Screening of barnyard millet (*Echinochloa frumentacea*) germplasm for salinity tolerance

N. Arthi, B. Rajagopal, S. Geethanjali, A. Nirmalakumari and N. Senthil

ISSN: 0975-928X

Volume: 10

Number:2

EJPB (2019) 10(2):659-666

DOI:10.5958/0975-928X.2019.00083.8

Electronic Journal of Plant Breeding, 10 (2): 659-666 (Jun 2019) DOI: 10.5958/0975-928X.2019.00083.8
ISSN 0975-928X

Research Article

Screening of barnyard millet (*Echinochloa frumentacea*) germplasm for salinity tolerance

N. Arthi[1], B. Rajagopal[2], S. Geethanjali[3], A. Nirmalakumari[4] and N. Senthil[5*]
[1]Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India.
[2]Department of Fruit Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, India.
[3]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India.
[4]Center of Excellance in Millets, Tamil Nadu Agricultural University, Athiyanthal, Thiruvannamalai, India.
[5]Department of Plant Molecular Biology and Bioinformatics,Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India.
***E-Mail**: senthil_natesan@tnau.ac.in

(Received: 12 Jun 2019; Revised: 14 Jun 2019; Accepted: 14 Jun 2019)

Abstract
Indian Barnyard millet (*Echinochloa frumentacea*) is a climate resilient crop known for high degree of tolerance to salinity. The present study was undertaken to investigate the nature of genetic variation existing in the barnyard millet germplasm for salt tolerance. Different levels of salt stress (50 mM, 100 mM, 150 mM and 200 mM) were imposed on germination stage. The barnyard millet genotypes are able to tolerate upto 200 mM concentration during germination when compared to the tolerant rice genotype FL478. Among plant characters studied the germination percentage, root length, shoot length, fresh and dry weight, relative root length, relative shoot length, relative germination percentage, vigour index were affected by salinity. The vigour index and germination percentage during 200 mM stress helped to differentiate the salt tolerance genotypes *viz*., CO (KV) 2, MDU-1, PRJ1, TNEf 301, TNEf 204, TNEf 361, TNEf 364, VL 29 from other germplasm.

Keywords
Germplasm screening, Salinity tolerance, EST- SSR markers, Barnyard millet

Introduction

Among various abiotic stress factors affecting cereal food production, soil salinity is one of the most severe environmental stresses limiting the productivity of 20% of total cultivated land and 33% of irrigated lands worldwide (Shrivastava and Kumar, 2015). Hence, developing suitable crop plants for salinity tolerance is essential to feed the global population. Due to changing climatic conditions among the cereals the barnyard millet is one the candidate. Indian Barnyard millet *Echinochloa frumentacea* (2n=36, 54) is second most important millet (Padulosi *et al*. 2009). It is one of early domesticated millets in India. In India, barnyard millet is grown in mid hills of Himalayan region of Uttarkand in the North and popular millet in Tamil Nadu. Compared to other millets barnyard millet is suitable for growing in marginal environments like dry farming area, coastal area (Sood *et al*. 2015). It has been used for reclamation of sodicity, arsenic and cadmium affected soils (Sherif, 2007). It has high degree of tolerance against salinity, drought, water logging, heat, floods and has climate resilient capacity (Singh *et al.* 2010, Gupta *et al*. 2010). The present study is formulated to study the variation among germplasm lines for tolerance to salinity. Among the salt screening method the present study focus the barnyard millet germplasm for its salt tolerance mechanism at germination stage.

Materials and Methods

A total of 89 accessions of barnyard millet were obtained from the Centre of excellence in millets, Athiyandhal and two rice genotypes used as control were obtained from Paddy Breeding Station, TNAU, Coimbatore – 3 were used for salt tolerance screening . Ten seeds of each genotypes of barnyard millet were allowed to germinate in different concentration of NaCl solution (0 mM, 50 mM, 100 mM, 150mM and 200 mM) in petri-dishes. The plates were placed in laboratory condition of 25±2 °C. The experiment was conducted by factorial completely randomized design (Factorial CRD) with genotype as first factor and treatment as second factor with three replications. Seeds were considered to be germinated when both plumule and radicle were emerged (more than 2 mm). Physio-morphological parameters like root length, shoot length, fresh weight and dry weight were recorded. For dry weight, Germination percentage, Relative germination percentage, Relative root length,

Relative shoot length, Vigour index was calculated. Based on the dry weight, genotypes were classified into ten groups on scale 0-9 (Ashraf and Waheed, 1990). Germination percentage was calculated based on the number of seeds successfully germinated and vigor index was calculated based on the shoot length and root length measured on 10^{th}day of germination using the formula described by Bewley and Black (2012) and Abdul-Baki and Anderson (1973) respectively are Germination percentage = (No of seeds germinated/ No of seeds sown) X 100 and Vigour index = (Shoot length + Root length) X Germination percent.

Genomic DNA was isolated from 89 barnyard millet genotypes by CTAB method. The DNA was diluted to 25 ng/ µl concentration and genotyped with EST-SSR markers developed by Jayakodi *et al*. 2019, Manimekalai *et al.* 2018. PCR reaction was performed with reaction volume of 10 µl containing 2 µl (50ng) of template DNA, l µl of forward and reverse primer (10 µM) and 1X Master mix (SmARTPrime). PCR profile was set with initial denaturation for 5 min at 94ºC followed by 35 cycles of denaturation of 94ºC for 30 s, annealing for 30 s, extension of 72 ºC for 45 s and final extension of 72 ºC for 7 min. The PCR product was separated using 3 % Agarose gel electrophoresis at 120 V for 2 hrs.

Results and Discussion

EST-SSR markers developed from barnyard millet transcriptome were utilized for studying the genetic diversity in the barnyard millet germplasm. EST-SSR markers showed polymorphism among the germplasm shown in Fig 1. Totally 18 EST-SSR markers were used for screening. Out of 18, 3 markers BMESSR 2, BMESSR 8 and BMESSR 35 (16%) showed polymorphism in the germplasm of 89 genotypes with the allele size ranging from 100-200 bp with two alleles per loci. BMESSR 2 marker differentiated the genotypes DHBM 33, ELSG 35, ELSG104, TNEf 371, TNEf 374 with allele size of 150 bp. BMESSR 8 differentiated genotype CO (KV) 2 from other genotypes, BMESSR 35 differentiated genotypes BYNDL-1, TNEf 307, ELSG 73 showing polymorphism with allele size of 160bp.

Eighty nine barnyard millet accessions showing diversity in EST-SSR were taken for salt screening. Genotypes were initially screened with two different salt concentrations (Control, 150 mM and 300 mM). All the accessions (38 genotypes in Fig 1.) which had germination percentage of >80% in control and showing variation in germination at 150 mM were selected and screened with different NaCl concentrations of 0 mM, 50 mM, 100 mM, 150mM and 200 mM along with rice genotypes White Ponni (Salt susceptible) and FL478(Salt tolerant) as control (Fig 2.).Effect of different salt concentration on seed germination is shown in the Fig 3. There is negative correlation between the treatments and germination percentage. As the salt concentration increases, there is gradual decrease in germination of seeds indicating salt induced inhibition of seed germination. During germination, salinity creates osmotic stress or ion toxicity which reduces water absorption capacity of seeds and then affects the hydrolysis of seed reserves delaying or causing death of seeds (Begum *et al*. 2010). Similar results were reported in Pearl millet (Ali, S.A and Idris, A.Y. 2015), Foxtail millet (Ardie *et al*. 2015), Maize (Hoque *et al*. 2015), Finger millet (Rahman *et al*. 2014) and Broomcorn millet (Liu *et al*. 2015).

Comparing barnyard millet with rice, rice did not germinate in higher salt concentrations (150 mM and 200 mM) whereas, barnyard millet showed germination even in 150mM and 200mM indicating the inherent capacity of barnyard millet genotypes for salt tolerance. Among the barnyard millet genotypes there is variation in germination indicating the genetic potential of salt tolerant genotypes. Genotypes CO (KV)2, TNEf 204. PRJ1, TNEf 301, TNEf 361, TNEf 364, VL29, MDU-1 showed germination in 200 mM salt concentration. Germination percentage was significantly decreased ($P< 0.05$) from 80% in control to 6% in 200mM salt concentration. Effect of salinity on relative germination percentage of genotypes shown in Fig 4.

Seedling root length and shoot length were also affected by salinity. There is significant reduction in the shoot and root length of the barnyard millet genotypes with the increasing salt concentration. Shoot growth was much affected in barnyard millet than root length with relative shoot length of 0.85% and relative root length 2.0% at 150 mM concentration of salt. Similar results were reported in wheat (Gupta and Srivastava, 1989). Reduction in shoot length was due to reduced supply of metabolites and nutrients to the growing shoots asions compete with the nutrient absorption. Genotypes CO (KV) 2, ELB 114, TNEf 307, TNEf 204, DHBM 19-7, ELB 114, BYNDL-1, VL 254 showed increased shoot length (> 1.5 cm) under stressed condition. Root length was also affected by salt causing toxic effect on roots. Genotypes BYNDL-1, VL 254, PRJ1, TNEf 204, TNEf 301, TNEf 304 showed increased root length (> 1.5 cm). Vigour index decreased significantly with the increasing salinity level shown in the Fig 3. Reduction in vigour index of 99% was observed in 150 mM concentration. Salinity stress affects the

metabolisms in plants which ultimately lead to reduction in growth and productivity of plants (Shafi *et al*. 2009).Genotypes CO (KV)1, CO (KV)2, BYNDL-1, DHBM 99-6-1, TNEf307, PRJ1, VL29, TNEf 354 have vigour index more than 220 compared to other genotypes.

Salinity also affects fresh and dry weight of seedlings. Fresh weight and dry weight were decreased significantly ($P<0.05$) with increased salinity level. At 150 mM concentration of salt, relative fresh weight and relative dry weight were 26.58% and 75.04% respectively. Decrease in the weight of the seedlings is due reduction in water uptake by the seedlings due to ions present in the solution (El-Kader *et al*. 2006). Similar decrease in fresh and dry weight under salinity has been reported in Maize seedlings (Cha-Um and Kirdmane, 2009) where nutrient absorption, utilization and photosynthesis were affected by salt (Jafari *et al*. 2009). Based on relative total dry matter the genotypes were classified into ten groups on scale 0-9 (Ashraf and Waheed, 1990).At 150 mM concentration of NaCl, 6 genotypes were classified as tolerant, 19 genotypes classified as moderately tolerant, 12 genotypes were susceptible and 1 genotype was highly susceptible (Table 1). Salt damage index calculated based on the germination percentage was 84%, 96%, 99% and 99% at 50 mM, 100 mM, 150 mM and 200 mM NaCl, respectively. All the characters *viz*., germination percentage, root length, shoot length, relative root length, relative shoot length, fresh weight, dry weight, relative fresh weight, relative dry weight, vigour index were significantly affected in genotypes and genotype X treatment interaction at $p< 0.01$ with % CV ranging from 2.4% to 18.95% given in table 2.

The diverse barnyard millet genotypes showed wide variation in term of EST SSR profile and salt tolerance. The barnyard millet showed higher salt tolerance capacity upto 200 mM when compared to rice (upto 100 mM FL 478 highly tolerant cultivars). The highly tolerant barnyard millet germplasm CO (KV) 2, MDU-1, PRJ1, TNEf 301, TNEf 204, TNEf 361, TNEf 364, VL 29 will be used for the further salt screening using hydroponics to confirm the results obtained during the germination stage.

Acknowledgement
We thank Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore for providing lab facilities.

References

Abdul-Baki, A. A. and Anderson, J. D. 1973. Vigor determination in soybean seed by multiple criteria 1. *Crop sci.*, **13(6)**, 630-633.

Ali, S. A. and Idris, A. Y. 2015. Effect of Seed Size and Sowing Depth on Germination and Some Growth Parameters of Faba Bean (*Viciafaba* L.). *Agric. and Biol. Sci J*, **1(1)**: 1-5.

Ardie, S. W., Khumaida, N., Nur, A. and Fauziah, N. 2015. Early identification of salt tolerant foxtail millet (*Setaria italica* L. Beauv). *Procedia Food Science*, **3**: 303-312.

Ashraf, M. and Waheed, A. 1990. Screening of local/exotic accessions of lentil (Lens culinaris Medic.) for salt tolerance at two growth stages. *Plant and Soil*, **128(2)**, 167-176.

Begum, F., Ahmed, I., Nessa, A. and Sultana, W. 2010. The effect of salinity on seed quality of wheat. *J. of the Bangladesh Agric. Univ.*, **8(1)**, 19-22.

Bewley, J. D. and Black, M. 2012. Physiology and biochemistry of seeds in relation to germination: volume 2: viability, dormancy, and environmental control. Springer Science & Business Media. New York.

Cha-Um, S. and Kirdmanee, C. 2009. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pak. J. Bot.*, **41(1)**: 87-98.

El-Kader, A., Mohamedin, A. and Ahmed, M. 2006. Growth and yield of sunflower as affected by different salt affected soils. *Int. J. Agric. Biol.*, **8(5)**: 583-587.

Gupta, A., Joshi, D., Mahajan, V., & Gupta, H. 2010. Screening barnyard millet germplasm against grain smut (*Ustilago panici-frumentacei* Brefeld). *Plant Gen. Res.*, **8(1)**: 52-54.

Gupta, S. and Srivastava, J. 1989. Effect of salt stress on morpho-physiological parameters in wheat *(Triticum aestivum L.)*. No. REP-4695. CIMMYT.

Hoque, M. M. I., Jun, Z. and Guoying, W. 2015. Evaluation of salinity tolerance in maize (*Zea mays* L.) genotypes at seedling stage. *J. of BioSci. & Biotech.*, **4(1)**.

Jafari, M. H. S., Kafi, M. and Astaraie, A. 2009. Interactive effects of NaCl induced salinity, calcium and potassium on physiomorphological traits of sorghum (*Sorghum bicolor* L.). *Pakistan J. of Bot.*, **41(6)**: 3053-3063.

Jayakodi, M., Madheswaran, M., Adhimoolam, K., Perumal, S., Manickam, D., Kandasamy, Thangaraj, K., Tae-Jin- Yang. and Natesan, S. 2019. Transcriptomes of Indian barnyard millet

and barnyardgrass reveal putative genes involved in drought adaptation and micronutrient accumulation. *Acta Physiologiae Plantarum*, **41(5**): 66.

Liu, M., Qiao, Z., Zhang, S., Wang, Y. and Lu, P. 2015. Response of broomcorn millet (*Panicum miliaceum* L.) genotypes from semiarid regions of China to salt stress. *The Crop J.*, **3(1)** :57-66.

Manimekalai, M., Dhasarathan, M., Karthikeyan, A., Murukarthick, J., Renganathan, V., Thangaraj, K., Vellaikumar, S., Vanniarajan, C. and Senthil, N. 2018. Genetic diversity in the barnyard millet (*Echinochola frumentacea*) germplasms revealed by morphological raits and simple sequence repeat markers. *Curr. Plant Biol.*, **14**: 71-78.

Padulosi, S., Mal, B., Bala Ravi, S., Gowda, J., Gowda, K., Shanthakumar, G., Yenagi.N. and Dutta, M. 2009. Food security and climate change: role of plant genetic resources of minor millets. *Indian J. of Plant Gen. Res.*, **22**(1): 1.

Rahman, H., Jagadeeshselvam, N., Valarmathi, R., Sachin, B., Sasikala, R., Senthil, N., Sudhagar, D., Robin, S. and Muthurajan, R. 2014. Transcriptome analysis of salinity responsiveness in contrasting genotypes of finger millet (*Eleusine coracana* L.) through RNA-sequencing. *Plant mol. Biol.*, **85**(4-5), 485-503.

Shafi, M., Bakht, J., Hassan, M. J., Raziuddin, M. and Zhang, G. 2009. Effect of cadmium and salinity stresses on growth and antioxidant enzyme activities of wheat (Triticum aestivum L.). *Bull. of environ. Contam. and toxicol*., **82**(6), 772-776.

Sherif, A. and Ali, E. 2007. *Echinochloa colona* (L.) Link., A Promising species to cultivate Salt Affected Soils in Arid lands. *American-EuroAsian J. Agric. & Environ. Sci.*, **2**(6).

Shrivastava, P. and Kumar, R. 2015. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. of biol. sci.*, **22**(2), 123-13.

Singh, K., Mishra, H. and Saha, S. 2010. Moisture-dependent properties of barnyard millet grain and kernel. *J. of Food Eng.*, **96(**4), 598-606.

Sood, S., Khulbe, R., K. Gupta, A. K., Agrawal, P. K., Upadhyaya, H. D. and Bhatt, J. C. 2015. Barnyard millet–a potential food and feed crop of future. *Plant Breed.*, **134**(2): 135-147.

Table 1. Classification based on Relative total dry matter (Ashraf and Waheed, 1990)

Scale	RDM (%)	Tolerance group	Genotypes (150mM)
0 1 2 3	>120 110-120 100-110 90-100	Tolerant	CO (KV)1, CO (KV)2, K1, ELSG104, TNEf 364, MDU-1 ,
4 5	80-90 70-80	Moderately Tolerant	DHBM 33, VB-13-32, DHBM 19-7, VL 254, DHBM 99-6-1, TNEf 204, TNEf 301, RAU 3, PRJ1, VL29, ELB36, TNEf 354, TNEf 358, TNEf 359, TNEf 366, TNEf 380, TNEf 381, ACM-10-82, ACM-10- 161
6 7	60-70 50-60	Susceptible	BYNDL-1, DHBM 99-6, TNEf 307, RAU 11, ANURAG, ELB114, TNEf 313, TNEf 320, TNEf 351, TNEf 361, TNEf 370, TNEf 374,
8 9	40-50 < 40	Highly Susceptible	TNEf 356

Table 2. F-value and probability level of traits

	Genotype	Treatment	Genotype X Treatment	CV
Germination percentage	21.75**	1155.18**	6.68**	18.95%
Root length	363.25**	56938.06**	1639.38**	5.64%
Relative root length	2840.78**	47845.29**	2214.20**	5.98%
Shoot length	643.84**	134912.38**	401.44**	4.64%
Relative shoot length	1310.10**	106376.70**	1411.98**	4.73%
Fresh weight	1083.9**	73511**	515.78**	2.92%
Relative fresh weight	1054.82**	35005.14**	222.08**	3.32%
Dry weight	2759.81**	2658.60**	238.12**	2.67%
Relative dry weight	160.55**	202.16**	118.62**	2.72%
Vigour index	585.36**	183284**	445.26**	4.48%
Reduction over control	28.15**	1204.98**	27.79**	2.40%
Salt damage index	25.40**	1082.06**	25.13**	2.57%

* $p<0.05$ Significant; ** $p<0.01$ Highly significant

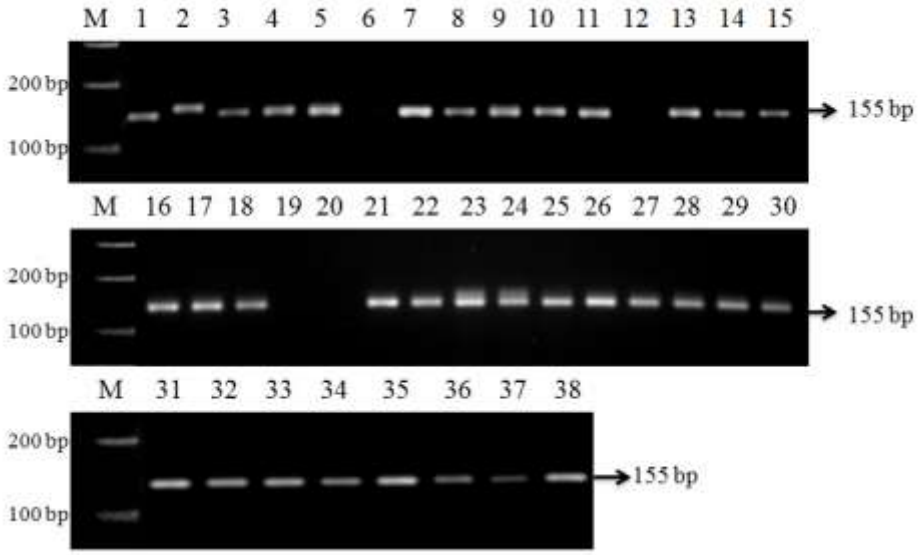

Fig 1. BMESSR 8 marker showing polymorphism in the germplasm.

(M: Marker 100bp, 1- CO (KV) 1, 2- CO(KV) 2, 3- DHBM 33, 4-BYNDL-1, 5-DHBM 99-6 , 6-VB-13-32 , 7-DHBM 19-7, 8- VL 254, 9- DHBM 99-6-1, 10- TNEf307, 11- TNEf204, 12- TNEf301, 13-RAU 11, 14- RAU 3, 15-ANURAG, 16-PRJ1, 17-K1, 18-VL29, 19-ELB36 , 20-ELB114, 21-ELSG104, 22-TNEf313, 23-TNEf320 , 24-TNEf351, 25-TNEf354, 26-TNEf356, 27-TNEf358, 28-TNEf359, 29-TNEf361, 30-TNEf364, 31- TNEf366 , 32-TNEf370, 33-TNEf374 , 34-TNEf380, 35-TNEf381, 36-MDU-1, 37-ACM-10-82 , 38-ACM-10-161)

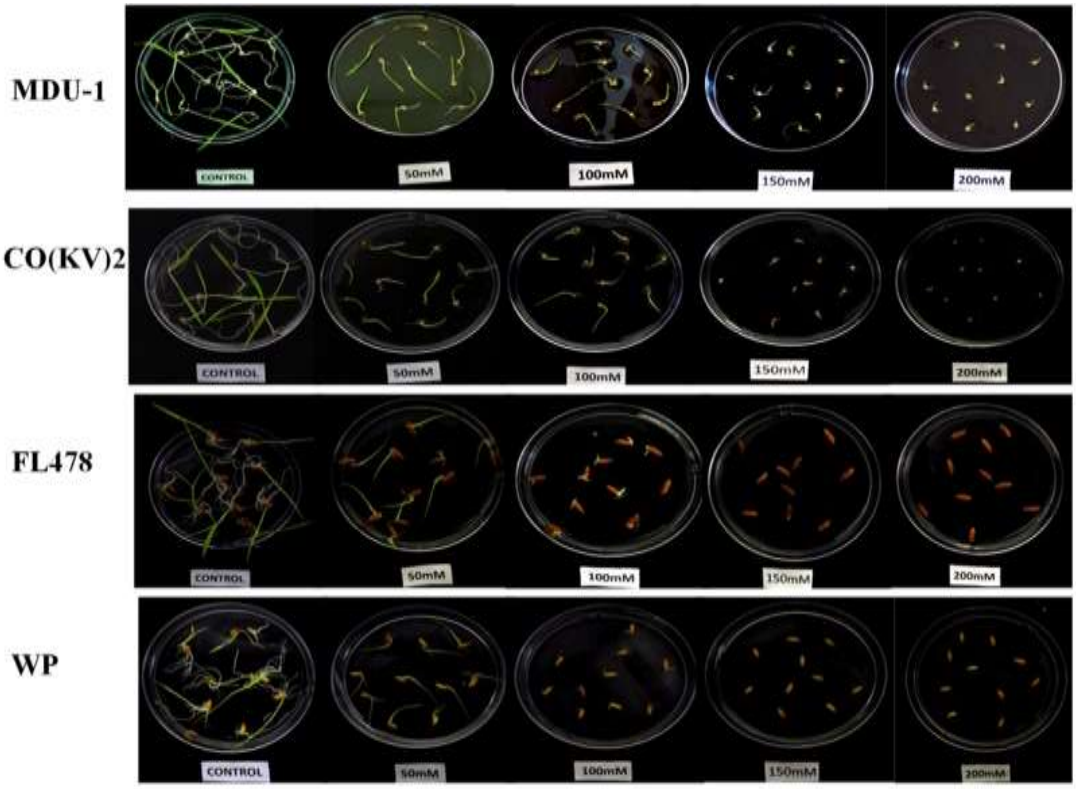

Fig 2. Screening of barnyard millet genotypes with different concentrations of NaCl (0mM, 50 mM, 100 mM, 150mM and 200 mM) along with rice.

Electronic Journal of Plant Breeding, 10 (2): 659-666 (Jun 2019) DOI: 10.5958/0975-928X.2019.00083.8
ISSN 0975-928X

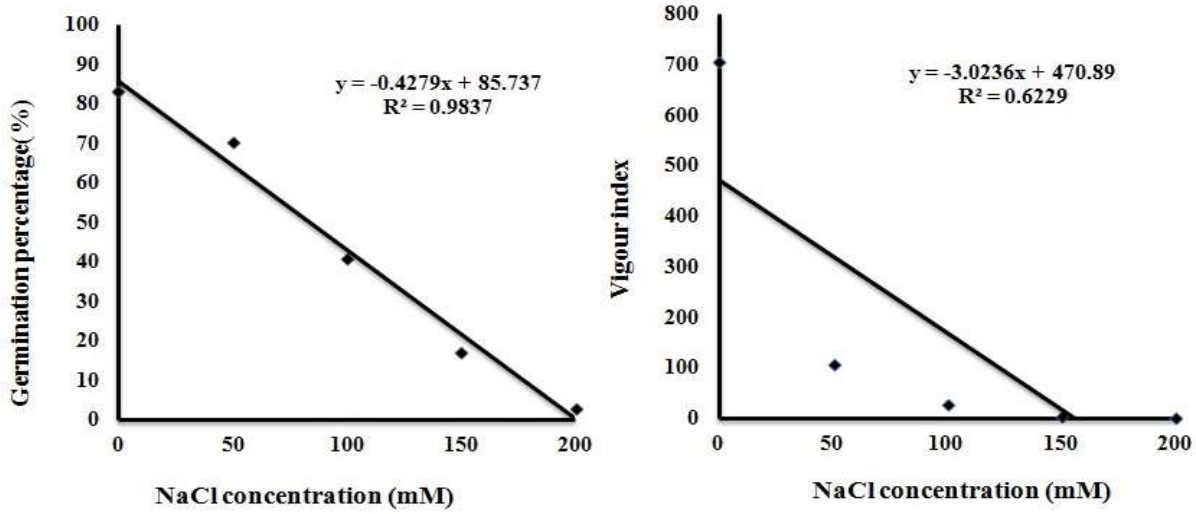

Fig 3. Effect of NaCl concentration on Germination percentage and Vigour index.

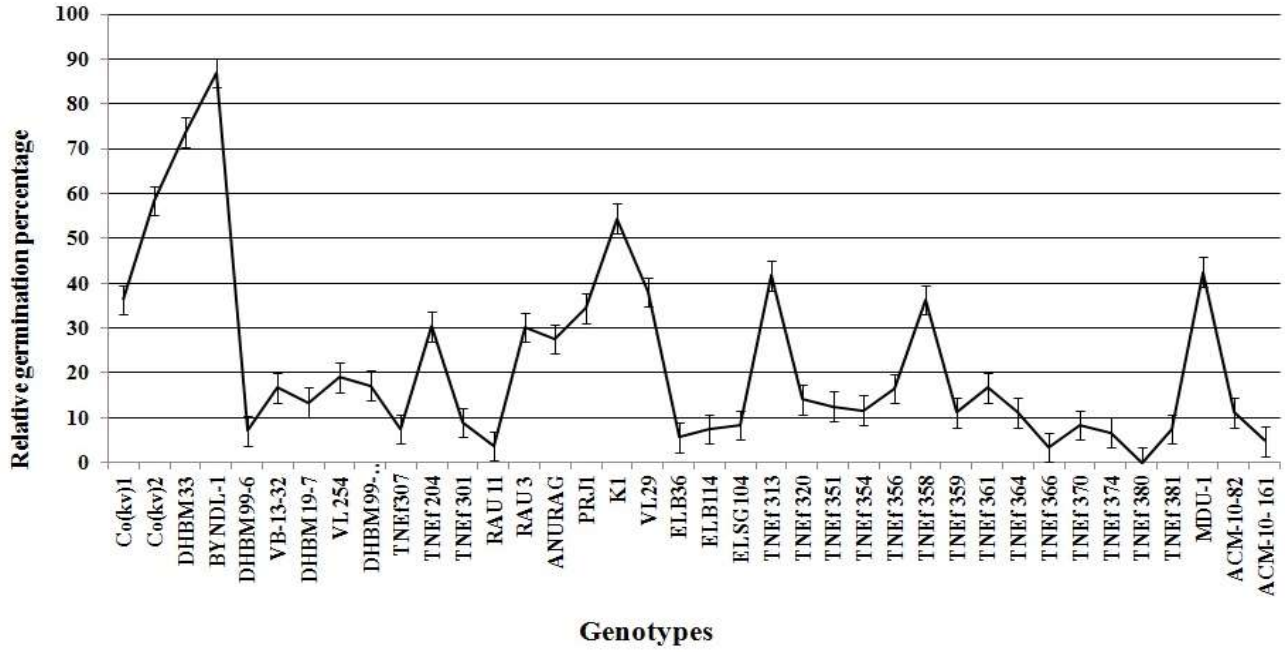

Fig 4. Effect of salinity on relative germination percentage of different genotypes at 150 mM NaCl.

https://ejplantbreeding.org