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Molecular and Morphological Characterization of near Isogenic Lines Developed for Major Abiotic Stresses of Rice (*Oryza sativa* L.)

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A B S T R A C T

In the present investigation efforts were made to identify the distinct, uniform and stable morphological characters and molecular markers between NILs developed for *SUB1* in background of Pushyami (MTU 1075), Amara (MTU 1064), *SALTOL* in Cotton dora sannalu (MTU 1010) and lodging resistance in Swarna (MTU 7029) and Indra (MTU 1061) and their respective recurring parents. Rice productivity is limited by major abiotic stresses. Incorporation of stress tolerance gene/Qtls into popular varieties is one of the breeding strategies to combat adverse effects of climate changes. Characterization of rice genotypes is necessary for identification and protection of varieties under PPV. Results of molecular characterization showed genetic diversity among 24 entries. PIC value ranged from 0.08 (RM 6006) to 0.70 (RM 2229). Cluster analysis grouped 24 entries into two distinct clusters at similarity coefficient of 33 %. The results revealed that grouping of clusters using molecular markers is in accordance with parental ancestry and morphological traits. Graphical genotyping revealed that genome recovery of recurring parent in developed NILs ranged from 75.1 % (MTU 2546A-12-18-1) to 96.4 % (MTU 2336-70-46-25-44). Most of the agro morphological characters were found to be similar between near isogenic lines and respective recurrent parent.

Introduction

Rice production and productivity is limited by major abiotic stresses like submergence, salinity, lodging in coastal irrigated ecosystem. Unabated efforts of researches resulted identification of *Sub1A* for flash flood tolerance (Xu and Mackill 1996; Nandi *et al.*, 1997; Toojinda *et al.*, 2003, Xu *et al.*, 2006), *Saltol* for seedling stage salinity tolerance (Gregorio, 1997), *SCM2* for lodging resistance (Ookawa *et al.*, 2010). Marker assisted breeding played a vital role in development of Near Isogenic Lines (NILs) for major abiotic stress like salinity, submergence into widely adopted varities (Hoque *et al.*, 2015; Renu Singh *et al.*, 2015; Girijarani *et al.*, 2015b; Iftekharuddaula *et al.*, 2016). There is a need to characterize rice varieties according to DUS (Distinctiveness, Uniformity and Stability) test guidelines prescribed by Protection of Plant Varieties and Farmers' Rights Act Authority to protect varieties and varietal identification. Agro morphological characterization provides

descriptors to distinguish one genotype from other. Techniques, such as plant characterization have been successfully used in recent years to help in identifying elite individuals. It is an indispensable tool for selecting varieties or lines based on agronomical, morphological, genetic or physiological characters (Ndour, 1998).

DNA fingerprinting with molecular markers allows precise, objective and rapid cultivar identification, it has been proved to be an efficient tool for characterization and management (Chakravarthi and Naravaneni, 2006). Simple sequence repeat (SSR) markers have been widely used for genetic analysis and cultivar identification by virtue of their abundance, co-dominant inheritance, high polymorphism, reproducibility and ease of assay by polymerase chain reaction (PCR) (Kuleung *et al.,* 2004). It is essential to build the fingerprinting database of the main commercial cultivars in the market for rapid and unambiguous cultivar identification (Zhu *et al.,* 2012). Background selection is the process of using markers covering all chromosomes and to accelerate the recovery of the recurrent parent genome during backcrossing (Hospital and Charcosset, 1997).

Characterization of NILs in comparison with recurrent parent is necessary for identification and protection of variety. Present study aimed to characterize NILs developed for submergence, salinity and lodging resistance using morphological and molecular markers.

Materials and Methods

Experiment was carried out at Andhra Pradesh Rice Research Institute and Regional Agricultural Research Station (APRRI and RARS) of West Godavari, Maruteru during kharif 2016 for morphological characterization. Experimental material consisting of rice Near Isogenic Lines (NILs) along with their corresponding recipient and donar parents developed for submergence, lodging and salinity tolerance developed at APRRI and RARS, Maruteru, West Godavari District. The coding of the experimental material used for the present study was presented in the Table 1. Twenty four entries comprising of NILs developed for Sub1 (NIL 1 and NIL 2) in background of Pushyami, Amara (NIL 5, 6, 7) using donar Swarna Sub1. NILs developed by incorporation *SCM2* confers lodging resistance in Swarna (NIL 9, 10, 11) and Cottondora Sannalu (NIL 24) using donar II 110-9-1-1-1, Indra (NIL 16, 17, 18) using donar BPT 2270 and NILs of Cottondora Sannalu for *Saltol* (NIL 19, 20, 21) using FL478 as donar.

Experiment was layout in completely randomized block design in 2 replications. 25 old seedlings were transplanted with spacing of 20 cm between rows and 15cm between plants. Recommended dose of fertilizers 90:60:60 kg/ha was provided. Molecular characterization was carried out during Rabi 2016-17.

DNA isolation and PCR assay

The total genomic DNA was isolated from 25 days old leaf samples as per the protocol described by Zheng *et al.,* (1995) with some modifications. The quality and quantity of DNA was estimated using ND8000 eight-channel spectrophotometer. 10ulPCR reaction mixture consists of 1 µl of10X buffer (10 mM TrisHCl (pH 8.3), 50 mM KCl, 1.5mM $MgCl_2$, 0.01% gelatin), 0.5 µl of dNTPs (2.5 m M. L^{-1}), 1 µl (5 µ molar) each of forward and reverse primers, 1 µl Taq DNA polymerase (0.5 U/µl) (Banglore Genei Private Limited, Bangalore), 3 µl of template DNA (10 ng/µl) and 2.5 µl of sterilized distilled water. Amplification were performed using Eppendorf thermo cycler with the temperature profiles of initial denaturation at

94^{o}C for 5 min, denaturation at 94^{o}C for 0.5 min, annealing at 55^{o}C for 0.5 min, extension at 72^{o}C for1.0 min and final extension for 7 min at72^{o}C for 35 cycles. The PCR amplicons were electrophoresed on 3% agarose gel stained with ethidium bromide (10mg/ml) at 100volts for 1.5 hr in 1X TBE buffer. A 100 bp ladder (Genei) was used for appropriate sizing of the products. The gel images were captured under UV light using syngene Ingenius geldoc system.

Molecular data analysis for characterization

Total of sixty nine polymorphic markers were used for characterization of NILs along with their respective parents. The DNA banding patterns obtained from SSR analysis for each primer were scored by visual observation.

The 0/1 matrix was used to calculate the genetic similarity to estimate all pair-wise differences in the amplification products for all entries. The genetic similarity between these plants was evaluated by calculating the Jaccard similarity coefficient. Similarity coefficients were used for cluster analysis using sub program of NTSYS-PC (Rohlf, 2000). The dendrogram was constructed by unweighted pair group method with arithmetic averages (UPGMA) sub programme of NTSYS-PC.

Polymorphic information content (PIC) was calculated, according to the method of Anderson *et al.*, (1993).

$$PIC = 1 - \sum_{f=1}^{n} Pij^2$$

Where P*ij* is the frequency of the jth allele for the ith marker, and is summed over n alleles. The calculation was based on the number of alleles per locus.

Genome recovery percentage %

The twenty-four entries consist of NILs along with their parents were screened with identified polymorphic SSR markers to decipher the percentage of the recurrent parent genome recovered (RPG) using Graphical Genotyping 2.0 (Van Berloo, 2008). The background recovery was calculated by using formula (Sundaram *et al.*, 2008). In the present study background selection was performed using 58 polymorphic markers between Pushyami and Swarna Sub1 to assess genome recovery percentage of Pushyami. Sixty two polymorphic markers were used in the present study for background selection between recurrent parent Amara and donor Swarna Sub1. Background selection was performed using 57 polymorphic markers between Swarna and donor II 110-9-1-1-1-1 to assess genome recovery percentage of Swarna. A total of 50 polymorphic markers are used to assess genome recovery percentage of Indra. A total of 65 polymorphic markers are used to assess the genome recovery of Cottondora sannalu. Background selection was performed using 46 polymorphic markers between Cottondora sannalu and FL 478 to assess genome recovery of Cottondora sannalu.

$$G = [(X + 1/2Y) /100]/N$$

N = total number of parental polymorphic markers screened

X = number of markers showing homozygosity for recurrent parent allele

Y = number of markers showing heterozygosity for parental alleles

Characterization based on morphological traits

The characteristics and their state and stage of observation were given as per the National

Test Guidelines for Distinctness, Uniformity and Stability (DUS) (Shobarani *et al.,* 2006) were recorded at different stages of crop growth period.

Results and Discussion

A total of 69 SSR markers were used covering all the chromosomes of rice for their molecular characterization and discrimination of twenty four entries of rice. The number of alleles per locus generated by each marker ranged from 1 to 4 alleles with an average number of 2.36 alleles per locus. The highest number of alleles (4) was detected for markers RM 243, RM 2972, RM 5210, RM 2229 and the lowest number of alleles (2) was detected for most of the primers. List of polymorphic microsatellite markers with their chromosomal locations, number of alleles and PIC value are presented in Table 2. The polymorphism information content (PIC) value ranged from 0.08 (RM 6006, RM 5055, RM 6364 and RM 2851) to 0.70 (RM 2229) with an average PIC of 0.41.

Islam *et al.,* (2012) observed a range of PIC value from 0.21 to 0.76 with an average of 0.57 in fourteen stress tolerant rice varieties of Bangladesh using 40 SSR primers and also revealed that higher the PIC value of a marker indicates higher probability of detecting the number of alleles among the cultivars. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (Virk *et al.,* 1995). Markers with high PIC RM 5919, RM 212 and RM 2229 found to be useful in distinguishing NILs in present study.

Cluster analysis

Jaccard's coefficient value ranged from 0.212 to 1.00 among 24 entries studied (Table 3). Near Isogenic Lines MTU 2547A-78-19-1-1 and MTU 2547A-77-11-1 showed 100 % similarity. Genotypes with low similarity values are more divergent. Among the 24 entries FL 478 is more divergent with NILs of Pushyami with lower similarity value of 0.273, *Sub1* donor Swarna Sub1 (NIL 4) also divergent with recurrent parent Pushyami (NIL 3), Amara (NIL 8) and NIL of Amara (NIL 6) with lower similarity value of 0.235, 0.389 and 0.212 respectively. Swarna expressed lower similarity index with MTU 2244-119-59-63-40 (0.248), MTU 2244-119-83-65 (0.235) NILs of Amara and recurrent parent Amara (0.263). MTU 2546A-34-1-9-1, a NIL of Swarna expressed lower similarity value of 0.270 with DST 8-162-4 NIL of Cottondora sannalu for *Saltol*. MTU 2251A-136-11-1 NIL of Cottondora sannalu expressed lower similarity value of 0.265 with Swarna, II 110-9-1-1-1-1 (0.265), Indra (0.299), Bavapuri sannalu (0.263), it indicates these lines expressed diversity at molecular level.

Similar results were reported by Venuprasad *et al.,* (2011) studied polymorphism analysis with 491 SSRs revealed that two NIL pairs are at least 96% genetically similar. Further the results were in agreement with, Patel *et al.,* (2016) conducted molecular characterization of Near Isogenic Lines using 29 RAPD and SSR markers.

A dendrogram (Figure 1) indicated that there were two major clusters 'I' and 'II' at 33% similarly level. Major cluster 'I' divided into two sub cluster IA with 8 entries and IB with 10 entries. NILs of Pushyami (MTU 2336-62-25-38-16 and MTU 2336-70-46-25-44) and recurrent parent Pushyami (MTU1075), Swarna and NILs of Swarna for lodging resistance (MTU 2546A-13-1-6-1, MTU 2546A-12-18-1, MTU 2546A-34-1-9-1) and Swarna Sub1 were grouped in cluster IA. The cluster IB consist of Amara and its *Sub1* NILs

(MTU 2244-119-59-63-40, MTU 2244-119-83-65, MTU 2241-39-10-44-1), Indra along with its NILs for lodging resistance (MTU 2547A-78-19-1-1, MTU 2547A-77-11-1, MTU 2547A-95-1-11-1), Bhavapuri sannalu and II 110-9-1-1-1-1 (Table 4). Major cluster 'II' divided into two sub cluster IIA with 5 entries and IIB with one entry. Cluster IIA consisted of Cottondora sannalu along with NILs for salinity tolerance (DST 8-162-4, DST 9-157-7, and DST 8-4-4) and a NIL for lodging (MTU 2251A-136-11-1). IIB consists of FL 478, a *saltol* donor.

Cluster IA consists of Swarna and its derived lines and Pushyami and its derived lines. Grouping of this cluster clearly indicated that Pushyami and Swarna have genome relationship in their ancestry because Swarna was derived from Vasista and Mashuri. Pushyami was derived from MTU 2716 and MTU 1010. While MTU 2716 was developed from Mashuri and Vijaya. This clearly demonstrated that Mashuri parent is common ancestry parent has contributed maximum genome inheritance in the development of Swarna and Pushyami and their respective Near Isogenic Lines.

Whereas cluster IB comprises most of Indra and Amara derived lines. It clearly indicated that Indra and Amara have same parents of PLA 1100 and MTU 1010. PLA 1100 intern derived from Mashuri and Vijaya. Bavapuri sannalu is derived from BPT 5204 and CR15MR1523 and BPT 5204 has Mashuri as one of the parent. II 110-9-1-1-1-1 derived from a cross between [(BPT 5204 / IET 9762)/ Swarna]/MTU2716 in which the parents BPT 5204, Swarna, MTU 2716 has common parent of Mashuri. Entries grouped in cluster I are late in duration with intermediate height. Cluster IIA consists of MTU 1010 and its derived lines and IIB consist of FL 478, donor for salinity tolerance. All the lines are dwarf and early in duration.

The above results revealed that grouping of cluster I using molecular markers is in accordance with parental ancestry and morphological traits. This indicated that markers used in this study can be useful to distinguish entries studied.

Genome recovery percentage

The results of graphical genotyping revealed that genome recovery percentage of NILs ranged from 75.1 % (MTU 2546A-12-18-1) to 96.4 % (MTU 2336-70-46-25-44). The genome recovery percentages of NILs were presented in the Table 5.

NIL MTU 2336-70-46-25-44 showed highest genome recovery percentage of 96.4 % among *Sub1* introgressed lines of Pushyami.

NILs of Amara, MTU 2244-119-59-63-40 and MTU 2244-119-83-65 showed highest genome recovery percentage of 96.3 % and 96.2 % respectively among the *Sub1* introgressed lines of Amara.

It indicates that this NILs are better recovered from respective recurrent parent Pushyami and Amara with *Sub1* locus. These NILs would be adopted by farmers in flood prone areas after intensive evaluation and testing.

Similar results were reported by Renu singh *et al.*, (2015) in 18 advanced backcross lines (M1–M17 and M20) from MTU 1075/Swarna-Sub1 cross at BC_3F_4 generation, showed 91.88–90.29 % overall recipient genome recovery and 85.35–88.45 %.

Three best plants were selected and their recipient genome recovery percentages were 86.84 %, 85.13 % and 85.0 % in Sub1 lines of BRRI dhan49 (Ara *et al.*, 2015). Ahmed *et al.*, (2016) background analysis of Sub1 incorporated lines of MR219 revealed genome recovery of 95.37 % at BC_2F_2 generations.

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

CODE	DESIGNATION	CROSS COMBINATION
NIL 1	MTU 2336-62-25-38-16	MTU 1075/SWARNA SUB1//*3 MTU 1075
NIL 2	MTU 2336-70-46-25-44	MTU 1075/SWARNA SUB1//*3 MTU 1075
NIL 3	Pushyami (MTU 1075)	RECURRENT PARENT
NIL 4	SWARNA SUB1	DONAR PARENT
NIL 5	MTU 2244-119-59-63-40	MTU 1064/SWARNA SUB1//*3 MTU 1064
NIL 6	MTU 2244-119-83-65	MTU 1064/SWARNA SUB1//*3 MTU 1064
NIL 7	MTU 2244-39-10-44-1	MTU 1064/SWARNA SUB1//*3 MTU 1064
NIL 8	Amara (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	Swarna (MTU 7029)	RECURRENT PARENT
NIL 13	II 110-9-1-1-1-1	DONAR PARENT
NIL 14	Indra (MTU 1061)	RECURRENT PARENT
NIL 15	Bavapuri sannalu (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	Cottondora sannalu (MTU 1010)	RECURRENT PARENT
NIL 24	MTU 2251A-136-11-1	MTU 1010/ II 110-9-1-1-1-1//*3 MTU 1010

Table.2 List of polymorphic microsatellite markers among 24 rice entries

S. No.	SSR Markers	Chromosomal number	Number of alleles	PIC Value
1	RM 243	1	4	0.64
2	RM 5919	1	3	0.66
3	RM 212	1	3	0.66
4	RM 246	1	2	0.50
5	AP 3206	1	2	0.50
6	RM 10694	1	2	0.38
7	RM 3412	1	2	0.15
8	RM 10855	1	3	0.66
9	RM 6334	1	2	0.33
10	RM 3865	2	3	0.54
11	RM 106	2	2	0.47
12	RM 5210	2	4	0.60
13	RM 3340	2	2	0.49
14	RM 6933	2	3	0.47
15	RM 1230	3	2	0.28
16	RM 5924	3	3	0.60
17	RM 1350	3	2	0.22
18	RM 5474	3	2	0.15
19	RM 3524	4	2	0.47

20	RM 335	4	3	0.49
21	RM 303	4	3	0.55
22	RM 241	4	2	0.50
23	RM 6006	4	2	0.08
24	RM 169	5	2	0.49
25	RM 249	5	2	0.22
26	RM 163	5	2	0.47
27	RM 6024	5	2	0.08
28	RM 8107	6	2	0.49
29	RM 3	6	2	0.54
30	RM 225	6	2	0.44
31	RM 19382	6	2	0.44
32	RM 2229	6	4	0.70
33	RM 253	6	2	0.29
34	RM 20547	6	2	0.28
35	RM 5509	6	3	0.63
36	RM 20557	6	2	0.33
37	RM 340	6	2	0.38
38	RM 23865	6	3	0.57
39	RM 20648	6	3	0.52
40	RM 464	6	2	0.49
41	RM 23805	6	2	0.44
42	RM 23869	6	2	0.22
43	Sub1BC2	6	2	0.22
44	RM 30	6	2	0.38
45	RM 320	7	3	0.60
46	RM 478	7	2	0.15
47	RM 5055	7	2	0.08
48	RM 5720	7	3	0.57
49	RM 149	8	2	0.33
50	RM 1111	8	2	0.44
51	RM 566	9	3	0.63
52	RM 524	9	3	0.52
53	RM 528	9	2	0.22
54	RM 23915	9	2	0.33
55	RM 23788	9	2	0.22
56	RM 6100	10	2	0.50
57	RM 484	10	2	0.15
58	RM 6364	10	2	0.08
59	RM 5926	11	2	0.38
60	RM 286	11	2	0.41
61	RM 6925	11	3	0.53
62	RM 5766	11	2	0.28
63	RM 6293	11	2	0.50
64	RM 2972	12	4	0.58
65	RM 309	12	2	0.49
66	RM 5939	12	3	0.58
67	RM 1227	12	2	0.41
68	RM 2529	12	2	0.49
69	RM 2851	12	2	0.08
	Average		2.36	0.41

Table.3 Jaccard's similarity coefficient matrix of 24 entries

	NIL1	NIL2	NIL3	NIL4	NIL5	NIL6	NIL7	NIL8	NIL9	NIL10	NIL11	NIL12	NIL13	NIL14	NIL15	NIL16	NIL17	NIL18	NIL19	NIL20	NIL21	NIL22	NIL23	NIL24
NIL1	**1.000**																							
NIL2	0.775	**1.000**																						
NIL3	0.703	0.658	**1.000**																					
NIL4	0.385	0.416	0.235	**1.000**																				
NIL5	0.355	0.326	0.432	0.248	**1.000**																			
NIL6	0.313	0.340	0.416	0.212	0.853	**1.000**																		
NIL7	0.400	0.400	0.465	0.260	0.595	0.615	**1.000**																	
NIL8	0.302	0.302	0.389	0.214	0.761	0.838	0.689	**1.000**																
NIL9	0.416	0.416	0.448	0.400	0.370	0.355	0.615	0.437	**1.000**															
NIL10	0.340	0.432	0.416	0.400	0.340	0.355	0.537	0.389	0.615	**1.000**														
NIL11	0.355	0.385	0.340	0.595	0.260	0.248	0.400	0.276	0.556	0.615	**1.000**													
NIL12	0.355	0.385	0.340	0.615	0.248	0.235	0.385	0.263	0.537	0.636	0.853	**1.000**												
NIL13	0.292	0.363	0.363	0.255	0.278	0.319	0.430	0.295	0.398	0.482	0.352	0.337	**1.000**											
NIL14	0.340	0.340	0.313	0.248	0.416	0.500	0.556	0.506	0.465	0.370	0.273	0.260	0.430	**1.000**										
NIL15	0.359	0.276	0.330	0.238	0.404	0.389	0.506	0.425	0.359	0.344	0.289	0.302	0.402	0.437	**1.000**									
NIL16	0.326	0.326	0.299	0.235	0.400	0.482	0.537	0.488	0.448	0.355	0.286	0.273	0.414	0.969	0.420	**1.000**								
NIL17	0.326	0.326	0.299	0.235	0.400	0.482	0.537	0.488	0.448	0.355	0.286	0.273	0.414	0.969	0.420	1.000	**1.000**							
NIL18	0.370	0.370	0.340	0.273	0.385	0.432	0.518	0.437	0.465	0.340	0.326	0.313	0.398	0.881	0.404	0.909	0.909	**1.000**						
NIL19	0.351	0.337	0.380	0.283	0.309	0.270	0.283	0.260	0.323	0.323	0.270	0.296	0.302	0.351	0.286	0.366	0.366	0.411	**1.000**					
NIL20	0.326	0.313	0.340	0.286	0.299	0.286	0.273	0.276	0.299	0.313	0.273	0.299	0.292	0.340	0.344	0.355	0.355	0.370	0.868	**1.000**				
NIL21	0.400	0.385	0.416	0.299	0.313	0.326	0.340	0.316	0.340	0.340	0.313	0.340	0.348	0.355	0.316	0.370	0.370	0.416	0.814	0.775	**1.000**			
NIL22	0.273	0.273	0.273	0.260	0.313	0.326	0.340	0.330	0.370	0.313	0.299	0.299	0.292	0.370	0.330	0.385	0.385	0.400	0.512	0.595	0.518	**1.000**		
NIL23	0.432	0.416	0.448	0.326	0.340	0.326	0.340	0.316	0.340	0.355	0.313	0.340	0.333	0.355	0.302	0.370	0.370	0.416	0.841	0.800	0.909	0.465	**1.000**	
NIL24	0.385	0.370	0.400	0.286	0.313	0.299	0.299	0.289	0.299	0.313	0.273	0.299	0.265	0.299	0.263	0.313	0.313	0.355	0.716	0.680	0.775	0.385	0.853	**1.000**

Table.4 Clustering pattern of the rice entries based on molecular data

Main cluster	Sub cluster	No. of entries	Designation of entries
I	IA	8	MTU2336-62-25-38-16, MTU2336-70-46-25-44, MTU1075, MTU2546A-13-1-6-1, MTU2546A-12-18-1, MTU2546A-34-1-9-1, MTU7029, Swarna Sub1.
	IB	10	MTU 2244-119-59-63-40, MTU 2244-119-83-65, MTU 2241-39-10-44-1, MTU 1064, MTU 2547A-78-19-1-1, MTU 2547A-77-11-1, MTU 2547A-95-1-11-1, MTU1061, BPT2270, II 110-9-1-1-1-1
II	IIA	5	DST 8-162-4, DST 9-157-7, DST 8-4-4, MTU 2251A-136-11-1, MTU1010.
	IIB	1	FL 478.

Table.5 Genome recovery percentage of Near Isogenic Lines (NILs) developed for submergence, lodging and salinity

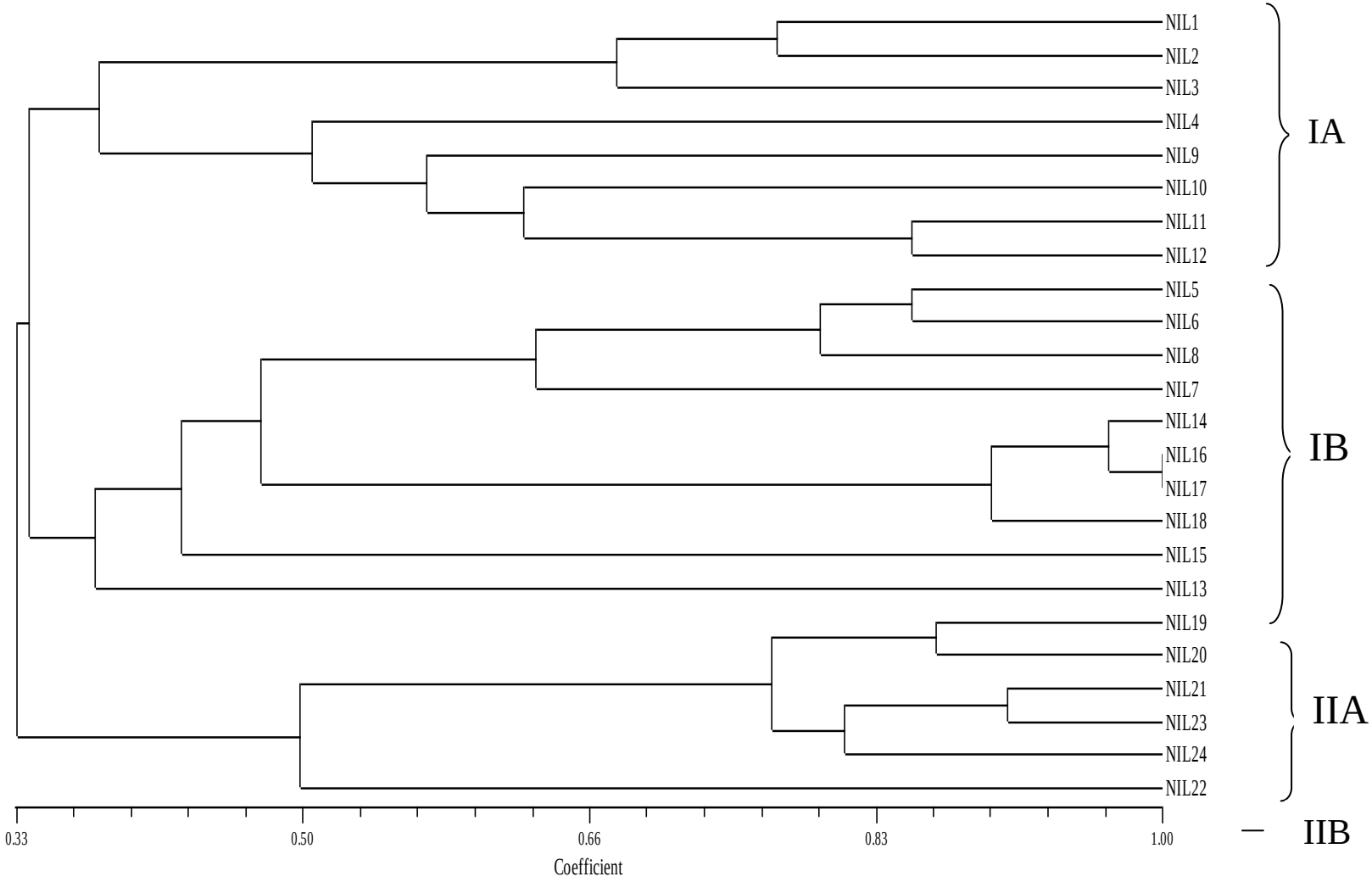
S. No.	Entry	Designation	Genome recovery %
1	NIL of Pushyami for *Sub1*	MTU 2336-62-25-38-16	89.9 %
2	NIL of Pushyami for *Sub1*	MTU 2336-70-46-25-44	96.4 %
3	Recurrent parent	Pushyami (MTU 1075)	
4	Donar parent	Swarna Sub1	
5	NIL of Amara for *Sub1*	MTU 2244-119-59-63-40	96.3 %
6	NIL of Amara for *Sub1*	MTU 2244-119-83-65	96.2 %
7	NIL of Amara for *Sub1*	MTU 2244-39-10-44-1	86.4 %
8	Recurrent parent	Amara (MTU 1064)	
9	NIL of Swarna for *SCM2*	MTU 2546A-13-1-6-1	79.3 %
10	NIL of Swarna for *SCM2*	MTU 2546A-12-18-1	75.1 %
11	NIL of Swarna for *SCM2*	MTU 2546A-34-1-9-1	94.1 %
12	Recurrent parent	Swarna (MTU 7029)	
13	Donar parent	II 110-9-1-1-1-1	
14	Recurrent parent	Indra (MTU 1061)	
15	Donar parent	Bavapuri sannalu (BPT 2270)	
16	NIL of Indra for *SCM2*	MTU 2547A-78-19-1-1	93.7 %
17	NIL of Indra for *SCM2*	MTU 2547A-77-11-1	88.4 %
18	NIL of Indra for *SCM2*	MTU 2547A-95-1-11-1	90.2 %
19	NIL of Cottondora sannalu for *Saltol*	DST 8-162-4	91.0 %
20	NIL of Cottondora sannalu for *Saltol*	DST 9-157-7	78.3 %
21	NIL of Cottondora sannalu for *Saltol*	DST 8-4-4	89.1 %
22	Donar parent	FL 478	
23	Recurrent parent	Cottondora sannalu (MTU 1010)	
24	NIL of MTU 1010 for *SCM2*	MTU 2251A-136-11-1	93.8 %

Table.6 Summary results of morphological and abiotic stress tolerance among 24 entries of rice

S. No.	Recurrent parent	Targeted trait	Near Isogenic Lines of recurrent parent	Distinct morphological character from recurrent parent	Tolerance to other abiotic stress
1.	Pushyami (MTU 1075)	Submergence tolerance	MTU 2336-62-25-38-16	1. Dark green intensity of leaf colour 2. Medium leaf senescence	Stagnant flood tolerance and lodging resistance
			MTU 2336-70-46-25-44	1. Dark green intensity of leaf colour 2. Medium leaf senescence	Stagnant flood tolerance and lodging resistance
2.	Amara (MTU 1064)	Submergence tolerance	MTU 2244-119-59-63-40	1. Weak pubescence of blade surface 2. Medium leaf senescence 3. Medium gelatinizing temperature	Stagnant flood tolerance, lodging resistance and moderate salinity tolerance.
			MTU 2244-119-83-65	1. Weak pubescence of blade surface 2. Long panicle length (<25 cm) 3. Straw colour lemma and palea 4. Presence of panicle awns 5. Medium leaf senescence 6. Very low 1000 grain weight (<15 g) 7. Narrow grain and decorticated grain width. 8. Medium gelatinizing temperature	Stagnant flood tolerance, lodging resistance and moderate salinity tolerance.
			MTU 2244-39-10-44-1	1. Weak pubescence of blade surface 2. Medium leaf senescence 3. Medium gelatinizing temperature	Stagnant flood tolerance, lodging resistance and moderate salinity tolerance.
3.	Swarna (MTU 7029)	Lodging resistance	MTU 2546A-13-1-6-1	1. Medium pubescence of blade surface 2. Thick stem thickness 3. High medium gelatinizing temperature 4. Medium leaf senescence	Stagnant flood tolerance, moderate tolerance to anaerobic germination
			MTU 2546A-12-18-1	1. Medium pubescence of blade surface 2. Thick stem thickness 3. long panicle length 4. High medium gelatinizing temperature 5. Medium leaf senescence	Stagnant flood tolerance, moderate tolerance to Anaerobic germination

S.No.	Recurrent parent	Targeted trait	Near Isogenic Lines of recurrent parent	Distinct morphological character from recurrent parent	Tolerance to other abiotic stress
			MTU 2546A-34-1-9-1	1. Medium pubescence of blade surface 2. Thick stem thickness 3. Medium leaf senescence	Moderate tolerance to anaerobic germination
4.	Indra (MTU 1061)	Lodging resistance	MTU 2547A-78-19-1-1	No variation is observed	Moderate salinity tolerance
			MTU 2547A-77-11-1	1. Medium 1000 grain weight	Anaerobic germination
			MTU 2547A-95-1-11-1	No variation is observed	Moderate salinity tolerance
5.	Cottondora sannalu MTU (1010)	Salinity tolerance	DST 8-162-4	1. Strong pubescence of blade surface 2. Thick stem thickness 3. Long panicle length 4. Medium panicle number per plant (>11)	Lodging resistance, Anaerobic germination
			DST 9-157-7	1. Strong pubescence of blade surface 2. Thick stem thickness 3. Long panicle length 4. Medium panicle number per plant (>11) 5. High 1000 grain weight 6. Medium grain and decorticated grain width.	Lodging resistance, Anaerobic germination, flash flood tolerance
			DST 8-4-4	1. Strong pubescence of blade surface 2. Thick stem thickness 3. Long panicle length 4. Medium panicle number per plant (>11) 5. High 1000 grain weight 6. Medium grain and decorticated grain width.	Lodging resistance, Anaerobic germination, stagnant flood tolerance
		Lodging resistance	MTU 2251A-136-11-1	1. Strong pubescence of blade surface 2. Thick stem thickness	

Fig.1 Dendogram depicting clustering pattern of the twenty four entries of rice using SSR markers. The scale at the bottom is Jaccard's similarity coefficient of genetic similarity



MTU 2546A-34-1-9-1 NIL of Swarna with non-lodging trait showed highest genome recovery of 94.1 %. NILs MTU 2547A-78-19-1-1 and MTU 2547A-95-1-11-1 showed highest genome recovery 93.7 % and 90.2 % respectively for recurrent parent Indra. MTU 2251A-136-11-1, NIL of Cottondora sannalu showed genome recovery of 93.8 %.

This NILs with targeted trait of lodging in the background of highly adaptive varieties Swarna (MTU 7029), Cottondora sannalu (MTU 1010) and popular variety Indra (MTU 1061) would withstand adverse effects of cyclones or heavy rains and provide higher yield. Introgression of *SCM2* in Swarna and Indra reported by Girijarani *et al.,* (2015a)

NIL of Cottondora sannalu, DST 8-162-4 showed highest genome recovery of 91.0 %. Above NIL with targeted trait of salinity in the background of highly adaptive variety Cottondora sannalu would perform better in coastal saline soils. Similarly, Hoque *et al.,* (2015) introgressed *Saltol* QTL into the genetic background of BRRI dhan49 using FL 478 as a donor parent through marker-assisted backcrossing and used 56 polymorphic markers for background selection.

Recently, Nareshbabu *et al.,* (2017) transferred a quantitative trait locus (QTL), *Saltol,* using FL 478 as donor into Pusa Basmati 1121 through marker assisted backcrossing.

The background genome recovery in the NILs ranged from 93.3 to 99.4%. The improved NILs were either similar or better than the recurrent parent PB1121 for yield, grain and cooking quality and duration.

Among the 24 entries evaluated, NIL MTU 2336-62-25-38-16 of Pushyami, MTU 2244-119-59-63-40, MTU 2244-119-83-65 of Amara were identified as NILs with targeted trait *Sub1* possessing maximum recovery of respective parents. NILs of Swarna MTU 2546A-34-1-9-1, Indra MTU 2547A-78-19-1-1, MTU 2547A-95-1-11-1 and Cottondora sannalu MTU 2251A-136-11-1 exhibited maximum genome recovery of respective recurrent parent besides possessing targeted loci *SCM2* conferring lodging resistance. Only one NIL of Cottondora sannalu, DST 8-162-4 with *Saltol* loci developed as best line with more genome recovery of recurrent parent.

Agro morphological characterization

Summary results of morphological distinguished characters among NILs and their respective parents are presented in Table 6.

NIL of Pushyami MTU 2336-70-46-25-44 for *Sub1* showed dark green intensity of leaf colour, medium leaf senescence can be differentiated with recurrent parent Pushyami possessing light green intensity of leaf colour and early leaf senescence. Indentified best NIL of Amara MTU 2244-119-59-63-40 for *Sub1* exhibited weak pubescence of blade surface, medium leaf senescence, medium gelatinizing temperature can be differentiated with recurrent parent Amara possessing medium pubescence of blade surface, early leaf senescence, low gelatinizing temperature.

Identified best NIL of Swarna MTU 2546A-34-1-9-1 for *SCM2* showed medium pubescence of blade surface, thick stem thickness and medium leaf senescence can be differentiated with recurrent parent Swarna for absence in pubescence of blade surface, thin stem thickness, early leaf senescence. NIL of popular variety Indra MTU 2547A-78-19-1-1 for *SCM2* showed exhibited no variation in all the agro morphological characters studied with the recurrent parent Indra.

NIL of Cottondora sannalu DST 8-162-4 for *Saltol* showed strong pubescence of blade surface, thick stem thickness, long panicle length, medium panicle number per plant (>11) can be differentiated with recurrent parent Cottondora sannalu possessing weak pubescence of blade surface, medium stem thickness, medium panicle length, low panicle number per plant (<11). NIL of Cottondora sannalu MTU 2251A-136-11-1 for *SCM2* exhibited strong pubescence of blade surface, thick stem thickness can be differentiated with recurrent parent Cottondora sannalu for weak pubescence of blade surface, medium stem thickness.

In the present investigation, molecular markers showing PIC value >0.5 can be used to distinguish NILs from their respective parents apart from gene linked markers of targeted trait. Best NILs with maximum genome recovery of recurrent parent along with morphological distinguished characters were identified.

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