Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 4594-4597

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-7 pp. 4594-4597**
Journal homepage: http://www.ijcmas.com

Original Research Article

Rapid and Efficient Method for Pre-Field Screening of Mutants for Salt Tolerance in Rice

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A B S T R A C T

Rapid and Efficient method is presented that screen salt tolerance in rice at seedlings stage. The method is based *in vitro* hydroponics liquid nutrient media in which salt is added in solution at the seedlings having 2-3 leaves. Requirement of equipment for hydroponic is given in figure. Advice is given on seed sterilization for inhibition of algal growth that restrict even germination of test material. 150mM Salt concentration treated after seedling grow in hydroponics, at the 2–3 leaf stage. Survival of seedlings are compared with non-mutated genotype Rajendra Mahshuri-1. Visual symptoms of salinity stress include reduced leaf area, whitish appearance of lower leaves, leaf tip death, leaf rolling and seedling death. Scoring is carried out according to the standard evaluation system developed by the International Rice Research Institute (IRRI). Recommended test salt concentrations are given along with a method to recover selected seedlings and examples of use.

Keywords

Salinity stress, Gamma radiation, Mutation, rice

Introduction

Salinity stress is one of the most complex stress tolerances to breed for, as the type, timing in relation to plant growth stage and intensity of the stress can all vary considerably (Witcombe *et al.*, 2008). Salinity tolerance screening under field conditions is difficult due to spatial and stress heterogeneity of soil chemical-related stresses, and the significant impact of environmental factors such as relative humidity, temperature, and solar radiation. Numerous studies showed that rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage, becomes sensitive during pollination and fertilization, and then becomes increasingly more tolerant at maturity.

Hence, a rapid and efficient method is the prerequisite to screen a large number of rice mutants at early seedling stage.

Therefore, in present investigation a rapid and efficient method for pre-field screening of Mutants for salt tolerance in rice has been developed and discussed.

Materials and Methods

Gamma irradiated mutant rice was screened for salinity stress in hydrophobic system developed at Department of Molecular Biology and Genetic Engineering, Bihar Agricultural University, Sabour in year 2015-16.

For making apparatus for screening mutant rice for salinity stress, 40 rectangular holes (3 cm diameter in a rows of 8) were made using surgical blade in a rectangular plastic sheet (3 mm thick). Then nylon net was stitched with nylon thread at one side of the rectangular plastic sheet. This plastic sheet was floated on 1X Hoagland solution kept in a rectangular plastic tray of 12-L capacity having dimension of 50-x-42-x-16-cm (fig.1). The sterile seeds (30-40 seeds) of rice mutants and Rajendra Mahsuri-1 as check genotype were placed on the net for germination for 15 days.

Then, seedlings were given salt stress by replacing the 1X Hoagland solutions (Table 1.) with 1X Hoagland solution with 150 mM NaCl for 15 days.

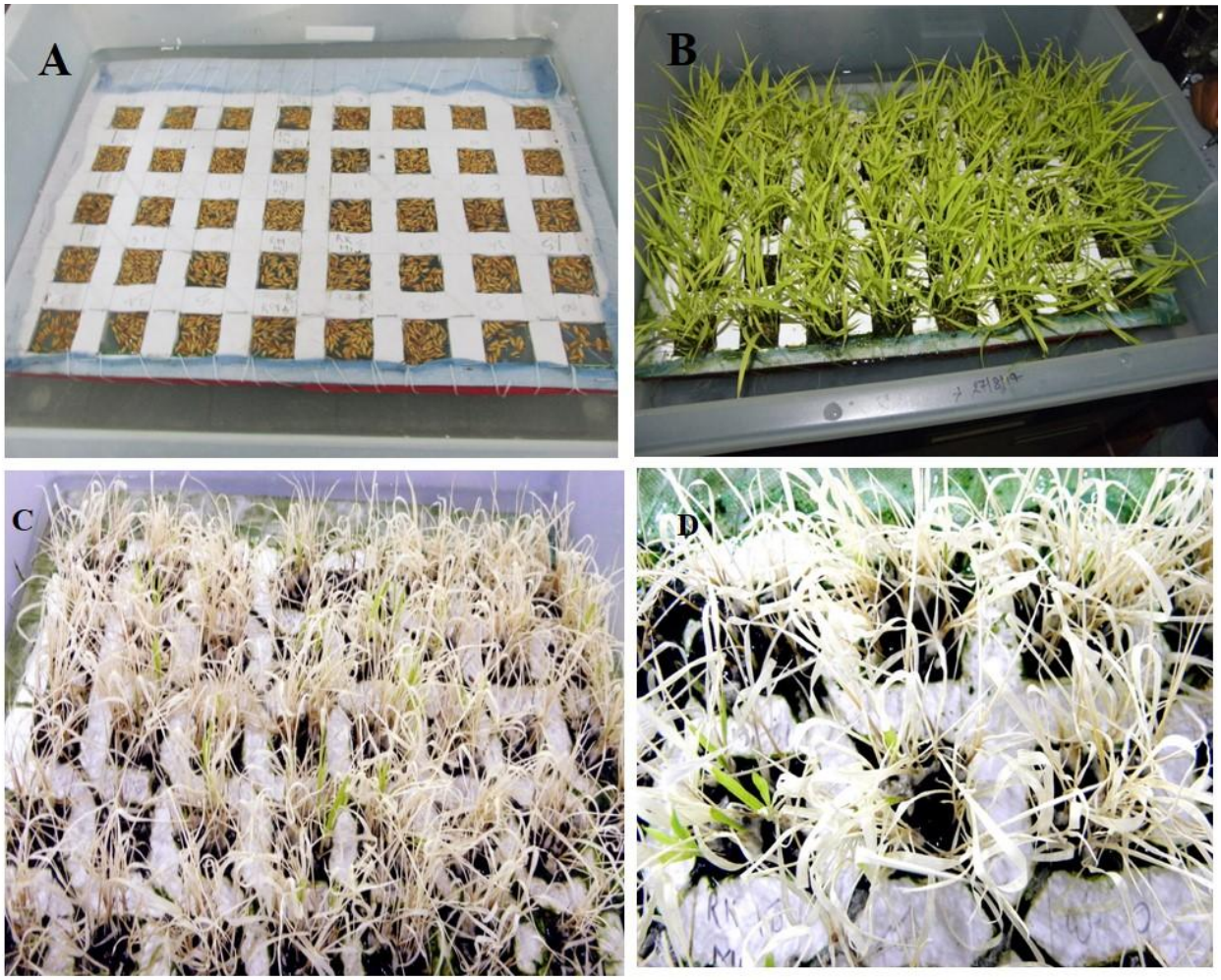

Figure 1. **Screening of mutants for salt tolerance: (A)** M4 seeds of Rajendra Mahsuri were placed on liquid medium for germination. (B) M4 seeds of Rajendra Mahsuri growing on liquid medium. (C) 150 mM NaCl treatment 15 days after treatment. (D) Survived plant

Results and Discussion

Rice is very sensitive to salinity at seedling stage. Salinity stress at early seedling stage manifest on the first leaf, followed by the second, and finally on the growing leaf. Salinity suppresses leaf elongation and emergence of new leaves. Photosynthetic function and chlorophyll content were inversely proportional to salinity level (Ota and Yasue 1962). The screening technique developed is based on the ability of seedlings to grow in salinized nutrient solution. In present investigation, 40 mutant M_4 mutant rice lines along with its parent Rajendra Mahsuri-1 were used for salt stress tolerance. The 150 mM of NaCl treatment for 15 days was found most effective as check variety (Rajendra Mahsuri-1) showed 100% mortality. Different mutant lines behaved differently on salt stress. Different mutant lines showed up to 40% survival on 150 mM of NaCl treatment for 15 days (fig. 2). The developed apparatus is robust, low cost and easy to make. This apparatus can be effectively used in screening rice mutants for salt stress tolerance.

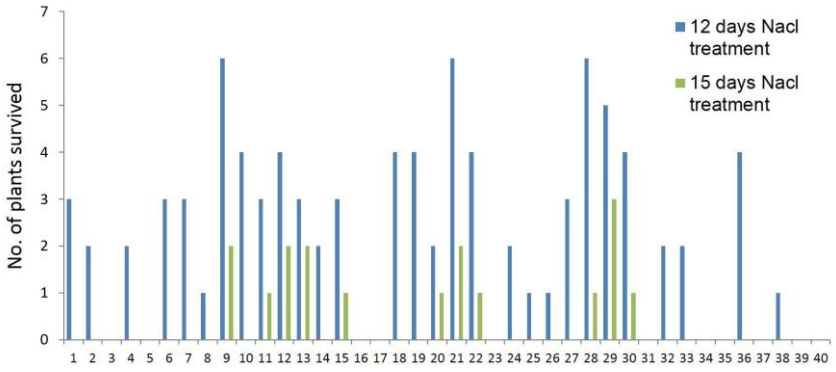

Figure 2. Scoring of live plants after 12 & 15 days of NaCl treatment, Total number of M4 seedlings screened: 1017, number of plants survived 11.

Table.1 Constitution of stock solutions of hydroponic

Stock no.	Chemical	Amounts/5 L
1	NH4NO3	457g
2	NaH2PO4H2O	201.5g
3	K2SO4	357g
4	CaCl2	443g
5	MgSO4 7H2O	1.620g
6	MnCl2 4H2O	7.5g
	(NH4)6 Mo7O24 4H2O	0.37g
	H3BO3	4.67g
	ZnSO4 7H2O	0.175g
	CuSO4 5H2O	0.155g
	FeCl3 6H2O	38.5g
	C6H8O7 H2O	59.5g
	1MH2SO4	250mL

References

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Witcombe JR, Hollington PA, Howarth CJ, Reader S and Steele KA (2008). Breeding for abiotic stresses for sustainable agriculture. Philosophical Transactions of the Royal Society B. 363: 703–716.