Saleh *et al.*, 2013). It contains 65% of carbohydrates, major propotion of which is in the form of dietary fibre and non-starchy poly saccharide,13.9% protein and essential micronutrients. Owing to their fast growing, early maturing ability and superior nutritional quality of crop straw Barnyard millet has been used as a fodder in United States, India and Japan and it can produce more than eight harvests per year (Obara, 1936).

In the current scenario, plants are exposed to various environmental changes. Environmental stress is one among the major areas of scientific concern because it affects crop growth and limits the productivity. Salinity is due to increase in dissolved inorganic salt concentration which includes cations like K^+, Mg^{2+}, Ca^{2+}, Na^+ and anions like NO^{3-}, HCO^{3-}, $SO4^{2-}$, Cl^-, and $CO3^{2-}$ in the soil solution. Soil salinization is favoured by arid and semi arid climates where evapotranspiration volume is greater than the precipitation volume (Bockheim and Gennadiyev, 2000). Irrigated systems are prone to salinization, more than half of the irrigated crops are affected by salinity or flooding (Hatfield, 2016). Salinity has affected beyond 30% of irrigated land and 6% of world's total land area (Parihar *et al.*, 2015). In order to overcome crop loss due to various stresses underutilized crops gains much more importance. Barnyard millet has a potential to withstand saline condition. It can be used as a reclamation crop for sodicity, arsenic and cadmium affected soils (Abe *et al.*, 2011).

This study aims to identify the existing genetic variability for salinity tolerance in 32 barnyard millet germplsam using various morpho-physiological parameters governing salt tolerance.

Materials and methods

Thirty two barnyard millet germplasm accessions consisting of 26 accessions originating from India, 4 from Japan (IEc531, IEc542, IEc558, IEc564) and 2 from Malawi (IEc348, IEC348) along with a check variety MDU1 were used. Accession numbers starts with IEc were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) genebank, Hyderabad, while remaining were from Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore and Department of Plant Breeding and

Genetics, Agricultural College and Research Institute, Madurai. The response of barnyard millet accessions for different levels of salinity was studied under *in vitro* condition at Plant Tissue Culture Laboratory, Agricultural College and Research Institute, Madurai.The seeds of 32 germplasm accessions used in this investigation was surface sterilized with 2.0% aqueous sodium hypochlorite for about 15 minutes at room temperature and then they are rinsed thoroughly using distilled water.

Salt stress in plants was imposed using various concentrations of NaCl *viz.*, 0mM, 50mM, 100mM, 150mM, 200mM and 250mM. The experimental design was laid out in Factorial Completely Randomized Design (Gomez *et al.*, 1984) with two replications and the following observations were made

Twenty five viable and disinfected seeds from each accessions (with two replications) which was treated with Bavistin for about 5 hours was placed on the filter paper bed and 10 ml of treatment solution were poured in each petri dish so that the seeds immersed partially for ensuring proper aeration. Then seeds were allowed to germinate at room temperature (25 ± 2 °C). Seeds were considered as germinated when the radicles measured 2 mm size. Germination count was taken on 5^{th},7^{th} and 9^{th} day and expressed in percentage.

Root length was measured from the base of the seed to the tip of its roots on 10 days old seedlings. Shoot length was measured from the seed to the tip of its leaf blade on 10 days old seedlings. Seedling length was the total measure of root and shoot length on 10 days old seedlings.

Vigour index:

Vigour index = (Average shoot length + Average root length) x Germination percentage

Relative germination rate (RGR):

The germination frequency of seeds in various salt treatments was measured as per (Li, 2008).

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

Relative salt injury rate (RSIR):

This indicates the effect of salinity on the rate of germination and it was calculated using the formula of (Li, 2008).

$$RSIR = \frac{(\text{Germination \% of control} - \text{Germination \% in treatment})}{(\text{Germination \% of control})}$$

Relative water content (RWC)

Relative water content indicates the measure of water content present in leaves. Fully expanded third leaf from the top of the plant is selected and used for analysis. Twenty-five leaf discs of uniform size were taken per accession in two replications and fresh weight (FW) these discs were recorded. After recording fresh weight, leaf discs were floated in distilled water for eight hours and turgid weight (TW) was recorded after surface drying with filter paper. Finally the leaf discs were then oven dried (65^0C) to record dry weight (DW). RWC was calculated using the following formula

$$RWC = \frac{\text{Fresh Weight (FW)} - \text{Dry Weight (DW)}}{\text{Turgid Weight (TW)} - \text{Dry Weight (DW)}}$$

The catalase activity in plant materials was estimated using the method of Luck (1974) with few modifications. Seeds were pre conditioned for this method. In this case, the seeds were soaked overnight in saline solution of different concentrations and control was also maintained with distilled water. One gram of pre conditioned seed was weighed and ground in a pestle and mortar along with 20 ml of 0.067 M phosphate buffer which was prepared by dissolving 3.522 g of KH_2 PO_4 and 7.268 g of Na_2HPO_4 $2H_2O$ in distilled water and then the volume was made up to one liter (Assay buffer should be diluted 10 times) and centrifuged at 15000 rpm (4^0C) for about five minutes. The supernatant collected was used for enzyme assay. In the experimental cuvette, 3 ml of H_2O_2 phosphate buffer (0.16 ml of H_2O_2 (10% w/v) was diluted to 100 ml using phosphate buffer – prepared fresh) and 0.02 ml of sample (1 ml of sample diluted to 10 ml) was added and mixed well with the help of glass rod. Time (Δt) required for the decrease in absorbance was noted at 240 nm in Cary UV spectrophotometer.

Peroxidase activity in plant materials were assessed by following the method of (Malik and Singh, 1980) with some modifications. Seeds were pre-conditioned. In this case, the seeds were soaked overnight in saline solution of different concentrations and control was also maintained with distilled water. One gram of preconditioned seeds were weighed and ground in a pestle and mortar along with three ml of 0.1 M phosphate buffer (pH 7). The homogenate was centrifuged at the rate of 10000 rpm for 10 min. The supernatant collected was the enzyme source. Add 0.1 ml of enzyme extract (sample) to 3 ml of H_2O_2 (0.142 ml of H_2O_2 diluted to 100 ml). Time (Δt) required for the increase in absorbance in UV spectrophotometer at 436 nm was noted. All statistical analysis was performed using AGRES software. Significant differences between the mean were compared using LSD test ($P<0.05$).

Results and Discussion

Seed germination plays a crucial role in plant's life cycle because it has a direct effect on crop stand (Hatfield and Prueger, 2015). Salt stress had a greater impact on seed germination. Imposing salt stress reduces germination. However, reduction in germination percentage induced by salt stress was considerably variable among different genotypes. Four barnyard millet accessions (ACM161, ACM295, ACM335, IEc167) recorded higher germination percentage (>70%) under higher levels of salt stress (250mM). Twelve accessions used in this study showed less than 35% of germination under salt stress (250mM) indicating that higher levels of salinity (Table 1) hinders germination by reducing the osmotic potential, accumulation of certain toxic ions and reduced nutrient uptake (Afzali *et al.*, 2011). These factors prevent aerobic respiration by altering the physiological and biochemical activity of the seeds.

Large variation for RGR was also observed among the entries indicating genotypic variation in germination as a response to salinity. This can be categorized based on the range used by Ardie *et al.*, 2015. Only one accession (ACM335) showed more than 0.8 at 250mM and it is tolerant to salinity. Three accessions (ACM161, ACM295, IEc167) can be grouped as moderately tolerant as they showed RGR (0.7 – 0.8). Sixteen accessions were grouped as moderately sensitive (0.4 – 0.7) and twelve accessions were sensitive (0.1 – 0.4) to salinity (Table1).

RSIR increased in all genotypes with increase in salinity level. Low RSIR (0.2) was reported in ACM335 (250mM) and 16 genotypes showed higher RSIR (0.5 – 1.0) in higher concentrations of salinity (250mM) and they were highly sensitive to saline stress (Table 1). These results were in agreement with previous reports in foxtail millet (Sreenivasulu *et al.*, 2000). This increase in injury may be due to disruption in cell membrane and reduced water uptake (Kumari *et al.*, 2013) whereas, there was a positive correlation between salt sensitivity and membrane damage (Sreenivasulu *et al.*, 2000).

The results from the present study at germination stage is in accordance with the previous reports in barnyard millet (Parkash *et al.*, 2018) and in other minor millets viz., kodo millet (Kumari *et al.*, 2013), finger millet (Toderich *et al.*, 2018), proso millet (Sabir and Ashraf, 2008) and foxtail millet (Ardie *et al.*, 2015). Tolerance to salinity at seedling stage indicates tolerance at vegetative and reproductive stage this has been explained in major crops such as rice (Hariadi *et al.*, 2015), wheat (Ali *et al.*, 2002), maize (Khan *et al.*, 2003), and sorghum (Bafeel, 2014). The morphological parameters like root length, shoot length and seedling length were declined significantly with rise in salt concentration. From this data it has been clearly noticed that both root and shoot growth got reduced due to salinity (Table 1). The growth of radical and plumule was greatly influenced by salinity because saline stress inhibits cell division and differentiation which ultimately reduces the plant growth (Tahjib-Ul-Arif *et al.*, 2018). Physiological indices, radical and plumule growth were negatively correlated with salinity (Liu *et al.*, 2005).

Salinity leads to physiological drought. Water loss within the plant therefore reduces the RWC. In this sense, this is one of the most reliable and extensively used indicator for salinity screening (Sánchez-Rodríguez *et al.*, 2010). Based on the experimental results the difference in RWC was observed between genotypes and with varied saline concentrations (Table 1). RWC decreased significantly when compared to control. Reduction in RWC was less upon exposure to lower levels of salinity, this may be due to osmotic adjustment of the plants under stressed conditions. The greatest decline in RWC was observed at 250 mM NaCl. The present investigation revealed that two barnyard millet accessions ACM331 and ACM161 exhibited higher RWC (>70%) at 250mM and were grouped as tolerant whereas, eight genotypes had low RWC (50%) at 250mM indicating the sensitivity towards salinity and inability to uptake water under stressed conditions. The results were in agreement with those of (Islam *et al.*, 2011) in foxtail millet and kodo millet. Reduction in RWC denotes loss of turgor which resulted in limited availability of water for cell extension (Katerji *et al.*, 1997). Under stressed conditions tolerant plants can maintain both leaf water relations, RWC in acclimated and non-acclimated regions (Khanna-Chopra and Selote, 2007).

Salinity induces ROS (Reactive Oxygen Species) production in plant cells (Banu *et al.*, 2010). Primarily they function as signaling molecules and mediates different physiological processes. Excess production of ROS causes toxic effect in plants by producing oxidative stress and leads to cell death (Banu *et al.*, 2010). The major scavengers of ROS were antioxidant enzymes such as Catalase (CAT), Peroxidase (POX) and Ascorbate peroxidase (APX). Based on the morpho physiological parameters studied, five tolerant, two moderately tolerant, three susceptible genotypes along with one check (MDU1) were selected in order to assess the relationship between salinity tolerance and antioxidant response. The activity of ROS scavenging antioxidants was measured in 11

selected genotypes viz., ACM161, ACM295, ACM333, ACM335, GECH10, T5, IEc134, IEc167, IEc348, IEc607 including check (MDU1). The enzyme activity varied significantly as a response to salinity. This study revealed that catalase and peroxidase activity increased with rise in salt level in tolerant genotypes (ACM161, ACM295, ACM335, GECH10, IEc167) but the enzyme activity in the salt sensitive genotypes (IEc134, IEc348, IEc607) declined with increase in salt concentration, when compared to control (Figure 1 and 2). Antioxidant enzyme activity were significantly affected in response to salt stress. Increased antioxidant response in plants seems to be positively related with reduced oxidative damage and better salinity tolerance (El-Shabrawi *et al.*, 2010). This trend was also supported by early researchers (Hasanuzzaman *et al.*, 2014); (Demiral and Türkan, 2005).

On the basis of morphological, physiological and biochemical assays performed in this study it is clearly evident that four accessions (ACM161, ACM295, ACM335 and IEc167) showed greater tolerance to salinity than the other genotypes used in this study. Among the four tolerant genotypes, three were ACM cultures (ACM161, ACM295 and ACM335) which are the selection from various local land races from Paramakudi, Kovilpatti, Kamudhi respectively. These three areas were located in the southern districts of Tamil Nadu. The identified resistant and susceptible genotypes to salinity from this study will help to formulate for further abiotic stress tolerant breeding programme.

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Table 1. Effect of salt stress on morpho physiological parameters governing salt tolerance induced by NaCl under *in vitro* condition

Parameters	Germination Percentage		RGR		Root Length		Shoot Length		Seedling Length		Vigour Index		RWC		RSIR	
	NaCl concentration		NaCl concentration		NaCl concentration		NaCl concentration		NaCl concentration		NaCl concentration		NaCl concentration		NaCl concentration	
Genotype	Control	250mM	Control	250mM	Control	250mM	Control	250mM	Control	250mM	Control	250mM	Control	250mM	Control	250mM
ACM110	99	58.88*	1	0.59*	9.3*	2.05*	7.35	2.16	16.65*	4.21*	1665*	247.8*	92.03*	65.88*	0	0.42
ACM161	99	75.63*	1	0.76*	10.85*	4.45*	8.35*	3.71*	19.2*	8.16*	1920*	616.7*	93.48*	70.92*	0	0.25
ACM295	100	78.88*	1	0.79*	9.45*	4.3*	7.59*	2.96*	17.04*	7.26*	1703.5*	572.69*	93.79*	69.02*	0	0.22
ACM331	100	68.88*	1	0.69*	9.6*	3.2*	7.12	3.18*	16.72*	6.38*	1671.5*	439.1*	92.84*	70.03*	0	0.32
ACM333	99	63.13*	1	0.64*	9.15*	2*	7.7*	2.92*	16.85*	4.92*	1685*	310.48*	92.83*	55.76*	0	0.37
ACM335	98.5	75.62*	1	0.81*	8.5*	2.7*	8.77*	3.09*	17.27*	5.79*	1726.5*	466.68*	90.76	68.77*	0	0.2
GECH10	99	65.63*	1	0.66*	7.75	3.75*	7.57*	3.16*	15.32	6.91*	1531.5	453.35*	93.01*	66.73*	0	0.35
GECH15	99	65.63*	1	0.66*	8.45	1.05	7.99*	3.05*	16.44*	4.1*	1643.5*	268.93*	89.74	65.87*	0	0.35
IEc747	98.5	64.38*	1	0.65*	8.05	1.2	7.34	3.23*	15.39	4.43*	1538.5	284.96*	93.2*	58.82*	0	0.36
TNEF199	98	44.38	1	0.45	7.6	1.15	7.54	2.92*	15.14	4.07*	1513.5	180.4	89.7	55.21*	0	0.56
TNEF203	99	21.88	1	0.22	8.05	1.3	7.09	2.74*	15.14	4.04*	1513.57	88.38	89.03	53.92*	0	0.79*
T5	100	64.38*	1	0.65*	9.8*	1.3	7.17	2.62*	16.97*	3.92*	1696.5*	252.15*	89.7	56.97*	0	0.36
IEc52	99	51.88*	1	0.52*	8.95*	1.65*	7.85*	1.27	16.8*	2.92	1680*	151.6	90.06*	62.44*	0	0.49
IEc58	99	43.75	1	0.44	8.05	0.55	7.07	2.19	15.12	2.74	1511.5	119.53	93.04*	53.76*	0	0.57
IEc134	97.5	0	1	0	8.05	0	7.65*	0	15.7	0	1570	0	85.19	0	0	1
IEc166	96.25	45.85	1	0.46	7.4	0.55	7.87*	0.71	15.27	1.26	1526.5	57.47	87.6	50.73	0	0.55
IEc167	99	71.88*	1	0.72*	8.6*	0.65	7.17	2.18	15.77	2.83	1576.5	203	91.82*	66.89*	0	0.29
IEc174	97.5	53.75*	1	0.54*	7.75	1	7.57*	2.68*	15.32	3.68	1531.5	198	87.33	58.08*	0	0.47
IEc179	99	53.75*	1	0.54*	8.35	1	7.45	3.61*	15.8	4.61*	1580	247.83*	90.06	61.99*	0	0.47
IEc348	97.5	0	1	0	7.55	0	7.72*	0	15.27	0	1526.5	0	88.97	0	0	1
IEc349	96.25	4.38	1	0.05	8.5*	0.5	7.19	0.66	15.69	1.16	1568.5	5.04	91.72*	46.77	0	0.96*
IEc365	99	34.38	1	0.35	7.4	1.15	6.87	2.26	14.27	3.41	1426.5	117.05	90.45	55.73	0	0.66*
IEc531	97.5	14.38	1	0.15	7.6	1.25	7.49	2.85*	15.09	4.1	1508.5	58.99	90.62	48.29	0	0.86*
IEc542	96.25	11.88	1	0.12	7.5	1.45	7.77*	2.73*	15.27	4.18*	1526.5	49.66	84.22	51.95	0	0.89*
IEc558	96.75	21.88	1	0.22	7.55	1	7.09	2.84*	14.64	3.84	1463.5	84.02	83.32	42.42	0	0.79*
IEc564	97.5	25.63	1	0.26	7.85	1.35	7.29	3.08*	15.14	4.43*	1513.5	113.28	91.8*	42.33	0	0.75*
IEc568	99	28.88	1	0.29	7.55	1.15	6.99	2.61*	14.54	3.76	1453.5	108.37	86.8	66.82*	0	0.72*
IEc574	97.5	0	1	0	7.65	0	7.17	0	14.82	0	1481.5	0	92.24*	0	0	1*
IEc607	93.63	0	1	0	8.35	0	6.59	0	14.94	0	1493.5	0	91.23*	0	0	1*
IEc672	100	56.25*	1	0.57*	9.55*	2.1*	7.74*	2.07	17.29*	4.17*	1728.5*	234.12*	90.57	64.24*	0	0.44
IEc675	97.5	41.88	1	0.42	8.35	2*	7.5	2.7*	15.85	4.7*	1585	196.93	90.01	62.95*	0	0.59
MDU1	99	65.63*	1	0.66*	9.4*	2.6*	7.73*	3.16*	17.13	5.76*	1713*	377.97*	92.11*	64.68*	0	0.35
Mean	98.24	42.92	1	0.43	8.39	1.51	7.48	2.29	15.87	3.8	1586.65	203.26	90.29	51.81	0	0.57

	SED	CD(0.05)	SED	CD(0.05)	SED	CD(0.05)	SED	CD(0.05)	SED	CD(0.05)	SED	CD(0.05)	SED	CD(0.05)	SED	CD(0.05)
Genotype	3.58	7.07	0.04	0.07	0.1	0.2	0.09	0.18	0.13	0.26	28.72	56.65	0.57	1.13	0.04	0.07
Treatment	1.55	3.06	0.02	0.03	0.04	0.09	0.04	0.08	0.06	0.11	12.44	24.53	0.25	0.49	0.02	0.03
Genotype x Treatment	8.78	17.31	0.09	0.17	0.25	0.5	0.23	0.45	0.32	0.63	70.36	138.77	1.4	2.76	0.09	0.17

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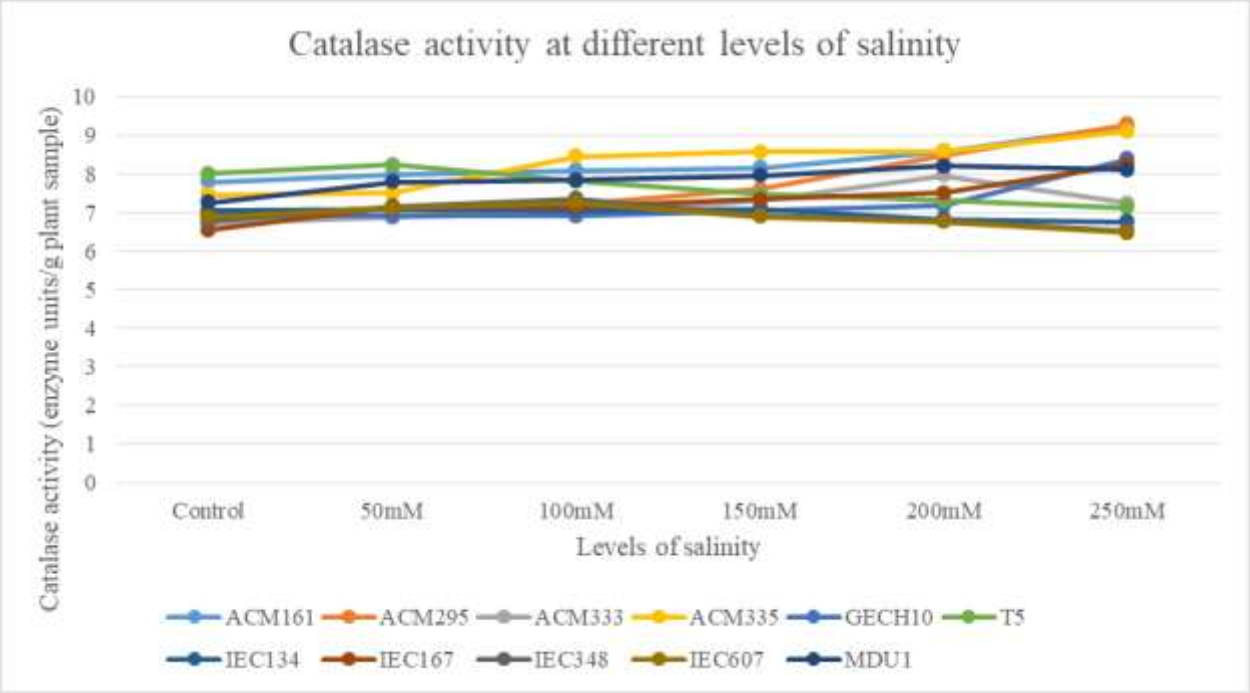

Fig. 1. Catalase activity at different levels of salinity

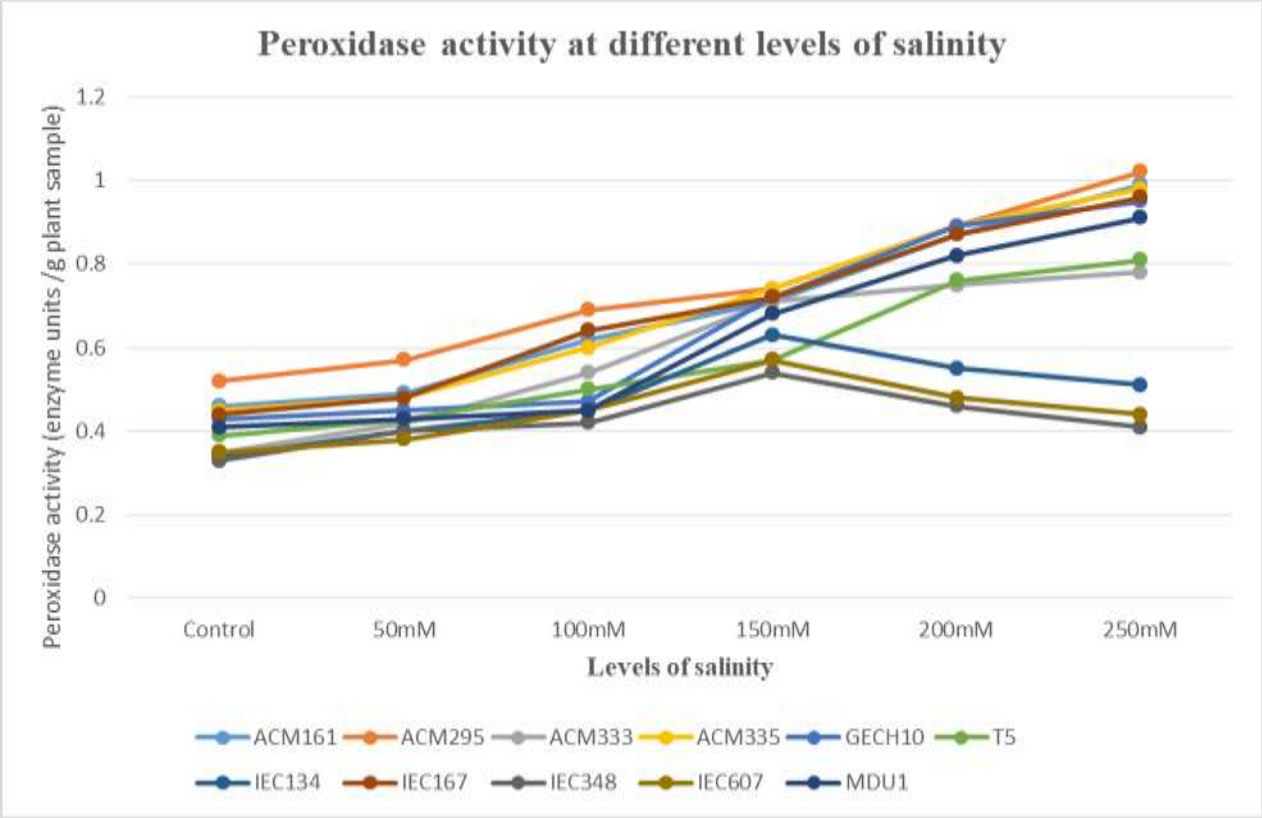

Fig. 2. Peroxidase activity at different levels of salinity

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