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Research Article

Evaluation of Near Isogenic Lines (NILs) of rice for major abiotic stresses of coastal areas

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Abstract
Rice crop in coastal areas is very often subjected to major abiotic stresses like submergence, lodging of the crop caused by heavy rains or cyclones and salinity. Identification of genotypes tolerance to multiple abiotic stresses is needed to sustain under climate changed conditions. NILs of Pushyami (MTU 2336-62-25-38-16, MTU 2336-70-46-25-44) with *Sub1* locus expressed tolerance to flash floods and also stagnant flooding and lodging resistance. NILs of Amara (MTU 2244-119-59-63-40, MTU 2244-119-83-65, MTU 2244-39-10-44-1) having *sub1* expressed tolerance to stagnant flooding, lodging resistance and moderate tolerance to salinity along with targeted trait of flash flood tolerance. NILs of Swarna (MTU 2546A-13-1-6-1, MTU 2546A-12-18-1, MTU 2546A-34-1-9-1) expressed moderate tolerance to anaerobic germination along with its targeted lodging related traits. NILs of Cottondora sannalu (DST 8-162-4, DST 9-157-7, DST 8-4-4) expressed lodging, flash flood, anaerobic germination along with targeted trait of seedling stage salinity tolerance. The results showed that NILs possessing multiple abiotic stress tolerance would sustain even under climate changed conditions.

Keywords
Near Isogenic Lines, Submergence, Lodging, Salinity, Anaerobic Germination

Introduction

Rice production is affected by many biotic and abiotic stresses throughout the world, among which abiotic stress alone contributes to about 50% of the total yield losses. Rice crop under coastal irrigated ecosystem prone to major abiotic stresses *viz*., floods, cyclones (causes lodging of the crop) and salinity resulting in decline in productivity. The world average rice yields from flooded area is 1.5 t/ha, with an area coverage of 11 million ha (Yamuna and Ashwini, 2016). Frequent floods are the major constraint in 5 million ha of cultivated area in India and flood prone area is increasing by the unprecedented cyclonic rains, heavy rains in short span of time and poor drainage system. Three different types of floods *viz*., submergence during germination (anaerobic germination), flash floods (complete submergence up to 2 weeks) and stagnant flooding (30-50 cm water depth) are mostly prevalent in coastal irrigated ecosystem (Reddy *et al*. 2015). Each type of flooding has different type of mechanisms to overcome it. Flash flooding leading to complete submergence of rice plants for 10–15 days is another major constraint prevailing mainly in rainfed lowlands. Most rice cultivars cannot survive complete submergence for more than a week. In India 30% of the rice growing area (12–14 M ha) is prone to flash flooding resulting in severe losses with average productivity of only 0.5–0.8 t ha^{-1} (Bhowmick *et al*., 2014). Stagnant flooding (SF; prolonged partial flooding; medium deep) occurs in areas when floodwater of 25–50 cm stagnates in the field from a few weeks to several months (Khush 1984; Singh *et al*., 2011).

The process by which the shoots of small grained cereals are displaced from their vertical stance is known as lodging. This usually occurs only after the ear or panicle has emerged and results in the shoots permanently leaning or lying horizontally on the ground. This can reduce yield by up to 80% and causes several knock-on effects including reduced grain quality, greater drying costs and slower harvest. It is a problem that limits cereal productivity in both developed and developing countries. There are two types of lodging stem lodging and root lodging. Stem lodging is classified into breaking and bending type. Breaking type of lodging occurs due to breaking of lower internodal and bending of uppernodes leads to bending type of lodging.

Salinity causes osmotic stress in plants, which is created by the accumulation of ions at the rhizosphere, is the first effect of salt stress which limits the water extraction ability of the roots that eventually leads to plant growth reduction. Ionic disequilibrium is the second effect of excess salt that quickly overtakes osmotic stress in rice and leads to nutrient starvation, enzyme inactivation, oxidative stress, and ionic toxicity in plant tissues. (Yeo *et al*., 1991; Roshandel and Flowers 2009;

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Turan *et al.*, 2012; Turan and Tripathy, 2013). Rice is relatively tolerant to stress for salinity during germination, active tillering, and maturity but is very sensitive at the early seedling stage and reproductive stage. The available three categories of alkali, saline and saline-sodic soils are problem soils and are generally also termed as salt affected soils. In India, about 8.6 m ha (18%) of rice area is affected by inland and coastal salinity. Of the two saline situations, inland salinity (mainly sodicity) is alarmingly increasing in the irrigation commands due to defective irrigation management and poor drainage.

In this present investigation, near isogenic lines developed for submergence in the background of Pushyami and Amara, lodging resistance for Swarna, Indra and Cottondora sannalu, salinity tolerance for Cotoondora sannalu were evaluated.

Materials and Methods

Screening for anaerobic germination tolerance was provided as per Reddy *et al*. (2015). Sterilized seeds were placed in petri dishes with moistened filter papers and incubated at 30 ^{0}C for 48 h for germination. Fifteen pre-germinated seeds at 3 days after incubation were sown into seedling trays (35.5×10×4.5cm) at about 1 cm soil depth in two replications per treatment. Each tray consists of three rows, 10 holes (2.5 cm) in each row. After sowing, the trays were submerged carefully in concrete tanks filled with 10 cm of water above the soil surface in the trays and was maintained at that depth for 14 days.

Number of seedlings survived 7 days after de-submergence was counted as anaerobic germination expressed in percentage relative to number of seeds sown to assess survival percentage.

$$\text{Anaerobic germination \%} = \frac{\text{Number of surviving seedlings 7 days after desubmergence}}{\text{pre germinated seeds just before submergence}} \times 100$$

Screening for flash flood tolerance was adopted as per Reddy *et al*. (2015). Sterilized seeds were placed in petri dishes with moistened filter papers and incubated at 30 ^{0}C for 48 h for germination. Before seeding, plastic cups were filled with soil. Pre-germinated (at 3 days after sowing) 25 seeds of each entry were placed, keeping almost equal distance and covered with dry soil in 2 replication by adopting completely randomized design. Seedlings were grown in plastic cups for 14 days and then, they were transferred to concrete water tank and were submerged by raising water depth up to 40 cm and maintained for 10 days. Number of seedlings survived 10 days after de-submergence was counted.

$$\text{Survival \%} = \frac{\text{Number of surviving plants 10 days after de submergence}}{\text{Plant numbers just before submergence}} \times 100$$

Thirty days old seedlings of twenty four entries were transplanted in cement lined submergence pond in randomized block design with two replication. Each entry was transplanted in 2 rows at spacing of 20×15 cm with a row length of 5.4 m. Flash floods were imposed by complete submergence for 14 days at 15 days after transplanting by maintaining 1 meter water depth. Number of seedlings survived 10 days after de submergence was counted.

Water depth of 40-50 cm was maintained in submergence pond immediately after recede of water from complete submergence up to harvesting stage for stagnant flood tolerance. At the time of harvesting plant survival (%) was recorded.

Twenty four entries were transplanted in shallow low land area in RBD with two replication. Each entry was transplanted in 2 rows at spacing of 20×15 cm with a row length of 3.6 m. Water depth of 40-50 cm was maintained in field upto harvesting stage for stagnant flood tolerance. At the time of harvesting plant survival (%) was recorded.

Screening for Seedling Stage Salinity Tolerance under Hydroponics study was performed as per protocol of Gregorio *et al.* (1997). Sterilized seeds were placed in petri dishes with moistened filter papers and incubated at 30 ^{0}C for 48 h for germination. Two pre-germinated seeds per hole were placed on the Styrofoam seedling float**.** The radicle was inserted through the nylon mesh. The Styrofoam seedling float was placed on the tray filled with distilled water. There are adequate nutrients in the endosperm for the seedlings to grow normally for three to four days. After three days, when seedlings were well established, distilled water was replaced with nutrient solution.

Initial salinity stress was imposed with EC=6 dsm^{-1} by adding 3g of NaCl/lt to nutrient solution. The solution was renewed eight days after initial salinization. The pH was monitored daily and was maintained at 5.0. After eight days of initial salinization, the EC was increased to 12 dsm^{-1} by adding 6g of NaCl/lt to nutrient solution. Initial scoring of the selected individual plants was recorded at 10 days after initial salinization as per SES of IRRI (2013). The description of the SES scale was presented in Table 2. The final score was recorded 16 days after initial salinization.

Lodging incidence was determined as per cent ratio of plants lodged as per IRRI, 2013 standard evaluation system (SES) under natural conditions at maturity stage.

Results and Discussion

Anaerobic germination percentage recorded lowest for II 110-9-1-1-1-1, MTU 2547A-95-1-11-1, NIL of Indra for lodging (0.00 %) and highest for MTU 1010, BPT 2270 (80.00 %) with a mean of 42.50 %. BPT 2270, MTU 1010 and NIL DST 9-152-7 expressed anaerobic germination more than 70 % indicating that these lines can survive upto 14 days under anaerobic conditions. Reddy *et al*., 2015 and Hendawy *et al*., 2012 also identified genotypes possessing plant survival >70 % as tolerant genotypes for anaerobic germination. (Table 2)

This trait exhibited lowest survival % for Amara, NILs of Swarna MTU 2546A-13-1-6-1, MTU 2546A-12-18-1, MTU 2546A-34-1-9-1, Swarna, II 110-9-1-1-1-1, NILs of Indra MTU 2547A-78-19-1-1, MTU 2547A-77-11-1 (0.00 %) and highest survival % was recorded for Swarna Sub1 (86.00 %) followed by NILs of Pushyami MTU 2336-70-46-25-44 (70.00 %) and MTU 2336-62-25-38-16 (63.75), with a mean of 24.43 %.

These results can be attributed that *Sub1* gene better expressed in NILs of Pushyami followed by Amara revealing that *Sub1* gene contributed for plant survival of 14 days old seedling but donor Swarna Sub1 showed relatively higher expression than NILs indicating that there is variation in expression of *Sub1*. *Saltol* introgressed lines showed significantly higher survival % than recurrent parent Cottondora sannalu it indicated that some genes in *Saltol* region also triggers expression of plant survival at seedling stage where as lodging resistance introgressed lines showed poor plant survival at seedling stage revealing that *SCM2* gene has no impact on plant survival at seedling stage. Sudhanshu *et al*., 2009 and Sudhanshu *et al*., 2014 also observed higher plant survival of *Sub1* lines than recurrent parent.

This trait exhibited lowest survival % for MTU 2547A-78-19-1-1, a NIL of Indra (9.93 %) and highest survival % was recorded for Swarna Sub1 (88.89 %) followed by MTU 2336-70-46-25-44 (85.42 %) and MTU 2336-62-25-38-16 (84.71 %), NILs of Pushyami with a mean of 46.10 %.

Sub1 version of Pushyami and Amara NILs expressed higher plant survival (more than 75 %) than recurrent parent indicated that *Sub1* was successfully introgressed and expressed at vegetative stage. II 110-9-1-1-1-1, tallest entry expressed higher pant survival (>75 %) by virtue of its elongation under submergence. Hendawy *et al*., 2014 and Samal *et al*., 2014 reported better plant survival of *Sub1* lines and their unraveled mechanism might be responsible for non *Sub1* lines for better survival.

This trait exhibited lowest survival % for MTU 2244-39-10-44-1, MTU 2546A-13-1-6-1 (0.00 %) and highest survival % was recorded for II 110-9-1-1-1-1 (66.45 %) with a mean of 28.04 %. Plants having moderate elongation of internodes survived better in this experiment.

Sub1 version of Pushyami (MTU 2336-62-25-38-16), (MTU 2336-70-46-25-44) and Amara (MTU 2244-119-59-63-40) and lodging donor II 110-9-1-1-1-1 expressed relatively better plant survival %. *Sub1* version of Swarna performed poor under stagnant flooding but *Sub1* version of Pushyami and Amara showed better performance because of moderate shoot elongation. Thus these three NILs can survive better under both flash flood and stagnant flooding. Reddy *et al*., 2015, Anshuman *et al.*, 2017 and Sandhya *et al*., 2017also developed and identified lines tolerant to both flash flood and stagnant flooding. *Sub1* version of Pushyami (MTU 2336-62-25-38-16), (MTU 2336-70-46-25-44) and Amara (MTU 2244-119-59-63-40) and lodging donor II 110-9-1-1-1-1 expressed relatively better plant survival %. Sub1 version of Swarna performed poor under stagnant flooding but *Sub1* version of Pushyami and Amara showed better performance because of moderate shoot elongation. Thus these three NILs can survive better under both flash flood and stagnant flooding. Reddy *et al*., 2015, Anshuman *et al.,* 2017 and Sandhya *et al*., 2017 also developed and identified lines tolerant to both flash flood and stagnant flooding.

Stagnant flooding plant survival % recorded lowest for MTU 2546A-13-1-6-1 (0.00 %) and highest (94.79 %) for Amara (MTU 1064), having stagnant flood tolerance with a mean of 49.87 %.

Sub1 version of Pushyami (MTU 2336-70-46-25-44 and MTU 2336-62-25-38-16) expressed better survival % than recurrent parent Pushyami under stagnant flooding. Recurrent parent Amara under stagnant flooding expressed higher survival by virtue of its elongation ability while respective NILs showed relatively lower % under stagnant flooding due to their lower elongation ability. Indentified two NILs of Swarna (MTU 2546A-12-18-1 and MTU 2546A-34-1-9-1), Indra (MTU 2547A-78-19-1-1) for lodging resistance, II 110-9-1-1-1-1 donor for lodging also expressed higher plant survival % under stagnant flooding indicated that these lines have plasticity to survive under stagnant flooding by virtue their elongation ability.

Salinity tolerance scores recorded lowest for FL 478 (2), salinity tolerance donor parent and highest for MTU 2546A-12-18-1, MTU 2546A-34-1-9-1, II 110-9-1-1-1-1, BPT 2270, MTU 1010 (9) with a mean of 6.20. *Saltol* was successfully introgressed in Cottondora sannalu (MTU 1010). Interestingly NILs of *Sub1* of Amara, lodging resistance NILs of Indra and their respective recurrent parent showed moderate level of salinity tolerance score (5) at seedling stage. Bhowmik *et al*. (2009), Amin *et al*. (2013) and Mohammadi Nejad *et al*. (2010) also identified salinity tolerant lines.

Per cent of lodging recorded lowest for MTU 2336-62-25-38-16, MTU 2336-70-46-25-44, MTU 1075, MTU 2244-119-83-65, II 110-9-1-1-1-1, BPT 2270, MTU 2547A-77-11-1, MTU 2547A-95-1-11-1 (0.00 %) and higher for Swarna Sub1 (99.12 %) with a mean of 30.60 %. Out of 24 entries, 15 entries are non lodging expressed lower per cent of lodging less than 25 %. Less than 25% can be indicated as lodging tolerant lines. Lower per cent of lodging of NILs than respective recurrent parent indicates these NILs expressed lodging resistance. These NILs can withstand adverse climatic conditions like cyclone or heavy rains at the time of reproductive stage. Girijarani *et al*., 2015 also observed variation in per cent of lodging among rice genotypes.

Intrestingly NILs of Amara and Pushyami with *Sub1* conferring flash flood tolerance also exhibited tolerance to stagnant flooding, lodging resistance and NILs of Amara also showed moderate level of salinity. NILs of Swarna, Indra for lodging resistance expressed anaerobic germination. NILs of Cottondora sannalu with *Saltol* also expressed anaerobic germination and flash flood tolerance. The identified NILs with multiple abiotic stresses would give sustainble yields under adverse climatic conditions.

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Table 1. Experimental material used for evaluation and characterization during *kharif* and *rabi* 2016-17.

CODE	DESIGNATION	CROSS COMBINATION
NIL 1	MTU 2336-62-25-38-16	MTU 1075/SWARNA SUB//*3 MTU 1075
NIL 2	MTU 2336-70-46-25-44	MTU 1075/SWARNA SUB//*3 MTU 1075
NIL 3	MTU 1075	RECURRENT PARENT
NIL 4	SWARNA SUB	DONAR PARENT
NIL 5	MTU 2244-119-59-63-40	MTU 1064/SWARNA SUB//*3 MTU 1064
NIL 6	MTU 2244-119-83-65	MTU 1064/SWARNA SUB//*3 MTU 1064
NIL 7	MTU 2241-39-10-44-1	MTU 1064/SWARNA SUB//*3 MTU 1064
NIL 8	MTU 1064	RECURRENT PARENT
NIL 9	MTU 2546A-13-1-6-1	MTU 7029/II 110-9-1-1-1-1//*3 MTU 7029
NIL 10	MTU 2546A-12-18-1	MTU 7029/II 110-9-1-1-1-1//*3 MTU 7029
NIL 11	MTU 2546A-34-1-9-1	MTU 7029/II 110-9-1-1-1-1//*3 MTU 7029
NIL 12	MTU 7029	RECURRENT PARENT
NIL 13	II 110-9-1-1-1-1	DONAR PARENT
NIL 14	MTU 1061	RECURRENT PARENT
NIL 15	BPT 2270	DONAR PARENT
NIL 16	MTU 2547A-78-19-1-1	MTU 1061/BPT 2270//*3 MTU 1061
NIL 17	MTU 2547A-77-11-1	MTU 1061/BPT 2270//*3 MTU 1061
NIL 18	MTU 2547A-95-1-11-1	MTU 1061/BPT 2270//*3 MTU 1061
NIL 19	DST 8-162-4	MTU 1010/FL 478//*3 MTU 1010
NIL 20	DST 9-157-7	MTU 1010/FL 478//*3 MTU 1010
NIL 21	DST 8-4-4	MTU 1010/FL 478//*3 MTU 1010
NIL 22	FL 478	DONAR PARENT
NIL 23	MTU 1010	RECURRENT PARENT
NIL 24	MTU 2251A-136-11-1	MTU 1010/PS 140-1//*3 MTU 1010

Table 2. Standard Evaluation Score (SES) OF Visual Salt Injury at Seedling Stage

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

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Table 3. Mean performance for different abiotic stresses among 24 entries of rice

S. No.	Character	Anaerobic germination %	Flash Floods		Flash flood + stagnant flooding plant survival %	Stagnant Flooding plant survival %	Salinity score	Per cent of lodging (%)
			Plant survival % at 34 DAS	Plant survival % at 34 DAT				
1	MTU 2336-62-25-38-16	46.67	63.75	84.71	48.46	70.74	7	0.00
2	MTU 2336-70-46-25-44	46.67	70.00	85.42	50.87	75.00	7	0.00
3	Pushyami (MTU 1075)	36.67	9.30	50.00	33.33	61.74	7	0.00
4	SWARNA SUB1	56.67	86.00	88.89	33.78	63.63	7	99.12
5	MTU 2244-119-59-63-40	36.67	48.44	77.78	61.42	67.71	5	1.77
6	MTU 2244-119-83-65	16.67	30.92	50.00	46.52	72.92	5	0.00
7	MTU 2244-39-10-44-1	26.67	28.13	29.17	0.00	67.67	5	13.24
8	Amara (MTU 1064)	43.33	0.00	47.22	43.93	94.79	5	41.48
9	MTU 2546A-13-1-6-1	46.67	0.00	22.73	0.00	0.00	7	9.71
10	MTU 2546A-12-18-1	26.67	0.00	29.80	25.86	78.13	9	13.82
11	MTU 2546A-34-1-9-1	30.00	0.00	48.61	36.39	77.08	9	11.18
12	Swarna (MTU 7029)	6.67	0.00	45.83	40.00	55.95	7	97.94
13	II 110-9-1-1-1-1	0.00	0.00	83.99	66.45	83.33	9	0.00
14	Indra (MTU 1061)	63.33	34.23	44.44	40.11	75.08	6	71.18
15	Bavapuri sannalu (BPT 2270)	80.00	43.40	54.29	28.93	17.71	9	0.00
16	MTU 2547A-78-19-1-1	30.00	0.00	9.93	7.35	76.04	5	3.53
17	MTU 2547A-77-11-1	60.00	0.00	11.67	6.49	1.10	7	0.00
18	MTU 2547A-95-1-11-1	0.00	9.11	15.00	9.63	3.60	5	0.00
19	DST 8-162-4	50.00	24.50	20.83	15.45	30.21	3	58.53
20	DST 9-157-7	70.00	66.09	22.50	9.33	9.38	3	74.67
21	DST 8-4-4	63.33	24.63	68.06	16.67	41.15	3	71.47
22	FL 478	60.00	12.80	29.67	13.30	39.58	2	66.76
23	Cotton dora sannalu (MTU 1010)	80.00	17.41	47.22	19.50	1.04	9	98.82
24	MTU 2251A-136-11-1	43.33	17.60	38.75	19.13	33.33	7	1.18
	Mean	42.50	24.43	46.10	28.04	49.87	6.21	30.60
	CV%	9.67	7.22	8.31	8.50	10.16	8.054	10.15
	CD	7.85	3.65	7.38	5.28	9.14	1.032	5.96
	SE(m)	2.67	1.24	2.51	1.79	3.11		2.02

C.V % = Coefficient of Variation percent C.D. = Critical Difference