



## Research Article

# A protocol for rapid screening of rice lines for seedling stage salinity tolerance

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(Received: 10 Jul 2018; Revised: 10 Sep 2018; Accepted: 21 Sep 2018)

### Abstract

Identification of tolerant genotypes based on phenotypic screening is one of the foremost requirements for studying genetics and molecular mapping of salinity tolerance trait in rice. For this to be accomplished efficiently, development of an efficient, reproducible, simple mass-screening protocol is imperative. The present study involves the development and validation of a modified, rapid, and reproducible protocol using sterilized silica sand as a base for growing the rice seedlings in the nutrient media replacing the generally deployed hydroponics based screening protocol where styrofoam is used as a base for seedling growth. Pre-germinated seeds were sown on silica sand filled plastic trays in three replications and modified Yoshida nutrient solution was periodically added for the first 21 days and appropriate quantities of NaCl was added to salinize the medium in different concentrations (60mM, 80mM, 100mM and 120mM). After a week of exposure to salinity, the seedlings were scored phenotypically and parameters associated with tolerance were recorded. The protocol was initially tested with known saline tolerant and sensitive rice genotypes and further validated in a set of introgressed Improved Samba Mahsuri breeding lines, possessing the QTL *Saltol*, which confers seedling stage tolerance to salinity. The modified protocol was able to clearly discriminate salt tolerant rice lines from sensitive ones and was superior to the routinely adopted hydroponics based protocol in terms of being more robust, reproducible, requires less nutrient solution and facilitating easy handling in screening of genotypes.

### Keywords

Silica sand, Yoshida media, NaCl, SES scale, Phenotypic screening

### Introduction

Major abiotic stresses that limit rice growth include salinity, temperature, drought, submergence and cold etc. (Shankar *et al* 2011). Salinity is the second most important constraint to sustainable agricultural production worldwide, after drought limiting the increase in rice productivity significantly (Gregorio *et al* 1997, Nejad *et al* 2008, Lutts *et al* 1995). Globally, salt affected area accounts for about 800 million ha of land which covers more than 6% of total global area. Of the 1500 million ha of dry land farming, 32 million ha (2%) are accounted to be salt effected at varying degrees by secondary salinity and to date more than 45 million ha (20%) of the total irrigated land (accounting about 230 million ha) is saline. (FAO 2008).

In India, about 8.4 million ha of rice growing area is affected by salinity (Tyagi *et al* 1998). Salt affected area is increasing at a significant rate across the country due to low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, over-exploitation of underground water and poor cultural practices. It

has been estimated that more than 50% of the arable land would be salinized by the year 2050 (Zeng *et al* 2000, Jamil *et al* 2011).

Salinity stress is complex as the sensitivity varies at various stages of plant growth. Among the cereals, rice is most sensitive to salinity during early seedling stage (2–3 leaf stage), but the crop comparatively gains tolerance during vegetative growth stage. However, it becomes sensitive during reproductive stage and again gains tolerance at later stages of maturity ( Rao *et al* 2008, Lafitte *et al* 2006 and Lutts *et al* 1995). At seedling stage, rice is ineffective in controlling influx of Na<sup>+</sup> ions across roots leading to rapid accumulation of toxic concentration in shoots (Yeo *et al* 1990; Roshandel and Flowers 2009; Singh and Flowers 2011), hence reducing the growth and survival of the seedlings, effecting the photosynthetic rate leading to yield reduction along with shrinkage in caloric and nutritional potential of agricultural products (Yokoi *et al* 2002) causing leaf injury or plant death exceeding capacity of salt compartmentalization in cytoplasm (Munns *et*

al 2006). Seedling stage salinity is one of the major constraints in many rice growing countries

including India. Salinity tolerance trait in rice is within the sensitive division from 0 dS m<sup>-1</sup> to 8 dS m<sup>-1</sup> (Mass 1986). Seedlings die at salt level of 10 dSm<sup>-1</sup> or more (Munns *et al* 2006), and yield loss can be as high as 90% at 3.5 dSm<sup>-1</sup> salt stress during the reproductive stage (Asch *et al* 2001). Salinity tolerance is a complex trait, and phenotypic responses of plants to salinity stress are highly affected by the environment (Gregorio and Senadhira *et al* 2002; Koyama *et al* 2001; Flowers 2004). The easiest and eco friendly way to address the problem is through development of salt tolerant varieties (Quijano-Guerta and Kirk 2002). The success of breeding programs depends on availability of a robust, reproducible screening protocol for identification of tolerant rice lines (Divyapriya *et al* 2017). Screening at field level has proved to be difficult due to soil heterogeneity, climatic factors and other environmental factors which may influence the physiological processes. Hence, screening under laboratory conditions is considered to be advantageous over field screening, particularly with respect to seedling stage salinity tolerance (Gregorio *et al* 1997).

Screening for salinity at seedling stage offers the possibility of pre-selection of breeding lines progeny and cultivars before field evaluation. Screening of salinity tolerance in breeding programs has been suggested in two steps: (i) screening seedling salinity tolerance for large segregating populations (early generation screening) under controlled conditions; and (ii) testing salinity tolerance of promising lines from the first round screening at reproductive stages under the field conditions as it helps in culling the number of genotypes for later stages of screening (Li *et al* 2011). In general, screening for seedling stage salinity tolerance is done through a protocol based on a glasshouse hydroponics test. In this protocol salt is added to the nutrient hydroponic solution in which the seedlings are grown in pre-determined quantities and seedlings are grown in this solution for a particular period (Bado *et al* 2016). However, apart from its numerous advantages being the most used protocol, it has its own disadvantages in terms of limitations in screening large number of samples, algal contamination of the hydroponic solution and differential growth of roots in hydroponic solution as compared to what is observed in soil. In the present study, we have attempted to develop a hydroponics-free protocol for rapid screening of seedlings for salinity tolerance, utilizing silica sand

base (Rajasree Corporation Ltd.) as a solid platform instead of styrofoam.

### Materials and Methods

Twelve pre-breeding lines possessing major QTL associated with seedling stage salinity tolerance, *viz.*, *Saltol* in the genetic background of improved Samba Mahsuri (ISM) were used as test material in the present experimental set up along with the tolerant and susceptible checks, *i.e.* FL478 (tolerant) and Improved Samba Mahsuri (Susceptible) respectively.

The equipment used in the experiment includes test trays, support media/base, stock solution storage containers, pH meter and EC meter. Plastic trays (Fig 1A) with outside dimensions of 40 X 20cm were used as test trays Silica sand (16 kg in a single tray) was used as the basal support media instead of Styrofoam base, which is generally used for salinity tolerance screening (Gregorio *et al.* 1997). Perforated PVC tubes with dimension 4 X 5mm were placed on each corner of the tray (Fig 1B) before filling up the tray with basal media (Silica sand). These tubes assisted in real-time monitoring of level of media in the tray and need-based supplementation of aqueous media (Yoshida media, Yoshida *et al* 1972). The silica sand of particle size 1mm and 2mm were used to serve as a solid support for the basal media.

Three fourth of the plastic trays were initially filled with approximately 16-17kg silica sand (Rajasree Corporation Ltd.) in each tray with perforated tubes placed on corners of the tray (Fig 1C). The seeds from all the twelve breeding lines along with the checks were selected and germinated in Petri plates for 3-4 days and were transferred to the silica sand containing trays. Screening was performed in controlled environmental condition in a glass house with 27-30°C/21-25°C of day/night temperature, relative humidity of 70% under natural daylight. The seedlings were supplemented with half strength Yoshida media for one week. After a week, seedlings were supplemented with full strength Yoshida's solution through perforated tubes placed on each corner of the tray, ensuring adequate supply of culture media (*i.e.* standard Yoshida medium without any extra salt) (Table 1). The P<sup>H</sup> of the media was regularly monitored and regular supply of the media may be necessary as there will be some evaporation of the media (Table 1). After 21 days, the seedlings were monitored carefully (Fig. 2B) and salt stress was imposed on healthy seedlings of the test entries and checks at various concentrations (*viz.*, 60mM, 80mM, 100mM, 120mM NaCl and control, *i.e.* no NaCl).

Visual scoring was done based on the IRRI SES score (IRRI, 2013) once in 7 day, 10 day and 15 days interval with final scoring observed when 90% leaves of the susceptible check (i.e. Improved Samba Mahsuri) were damaged.

### Results and Discussion

In the present experiment, silica sand of two different sizes (i.e., 1mm and 2mm) was used to grow rice seedlings. We have observed that the plant growth was not very good in 1mm size silica sand base as compared to 2 mm base, with the silica sand base of 1mm size observed to be compacted leading to lesser aeration for the seedlings and that of 2mm size base to be coarse, helping in proper aeration required for seedlings and roots. Based on these observations, 2mm size silica sand was chosen as favourable platform for the experimental set up.

Initially, the test entries along with the controls (i.e. the susceptible check, i.e. Improved Samba Mahsuri) were grown in silica sand as described in the materials and methods without application of any NaCl to ensure the vigorous growth of the seedlings and the seedlings were observed to be growing robustly in the silica sand base. When the seedlings of both the tolerant and susceptible rice genotypes were subjected to salt stress at different concentrations, no difference in reduction of plant growth was observed in 40 mM and 60mM NaCl concentrations and all the 12 lines analyzed along with checks were observed to be healthy with no symptoms of salt exposure. On the other hand, the application of 80mM and 100 mM NaCl caused some differences in the plant growth two weeks after exposure. Clear differences were observed in plant growth, when seedlings were exposed to 120 mM NaCl concentration for a period of two weeks indicating that a 120 mM NaCl concentration was the ideal one to clearly distinguish the lines based on their tolerance/susceptibility (Figure 3)

Using the protocol, when the 12 lines of Improved Samba Mahsuri (ISM) introgressed with *Saltol*, were screened at 120 mM NaCl, they were observed to be ranging from 1 (highly tolerant) to 3 (moderately tolerant) in terms of IRRI-SES scores, with three out of 12 lines noted to be highly tolerant (ISM-18-217-18-141, ISM-110-39-23-147, ISM-105-34-6-182) five as tolerant (ISM-19-15-178-142, ISM-102-179-13-179, ISM-10-12-67-157, ISM-84-7-12-47 and ISM-100-17-66-98) and four as moderately tolerant (ISM-9-5-50-160, ISM-22-102-179-143, ISM-3-181-90-196, ISM-82-79-45-76; Table 1). We have also observed clear morphological differences for both shoot and root

length between susceptible and tolerant parents as well as in the 12 *Saltol* containing genotypes (Fig 2B & D). Based on the results obtained, it can be concluded that silica sand could be used as base for salinity screening replacing the hydroponics, thus increasing the accuracy and throughput, which is lacking in hydroponic experiments.

Salinity is second most important abiotic stress and is a major constraint to rice productivity after drought (Gregorio *et al* 1997). Rice is a glycophyte by nature and is susceptible to salinity showing vivid response against the detrimental effects of increased salt accumulation (Ghosh *et al* 2016). Rice is more sensitive to salinity at seedling stage and reproductive stage, but the threshold sensitivity differs among the genotypes based on the concentration of NaCl and duration of exposure (Flowers and Yeo 1981, Pearson 1959). Salinity can limit growth and plant yield by reducing osmotic potential and creating ion toxicity. Seedling stage salinity exposure induces premature senescence of leaves (Sahu and Mishra 1987, Lutts *et al* 1996). Reproductive stage is also a very sensitive stage and the effect of salinity on yield is more pronounced at this stage (Pearson 1959). It has been estimated that yield loss due to salinity is to an extent of 30-50%, particular in endemic areas (Linghe *et al* 2000). Fortunately, rice crop has significant genetic variability with respect to tolerance to salinity and hence breeding for salinity tolerance is one of the key objectives of many rice breeding programs across the world. (Ashraf and LinWu 2011, Singh *et al* 2010). Even though protocols, based on hydroponics are available for screening of rice genotypes at seedling stage, they have many lacunae (Faiyue *et al* 2012). The present study was therefore carried out with an objective to develop a rapid, reliable and cheap protocol for mass screening of rice seedlings against salinity stress.

The most common approach to cope up with salinity constraint is through development of salinity tolerant varieties either through conventional or molecular breeding strategies. This requires availability of a rapid and reliable screening technique (Gregario *et al* 1997). However, efficient and rapid screening method is required to hasten the breeding program. Presently, phenotypic screening of rice genotypes is performed using hydroponics where a styrofoam base is used. This methodology has few limitations like algal contamination of the liquid growth medium, need for frequent replenishing of the growth media, sub-optimal growth of the seedlings in the culture media and requirement of controlled conditions for growing the seedlings. Considering



these points, we attempted to develop a rapid and reliable screening protocol for screening rice genotypes at seedling stage for salinity tolerance through the present study. We replaced silica sand as the base for growing rice seedlings, instead of a hydroponic solution in Styrofoam base. We have observed healthy growth of seedlings on silica sand base (2mm) and after treatment of seedlings with different concentrations of NaCl (60, 80,100 and 120mM). At 120mM, we are clearly able to identify susceptible genotypes from the tolerant ones based on the visual symptoms using SES scale (Table 2) (IRRI 2013). Based on the observations, silica sand based salinity screening method can be considered as rapid and reliable screening method for identification of salt tolerant genotypes which can be used for mapping studies and for large scale screening in the breeding programs. Another interesting feature noticed in the present study is that the 12 breeding lines of Improved Samba Mahsuri were showing differential levels of tolerance to salinity. This could be because, the extent of introgression of donor parent genome in these lines were ranging from 2 Mb (in the moderately tolerant lines) to 8 Mb (in most of the tolerant or highly tolerant lines; data not shown). It has been earlier reported that *Saltol* region consists of two distinct candidate genes, viz., one encoding a *SKC* gene and another encoding a *HKT1* gene (Cotsaftis *et al* 2012), with the region spanning several Mbs. This indicates that efforts for breeding *Saltol* in elite rice varieties should focus on introgression of the entire *Saltol* region using flanking markers instead of using a single marker specific for each of the candidate genomic regions.

The current experimental setup reduces the time, less expensive and moreover supports the breeding programs on large scale. Styrofoam bases are difficult to maintain and may have more chances for contamination with algae and other microbes. The solid support also accommodates more number of seedlings comparatively than Styrofoam. Moreover, the root parameters interpretation in the hydroponics is not similar to that of the field conditions as the nutrient media provision in the hydroponics set up leads to the rapid growth of the roots and also leads to false interpretation due to clogging of roots of two or more genotypes. In silica sand based setup, the interpretations will almost be similar to that of the soil conditions as silica sand is also a basal sand and no clogging of roots was observed between the genotypes.

Based on the observations from the present study we can conclude that silica base screening method was reliable, time saving and easy handling and

rapid method comparative to Styrofoam based hydroponic screening method for screening seedling stage salinity tolerance in rice genotypes. This methodology can be considered as most reliable method for performing screening with large scale populations in the current breeding programs so that we can identify tolerant genotypes and used as donors in the current breeding program. Moreover, this method is also useful for diversity analysis studies and also for mapping population screening.

#### Acknowledgements

Our sincere acknowledgements for the financial assistance provided by the ICAR-Indian Agricultural Research Institute with award Number: (F.No.F.3/CRPMB/Gen/2015-16/1714) and also to DST INSPIRE with award no DST/INSPIRE Fellowship/2013/303 for necessary support. We would also like to acknowledge our Director, ICAR-Indian Institute of Rice Research for contributing the required lab facilities

#### References

- Asch, F. Marco, C.S. Wopereis. 2001. Responses of field-grown irrigated rice cultivars to varying levels flood water salinity in a semi-arid environment. *Field Crops Research* **70**: 127-137.
- Ashraf, M. Lin Wu. 2011. Breeding for Salinity Tolerance in Plants. *Current review in plant sciences*.**13** (1): 17-42.
- Cotsaftis, O.D. Plett, N. Shirley, M. Tester, M. Hormova. 2012. A two-staged model of Na<sup>+</sup> exclusion in rice explained by 3D modeling of HKT transporter and alternative splicing. *PLoS One*. **7** (7): 39865
- FAO 2008. Land and Plant Nutrition Management Service. Retrieved: <http://www.fao.org/ag/agll/spush/>
- Flowers, T.J. Yeo, A.R. 1981. Variability in the resistance of Sodium chloride salinity within rice (*Oryza sativa* L.) varieties. *The New Phytologist* **88**:363-373.
- Flowers, T.J. 2004. Improving crop salt tolerance. *Journal of Experimental Botany*. **55**: 307-319
- Gregorio, G.B. Senadhira, D. Mendoza, R.D. 1997. Screening rice for salinity tolerance, IRRI discussion paper series no. 22.
- Gregorio, G.B. Senadhira, D. Mendoza, R.D. Manigbas, N.L. Roxas, J.P. Cuerta, C.Q. 2002. Progress in breeding for salinity tolerance and associated abiotic stresses in rice. *Field Crops*



- Res.* **76**:91–101. Doi: 10.1016/S0378-4290(02)00031.
- [http://www.knowledgebank.irri.org/ricebreedingcourse/documents/Screening\\_manual.pdf](http://www.knowledgebank.irri.org/ricebreedingcourse/documents/Screening_manual.pdf), 2013.
- Jamil, A. Riaz, S. Ashraf, M. Foolad. M.R. 2011. Gene expression profiling of plants under salt stress. *Crit. Rev. Plant Sci.* **30** (5): 435–458.
- Koyama, M.L. Levesley, A. Koebner, R. Flowers, T.J. Yeo, A.R. 2001. Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiol.* **125**:406–422. Doi: 10.1104/pp.125.1.406.
- Lafitte, H.R. Vijayakumar, C.H.M. Gao, Y.M. Shi, Y. Xu, J.L. Fu, B.Y. Yu, S.B. Ali, A.J. Domingo, J. Maghirang, R. Torres, R. Mackill, D. Li, Z.K. 2006. Improvement of rice drought tolerance through backcross breeding: evaluation of donors and results from drought nurseries. *Field Crops Research.* **97**: 77–86
- Li, H.W. Zang, B.S. Deng, X.W. Wang, X.P. 2011. Overexpression of the trehalose-6-phosphate synthase gene *OsTPS1* enhances abiotic stress tolerance in rice *Planta*, **234** (5):1007-1018.
- Linghe, Z. Shannon, M.C. Zeng, L.H. 2000. Salinity effects on seedling growth and yield components of rice. *Crop Science.* **40** (4): 996-1003.
- Lutts, S. Kinet, J.M. Bouharmont, J. 1995. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *J Exp Bot.* **46** (12): 1843-1852
- Lutts, S. Kinet, J.M. Bouharmont, J. 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of Botany.* **78** :389- 398.
- Mass, E.V. 1986. Salt tolerance of plants. *Appl Agric Res.* **1**: 12–26
- Munns, R. James, R. Lauchli, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany.* **57**: 1025–1043.
- Pearson, G.A. 1959. Factors influencing salinity of submerged soils and growth of Caloro rice. *Soil Science.* **87**: 198 -207.
- Quijano-Guerta, C. Kirk, G.J.D. 2002. Tolerance of rice Germplasm to salinity and other chemical stresses in tidal wetlands. *Field Crop Res.* **76**: 111–121. Doi:10.1016/S0378-4290 (02)00033-3.
- Rakesh Kumar Singh, Edilberto Redoña, Luzminda Refuerzo. 2010. Varietal Improvement for Abiotic Stress Tolerance in Crop Plants: Special Reference to Salinity in Rice. Chapter 18, DOI: 10.1007/978-90-481-3112-9\_18.
- Rao, S.P. Mishra, B. Gupta, S.R. Rathore, A. 2008. Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. *Plant Breeding.* **127**: 256–261.
- Roshandel, P. Flowers, T. 2009. The ionic effects of NaCl on physiology and gene expression in rice genotypes differing in salt tolerance. *Plant and Soil* **315**: 135–147.
- Sahu, A.C. Mishra, D. 1987. Changes in some enzyme activities during excised rice leaf senescence under NaCl stress. *Biochemie und Physiologie der Pflanzen.* **182**: 501-505.
- [Shanker AK, Venkateswarlu B \(2011\) Abiotic stress in plants – mechanisms and adaptations. InTech, Rijeka, p ix](#)
- Singh, R.K. Redoña, E. Refuerzo, L. 2010b. Varietal improvement for abiotic stress tolerance in crop plants: Special reference to salinity in rice. In: Pareek A., Sopory S.K., Bohnert H.J., (eds.) Abiotic stress adaptation in plants: Physiological, molecular and genomic foundation.
- Singh, R.K. Flowers, T.J. 2011. Physiology and molecular biology of the effects of salinity on rice. In Handbook of Plant and Crop Stress (ed. M. Pessarakli) 3rd edn, pp. 899–939. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA.
- Souleymane Bado, Brian Peter Forster, Roman Padilla-Alvarez, Christian Resch, Alessandro Migliori, Yacouba Diawara, Milko Jakšić, Abdelbagi Mukhtar Ali Ghanim, Stephen Nielsen, Margit Laimer. 2006. Prediction of Salt Tolerance in Rice (*Oryza Sativa*) based on Shoot Ion Content under Non-Stressed Conditions. *Journal of materials science and engineering A-6* (1-2): 1-6, Doi: 10.17265/2161-6213/2016.1-2.001.
- [Tyagi, N.K. 1998. Management of Salt Affected Soils. In: Singh, G.B., Sharma, B.R. \(Eds.\), 50 years of Natural Resource Management Research. Indian Council of Agricultural Research, New Delhi, pp. 363–401.](#)
- Yeo, A.R. Yeo, M.E. Flowers, S.A. Flowers, T.J. 1990. Screening of rice (*Oryza sativa*) genotypes for physiological characters contributing to salinity resistance and their relationship to overall performance. *Theor Appl Genet.* **79** (3): 377-384.



Yokoi, S. Quintero, F.J. Cubero B, Ruiz, M.T. Bressan, B.A. Hasegawa, P.M. Pardo, J.M. 2002. Differential expression and function of *Arabidopsis thaliana* NHX Na<sup>+</sup>/H<sup>+</sup> antiporters in the salt stress response. *Plant J.* **30**: 529–539.

Yoshida, S. Forno, D.A. Cock, J.H. 1972. Laboratory Manual for Physiological studies of rice. International Rice Research Institute. 2 nd Ed  
Zeng, L. Shannon, C. 2000. Salinity effects on seedling growth and yield components of rice. *Crop Sci.* **40**: 996–1003.



**Table 1. Composition of Yoshida medium**

Solution	Component	Stock solution (g/l)
A	NH <sub>4</sub> NO <sub>3</sub>	914
B	NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O	403
C	K <sub>2</sub> SO <sub>4</sub>	714
D	CaCl <sub>2</sub>	886
E	MgSO <sub>4</sub> .7H <sub>2</sub> O	3240
	MnCl <sub>2</sub> .4H <sub>2</sub> O	15
	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O	0.74
	H <sub>3</sub> BO <sub>3</sub>	9.34
F	ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.35
	CuSO <sub>4</sub> .5H <sub>2</sub> O	0.31
	FeCl <sub>3</sub> .6H <sub>2</sub> O	77
	Citric acid (Monohydrate)	119
	p <sup>H</sup>	5

S.No	Pre Breeding lines codes	Salt injury scores	Tolerance
1	FL478	1	HT
2	ISM	9	S
3	ISM-9-5-50-160	5	MT
4	ISM-19-15-178-142	3	T
5	ISM-18-217-18-141	1	HT
6	ISM-22-102-179-143	5	MT
7	ISM-102-179-13-179	3	T
8	ISM-110-39-23-147	1	HT
9	ISM-3-181-90-196	5	MT
10	ISM-82-79-45-76	5	MT
11	ISM-10-12-67-157	3	T
12	ISM-84-7-12-47	3	T
13	ISM-105-34-6-182	1	HT

**Table 2. Phenotypic scoring of genotypes based on IRRIS SES (Standard Evaluation Scale) 2013**

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14	ISM-100-17-66-98	3	T
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\*HT- Highly tolerant; MT-Moderately tolerant; T-Tolerant check; S-Sensitive check



**Fig. 1. Equipment used for salinity screening; (A) Plastic tray arranged with perforated tubes (B) Perforated tubes used for the experiment (C) Experimental setup used for salinity screening (D) Transferred seedlings from petri plates to silica sand based tray**



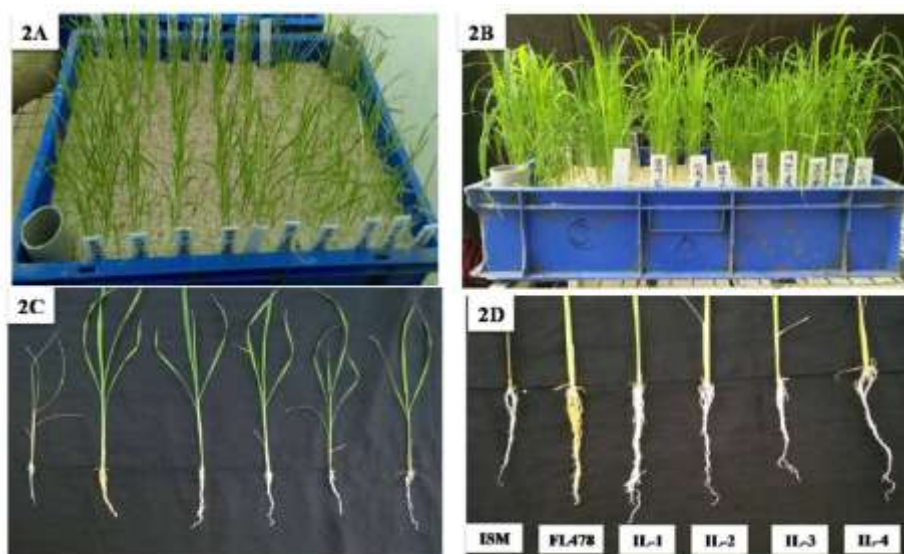


Fig. 2. A– Growth of rice seedlings on the silica sand base at 7 days after sowing B– Growth of rice seedlings on the silica sand base at 21 days after sowing without any salinity treatment 2C & D – Shoot and root morphology of checks ISM and FL478 along with introgressed lines IL-1 to IL-4 after 21 days of salinity treatment

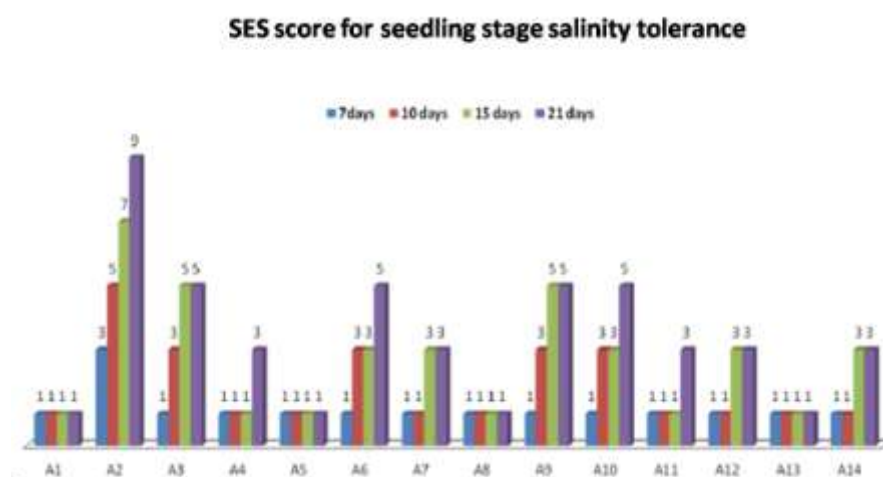


Fig. 3. Graphical representation of seedling stage salinity tolerance at different time intervals of salt treatments. A1- FL478 (Tolerant check); A2- ISM (Susceptible check); A3-A14-Introgressed lines of ISM introgressed with *Saltol* QTL.