Mass screening for salinity tolerance in rice (O*ryza sativa*. L) genotypes at early seedling stage by hydroponics

M. Amaravel, S. Merina Prem Kumari, M. Arumugam Pillai, S. Saravanan, M. L. Mini and Asish K. Binodh

ISSN: 0975-928X
Volume: 10
Number:1

EJPB (2019) 10(1):137-142
DOI: 10.5958/0975-928X.2019.00016.4

https://ejplantbreeding.org

Electronic Journal of Plant Breeding, 10 (1): 137 – 142 (Mar 2019) DOI: 10.5958/0975-928X.2019.00016.4
ISSN 0975-928X

Research Article

Mass screening for salinity tolerance in rice (*Oryza sativa*. L) genotypes at early seedling stage by hydroponics

M. Amaravel*, S. Merina Prem Kumari, M. Arumugam Pillai, S. Saravanan, M. L. Mini and Asish K. Binodh
Agricultural College and Research Institute, Killikulam.
*PG scholar, Dept. of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam, Vallanadu – 628 252.
***E-Mail**: amaravelbscagri@gmail.com

(Received: 11 Jan 2019; Revised: 12 Feb 2019; Accepted: 13 Feb 2019)

Abstract
Salinity is a major abiotic factor that affects rice cultivation in the world along the coastal areas. The salt affected areas in India is estimated to be around 8 to 10 million hectares that causes significant reduction in rice growth and yield. In order to improve the rice cultivars for salt tolerance, selection of the suitable genotypes is necessitated. Hence, in this experiment ninety seven rice genotypes including traditional landraces were collected and subjected to screening under salt stress by hydroponics system on Yoshida nutrient solution of EC 8 $dSm^{-1}$and 12 dSm^{-1} with 4 replications. The genotypes were scored on 15th day after sowing based on standard evaluation scoring system of IRRI. Among the 97 rice genotypes scored, 5% were tolerant, 21% were moderately tolerant, 54% genotypes were susceptible and 18% were highly susceptible. The rice genotypes *viz*., Pokkali, FL478, Kuliyadichan, Gurukot and IR12L-107 revealed significant level of tolerance to salt stress. The rice genotypes, Pokkali and FL478 showed no symptoms at 8 dSm^{-1} and leaf rolling alone at 12dSm^{-1}. Kuliyadichan, Gurukot and IR12L-107 showed leaf rolling at 8dSm^{-1} and leaf rolling and leaf tip burning at 12dSm^{-1}. Screening of the rice genotypes for salinity tolerance only by visual symptoms is not reliable and hence the selected rice genotypes will further be characterized based on the morphological and biochemical parameters under salt stress in order to select elite salt tolerant rice genotypes for use in developing salinity tolerant somaclones by *in vitro* culture.

Key words
Salinity stress tolerance, rice genotypes, hydroponics, Yoshida nutrient solution, Standard evaluation scoring system of IRRI.

Introduction

Rice (*Oryza sativa L.*) is the most abundant crop in the world and is the staple food of almost half of the world's population (Mohammadi *et al*.,2012). Asia contributes 90% of area that comes under rice cultivation. India is the second largest producer next to China. Rice cultivation is majorly affected by biotic and abiotic stresses. Abiotic stresses caused by drought, heat and salinity cause substantial loss of yield in rice. Next to drought, salinity is the major soil problem in the rice cultivating areas. Salinity stress causes reduction in production and productivity of rice (Takehisa *et al*., 2004). In agriculture, salinity is caused by low rainfall, high evaporation, depleted ground water and accumulation of sea salt in ground water table. In India 8 to 10 million ha of land is affected by salinity. Coastal areas are commonly affected by salinity, that noticeably decreases the growth and yield of rice. Rice is moderately sensitive to salinity and is tolerant to salinity during germination, tillering and late maturity stages and susceptible during seedling and reproductive stages (Lafitte *et al.*,2004), that is directly proportion to reduction in the yield (Flowers, 2004 and Zeng *et al.*, 2001).

There are certain landraces by nature, are highly tolerant to salinity compared to tolerant varieties (Bonila *et al.*, 2002) and selection of salt tolerant genotype is required for improvement of rice to salinity problem in soil. Hydroponic screening method is normally used to identify the saline tolerant genotypes. In early seedling stage, rice is highly sensitive to salinity and this stage is used for identification of the salinity tolerant rice genotype (Gregario *et al.*, 1997;Heenan *et al.*,1988; Pearson *et al.*, 1966; Lutts *et al.*, 1995; Senanayake *et al.*, 2017). The observation of visual symptoms of salinity is most commonly followed for mass screening(Gregorio *et al.*, 1997). In early stage, shoot and root of rice seedlings were abridged due to salt accumulation (Krishnamoorthy *et al.*, 2016). Also deposition of salt in the plant leads to reduced photosynthetic leaf area (Munns, 2002).Phenotypic scoring for salinity in rice seedling stage was done by phenotypic observations like leaf rolling, leaf tip burning, leaf drying and ultimate plant dying (Bhowmik *et al.,* 2009;Rubel *et al.,* 2014; Islam and Karim, 2010; Ravikiran *et al.,* 2018 and Banumathy *et al.,* 2018). The salinity scoring is given by Standard evaluation system of IRRI, that is used to categorize the genotypes as highly tolerant, tolerant, moderately tolerant, susceptible, and highly susceptible, based on the visual symptoms as mentioned earlier. Considering the importance of salinity stress in rice, selection of appropriate genotypes for its tolerance to saline soil condition is performed by screening rice genotypes at seedling stage and classifying based on their tolerance to salinity. The screening of rice genotypes is done by phenotypic scoring and further grouping will be made by observing the morphological and biochemical parameters for its further use in developing soma clonal variants for salt stress tolerance.

Materials and Methods

Rice seeds of ninety seven genotypes collected from the Department of Plant Breeding and Genetics, AC&RI, Killikulam, were utilized for salinity tolerance screening. Seedlings were raised in hydroponic system using Yoshida nutrients solution with different EC levels of 8 dS/m and 12 dS/munder the lab conditions. Yoshida nutrient solution comprised of macronutrients and micronutrients and was prepared as suggested by Yoshida *et al*. (1976).

The screening of rice genotypes for salt tolerance at seedling stage was conducted in the hydroponics system by following the IRRI standard protocol (Gregorio *et al., 1997*). This is a rapid screening technique that uses plastic trays with tight-fitting thermocol support platform with holes for placing the seeds. Seeds were sown in the nylon mesh placed just below the thermocol platform. The trays were filled with sterile distilled water until the water level was 1mm above the mesh (Plate.1). Seeds of 97 genotypes were surface sterilized by soaking in 2% sodium hypochlorite solution for 2 to 3 minutes, followed by three rinses in sterile distilled water and placed in each compartment of the test tray and allowed for initial germination for 3 days in the dark. After 3 days, water was drained and the container was filled with Yoshida nutrient solution with different salt concentrations for EC values of 8 dS/m and 12 dS/m by adding sea salt. Non- salinized setup was maintained using Yoshida medium without adding sea salt. Every two days the volume of the nutrient solution was brought back to the level of the nylon mesh and pH adjusted to 5.0 and EC value was also maintained. The screening experiment was done in glass house conditions with day/night temperature of 32/22°C and relative humidity of at least 50% during the day. Phenotypic scoring and observations were recorded on the 12th day of salt treatment in 15 days old seedlings grown both in non-salinized and various salinized conditions. Based on the visual symptoms like reduced leaf area, lower leaves become whitish, leaf tip death and leaf rolling, the rice seedlings were categorized as Highly tolerant, Tolerant, Moderately tolerant, Sensitive and Highly sensitive. The standard saline tolerant genotypes, Pokkali and FL478 were included as check in the test for comparison. The visual scoring for salinity tolerance was done as shown in Table.1 with the score of 1 for tolerant and 9 for sensitive (Al-Amin *et al.,* 2013).

Results and Discussion

The rice genotypes were categorized based on the Standard Evaluation Scoring system (SES) of IRRI. All genotypes grew normally in the non-salinized condition. In the salinized treatments, the genotypes showed variations in visual symptoms with score 1 to score 9 (Table.1). On the 12th day of salt treatment in both EC values of 8 dS/m and 12 dS/m, saline tolerant standards, Pokkali and FL478 showed normal growth whereas the susceptible standard, ASD16 showed dried leaves. The symptoms of the remaining 94 genotypes were compared to Pokkali, FL478 and ASD16 to estimate the degree of tolerance to salinity (Fig.1).

Among the 97 genotypes including the standards, 11 genotypes showed tolerance, 2 genotypes were highly tolerant, 44 genotypes were moderately tolerant and 5 genotypes were highly susceptible under EC 8 dS/m. However, at EC 12 dSm^{-1} no genotype showed highly tolerance and only five genotypes, namely Kuliyadichan, Gurukot, FL478, Pokkali and IR12L107 showed tolerance to salinity. These five genotypes were selected for saline tolerant character. Twenty one genotypes were moderately tolerant, fifty four genotypes were susceptible and seventeen genotypes were highly susceptible. Highly susceptible genotypes are ACK 14004, ASD16, ADT37, CO39, CO 45, CO51, CR1009, Poonkar, Virendira, Chinapunchai, Kottra samba, Purpleputtu, Thondi, Kichali samba, Annada, IR12L104 and IR 11L 433. The standards, Pokkali and FL478 showed high salinity tolerance at EC 8dS/m and tolerance at EC 12dS/m. Among the eleven rice genotypes that were tolerant at EC 8 dS/m, only three genotypes namely Kuliyadichan, Gurukot and IRL107 showed tolerance at EC 12 dS/m and the remaining 8 rice genotypes showed moderate tolerance at EC 12 dS/m. The rice genotypes, Kuliyadichan, Gurukot and IR12L107 were tolerant at both EC values of 8 dS/m and 12 dS/m (Table.2).

Gregorio *et al.* (1997) and Mondal and Borromeo (2016), also identified saline tolerant rice genotypes based on the Standard Evaluation Scoring system (SEC) of IRRI in seedling stage under hydroponic system. The salinity responses in Thai indigenous rice genotypes were investigated and improved salt tolerant varieties were identified by screening in seedling stage (Ninsuwan *et al.,* 2013). The rice genotypes CSR-13 and CSR-30 showed high tolerance to salinity and were suggested for use as donor parents for salinity tolerance in backcross breeding and for allele mining for salinity tolerance gene (Chhavi *et al.,* 2018). The rice genotypes were divided into three clusters, susceptible, moderately tolerant and tolerant, based on the morphological and biochemical parameters at germination stage following IRRI standard protocol at 12 dS/m salinity level (Tahjib-Ul-Arif *et al*., 2018). Wangsawang *et al.* (2018), have chosen Ouukan383 and Kanniho as salinity-tolerant and salinity-sensitive cultivars, respectively, according to visual symptoms under salinity stress. The results in this study revealed that salinity inhibits the rice seedling growth and higher concentration is detrimental to the growth. Also it is concluded in the present investigation that the rice genotypes, Pokkali, FL478, Kuliyadichan, Gurukot, Surakuruvai, Chinapunchai, Anjali, IR12L107. TPS4, IRBL5M, ADT41, CO43 and ADT42 showed salinity threshold tolerance at EC 8dS/m and five genotypes, Pokkali, FL478, Kuliyadichan, Gurukot, and IR12L107 determined its supremacy with salinity threshold tolerance at EC 12dS/m and all these five genotypes were selected as the prominent genotypes for further morphological and biochemical screening, since salinity tolerance is contributed by various plant attributes and thereafter to exploit the candidate rice genotypes to develop somaclonal variations for salt tolerance by *in vitro* culture. Somaclonal variations arise by mutation due to recurrent subculture of proliferated callus for a long period of time and these variations improve the crops for biotic and abiotic stress tolerance (Karp, 1995 and Abdullah, 2013). Hence, somaclonal variation is an additional technique of genetic alteration in order to create variability (Paolis 2019). The nutrient composition of MS media for callus induction, proliferation and regeneration influence the cells for somaclonal variation (Remee *et al.,* 2017) and create desirable changes in crop plants. The future work on developing somaclonal variants for sustaining saline condition during the growth period and augmenting higher productivity is well predicted.

Reference

Abdullah, S., Khan, F. A., Iftikhar, R., Raza, M. M., Aslam, R., Hammad, G. and Ijaz, U. 2013.Detection of Somaclonal Variation in Micropropagated Plants of Sugarcane and SCMV Screening through ELISA. *Journal of Agricultural Science*, **5**(4).

Al-Amin, M., Islam, M.M., Begum, S.N., Alam, M.S., Moniruzzaman, M. and Patwary, M.A.K. 2013.Evaluation of rice germplasm under salt stress at the seedling stage through SSR markers. *Int. J. Agril. Res. Innov. & Tech*, **3**(1**),** 52-59.

Banumathy, S., Veni, K., Anandhababu, R., Arunachalam, P., Raveendran, M., &Vanniarajan, C. 2018. Character association and stress indices for yield components in Saltolintrogressed backcross inbred lines of rice (*Oryza sativa L.*). *Indian Journal of Agricultural Research*, **52**(1), 28-33.

Bhowmik, S.K., Titov, S., Islam, M.M., Siddika, A., Sultana, S. and Haque, M.S. 2009. Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. *African Journal of Biotechnology*, **8**(23).

Bonilla, P.S., Dvorak, J., Mackell, D., Deal, K. and Gregorio, G. 2002. RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa L.*) using recombinant inbred lines. *Philippine Agricultural Scientist (Philippines)*, **85** (1): 64-74

Chhavi, D.K., Kumar, D.G., Singh, A. and Singh A.K. 2018.Screening of Rice (*Oryzasativa*L.) for Salinity Tolerance at Vegetative Stage under Hydroponic Condition. *Int. J. Curr. Microbiol. App.Sci.* **7**(06): 1539-1542.

Flowers, T.J. 2004.Improving crop salt tolerance. *Journal of Experimental botany*, **55**(396), 307-319.

Gregorio, G.B., Senadhira, D. and Mendoza, R.D. 1997. Screening rice for salinity tolerance .*IRRI discussion paper series*.**22**: 1-30

Heenan, D.P., Lewin, L.G. and McCaffery, D.W. 1988. Salinity tolerance in rice varieties at different growth stages. *Australian Journal of Experimental Agriculture*, **28**(3): 343-349.

Islam, M M. and Karim, M.A. 2010.Evaluation of rice (*Oryza sativa L.*) genotypes at germination and early seedling stage for their tolerance to salinity. *The agriculturists*, **8**(2), 57-65.

Karp, A. 1995.Somaclonal variation as a tool for crop improvement. *Euphytica*, **85**(1-3), 295-302.

Krishnamurthy, S.L., Sharma, P.C., Sharma, S.K., Batra, V., Kumar, V. and Rao, L. V. S. 2016.Effect of salinity and use of stress indices of morphological and physiological traits at the seedling stage in rice.

Lafitte, H.R., Ismail, A., and Bennett, J. 2004. Abiotic stress tolerance in rice for Asia: progress and the future. In :*New directions for a diverse planet: proceedings for the 4th International Crop Science Congress, Brisbane, Australia* **26**: 1-17.

Lutts, S., Kinet, J.M., andBouharmont, J. 1995. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *Journal of Experimental Botany*, **46**(12), 1843-1852.

Mohammadi-Nejad, G., Singh, R.K., Arzani, A., Sabouri, H., Gregorio, G.B., and Rezaie, A.M. 2012.Evaluation of salinity tolerance in rice genotypes. *International Journal of Plant Production*, **4(3),** 199-208.

Mondal, S. and Borromeo, T.H. 2016.Screening of salinity tolerance of rice at early seedling stage. *Journal of Bioscience and Agriculture Research*, **10**(01), 843-847.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant, cell & environment*, **25**(2), 239-250.

Ninsuwan, U.,Pornsirikarn, P., Jundee, N., andPuima, N. 2013. Identification of salt tolerance in thai indigenous rice on the basis of Na/K ratio and salt stress response. *Asian J Plant Sci*, **12**(6-8), 247-251.

Paolis, A. D., Frugis, G., Giannino, D., Iannelli, M. A., Mele, G., Rugini, E. and Nicolodi, C. 2019. Plant Cellular and Molecular Biotechnology: Following Mariotti's Steps. *Plants*, **8**(1), 18.

Pearson, G.A., Ayers, A.D. and Eberhard, D.L. 1966. Relative salt tolerance of rice during germination and early seedling development. *Soil Science*, **102**(3), 151-156.

Ravikiran, K.T., Krishnamurthy, S.L., Warraich, A.S. and Sharma, P.C. 2018. Diversity and haplotypes of rice genotypes for seedling stage salinity tolerance analyzed through morpho-physiological and SSR markers. *Field Crops Research*, **220,** 10-18.

Remee R.N., Snigdha S.S., Islam M.M. and Mamun S.M.A. 2017.In vitro Morphogenesis in two indigenous rice cultivars through dehusked seed culture.*Khulna University Studies* Volume **14** (1 & 2): 15-26

Rubel, M.H., Hassan, L., Islam, M.M., Robin, A.H. K. and Alam, M. J. 2014.Evaluation of rice genotypes under salt stress at the seedling and reproductive stages using phenotypic and molecular markers. *Pak. J. Bot*, **46**(2), 423-432.

Senanayake, R,M,N,H,. Herath, H,M,V,G. and Wickramesinghe, I,P,. 2017. Phenotypic screening of rice varieties for tolerant to salt stress at seed germination, seedling and maturity stages. *Tropical agriculture research vol*. **29** (1): 90-100 (2017)

Tahjib-Ul-Arif, M., Sayed, M.A., Islam, M.M., Siddiqui, M.N., Begum, S.N. and Hossain, M.A. 2018.Screening of rice landraces (*Oryza sativa L.*) for seedling stage salinity tolerance using morpho-physiological and molecular markers. *ActaPhysiologiaePlantarum*, **40**(4), 70.

Takehisa, H., Shimodate, T., Fukuta, Y., Ueda, T., Yano, M., Yamaya, T. and Sato, T. 2004. Identification of quantitative trait loci for plant growth of rice in paddy field flooded with salt water. *Field Crops Research*, **89**(1), 85-95.

Wangsawang, T., Chuamnakthong, S., Kohnishi, E., Sripichitt, P., Sreewongchai, T. and Ueda, A. 2018. A salinity-tolerant japonica cultivar has Na+ exclusion mechanism at leaf sheaths through the function of a Na+ transporter Os HKT 1; 4 under salinity stress. *Journal of Agronomy and Crop Science*, **204**(3), 274-284.

Yoshida,S., D.A. Forno, J.H., Cock and K. A.Gomez. 1976. Laboratory Manual for Physiological studies of rice. 3rd edition.IRRI, Los Babos, Philippines.pp.62-66

Zeng, L., Shannon, M. C.and Lesch, S. M. 2001. Timing of salinity stress affects rice growth and yield components. *Agricultural Water Management*, **48**(3), 191-206.

Table1. Standard evaluation scoring system (SES) of IRRI for visual salt injury symptoms in rice seedlings

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish & rolled	Tolerant
5	Growth severely retarded most leaves are rolled & few elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry: some plants dying	Sensitive
9	Almost all plant dead or dying	Highly sensitive

Table2. Categorization of rice genotypes for salt tolerance using visual symptoms based on the Standard Evaluation Scoring system (SES) of IRRI

Salinity tolerance categories	Phenotypic scoring of rice genotypes at EC 8 dS/m	Phenotypic scoring of rice genotypes at EC 12 dS/m
Highly tolerant (score 1)	Pokkali, FL478	None
Tolerant (score 3)	Surskuravai, Chiunapunchai, Anjali, Karukot, Kuliyadichan, TPS4, IRBL5M, IR12L107, ADT42, ADT41, CO43,	Pokkali, FL478, Kuliyadichan, Karukot, IR12L107
Moderately tolerant (score 5)	Kattanur, Jaya, Veethirupa, Abya, Seeraga Samba, Kalakeri, Whiteponni5 -35, Maranellu, Kotrasamba, Dhalaheera, Purpleputtu, Meikuruvai, Krishnahemavathi, Kadaikannan, Sadabahar, IRBLTARPI, LFR293, PY5, ADT43, IR12L104, ASD18, CO49, MDU5, IR12L115, IR11L114, IR50, IR12L138, ADT46, BPT5204, IR12L110, IR12L214, ADT39, ADT48, ACK12001, IRDLL25CA, IR12L138, IR11L433, CO45, IR10A240, JGL1798, TN1, PY2, CO50,	ADT43, CO49, ADT42, IRT11L114, IR50, BPT5204, IR12L110, IR12L214, ADT48, CO50, Maranellu, Krishnahemavathi, Sadabahar, PY2, Kattanur, Kalinga, Veethirupa, Abya, Kalakeri, CO43, Whiteponni5-35
Susceptible (score 7)	Navara, Adukan, Athira, Poonkar, Virendira, Thondi, Vanapraba, Sabagidhan, Kichalisamba, Annada, Kayuma, Pusabasmati, Kuruvaikar, Anna4, Karsamba, Barathi, Jaisree Ram, Kavya, CR1009, Uma, Rajalaxsmi, Kullakar, Kattisamba, Mulampunchan, Whiteponni 5-36, Karudansamba, IR11L465, IRRI163, IR64, IR11L193, ASD16, IR08L150, BB8, IRRI134, IR72, CO39, CO51,	Navara, Adukan, Athira, Surakuruvai, Anjali, Dhalaheera, Meikuruvai, Vanapraba, Sabagidhan, Kadaikannan, Kayuma, Pusabasmati,Tn1, Kuruvaikar, Jaya, Anna4, Karsamba, Seeragasamba, Barathi, Jaisree Ram, Kavya, Uma, Rajalaxmi, Kullakar, Kattisamba, Mulampunchan, Whiteponni5-36, Karudan Samba, IRBLTARPI, LFR293, TPS4, PY5, ASD18, IRBL5M, MDU5, IR12L115, IR11L465, IRRI163, IR64, IR12L138, ADT46, IR11L193, ADT39, ACK12001, IRDL25CA, IR08L150, BB8, IRRI134, IR72, IR12L138, ADT41, IR10A240, JGL1798
Highly susceptible (score 9)	ACK14004, ADT37, Kalinga, Navara,	IR12L104, ACK14004, ASD16, ADT37, CO39, IR11L433, CO45, CO51, Poonkar, Virendira, Chinapunchai, Kotrasamba, Purpleputtu, Thondi, Kichalisamba, Annada, Navara, CR1009,

Electronic Journal of Plant Breeding, 10 (1): 137 – 142 (Mar 2019)
ISSN 0975-928X
DOI: 10.5958/0975-928X.2019.00016.4

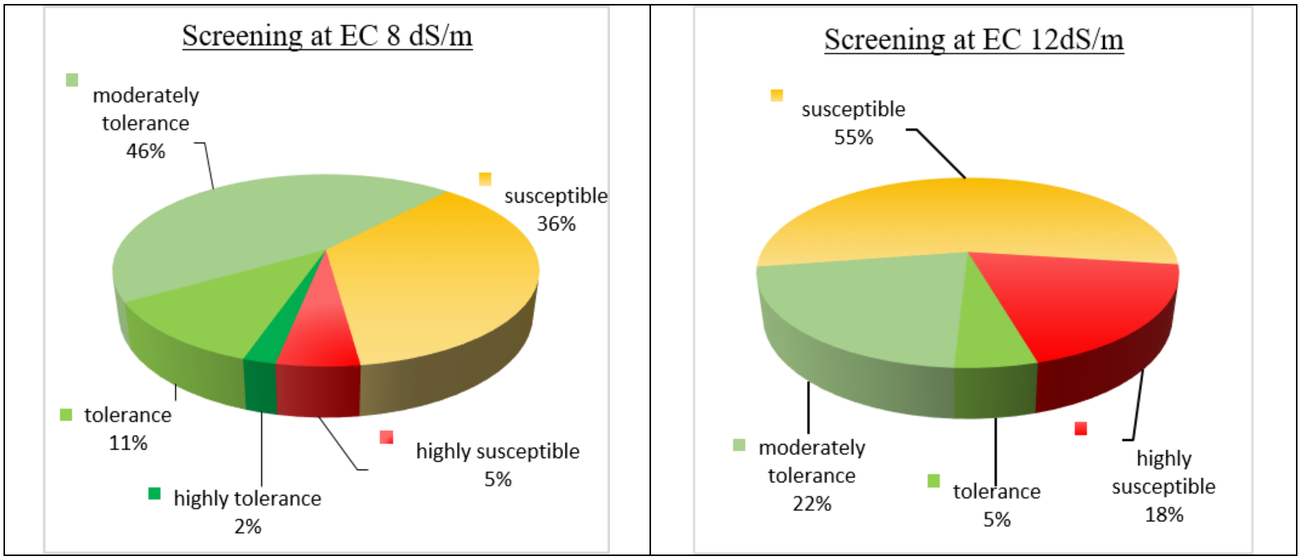

Fig.1. Percentage of rice genotypes showing differing salinity tolerance at EC 8 dS/m and 12 dS/m

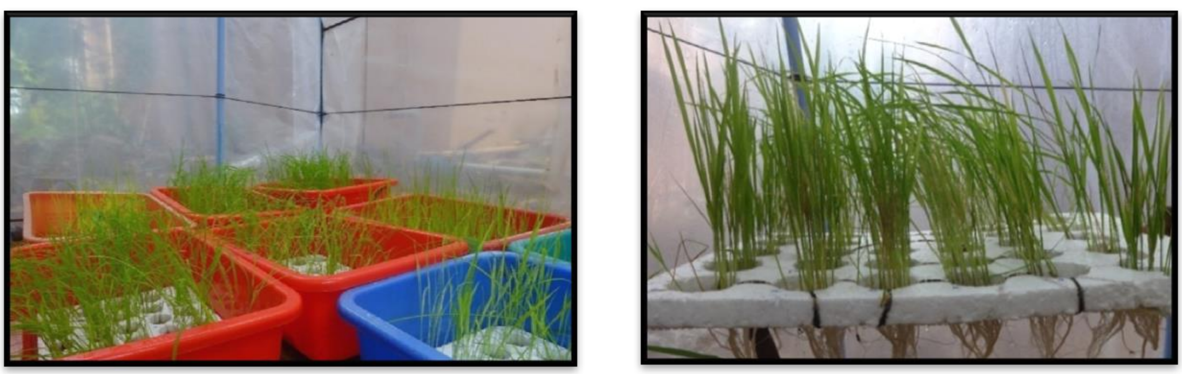

Plate 1. Experimental set up for screening 97 rice genotypes under hydroponics

INDIAN SOCIETY OF PLANT BREEDERS

ESTD 1995

https://ejplantbreeding.org