

SSR-BASED MOLECULAR PROFILING OF SELECTED DONORS OF WIDE COMPATIBILITY, ELONGATED UPPERMOST INTERNODE, STIGMA EXSERTION AND SUBMERGENCE TOLERANCE TRAITS AND PARENTAL LINES OF COMMERCIAL RICE (*O. SATIVA* L.) HYBRIDS

RAHUL PRIYADARSHI¹, AKHILESH KUMAR SINGH², DURGA KHANDEKAR³, PRANITHA KORADI⁴,
DEMUDUNAIDU P⁵, PAWAN KHERA⁶, HARIPRASAD AS⁷, K. ULAGANATHAN⁸ & VINAY SHENOY⁹

^{1,2,3,4,5,6,9} Barwale Foundation Research Centre, Hyderabad, Telangana, India

^{1,8} Osmania University, Hyderabad, Telangana, India

⁵ Directorate of Oilseeds Research, Hyderabad, Telangana, India

⁶ International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, Telangana, India

⁷ Indian Institute of Rice Research (Formerly Directorate of Rice Research), Hyderabad, Telangana, India

ABSTRACT

Molecular breeding plays an important role in sustainable agriculture development. Hybrid rice technology aims to increase the yield potential of rice beyond the level of inbred high-yielding varieties (HYVs) by exploiting the phenomenon of hybrid vigour or heterosis. Improvement of hybrid rice parental line is necessary to meet the food security problem. Parental polymorphism was carried with 215 SSR markers between five recurrents and ten donors. During the foreground selection, both reported markers (S5-Indel and BF-S5) were validated for wide compatibility, 2 out of 14 (ART5 and SC3) validates for submergence tolerance, one out of two (RM5) validate for stigma exsertion, whereas 2 of 3 markers (RM5970, RM3476) validated for elongated uppermost internode traits between recurrents and donors. For background selection, maximum polymorphic markers (112) between IR58025eB i.e improved maintainer line with elongated uppermost internode and *Oryza meridionalis* and minimum polymorphic markers (42) between IR79156B and IR91-1591-3 were found. Marker-assisted backcrossing accelerate, the transfer of gene of interest in desirable genetic background. Genotypes IR58025B and IR58025eB emerged as genetically most similar with a value of 97%. The genotypes IR64 Sub1 and *Oryza meridionalis* were found most divergent showing 33% genetic similarity. Dissimilarity coefficient of the generated information obtained on genetic relatedness would be supportive in further rice breeding program.

KEYWORDS: Backcrossing, Parental Polymorphism, Simple Sequence Repeats (SSR), Genetic Diversity

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops of the world and is a primary source of food for more than half of the world's population. India needs to increase its rice production by 2.5 million tonnes a year to meet its requirement in 2050. During the past three decades, hybrid rice has made great contributions to the increase in rice yield (Virmani, 1996; Yuan, 2004). With present and anticipated global food demand further necessitates a significant increase in hybrid rice production to solve food security problem. This can be achieved by improvement of hybrid rice parental lines. It has been reported that magnitude of heterosis, for instance, is in the order of *indica/japonica* > *indica/javanica* > *japonica/javanica* > *indica/indica* > *japonica/japonica* (Yuan, 1994). However, the major problem in the exploitation of

indica/japonica hybrids is the hybrid sterility, which has been the subject of intensive study for over 50 years. Often, donors of WC gene(s) can not directly be utilized as agronomic base for development of commercial varieties/hybrids, mainly because of the fact that they may not combine all the desired agronomic traits. This warrants, in the first place, the need to transfer the targeted WC gene(s) into suitable agronomic bases through adoption of appropriate breeding/selection approaches. Conventional backcross breeding and selection based on phenotype is cumbersome, time-consuming, and not always reliable. If such genes are tagged to reliably detectable DNA markers, transfer of them to desired agronomic bases could be achieved with ease and certainty (Siddiq *et al.*, 2005). Discovery of wide compatibility in rice offers an opportunity for overcoming the reproductive barrier exhibited in hybrids between *indica* and *japonica*, and thereby for using the strong heterosis derived from intersubspecific crosses.

The side effects of the male sterility usually create barriers for cross-pollination of the female parent including incomplete exertion of panicle which prevents access to about 20% of the spikelets and the failure of about 20% of spikelets to open at all (Yan and Li, 1987; Tian, 1991). Thus up to 40% of spikelets may not be available for pollination and subsequent seed production if gibberellin (GA3) technology is not adapted (Yuan and Fu, 1995). Being a self-pollinated crop, commercial production of hybrid seed plays a key role in successful implementation of hybrid rice (Yan *et al.*, 2009). Previous studies have demonstrated that the stigma exertion rate of the male sterile line, the female parent in production of hybrid seed, is a key factor contributing to the efficient improvement of hybrid seed production, since exerted stigmas remain viable up to about 4 days and could continue to accept pollens (Long and Shu, 2000; Tian *et al.*, 2004). Incomplete exertion, known to be caused by reduced level of endogenous GA3 synthesis, is a major bottleneck in obtaining higher seed yield in hybrid rice seed production plots. This problem is usually overcome by application of GA3 (50-100 g/ha in India and 200-250 g/ha in China) which in turn increases the seed production cost and adversely affect the quality of hybrid seed through reduced dormancy and storage life (Honnaiah, 2003). The elongated uppermost internode (*eui*) gene provides a genetic alternative for GA3 application in hybrid rice seed production by overcoming incomplete panicle exertion of cytoplasmic male sterile (CMS) lines. GA3 increase panicle length due to cell division and elongation (Yuan *et al.*, 2003; Tiwari *et al.*, 2011). Two recessive genes (*eui-1* and *eui-2*) mapped on chromosome 5 and 10 respectively have so far been reported to control the internode elongation in rice (Yang *et al.*, 2001; Ma *et al.*, 2004). Submergence is one of the most abiotic constraints for rice production in especially in rainfed low land areas, seriously affects the growth and yield of rice crop. The effect of this stress has intensified in past decades and is predicted to increase in future as a result of global climate change. Developing rice cultivars with tolerance of submergence and with agronomic and quality traits acceptable to farmers is a feasible approach to address this problem. Because of its agricultural importance, rice has been bred intensively resulting in the doubling of the production by adopting high-yielding varieties/hybrids. Wild species of *Oryza* offers a good source of beneficial alleles and can be transferred into cultivars for enhancing yield both in natural and stress conditions (McCouch *et al.*, 2007; Price *et al.*, 2002). Sun *et al.*, (2001) reported that cultivated rice has only 60% of the alleles from wild rice. *Oryza meridionalis* Ng. is an annual diploid (2n=24) wild species, endemic to northern Australia (Ng *et al.*, 1981) and some parts of Irian Jaya and Indonesia (Lu and Silitonga, 1999). Earlier reports on *O. meridionalis* indicates that it has adaptation to arid climatic conditions (Second, 1988), has drought avoidance traits (Brar and Kush, 1997; Somanthri, 2001), and good levels of tolerance to heat compared with *O. sativa* (Andrew *et al.*, 2009), yet it has not been exploited very much in rice breeding programs.

Plant breeding creates novel combinations of genes and develops new crop varieties of economic value. Rate of increase in crop yield is currently declining because production potential of modern cultivars has been remained stagnant

due to narrow genetic base and constraints due to biotic and abiotic stresses. Backcrossing has been a widely used technique in plant breeding for almost a century. The employment molecular markers for marker-assisted backcross breeding (MABB) can enhance the efficiency of introgression of target trait as compared to conventional breeding. Among different classes of available molecular markers, simple sequence repeats (SSRs) are useful for a variety of applications in plant genetics and breeding because of their reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and good genome coverage. SSR markers have been more useful for parental polymorphism study. In rice, microsatellites have been classified into two groups based on length of SSR motif and their potential as informative markers: Class I microsatellites contain perfect SSRs >20 nucleotides in length and Class II contains perfect SSRs >12 nucleotides and <20 nucleotides in length. Class I markers are reported to be highly variable (Cho *et al.*, 2000), whereas Class II SSRs are less variable owing to limited expansion of microsatellite repeat motif during slipped-strand mispairing over the shorter SSR template (Temnykh *et al.*, 2001). As of now, 18 828 Class I microsatellite markers have been identified (IRGSP, 2005) and a high-density SSR map with genome coverage of approximately two SSRs per centimorgan (cM) has been constructed in rice (McCouch *et al.*, 2002).

MABB most commonly used to incorporate one or a few genes into an adapted or elite variety or parental line. In this method first level, markers can be used in combination with or to replace screening for the target gene or QTL. This is referred to as 'foreground selection' (Hospital & Charcosset, 1997). The second level involves selecting BC progeny with the target gene and recombination events between the target locus and linked flanking markers- we refer to this as 'recombinant selection'. The purpose of recombinant selection is to reduce the size of the donor chromosome segment containing the target locus (i.e. size of the introgression). This is important because the rate of decrease of this donor fragment is slower than for unlinked regions and many undesirable genes that negatively affect crop performance may be linked to the target gene from the donor parent-this is referred to as 'linkage drag' (Hospital, 2005). The third level of MABB involves selecting BC progeny with the greatest proportion of recurrent parent (RP) genome, using markers that are unlinked to the target locus-we refer to this as 'background selection'. Background selection refers to the use of tightly linked flanking markers for recombinant selection and unlinked markers to select for the RP (Hospital & Charcosset, 1997; Frisch *et al.*, 1999). Three line system of hybrid rice consist of maintainer line, cytoplasmic male sterile (CMS) line and restorer line. In this study, microsatellite markers were used to (i) Identification of markers for foreground selection among selected parents of rice genotypes; (ii) Identification of markers for background selection among selected parents of rice genotypes; (iii) Genetic diversity and population structure analysis.

MATERIALS AND METHODS

Plant Material

The present study was conducted at Barwale Foundation Research Centre, Hyderabad, India. For this study, 215 SSR markers were surveyed on 15 rice genotypes. Plant material for this study was mentioned in Table 1.

Table 1: List of Recipients and Donors

Genotype	Trait	Marker	Features	References
Donor Lines				
1. <u>Dular</u>	Wide compatibility (WC)	S5-InDe1, BF-S5	Five different wide compatibility genes	Pawan Khera, 2011, Sundaram <i>et al.</i> , 2010, Wang <i>et al.</i> , 1998
2. Samba Mahsuri Sub1	Submergence tolerance	ART5, SC3	Elite fine-grained high yielding and submergence tolerant	Sarkar <i>et al.</i> , 2011; Septiningsih, 2009
3. IR64 Sub1	Submergence tolerance	ART5, SC3	Semidwarf, high-yielding variety and submergence tolerant	Sarkar <i>et al.</i> , 2011; Septiningsih, 2009
4. BR-11 Sub1	Submergence tolerance	ART5, SC3	Widely grown mega-variety of Bangladesh with <i>Sub1</i> gene	Sarkar <i>et al.</i> , 2011; Septiningsih, 2009
5. TDK-1 Sub1	Submergence tolerance	ART5, SC3	Improved variety with <i>Sub1</i> gene	Sarkar <i>et al.</i> , 2011; Septiningsih, 2009
6. CR-1009 Sub1	Submergence tolerance	ART5, SC3	Lowland, photosensitive and late maturing variety with <i>Sub1</i> gene	Sarkar <i>et al.</i> , 2011; Septiningsih, 2009
7. <u>Swarna</u> Sub1	Submergence tolerance	ART5, SC3	First high-yielding submergence tolerant mega variety of India	Sarkar <i>et al.</i> , 2011; Septiningsih, 2009; Neeraja <i>et al.</i> , 2007
8. BF16B	Stigma exsertion	RM5, RM44	High out-crossing potential	Priyadarshi <i>et al.</i> , 2014b; Yan <i>et al.</i> , 2009
9. <u>Oryza meridionalis</u>	Heat tolerance, drought avoidance traits and good source for root traits contributing to moisture stress	-	Adaptation to arid climatic conditions, has drought avoidance traits, good levels of tolerance to heat, good source for root traits contributing to moisture stress	Second, 1988; Brar and Kush, 1997; Somanthri, 2001; Andrew <i>et al.</i> , 2009; Kalmesher <i>et al.</i> , 2012; Mohan <i>et al.</i> , 2012
10. IR91-1591-3	Elongated uppermost internode	RM5970, RM3476	High outcrossing potential and elongated uppermost internode trait	Khera <i>et al.</i> , 2009
Recipient Lines				
1. IR58025B	Maintainer	-	Elite maintainer line of IR58025A	-
2. IR58025eB	Maintainer	-	Improved maintainer line with elongated uppermost internode	Singh <i>et al.</i> , 2007
3. IR79156B	Maintainer	-	Maintainer line of IR79156A	-
4. PRR78	Restorer	RM6100, RM1108	An elite basmati rice restorer line; Restorer for Pusa RH10 aromatic hybrid	Sattari <i>et al.</i> , 2008
5. KMR3	Restorer	RM6100, RM1108, RM7466	An elite <i>indica</i> rice restorer line. Restorer for KRH2 hybrid, one of the most popular public bred non-aromatic hybrids	Sheeba <i>et al.</i> , 2009; Sattari <i>et al.</i> , 2008

DNA Isolation and SSR Genotyping

Genomic DNA was isolated from young and healthy leaf samples following the protocol of Dellaporta *et al.* (1983). With respect to SSR markers, Polymerase Chain Reaction (PCR) was carried out using 15–20 ng of template DNA, 0.05 mM of dNTPs (Eppendorf, USA), 5pM of each forward and reverse primer, 0.5 units of *Taq* DNA polymerase (Bangalore Genei, India) and 1X PCR reaction buffer (Bangalore Genie, India) in a total volume of 15 µl. PCR was carried out using BIO-RAD MyCycler thermal cycler with initial denaturation at 94 °C for 5 min followed by 35 cycles of PCR amplification under the following parameters: 15 seconds at 94 °C, 30 seconds at 55 °C, and 45 seconds at 72 °C, followed by final extension at 72 °C for 6 min. PCR amplified products were resolved on 8% polyacrylamide gel in 0.5 X TBE buffer at 100 V and 1.5% agarose gel in 1X TAE buffer at 110 V. Following staining with ethidium bromide, the gels were visualized under UV light in a gel documentation system.

Cluster Analysis BASED on Molecular Marker Genotyping Data

For diversity analysis, the amplified DNA fragments were scored as alleles for each primer genotype combination. The data was entered into allelic format and subsequently analyzed using the computer package DARwin (V.6.0.5) (Perrier and Jacquemoud-Collet, 2006). The dissimilarity coefficients were calculated and used to ascertain the genetic interrelationship by (1) partitioning the variance of the data sets using principal component analysis (PCA) and (2) constructing a dendrogram using the unweighted pair group method of arithmetic mean average (UPGMA) cluster analysis. The software PowerMarker version 3.25 (Liu and Muse, 2005) was used to calculate the average number of alleles, allele frequency, allele diversity, and polymorphism information content (PIC) values.

Population Structure Analysis

The structure of the population was studied with Structure version 2.3.4 software (Pritchard *et al.*, 2000). Clustering methods with distinctive allele frequency were used to identify the optimum number of population (K)

subgroups. Each individual can be a member of multiple subpopulations with a different coefficient, with the sum of all being equal to 1 (Aranzana *et al.*, 2010). Using admixture model assumptions with correlated alleles, K was presumed to be 2-5, selected after five independent runs. Each run consisted of a burn-in period of 100,000 steps followed by 100,000 Monte Carlo Markov Chain replicates, as suggested by Pritchard and Wen (2003). The estimated log probability of data [LnP(D)] from Structure overestimates the number of subgroups when examining inbred lines. Hence, we also used an ad hoc method for ΔK (Evanno *et al.*, 2005) to estimate that number. The inferred ancestry of $\geq 80\%$ was used to assign the inbreds of the same subgroup, while $< 80\%$ was assigned to be an admixture group (Stich *et al.*, 2005).

RESULTS

Identification of Markers for Foreground Selection

The validation of reported markers for targeted traits is presented in Table 2.

Table 2: Molecular Profiling of Reported Markers for Foreground Selection between Recurrents and Donors

Reported Markers	IR58025B	IR58025eB	Dular	Samba Mahsuri sub1	IR64 sub1	BR-11 Sub1	TDK-1 Sub1	CR-1009 sub1	Swarna sub1	IR79156B	BF-16B	<i>Oryza meridionalis</i>	PRR78	KMR3	IR91-1591-3
Wide Compatibility															
S5-InDel	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
BF-S5	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
Submergence Tolerance															
ART5	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
SC3	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
Stigma Exsertion															
RM5	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Elongated Uppermost Internode															
RM5970	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
RM3476	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+

Wide Compatibility (WC) Trait

WC is a dominant trait. The major WC gene locus *S5ⁿ* in rice (*Oryza sativa* L.) located on chromosome 6. Reported gene-based markers (S5-InDel, BF-S5) were used for screening of *S5ⁿ* allele among 15 rice genotypes. Both markers validated between recurrents and donor (Dular). The present results showed the presence of *S5ⁿ* allele even in Swarna Sub 1. The representative gel of foreground selection was mentioned in Figure 1.

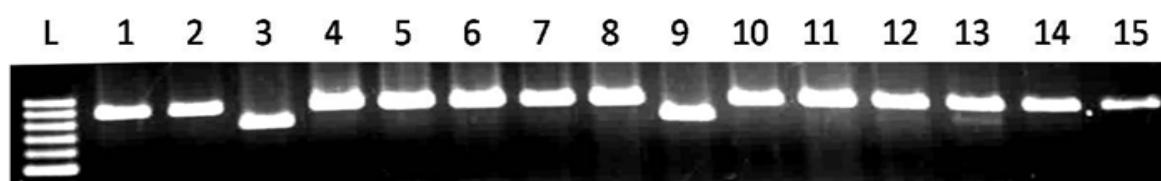


Figure 1: Amplification Profile of 15 rice genotypes with BF-S5 on Chromosome 6 for wide Compatibility Trait.

Amplicons were Resolved on 1.5% Agarosegel Electrophoresis(AGE) Stained with Ethidium Bromide. Details of Genotypes: L=100bp Ladder, 1= IR58025B, 2= IR58025eB, 3=Dular, 4=Samba Mahsuri Sub1, 5=IR64Sub1, 6=BR11Sub1, 6=BR-11Sub1, 7=TDK-1Sub1, 8=CR-1009Sub1, 9=SwarnaSub1, 10=IR79156B, 11=BF-16B, 12=Oryza Meridionalis, 13=PRR78, 14=KMR3, 15=IR91-1591-3

Submergence Tolerance (SUB 1) Trait

SUB 1 is a dominant trait. The major submergence tolerance gene (*Sub1*) in rice (*Oryza sativa* L.) located on chromosome 9. Molecular screening of *Sub1* trait was carried out with 14 reported markers. Only two markers (ART5, SC3) validated between recurrents and donors.

Stigma Exsertion (SE) Trait

SE is a dominant trait and plays a role in increasing the outcrossing potential. It has been reported on all 12 chromosomes. We used RM5 for single stigma exsertion and dual stigma exsertion on chromosome 1 and RM 44 for dual stigma exsertion on chromosome 8 among 15 rice genotypes. Out of two markers only RM5 validated between recurrents and donor.

Elongated Uppermost Internode (EUI) Trait

EUI is a recessive trait that helps increase the outcrossing potential in rice. This trait is useful for CMS line, where 20% of the spikelets remain within flag leaf. Three reported markers for EUI trait on chromosome 5 used among 15 rice genotypes. Only two markers, namely RM5970 and RM3476 validated among recurrents and donor.

IDENTIFICATION OF MARKERS FOR BACKGROUND SELECTION

The present study was conducted to screening of background markers using 215 microsatellite markers across the genome. For background selection, maximum polymorphic markers (112) between IR58025eB i.e improved maintainer line with elongated uppermost internode and *Oryza meridionalis* and minimum polymorphic markers (42) between IR79156B and IR91-1591-3 were found. Details on number of polymorphic markers are mentioned in Table 3.

Table 3: List of Polymorphic Markers among 15 Rice Genotypes with 215 SSR Markers

Genotypes	IR58025B	IR58025eB	Dular	Samba Mahsuri Sub1	IR64 Sub1	BR-11 Sub1	TDK-1 Sub1	CR-1009 Sub1	Swarna Sub1	IR79156B	BF-16B	<i>Oryza meridionalis</i>	PRR78	KMR3	IR91-1591-3
IR58025B	0														
IR58025eB	4	0													
Dular	96	95	0												
Samba Mahsuri Sub1	62	61	92	0											
IR64 Sub1	99	94	119	97	0										
BR-11 Sub1	57	53	99	65	98	0									
TDK-1 Sub1	64	60	93	50	99	57	0								
CR-1009 Sub1	71	69	99	93	104	49	63	0							
Swarna Sub1	71	69	84	75	98	41	71	64	0						
IR79156B	31	30	90	51	87	46	58	62	68	0					
BF-16B	60	58	100	54	95	52	66	85	75	46	0				
<i>Oryza meridionalis</i>	111	112	110	114	120	117	113	116	110	106	113	0			
PRR78	63	62	103	74	85	61	71	77	68	50	66	107	0		
KMR3	65	65	73	69	100	59	68	62	61	61	74	110	68	0	
IR91-1591-3	46	43	84	74	103	58	72	67	67	42	55	111	41	34	0

Further, list of polymorphic markers is presented in Supplementary Table 8. However, the polymorphism survey for selected recurrents and donors were carried further and the details on the primers used is mentioned in Table 4.

Table 4: List of Polymorphic Markers among Selected Recurrents and Donors

S. No.	Combination	No. Of Markers Used	Polymorphic Markers
1	IR58025eB/Dular	486	259
2	IR58025B/Dular	486	259
3	IR58025eB/Swarna Sub1	486	160
4	IR58025B/ Swarna Sub1	486	160
5	IR58025B/BF-16B	540	181

The representative gel of background selection is depicted in Figure 2.

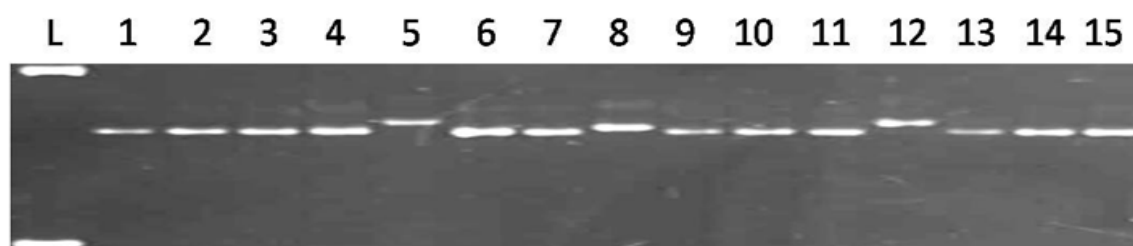


Figure 2: Amplification Profile of 15 Rice Genotypes with RM22188 on Chromosome 7. Amplicons were Resolved on 8% Non-Denaturation PAGE Stained with Ethidium Bromide. Details of Genotypes: L-100 bp Ladder, 1=IR58025B, 2=IR58025eB, 3=Dular, 4=Samba Mahsuri Sub1, 5=IR64 Sub1, 6=BR-11 Sub1, 6=BR-11 Sub1, 7=TDK-1 Sub1, 8=CR-1009 Sub1, 9=Swarna Sub1, 10=IR79156B, 11=BF-16B, 12=Oryza Meridionalis, 13=PRR78, 14=KMR3, 15=IR91-1591-3

GENETIC DIVERSITY AND POPULATION STRUCTURE ANALYSIS

Selection of SSR Markers for Genetic Diversity

Of the 215 SSR marker used, 117 were polymorphic with an average of 17.91 markers per chromosome. The amplified fragments ranged from 80 to 885 bp in size. Further, a total of 510 alleles were detected with an average 2.88 alleles per locus during the study. The Polymorphism Information Content (PIC) values for all the polymorphic primers across 15 rice genotypes varied from 0.12 to 0.77, with an average of 0.36. Detailed information regarding the 177 polymorphic SSRs along with their PIC values is given in Table 5.

Table 5: Selected Markers from Different Chromosomes and their PIC Values

S. No.	Primer	Chr.	Position	Amp. Size (Bp)	Annealing Temp.	Major Allele Frequency	Alleleno.	Gene Diversity	Pic
1	RM495	1	0.21	130-140	58	0.53	2.00	0.50	0.37
2	RM3148	1	0.74	150-175	58	0.60	4.00	0.59	0.55
3	RM1	1	4.63	80-115	58	0.53	5.00	0.65	0.61
4	RM283	1	4.88	160-165	58	0.93	2.00	0.12	0.12
5	RM7466	1	5.78	140-145	58	0.73	2.00	0.39	0.31
6	RM272	1	5.94	130-135	58	0.67	2.00	0.44	0.35
7	RM1360	1	6.21	275-280	58	0.73	2.00	0.39	0.31
8	RM5800	1	7.39	160-180	58	0.53	4.00	0.62	0.57
9	RM576	1	8.13	120-135	58	0.47	4.00	0.65	0.59
10	RM294A	1	10.07	150-170	58	0.67	3.00	0.48	0.41
11	RM493	1	12.28	175-205	58	0.27	6.00	0.80	0.77
12	RM9	1	23.32	145-180	58	0.87	3.00	0.24	0.23
13	RM5	1	23.97	110-125	58	0.47	4.00	0.67	0.61
14	RM39	1	28.13	110-120	58	0.33	4.00	0.74	0.69

Table 5: Contd.,

15	RM443	1	28.33	120-125	58	0.67	2.00	0.44	0.35
16	RM11549	1	28.63	190-195	58	0.53	2.00	0.50	0.37
17	RM11558	1	28.95	85-95	58	0.87	3.00	0.24	0.23
18	RM11573	1	29.32	170-180	58	0.80	3.00	0.34	0.31
19	RM1152	1	30.09	290-300	58	0.47	3.00	0.63	0.56
20	RM6436	1	30.26	140-145	58	0.87	2.00	0.23	0.20
21	RM11629	1	30.47	290-310	58	0.67	3.00	0.48	0.41
22	RM128	1	30.73	160-170	58	0.87	3.00	0.24	0.23
23	RM11649	1	30.76	190-250	58	0.80	2.00	0.32	0.27
24	RM11687	1	31.76	295-310	58	0.60	4.00	0.58	0.53
25	RM3632	1	31.91	295-300	58	0.93	2.00	0.12	0.12
26	RM297	1	32.09	150-180	58	0.67	4.00	0.51	0.46
27	RM11748	1	33.24	175-185	58	0.73	4.00	0.44	0.41
28	RM11722	1	32.44	175-180	58	0.80	3.00	0.34	0.31
29	RM11734	1	32.85	130-135	58	0.87	2.00	0.23	0.20
30	RM472	1	37.88	220-290	58	0.87	3.00	0.24	0.23
31	RM165	1	40.10	95-105	58	0.93	2.00	0.12	0.12
32	RM104	1	40.16	135-140	58	0.87	3.00	0.24	0.23
33	RM3340	2	0.38	115-135	58	0.53	4.00	0.62	0.57
34	RM12351	2	1.05	145-150	58	0.60	2.00	0.48	0.36
35	RM7033	2	1.66	180-190	58	0.67	4.00	0.51	0.46
36	RM12435	2	2.08	250-260	58	0.87	2.00	0.23	0.20
37	RM12492	2	3.16	190-200	58	0.53	2.00	0.50	0.37
38	RM12624	2	5.00	380-385	58	0.93	2.00	0.12	0.12
39	RM12646	2	5.21	150-155	58	0.93	2.00	0.12	0.12
40	RM12673	2	5.67	100-105	58	0.93	2.00	0.12	0.12
41	RM12696	2	6.14	300-305	58	0.73	2.00	0.39	0.31
42	RM322	2	7.43	105-110	58	0.53	2.00	0.50	0.37
43	RM12939	2	9.86	190-200	58	0.53	3.00	0.55	0.46
44	RM561	2	18.76	195-200	58	0.80	2.00	0.32	0.27
45	RM341	2	19.34	160-185	58	0.40	5.00	0.74	0.70
46	RM475	2	20.40	195-205	58	0.67	3.00	0.48	0.41
47	RM262	2	20.79	155-170	58	0.47	3.00	0.60	0.52
48	RM263	2	25.86	160-195	58	0.33	5.00	0.73	0.68
49	RM450	2	28.62	130-140	58	0.87	3.00	0.24	0.23
50	RM240	2	31.49	125-140	58	0.67	3.00	0.50	0.44
51	RM13992	2	32.12	200-210	58	0.80	3.00	0.34	0.31
52	RM14285	3	0.60	85-95	58	0.87	3.00	0.24	0.23
53	RM14302	3	0.86	80-95	58	0.60	3.00	0.56	0.50
54	RM6297	3	1.76	130-150	58	0.80	3.00	0.34	0.31
55	RM231	3	2.45	180-190	58	0.53	3.00	0.55	0.46
56	RM489	3	4.33	195-210	58	0.87	2.00	0.23	0.20
57	RM157B	3	9.49	85-105	58	0.87	2.00	0.23	0.20
58	RM14796	3	10.36	90-95	58	0.73	2.00	0.39	0.31
59	RM14810	3	10.65	150-155	58	0.93	2.00	0.12	0.12
60	RM564	3	18.58	250-260	58	0.93	2.00	0.12	0.12
61	RM14234	3	21.20	190-195	58	0.80	2.00	0.32	0.27
62	RM411	3	21.42	185-190	58	0.60	2.00	0.48	0.36
63	RM16	3	23.12	180-190	58	0.87	2.00	0.23	0.20
64	RM15573	3	25.01	395-410	58	0.67	3.00	0.48	0.41
65	RM426	3	27.58	170-185	58	0.40	4.00	0.69	0.64
66	RM335	4	0.68	135-150	58	0.47	4.00	0.61	0.54
67	RM32	4	8.04	150-165	58	0.93	2.00	0.12	0.12
68	RM16792	4	18.01	190-220	58	0.53	4.00	0.60	0.53
69	RM6314	4	18.44	160-185	58	0.40	3.00	0.66	0.58

Table 5: Contd.,

70	RM16830	4	18.68	145-150	58	0.67	2.00	0.44	0.35
71	RM16843	4	18.90	130-135	58	0.93	2.00	0.12	0.12
72	RM16852	4	19.01	105-120	58	0.53	4.00	0.62	0.57
73	RM3742	4	19.74	155-160	58	0.87	3.00	0.24	0.23
74	RM1359	4	19.86	105-140	58	0.80	4.00	0.35	0.33
75	RM119	4	21.24	180-185	58	0.53	2.00	0.50	0.37
76	RM3866	4	23.17	170-250	58	0.73	4.00	0.44	0.41
77	RM6540	4	23.44	90-95	58	0.87	2.00	0.23	0.20
78	RM17155	4	24.11	80-85	58	0.93	2.00	0.12	0.12
79	RM1388	4	25.03	95-220	58	0.67	3.00	0.50	0.44
80	RM241	4	26.85	140-150	58	0.93	2.00	0.12	0.12
81	RM317	4	29.06	130-180	58	0.67	4.00	0.51	0.46
82	RM17479	4	30.90	90-100	58	0.53	3.00	0.59	0.51
83	RM17506	4	31.52	190-195	58	0.60	2.00	0.48	0.36
84	RM17521	4	31.86	160-170	58	0.47	3.00	0.60	0.52
85	RM3335	4	32.84	250-260	58	0.93	2.00	0.12	0.12
86	RM13	5	2.99	95-160	58	0.33	7.00	0.79	0.76
87	RM574	5	3.45	270-280	58	0.67	3.00	0.50	0.44
88	RM509	5	16.32	160-180	58	0.93	2.00	0.12	0.12
89	RM6054	5	22.77	175-180	58	0.53	2.00	0.50	0.37
90	RM5970	5	23.94	120-128	58	0.47	4.00	0.61	0.54
91	RM31	5	28.61	145-165	58	0.40	3.00	0.66	0.58
92	RM204	6	3.16	105-120	58	0.73	3.00	0.42	0.37
93	RM225	6	3.41	100-120	58	0.47	4.00	0.68	0.62
94	RM19462	6	4.00	330-345	58	0.93	2.00	0.12	0.12
95	RM19521	6	4.71	130-135	58	0.87	2.00	0.23	0.20
96	RM111	6	5.09	125-130	58	0.73	2.00	0.39	0.31
97	RM19552	6	5.21	100-115	58	0.60	4.00	0.58	0.53
98	RM19592	6	5.66	105-180	58	0.87	2.00	0.23	0.20
99	S5-t1	6	5.75	381-517	58	0.87	2.00	0.23	0.20
100	BF S5	6	5.75	739-885	58	0.87	2.00	0.23	0.20
101	S5 InDel	6	5.75	281-417	58	0.87	2.00	0.23	0.20
102	RM19614	6	6.04	330-345	58	0.47	3.00	0.56	0.46
103	RM276	6	6.23	95-150	58	0.47	4.00	0.67	0.61
104	RM50	6	6.37	95-100	58	0.80	2.00	0.32	0.27
105	RM136	6	8.75	100-105	58	0.80	2.00	0.32	0.27
106	RM3207	6	17.73	205-210	58	0.93	2.00	0.12	0.12
107	RM275	6	24.32	120-125	58	0.53	2.00	0.50	0.37
108	RM412	6	30.32	180-200	58	0.93	2.00	0.12	0.12
109	RM20705	6	30.44	150-155	58	0.87	2.00	0.23	0.20
110	RM20712	6	30.58	365-370	58	0.93	2.00	0.12	0.12
111	RM141	6	31.00	100-105	58	0.93	2.00	0.12	0.12
112	RM20827	7	0.52	90-100	58	0.60	3.00	0.52	0.44
113	RM20847	7	0.76	180-185	58	0.67	2.00	0.44	0.35
114	RM20884	7	1.30	160-170	58	0.47	3.00	0.63	0.56
115	RM21183	7	5.77	150-155	58	0.93	2.00	0.12	0.12
116	RM21194	7	6.03	95-105	58	0.67	3.00	0.48	0.41
117	RM5672	7	6.37	95-115	58	0.87	3.00	0.24	0.23
118	RM21242	7	6.76	145-155	58	0.87	3.00	0.24	0.23
119	RM445	7	17.46	180-190	58	0.67	3.00	0.48	0.41
120	RM432	7	18.95	165-300	58	0.53	4.00	0.62	0.57
121	RM560	7	19.58	270-300	58	0.93	2.00	0.12	0.12
122	RM234	7	25.47	145-170	58	0.40	5.00	0.67	0.61
123	RM10	7	22.18	150-155	58	0.67	2.00	0.44	0.35
124	RM22081	7	27.65	140-190	58	0.80	3.00	0.34	0.31

Table 5: Contd.,

125	RM22143	7	28.98	185-195	58	0.53	4.00	0.60	0.53
126	RM22153	7	29.06	125-130	58	0.93	2.00	0.12	0.12
127	RM22168	7	29.48	185-380	58	0.80	4.00	0.35	0.33
128	RM22188	7	29.66	175-185	58	0.80	3.00	0.34	0.31
129	RM25	8	4.37	140-150	58	0.80	3.00	0.34	0.31
130	RM544	8	5.10	190-195	58	0.93	2.00	0.12	0.12
131	RM44	8	11.75	100-110	58	0.80	2.00	0.32	0.27
132	RM23077	8	19.64	185-200	58	0.53	4.00	0.62	0.57
133	RM342	8	19.96	125-150	58	0.40	6.00	0.71	0.67
134	RM284	8	21.14	150-155	58	0.93	2.00	0.12	0.12
135	RM210	8	22.47	130-155	58	0.60	5.00	0.60	0.56
136	RM281	8	27.89	130-150	58	0.73	4.00	0.44	0.41
137	RM23683	9	0.97	295-300	58	0.73	2.00	0.39	0.31
138	RM5799	9	3.80	90-100	58	0.87	2.00	0.23	0.20
139	RM23805	9	4.50	260-270	58	0.60	3.00	0.56	0.50
140	RM23887	9	6.54	250-260	58	0.67	3.00	0.48	0.41
141	RM23917	9	7.30	260-265	58	0.87	2.00	0.23	0.20
142	RM23958	9	7.99	170-190	58	0.53	4.00	0.62	0.57
143	RM105	9	12.55	135-145	58	0.60	3.00	0.55	0.48
144	RM524	9	12.92	180-190	58	0.87	2.00	0.23	0.20
145	RM566	9	14.70	250-260	58	0.87	3.00	0.24	0.23
146	RM278	9	19.32	155-170	58	0.80	3.00	0.34	0.31
147	RM160	9	19.78	90-120	58	0.80	3.00	0.34	0.31
148	RM107	9	20.06	295-305	58	0.47	3.00	0.63	0.56
149	RM216	10	5.35	185-195	58	0.73	3.00	0.42	0.37
150	RM25103	10	5.79	100-110	58	0.93	2.00	0.12	0.12
151	RM184	10	16.35	200-205	58	0.73	2.00	0.39	0.31
152	RM258	10	18.01	155-165	58	0.60	3.00	0.55	0.48
153	RM269	10	18.02	180-190	58	0.80	3.00	0.34	0.31
154	RM6737	10	18.71	165-205	58	0.33	6.00	0.79	0.76
155	RM5373	10	18.72	110-120	58	0.67	3.00	0.50	0.44
156	RM25651	10	18.80	295-300	58	0.60	2.00	0.48	0.36
157	RM25653	10	18.80	140-155	58	0.40	3.00	0.66	0.58
158	RM6100	10	18.81	175-195	58	0.40	4.00	0.68	0.62
159	RM171	10	19.04	295-300	58	0.67	2.00	0.44	0.35
160	RM25664	10	19.04	180-195	58	0.80	3.00	0.34	0.31
161	RM25669	10	19.13	150-155	58	0.53	2.00	0.50	0.37
162	RM25670	10	19.13	185-195	58	0.67	3.00	0.48	0.41
163	RM1108	10	19.16	125-130	58	0.53	2.00	0.50	0.37
164	RM228	10	22.24	120-150	58	0.47	6.00	0.71	0.68
165	RM7203	11	1.08	95-110	58	0.80	2.00	0.32	0.27
166	RM441	11	6.08	195-200	58	0.93	2.00	0.12	0.12
167	RM26370	11	8.24	165-170	58	0.87	2.00	0.23	0.20
168	RM26384	11	8.55	280-290	58	0.53	3.00	0.59	0.51
169	RM26464	11	10.34	100-110	58	0.93	2.00	0.12	0.12
170	RM7226	11	14.05	160-190	58	0.40	4.00	0.66	0.59
171	RM287	11	16.76	100-120	58	0.67	2.00	0.44	0.35
172	RM457	11	19.06	245-250	58	0.73	2.00	0.39	0.31
173	RM206	11	22.01	145-170	58	0.53	6.00	0.66	0.62
174	RM7003	12	6.77	95-105	58	0.87	3.00	0.24	0.23
175	RM28561	12	24.04	285-290	58	0.60	2.00	0.48	0.36
176	RM28585	12	24.38	150-180	58	0.87	3.00	0.24	0.23
177	RM28669	12	25.52	185-195	58	0.73	4.00	0.44	0.41
Mean						0.70	2.88	0.41	0.36

Further, a dendrogram was constructed using UPGMA (Unweighted Pair Group Method with Arithmetic Averages) method comprising three main groups that is, A, B and C (Figure 3).

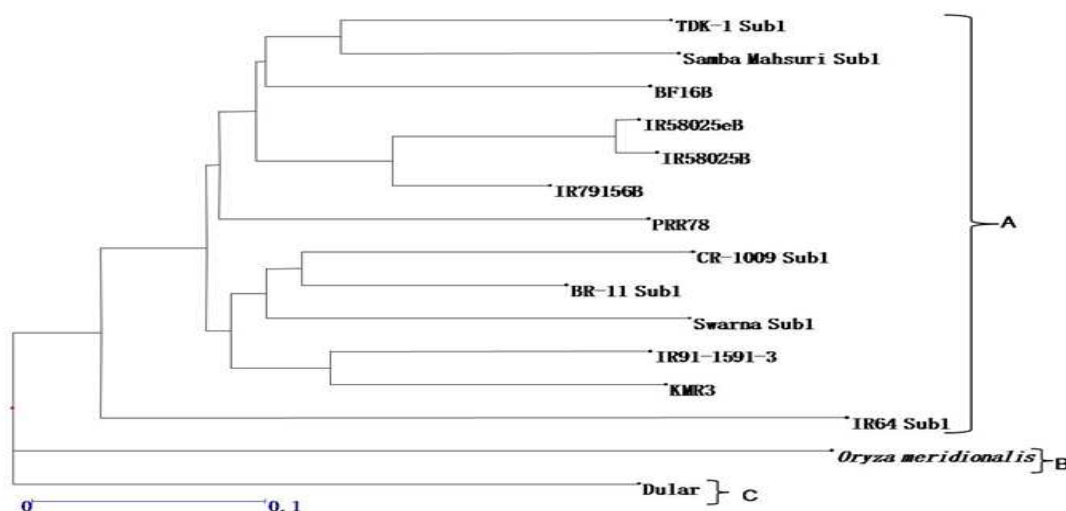


Figure 3: Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Cluster Tree of 15 Rice Genotypes by 177 Microsatellites Markers

Genotypes IR58025B and IR58025eB emerged as genetically most similar with a value of 97%. However, IR64 Sub1 and *Oryza meridionalis* were found most divergent with 33% similarity. Details of genetic dissimilarity among the parents is given in Table 6.

Table 6: Average Estimates of Genetic Dissimilarity between 15 Rice Genotypes using 177 SSR Markers

Genotypes	IR58025B	IR58025eB	Dular	Samba Mahsuri Sub1	IR64 Sub1	BR-11 Sub1	TDK-1 Sub1	CR-1009 Sub1	Swarna Sub1	IR79156B	BF-16B	<i>Oryza Meridionalis</i>	PRR78	KMR3
IR58025eB	0.03													
Dular	0.56	0.55												
Samba Mahsuri Sub1	0.36	0.36	0.52											
IR64 Sub1	0.56	0.55	0.67	0.55										
BR-11 Sub1	0.32	0.31	0.54	0.36	0.55									
TDK-1 Sub1	0.36	0.34	0.52	0.29	0.56	0.32								
CR-1009 Sub1	0.41	0.41	0.56	0.38	0.58	0.28	0.36							
Swarna Sub1	0.42	0.41	0.49	0.44	0.56	0.31	0.40	0.37						
IR79156B	0.18	0.18	0.53	0.29	0.51	0.27	0.33	0.36	0.40					
BF-16B	0.34	0.33	0.56	0.31	0.57	0.32	0.38	0.40	0.43	0.27				
<i>Oryza meridionalis</i>	0.63	0.63	0.62	0.65	0.67	0.64	0.63	0.65	0.62	0.60	0.63			
PRR78	0.35	0.35	0.60	0.43	0.49	0.36	0.41	0.45	0.41	0.29	0.37	0.60		
KMR3	0.39	0.38	0.54	0.41	0.58	0.35	0.41	0.37	0.36	0.35	0.42	0.62	0.38	
IR91-1591-3	0.39	0.37	0.49	0.43	0.59	0.33	0.42	0.39	0.39	0.34	0.34	0.63	0.35	0.28

Principal Component Analysis (PCA) based on SSR Data

PCA was utilized to derive a 2-dimensional scatter plot of individuals, such that the geometrical distances among individuals in the plot reflect the genetic distances among them with minimal distortion (Figure 4). However, the PCA was not completely compatible with that of UPGMA dendrogram.

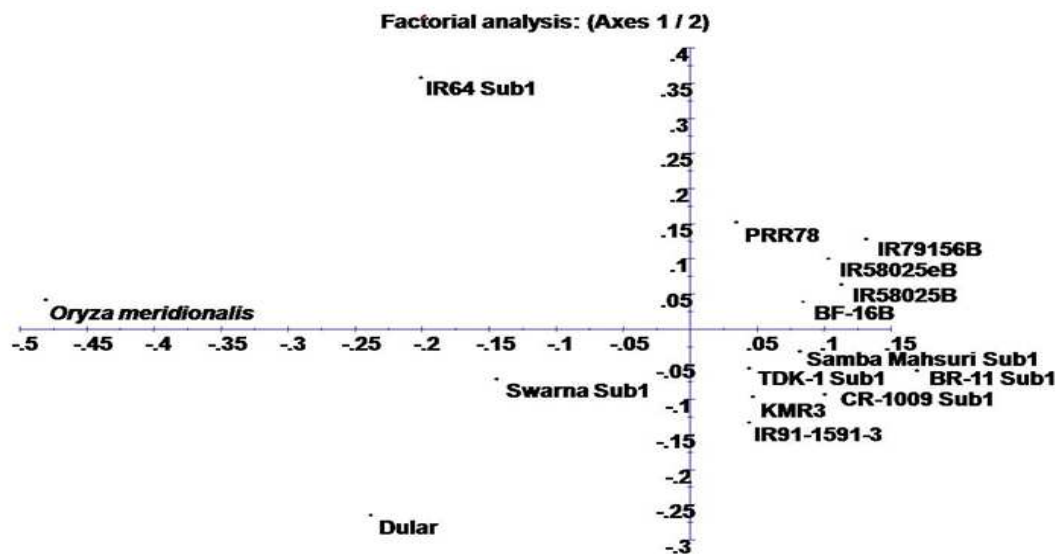


Figure 4: Principal Component Analysis (PCA) of 15 Genotypes of Rice Based on Polymorphism Displayed by 177SSR Markers

Population Structure Analysis

Structure is the most extensively used clustering software applied to detect population genetic structure (Pritchard *et al.*, 2000). Inferred ancestry of individuals has three clusters (Table 7).

Table 7: Inferred Ancestry of Individuals Included in Structure

S. No.	Label (%Miss)	Cluster 1	Cluster 2	Cluster 3
1	IR58025B (0)	0.000	0.000	0.999
2	IR58025eB (0)	0.000	0.000	0.999
3	Dular (0)	0.000	0.999	0.001
4	Samba Mahsuri sub1 (0)	0.001	0.002	0.997
5	IR64 sub1 (0)	1.000	0.000	0.000
6	BR-11 Sub1 (0)	0.000	0.000	0.999
7	TDK-1 Sub1 (0)	0.001	0.003	0.995
8	CR-1009 sub1 (0)	0.000	0.001	0.999
9	Swarna sub1 (0)	0.003	0.172	0.825
10	IR79156B (0)	0.001	0.000	0.998
11	BF-16B (0)	0.000	0.000	0.999
12	<i>Oryza meridionalis</i> (0)	0.000	0.999	0.000
13	PRR78 (0)	0.083	0.001	0.916
14	KMR3 (0)	0.000	0.001	0.999
15	IR91-1591-3 (0)	0.000	0.002	0.998

Note: % Miss denotes no gap with markers profiling among parents

DISCUSSIONS

From application angle, DNA markers are widely used in marker-assisted breeding/selection. For effective use of breeding identification of appropriate markers for foreground and background selection is equally important. It facilitates speedy and reliable recovery of recurrent genome. Two hundred and fifteen SSR primers used in the present investigation are distributed across the genome. The outcome of the present study can be used for further introgression of targeted traits through MABB. Previous reports mentioned many success story for transfer of gene of interest through marker-assisted backcrossing. Priyadarshi *et al.*, (2014a) reported about transfer of *S5ⁿ* allele from Dular (*Aus indica*) into IR58025eB, a

maintainer line having elongated uppermost internode (EUI) trait by marker-assisted backcrossing. Transfer of stigma exsertion (SE) trait from BF-16B (90% total SE) into IR58025B (35% total SE) by marker-assisted backcrossing (Priyadarshi *et al.*, 2014b). Transfer of stigma exsertion trait from *indica* cultivar BF-16B (90% total SE) into IR79156B (46% total SE), a wild-abortive maintainer line of rice hybrid parent, via marker-assisted backcross breeding (Koradi *et al.*, 2015). The evaluation of improved IR58025eA having 'eui' gene indicated a significant improvement of panicle exsertion, panicle length and number of spikelets per panicle to the tune of 20.8, 10.7 and 13.6%, respectively (Priyadarshi *et al.*, 2012). An increase of 30%, 15% and 5% for stigma exsertion, total stigma length and style length was observed in CMS line than its maintainer lines. Furthermore, stigma width was decreased by 10% in CMS lines, than its male counterpart. Highest stigma exsertion percentage was observed in wild relatives, followed by landraces, maintainers and CMS lines. Stigma exsertion was found to be positively correlated with panicle length, grain length and ratio of grain length to grain width, while it was negatively correlated with spikelet width (Singh *et al.*, 2012). In rice, the feasibility of marker assisted back cross breeding (MABB) to pyramid BB resistance genes has been well demonstrated (Sundaram *et al.*, 2008). Yang *et al.*, (1997) have developed wide compatible restorers by anther culture technique using *indica* restorer/WCV and *japonica* restorers/WCV for utilization in restoration of fertility of both WA and BT cytoplasm. Neeraja *et al.*, (2007) has been successfully introgressed SUB 1 through marker-assisted backcrossing (MAB) into a popular high-yielding variety from India, Swarna, within a 2-year time frame.

The design of marker-assisted backcrossing programs was studied with respect to the introgression of single dominant and recessive genes (Hospital *et al.*, 1992), two genes (Frisch and Melchinger, 2001), and favorable alleles at quantitative trait loci (Bouchez *et al.*, 2002). More recently, marker-assisted backcrossing for developing libraries of near-isogenic lines was studied (Falke and Frisch, 2011). These studies have mainly focused on optimizing the number of genotyped individuals as well as the positions and density of background selection markers with respect to the required number of marker data points. The optimizations have been carried out assuming marker systems in which each marker locus is analyzed in a separate assay (Prigge *et al.*, 2009). Marker-assisted selection has been more widely employed for simply inherited traits than for polygenic traits, although there are a few success stories in improving quantitative traits through MAS (Babu *et al.*, 2004).

The SSRs are ubiquitous in their distribution in plant genomes and they exhibit higher level of polymorphism, which is attributed to the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Powell *et al.*, 1996). Dendrogram constructed using data of 177 SSR loci by DARwin (V.6.0.5) software package and based on UPGMA method. The three main groups, that is, A, B and C of genotypes have been obtained from the resulting dendrogram. Cluster A was the major one comprising of 13 tested genotypes (TDK-1 Sub1, Samba Mahsuri Sub1, BF-16B, IR58025eB, IR58025B, IR79156B, PRR78, CR-1009 Sub1, BR-11 Sub1, Swarna Sub1, IR91-1591-3, KMR3 and PRR78). Genotype *Oryza meridionalis* present in group B, where as Dular present in group C. IR58025B and IR58025eB emerged as genetically most similar with a value of 97%. The genotypes IR64 Sub1 and *Oryza meridionalis* were found most divergent showing 33% genetic similarity. Dissimilarity coefficient of the generated information obtained on genetic relatedness would be supportive in further breeding of rice, the selection of parents for crossing and will also be helpful in widening the genetic base of breeding materials. The principal component analysis (PCA) did show some degree of similarities with UPGMA cluster analysis. This may be due to the fact that PCA is more informative in differentiation among major groups while the UPGMA cluster analysis provides higher resolution among closely related populations. Mohan *et al.*, 2012 reported that *O. meridionalis* can be novel source of natural genetic variation for the improvement of

rice under irrigated as well as under aerobic condition.

CONCLUSIONS

The polymorphic markers identified among recurrent and donor parents in the present investigation would help in easy introgression of targeted trait through marker assisted back cross breeding, which in turn will improve the parental lines and enhance productivity of hybrid rice.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the Executive Director Barwale Foundation, Hyderabad and Dr. Usha B. Zehr, Director, Barwale Foundation, for providing all the facilities for execution of this research work.

REFERENCES

1. Andrew PS, Paul AH, Brian J, Atwell (2009) Physiological and molecular changes in *Oryza meridionalis* Ng., a heat-tolerant species of wild rice. J Exp Bot 61: 191-202
2. Aranzana MJ, Abbasi E, Howard W, Arus P (2010) Genetic variation, population structure and linkage disequilibrium in peach commercial varieties. BMC Genet 11: 69
3. Babu R, Nair SK, Prasanna BM, Gupta HS (2004) Integrating markerassisted selection in crop breeding- Prospects and challenges. Curr Sci 87: 607-619
4. Bouchez A, Hospital F, Causse M, Gallais A, Charcosset A (2002) Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. Genetics 162: 1945-1959
5. Brar DS, Khush GS (1997) Alien introgression in rice. Plant Mol Biol 35: 35-47
6. Cho YG, Ishii T, Temnykh S *et al.* (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100: 713-722
7. Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA miniprep: version II. Plant Molecular Biology Reporter 1: 19-21
8. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. Mol Ecol 14: 2611-2620
9. Falke KC, Frisch M (2011) Power and false positive rate in QTL detection with near-isogenic line libraries. Heredity 106: 576-584
10. Frisch M, Bohn M, Melchinger AE (1999) Comparison of selection strategies for marker-assisted backcrossing of a gene. Crop Sci 39: 1295-1301
11. Frisch M, Melchinger AE (2001) Marker-assisted backcrossing for introgression of a recessive gene. Crop Sci 41: 1485-1494
12. Honnaiah M (2003) Economics of hybrid rice cultivation and seed production. In Winter school on advances in hybrid rice technology (eds): Viraktamath BC, Ahmed MI, Mishra, B Directorate of Rice Research, Hyderabad, India pp. 391-398

13. Hospital F (2005) Selection in backcross programmes. *Phil Trans R Soc* 360: 1503-1511
14. Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. *Genet* 147: 1469-1485
15. Hospital F, Chevalet C, Mulsant P (1992) Using markers in gene introgression breeding programs. *Genetics* 132: 1199-1210
16. IRGSP (2005) International Rice Genome Sequencing Project. The map-based sequence of the rice genome. *Nat* 436(7052): 793-800
17. Kalmeshwer Gouda P, Mohan Kumar Varma C, Saikumar S *et al.* (2012) Direct selection for grain yield under moisture stress in *Oryza sativa* cv. IR58025B x *Oryza meridionalis* population. *Crop Sci* 52: 501-510
18. Khera P (2011) Genetics and molecular mechanism of wide compatibility genes for exploiting inter-subspecific heterosis in rice (*Oryza sativa* L.). Dissertation, Osmania University, Hyderabad
19. Khera P, Gangashetti MG, Singh S *et al.* (2009) Identification and genetic mapping of elongated uppermost internode gene 'eui' with microsatellite markers in rice (*Oryza sativa* L.). *Journal of Plant Breeding and Crop Science* 1(10): 336-342
20. Koradi P, Singh AK, Priyadarshi R, Khandekar D *et al.* (2015) Introgression of stigma exsertion trait into a maintainer line IR79156B by marker assisted backcross breeding. Accepted for 5th International Conference on Next Generation Genomics and Integrated Breeding for Crop Improvement, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India
21. Liu K, Muse S (2005) Power Marker: new genetic data analysis software, version 3.25. <http://www.powermarker.net>. Accessed 10 Dec 2014
22. Long LH, Shu K (2000) Increasing outcrossing rate of indica hybrid rice. *J Hunan Agric Univ* 26: 205-208 (Chinese with English abstract)
23. Lu BR, Silitonga TS (1999) Wild rice *Oryza meridionalis* was first found in Indonesia (notes from field). *Int Rice Res Notes* 24: 28
24. Ma HL, Zhang SB, Lu Q, Fang XJ, Yang SL and Yang RC (2004) Genetic analysis and molecular mapping of *eui1* gene conferring elongated uppermost internode in the indica rice mutant Xieqingzao eB1 (English). *Chinese J Agr Biotechnol* 1(2): 103-107
25. McCouch SR, Sweeney M, Li J, Jiang H, Thomson M (2007) Through the genetic bottleneck: *O. rufipogonas* a source of trait-enhancing alleles for *O. sativa*. *Euphytica* 154: 317-339
26. McCouch SR, Teytelman L, Xu Y *et al.* (2002) Development and Mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Res* 9: 199-207
27. Mohan Kumar Varma C, Kalmeshwer Gouda P, Saikumar S *et al.* (2012) Transgressive Segregation for Yield Traits in *Oryza sativa* IR58025B X *Oryza meridionalis* Ng. BC₂F₃ Population under Irrigated and Aerobic Conditions. *J Crop Sci Biotech* 15(3): 231-238

28. Neeraja CN, Maghirang-Rodriguez R, Pamplona A *et al.* (2007) A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theor Appl Genet* 115: 767-776
29. Ng NQ, Hawkes JG, Williams JT, Chang TT (1981) The recognition of a new species of rice (*Oryza*) from Australia. *Bot J Linn Soc* 82: 327-33
30. Perrier X, Jacquemoud-Collet JP (2006) DARwin software. <http://darwin.cirad.fr/darwin>. Accessed 10 Dec 2014
31. Powell W, Machray G, Provan J (1996) Polymorphism revealed by simple sequence repeats. *Trends Plant Sci* 1: 215-222
32. Price AH, Townend J, Jones MP, Audebert A, Courtois B (2002) Mapping QTL associated with drought avoidance in upland rice grown in the Philippines and West Africa. *Plant Mol Biol* 48: 683-695
33. Prigge V, Melchinger AE, Dhillon BS, Frisch M (2009) Efficiency gain of marker-assisted backcrossing by sequentially increasing marker densities over generations. *Theor Appl Genet* 119: 23-32
34. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959
35. Pritchard JK, Wen W (2003) Documentation for the Structure software Version 2.3.4 Chicago. http://www.pritch.bsd.uchicagoedu/software/structure2_1.html. Accessed 9 Dec 2014.
36. Priyadarshi R, Singh AK, Khandekar D *et al* (2014a) Stacking of wide compatibility (WC) and elongated uppermost internode (*EUI*) traits in IR58025B, a maintainer line of rice by marker assisted introgression. 3rd International Conference on Agriculture & Horticulture, International Convention Centre (HICC), Hyderabad, India pp.194.
37. Priyadarshi R, Singh AK, Koradi P *et al* (2014b) Introgression of stigma exertion trait into IR58025B, an elite maintainer line of rice (*Oryza sativa* L.). 3rd International Conference on Agriculture & Horticulture, International Convention Centre (HICC), Hyderabad, India pp.152
38. Priyadarshi R, Singh A, Khera P, Khandekar D, Allu RK, Shenoy V (2012) Marker assisted introgression of elongated uppermost internode gene in IR58025A. 6th International Hybrid Rice Symposium, 10-12 September 2012, Hyderabad, India, pp. 96
39. Sarkar RK, Bhattacharjee B (2011) Rice Genotypes with SUB1 QTL Differ in Submergence Tolerance, Elongation Ability during Submergence and Re-generation Growth at Re-emergence. *Rice* 5(7): 1-11
40. Sattari M, Kathiresan A, Glenn B Gregorio, Virmani SS (2008) Comparative genetic analysis and molecular mapping of fertility restoration genes for WA, Dissi, and Gambiaca cytoplasmic male sterility systems in rice. *Euphytica* 160(3): 305-315
41. Second G (1988) Field notes and observations on, a collection of wild rice species in Australia, 3-31 May 1987
42. Septiningsih EM, Pamplona AM, Sanchez DL *et al.* (2009) Development of submergence-tolerant rice cultivars: the *Sub1* locus and beyond. *Annals of Botany* 103: 151-160
43. Sheeba NK, Viraktamath BC, Sivaramakrishnan S *et al.* (2009) Validation of molecular markers linked to fertility

- restorer gene(s) for WA-CMS lines of rice. *Euphytica* 167: 217-227
44. Siddiq EA, Singh S (2005) Wide-compatibility system for yield enhancement of tropical rice through inter-subspecific hybridization. *Advances in Agronomy* 87: 157-209
 45. Singh A, Priyadarshi R, Khandekar D, Allu RK, Shenoy V and Khera P (2012) Validation of molecular markers for stigma exsertion trait in rice. 6th International Hybrid Rice Symposium, 10-12 September 2012, Hyderabad, India, pp. 103
 46. Singh S, Gangashetti MG, Khera P *et al.* (2007) Development of elongated uppermost internode CMS lines for hybrid rice breeding in India. *Molecular Plant Breeding* 5(2): 215-216
 47. Somanthri IH (2001) Wild rice (*Oryza* spp.): their existence and research in Indonesia. *Bull Agro Bio* 5: 14-20
 48. Stich B, Melchinger AE, Frisch M *et al* (2005) Linkage disequilibrium in European elite maize germplasm investigated with SSRs. *Theor Appl Genet* 111: 723-730
 49. Sun CQ, Wang XK, Li ZC, Yoshimura A, Iwata N (2001) Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*Oryza sativa* L.) using RFLP markers. *Theor Appl Genet* 102: 152-162
 50. Sundaram RM, Priya MRV, Biradar SK, Laha GS, Reddy GA, Shobha Rani N, Sarma NP, Sonti RV (2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica* 160: 411-422
 51. Sundaram RM, Sakthivel K, Hariprasad AS *et al.* (2010) Development and validation of a PCR-based functional marker system for the major wide-compatible gene locus *S5* in rice. *Mol Breeding* 26: 719-727
 52. Temnykh SG, Declerck A, Lukashova L *et al.* (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations and genetic marker potential. *Genome Res* 11(8): 1441-52
 53. Tian DC (1991) Cultivation for out-crossing in rice-principles and techniques to achieve high yield in hybrid seed production. Sichuan Press of Sci & Tec, Chengdu (Chinese)
 54. Tian DC, Huang SK, Duan YG, Wang YH (2004) The relationship between flowering and pollination time and outcrossing rate of male sterile lines in hybrid rice seed production. *Hybrid Rice* 19: 50-54 (Chinese with English abstract)
 55. Tiwari DK, Pandey P, Giri SP, Dwivedi JL (2011) Effect of GA3 and other plant growth regulators on hybrid rice seed production. *Asian journal of plant sciences* 10(2): 133-139
 56. Virmani SS (1996) Hybrid rice. *Adv Agron* 57: 377-462
 57. Wang J, Liu KD, Xu CG, Li XH, Zhang Q (1998) The high level of wide compatibility of variety ‘‘Dular’’ has a complex genetic basis. *Theor Appl Genet* 97: 407-411
 58. Yan WG, Li SF (1987) Study on out-crossing characteristics among male sterile lines containing same nucleus in rice. *Hybrid Rice* 4: 8-11 (Chinese)

59. Yan WG, Li Y, Agrama HA *et al.* (2009) Association mapping of stigma and spikelet characteristics in rice (*Oryza sativa* L.). *Mol Breeding* 24: 277
60. Yang JC, Zhu QS, Wang ZQ, Lang YZ (1997) Photosynthetic characteristics, dry matter accumulation, and its translocation in inter sub-specific hybrid rice. *Acta Agronomica Sinica* 23(1): 82–88
61. Yang SL, Ma HL, Xue QP *et al.* (2001) Genetic and microsatellite analysis of a new elongated uppermost internode gene *eui2* of rice. *Acta Botanica Sinica* 43: 67-71
62. Yuan L, Wu X, Liao F, Ma G, Xu Q (2003) *Hybrid Rice Technology*. China Agricultural Press. Beijing, China. pp.132
63. Yuan LP (1994) Increasing yield potentials in rice by exploitation of heterosis. In “Hybrid Rice Technology: New Development and Future Prospects” (S. S. Virmani, Ed.), IRRI, Manila, Philippines pp. 1-6
64. Yuan LP (2004) Hybrid rice technology for food security in the world. *Crop Res* 18: 185-186
65. Yuan LP, Fu XQ (1995) Technology of hybrid rice production. Food and Agriculture Organization of the United Nations, Rome, Italy

APPENDICES

Supplementary

Table 8: List of Polymorphic Markers for Background Selection among Recurrents

S. No.	Combination	Polymorphic Primers
1.	IR58025B/Dular	RM495, RM335, RM11549, RM157B, RM7466, RM11722, RM576, RM1, RM11573, RM11748, RM3148, RM11687, RM11629, RM443, RM1152, RM128, RM5800, RM5, RM493, RM12351, RM7033, RM12492, RM12673, RM263, RM12939, RM240, RM341, RM561, RM489, RM14234, RM14302, RM15573, RM564, RM6314, RM16830, RM16843, RM16852, RM16792, RM1359, RM17506, RM17521, RM3866, RM119, RM6540, RM17479, RM317, RM231, RM13, RM31, RM39, RM19462, RM19521, RM136, RM276, RM204, RM275, RM19552, RM50, RM225, RM234, RM10, RM20884, RM432, RM445, RM22081, RM20827, RM22143, RM23077, RM281, RM210, RM44, RM25, RM5970, RM107, RM23958, RM105, RM216, RM25653, RM269, RM228, RM6737, RM25664, RM25669, RM6100, RM294A, RM25103, RM1108, RM171, RM184, RM206, RM7226, RM457, RM287, RM7203, RM28585, RM28669
2.	IR58025B/Samba Mahsuri Sub1	RM157B, RM7466, RM1360, RM1, RM11748, RM3148, RM11629, RM443, RM1152, RM5800, RM5, RM493, RM12435, RM12492, RM262, RM12939, RM322, RM489, RM14302, RM15573, RM6314, RM16792, RM17506, RM1388, RM17479, RM231, RM13, RM31, RM39, RM19614, RM111, RM276, RM275, RM225, RM234, RM20884, RM445, RM20847, RM3077, RM342, RM210, RM25, RM23683, RM5799, RM216, RM269, RM228, RM258, RM6737, RM25664, RM25651, RM25669, RM5373, RM6100, RM294A, RM1108, RM171, RM206, RM7226, RM7203, RM28561, RM28585
3.	IR58025B/ IR64 Sub1	RM495, RM11734, RM3632, RM272, RM11549, RM9, RM11558, RM11722, RM1360, RM576, RM1, RM11573, RM297, RM11748, RM11687, RM1152, RM6436, RM128, RM5800, RM5, RM12492, RM12646, RM262, RM263, RM12696, RM13992, RM12939, RM240, RM341, RM561, RM322, RM475, RM489, RM426, RM14285, RM14302, RM14810, RM15573, RM411, RM16, RM3742, RM1359, RM17506, RM1388, RM6540, RM17479, RM317, RM13, RM31, RM6054, RM19521, RM19614, RM136, RM412, RM20705, RM276, RM141, RM275, RM19552, RM225, RM3207, RM234, RM21194, RM21242, RM20884, RM432, RM5672, RM445, RM22081, RM22168, RM20847, RM22188, RM22143, RM23077, RM342, RM210, RM5970, RM23683, RM107, RM23958,

		RM23887, RM566, RM23805, RM160, RM278, RM25653, RM269, RM228, RM258, RM6737, RM25651, RM25669, RM5373, RM6100, RM184, RM206, RM26370, RM28561, RM28585
4.	IR58025B/ BR-11 Sub1	RM495, RM104, RM335, RM272, RM11549, RM576, RM1, RM11748, RM493, RM12351, RM263, RM12939, RM341, RM561, RM322, RM489, RM426, RM14302, RM14796, RM411, RM6314, RM16792, RM17506, RM17521, RM3335, RM119, RM1388, RM231, RM574, RM31, RM6054, RM19614, RM276, RM275, RM225, RM234, RM10, RM21194, RM20884, RM20827, RM3340, RM23077, RM342, RM23958, RM105, RM25653, RM269, RM228, RM258, RM6737, RM25651, RM25669, RM206, RM26384, RM7226, RM7203, RM28585
5.	IR58025B/ TDK-1 Sub1	RM495, RM335, RM7466, RM1360, RM1, RM11748, RM3148, RM11629, RM1152, RM5800, RM493, RM12351, RM12492, RM262, RM263, RM12939, RM561, RM322, RM489, RM426, RM14302, RM14796, RM6314, RM16792, RM17506, RM17521, RM1388, RM17479, RM13, RM32, RM31, RM39, RM275, RM50, RM225, RM19592, RM234, RM21194, RM20884, RM432, RM445, RM20847, RM3340, RM281, RM342, RM210, RM44, RM105, RM566, RM269, RM228, RM6737, RM25664, RM25651, RM25669, RM5373, RM6100, RM171, RM206, RM7226, RM457, RM287, RM7203, RM28585
6.	IR58025B/ CR-1009 Sub1	RM495, RM335, RM272, RM576, RM297, RM11748, RM3148, RM11687, RM11629, RM11649, RM1152, RM5800, RM493, RM12351, RM262, RM263, RM12939, RM561, RM475, RM489, RM426, RM6297, RM14796, RM411, RM6314, RM16792, RM1359, RM17506, RM3866, RM1388, RM17479, RM231, RM574, RM13, RM31, RM39, RM6054, RM19614, RM276, RM204, RM275, RM225, RM234, RM20884, RM445, RM22168, RM20847, RM22188, RM22143, RM3340, RM23077, RM281, RM342, RM23683, RM5799, RM107, RM105, RM25653, RM269, RM228, RM6737, RM25670, RM6100, RM1108, RM171, RM206, RM26384, RM7226, RM441, RM7203, RM28585
7.	IR58025B/Swarna Sub1	RM495, RM335, RM272, RM11549, RM576, RM1, RM297, RM11748, RM11687, RM11629, RM443, RM1152, RM5800, RM5, RM493, RM12696, RM12939, RM240, RM561, RM489, RM426, RM14234, RM6297, RM14302, RM14796, RM411, RM6314, RM16830, RM16852, RM16792, RM17506, RM17521, RM119, RM1388, RM17479, RM13, RM6054, RM19614, RM276, RM275, RM19552, RM225, S5-t1, BF S5, S5 InDel, RM234, RM10, RM21194, RM20884, RM5672, RM445, RM20827, RM3340, RM23077, RM342, RM25, RM107, RM105, RM25653, RM269, RM228, RM6737, RM25651, RM5373, RM6100, RM1108, RM7226, RM287, RM7203, RM28561, RM28585
8.	IR58025B/ BF-16B	RM283, RM272, RM11549, RM9, RM472, RM576, RM1, RM11748, RM3148, RM11629, RM5800, RM493, RM7033, RM12435, RM12492, RM12624, RM262, RM341, RM561, RM475, RM489, RM14302, RM6314, RM16852, RM16792, RM17506, RM17521, RM119, RM1388, RM17479, RM231, RM574, RM13, RM31, RM39, RM19614, RM111, RM19552, RM225, RM234, RM445, RM20847, RM22143, RM3340, RM23077, RM342, RM210, RM278, RM216, RM25653, RM269, RM228, RM258, RM25651, RM25669, RM294A, RM206, RM7203, RM7003, RM28585
9.	IR58025B/ <i>Oryza meridionalis</i>	RM11734, RM104, RM335, RM11549, RM9, RM11558, RM11722, RM472, RM576, RM1, RM11573, RM165, RM297, RM11748, RM11687, RM11629, RM11649, RM443, RM1152, RM5800, RM5, RM493, RM7033, RM262, RM263, RM13992, RM240, RM341, RM561, RM322, RM450, RM489, RM426, RM14234, RM14285, RM6297, RM14302, RM15573, RM6314, RM16830, RM16852, RM3742, RM17521, RM3866, RM1388, RM17479, RM241, RM17155, RM317, RM231, RM574, RM13, RM31, RM509, RM136, RM111, RM276, RM204, RM19552, RM50, RM225, RM234, RM560, RM21194, RM21242, RM20884, RM432, RM445, RM22081, RM22168, RM21183, RM22188, RM22143, RM22153, RM3340, RM23077, RM281, RM210, RM284, RM524, RM107, RM23958, RM23887, RM105, RM566, RM23805, RM160, RM278, RM216, RM25653, RM269, RM228, RM258, RM6737, RM25651, RM25670, RM294A, RM1108, RM171, RM184, RM206, RM26370, RM26384, RM26464, RM7226, RM457, RM287, RM7203, RM7003, RM28585, RM28669

10.	IR58025B/ IR91-1591-3	RM11549, RM9, RM576 RM1, RM11748, RM443, RM5800, RM5, RM493, RM12351, RM7033, RM12492, RM263, RM12696, RM12939, RM341, RM15573, RM16852, RM119, RM1388, RM17479, RM574, RM13, RM31, RM39, RM6054, RM19614, RM432, RM20827, RM3340, RM23077, RM342, RM107, RM23887, RM25653, RM269, RM6737, RM25670, RM6100, RM294A, RM206, RM26384, RM7226, RM287, RM28585, RM28669
11.	IR58025eB/Dular	RM495, RM335, RM11549, RM157B, RM7466, RM11722, RM576, RM1, RM11573, RM3148, RM11687, RM11629, RM443, RM1152, RM128, RM5800, RM5, RM493, RM12351, RM7033, RM12492, RM12673, RM263, RM12939, RM240, RM341, RM561, RM489, RM14234, RM14302, RM15573, RM564, RM6314, RM16830, RM16843, RM16852, RM16792, RM1359, RM17506, RM17521, RM3866, RM119, RM1388, RM6540, RM17479, RM317, RM231, RM13, RM31, RM39, RM19462, RM19521, RM136, RM276, RM204, RM275, RM19552, RM50, RM225, RM234, RM10, RM20884, RM432, RM445, RM22081, RM20827, RM22143, RM23077, RM281, RM210, RM44, RM25, RM5970, RM107, RM23958, RM105, RM216, RM25653, RM269, RM228, RM6737, RM25664, RM25669, RM6100, RM294A, RM25103, RM1108, RM171, RM184, RM206, RM7226, RM457, RM287, RM7203, RM28669
12.	IR58025eB/Samba Mahsuri Sub1	RM157B, RM7466, RM1360, RM1, RM3148, RM11629, RM443, RM1152, RM5800, RM5, RM493, RM12435, RM12492, RM262, RM12939, RM322, RM489, RM14302, RM15573, RM6314, RM16792, RM17506, RM17521, RM1388, RM17479, RM231, RM13, RM31, RM39, RM19614, RM111, RM276, RM275, RM225, RM234, RM20884, RM445, RM20847, RM23077, RM342, RM210, RM25, RM23683, RM5799, RM216, RM269, RM228, RM258, RM6737, RM25664, RM25651, RM25669, RM5373, RM6100, RM294A, RM1108, RM171, RM206, RM7226, RM7203, RM28561
13.	IR58025eB/ IR64 Sub1	RM495, RM11734, RM3632, RM272, RM11549, RM9, RM11558, RM11722, RM1360, RM576, RM1, RM11573, RM297, RM11748, RM1168, RM1152, RM6436, RM128, RM5800, RM5, RM12492, RM12646 RM262, RM263, RM12696, RM13992, RM12939, RM240, RM341, RM561, RM322, RM475, RM489, RM426, RM14285, RM14302, RM14810, RM15573, RM411, RM16, RM3742, RM1359, RM17506, RM17521, RM6540, RM17479, RM317, RM13, RM31, RM6054, RM19521, RM19614, RM136, RM412, RM20705, RM276, RM141, RM275, RM19552, RM225, RM3207, RM234, RM21194, RM21242, RM20884, RM432, RM5672, RM445, RM22081, RM22168, RM20847, RM22188, RM22143, RM23077, RM342, RM210, RM23683, RM107, RM566, RM160, RM278, RM25653, RM269, RM228, RM258, RM6737, RM25651, RM25669, RM5373, RM6100, RM184, RM206, RM26370, RM28561
14.	IR58025eB/ BR-11 Sub1	RM495, RM104, RM335, RM272, RM11549, RM576, RM1, RM493, RM12351, RM263, RM12939, RM341, RM561, RM322, RM489, RM426, RM14302, RM14796, RM411, RM6314, RM16792, RM17506, RM17521, RM3335, RM119, RM231, RM574, RM31, RM6054, RM19614, RM276, RM275, RM225, RM234, RM10, RM21194, RM20884, RM20827, RM3340, RM23077, RM342, RM105, RM25653, RM269, RM228, RM258, RM6737, RM25651, RM25669, RM206, RM26384, RM7226, RM7203
15.	IR58025eB/ TDK-1 Sub1	RM495, RM335, RM7466, RM1360, RM1, RM3148, RM11629, RM1152, RM5800, RM493, RM12351, RM12492, RM262, RM263, RM12939, RM561, RM322, RM489, RM426, RM14302, RM14796, RM6314, RM16792, RM17506, RM17479, RM13, RM32, RM31, RM39, RM275, RM50, RM225, RM19592, RM234, RM21194, RM20884, RM432, RM445, RM20847, RM3340, RM281, RM342, RM210, RM44, RM105, RM566, RM269, RM228, RM6737, RM25664, RM25651, RM25669, RM5373, RM6100, RM171, RM206, RM7226, RM457, RM287, RM7203
16.	IR58025eB/ CR-1009 Sub1	RM495, RM335, RM272, RM576, RM297, RM3148, RM11687, RM11629, RM11649, RM1152, RM5800, RM493, RM12351, RM262, RM263, RM12939, RM561, RM475, RM489, RM426, RM6297, RM14796, RM411, RM6314, RM16792, RM1359, RM17506, RM17521, RM3866, RM17479, RM231, RM574, RM13, RM31, RM39, RM6054, RM19614, RM276, RM204, RM275, RM225,

		RM234, RM20884, RM445, RM22168, RM20847, RM22188, RM22143, RM3340, RM23077, RM281, RM342, RM23683, RM5799, RM107, RM105, RM25653, RM269, RM228, RM6737, RM25670, RM6100, RM1108, RM171, RM206, RM26384, RM7226, RM441, RM7203
17.	IR58025eB/Swarna Sub1	RM495, RM335, RM272, RM11549, RM576, RM1, RM297, RM11748, RM11687, RM11629, RM443, RM1152, RM5800, RM5, RM493, RM12696, RM12939, RM240, RM561, RM489, RM426, RM14234, RM6297, RM14302, RM14796, RM411, RM6314, RM16830, RM16852, RM16792, RM17506, RM17521 RM119, RM17479, RM13, RM6054, RM19614, RM276, RM275, RM19552, RM225, S5-t1, BF-S5, S5-InDel, RM234, RM10, RM21194, RM20884, RM5672, RM445, RM20827, RM3340, RM23077, RM342, RM25, RM107, RM105, RM25653, RM269, RM228, RM6737, RM25651, RM5373, RM6100, RM1108, RM7226, RM287, RM7203, RM28561
18.	IR58025eB/ BF-16B	RM283, RM272, RM11549, RM9, RM472, RM576, RM1, RM3148, RM11629, RM5800, RM493, RM7033, RM12435, RM12492, RM12624, RM262, RM341, RM561, RM475, RM489, RM14302, RM6314 RM16852, RM16792, RM17506, RM17521, RM119, RM17479, RM231, RM574, RM13, RM31, RM39, RM19614, RM111, RM19552, RM225, RM234, RM445, RM20847, RM22143, RM3340, RM23077, RM342, RM210, RM5970, RM278, RM216, RM25653, RM269, RM228, RM258, RM25651, RM25669, RM294A, RM206, RM7203, RM7003
19.	IR58025eB/ <i>Oryza meridionalis</i>	RM11734, RM104, RM335, RM11549, RM9, RM11558, RM11722, RM472, RM576, RM1, RM11573, RM165, RM297, RM11748, RM11687, RM11629, RM11649, RM443, RM1152, RM5800, RM5, RM493, RM7033, RM262, RM263, RM13992, RM240, RM341, RM561, RM322, RM450, RM489, RM426, RM14234, RM14285, RM6297, RM14302, RM15573, RM6314, RM16830, RM16852, RM3742, RM17521, RM3866, RM1388, RM17479, RM241, RM17155, RM317, RM231, RM574, RM13, RM31, RM509, RM136, RM111, RM276, RM204, RM19552, RM50, RM225, RM234, RM560, RM21194, RM21242 RM20884, RM432, RM445, RM22081, RM22168, RM21183, RM22188, RM22143, RM22153, RM3340, RM23077, RM281, RM210, RM284, RM5970, RM524, RM107, RM23958, RM23887, RM105, RM566, RM23805, RM160, RM278, RM216, RM25653, RM269, RM228, RM258, RM6737, RM25651, RM25670, RM294A, RM1108, RM171, RM184, RM206, RM26370, RM26384, RM26464, RM7226, RM457, RM287, RM7203, RM7003, RM28585, RM28669
20.	IR79156B/Dular	RM495, RM335, RM11549, RM157B, RM7466, RM11722, RM576, RM1, RM11573, RM3148, RM11687, RM11629, RM443, RM1152, RM128, RM5800, RM5, RM493, RM12351, RM7033, RM12492, RM12673, RM262, RM263, RM12939, RM240, RM475, RM14234, RM15573, RM564, RM6314, RM16830, RM16843, RM16852, RM16792, RM1359, RM17506, RM17521, RM3866, RM1388, RM6540, RM17479, RM317, RM13, RM31, RM19462, RM19521, RM136, RM276, RM204, RM275, RM19552, RM50, RM225, RM234, RM10, RM20884, RM432, RM445, RM22081, RM20827, RM22143, RM23077, RM281, RM210, RM44, RM25, RM5970, RM107, RM23958, RM105, RM216, RM25653, RM228, RM6737, RM25664, RM25651, RM6100, RM294A, RM25103, RM1108, RM171, RM184, RM206, RM26384, RM7226, RM457, RM287, RM28561, RM28669
21.	IR79156B/Samba Mahsuri Sub1	RM157B, RM7466, RM1360, RM576, RM3148, RM443, RM5, RM493, RM12435, RM12492, RM263, RM12939, RM341, RM561, RM322, RM475, RM15573, RM6314, RM16792, RM17506, RM119, RM1388, RM17479, RM317, RM13, RM19614, RM111, RM276, RM275, RM225, RM234, RM20884, RM445, RM20847, RM342, RM210, RM25, RM23683, RM5799, RM216, RM228, RM258, RM6737, RM25664, RM5373, RM6100, RM294A, RM1108, RM171, RM26384, RM7226
22.	IR79156B/ IR64 Sub1	RM495, RM11734, RM3632, RM272, RM11549, RM9, RM11558, RM11722, RM1360, RM1, RM11573, RM297, RM11748, RM11687, RM11629, RM6436, RM128, RM5800, RM5, RM12492, RM12646, RM262, RM263, RM12696, RM13992, RM12939, RM240, RM341, RM322, RM475, RM426, RM14285, RM14810, RM15573, RM411, RM16, RM3742, RM1359, RM17506, RM119,

		RM6540, RM17479, RM317, RM231, RM13, RM31, RM39, RM6054, RM19521, RM19614, RM136, RM412, RM20705, RM276, RM141, RM275, RM19552, RM225, RM3207, RM234, RM21194, RM21242, RM20884, RM432, RM5672, RM445, RM22081, RM22168, RM20847, RM22188, RM22143, RM342, RM210, RM23683, RM107, RM566, RM160, RM278, RM25653, RM228, RM258, RM5373, RM6100, RM184, RM26370, RM26384, RM7203
23.	IR79156B/ BR-11 Sub1	RM495, RM104, RM335, RM272, RM11549, RM576, RM11629, RM1152, RM5800, RM493, RM12351, RM262, RM12939, RM341, RM322, RM475, RM426, RM14796, RM411, RM6314, RM16792, RM17506, RM17521, RM3335, RM317, RM574, RM13, RM39, RM6054, RM19614, RM276, RM275, RM234, RM10, RM21194, RM20884, RM20827, RM3340, RM342, RM105, RM25653, RM228, RM258, RM6737, RM7226, RM28561
24.	IR79156B/ TDK-1 Sub1	RM495, RM335, RM7466, RM1360, RM576, RM3148, RM493, RM12351, RM12492, RM263, RM12939, RM341, RM322, RM475, RM426, RM14302, RM14796, RM6314, RM16792, RM17506, RM17521, RM119, RM17479, RM317, RM231, RM13, RM32, RM31, RM39, RM275, RM50, RM19592, RM234, RM21194, RM20884, RM432, RM445, RM20847, RM3340, RM23077, RM281, RM342, RM210, RM44, RM105, RM566, RM228, RM6737, RM25664, RM5373, RM6100, RM171, RM206, RM26384, RM7226, RM457, RM287, RM28561
25.	IR79156B/ CR-1009 Sub1	RM495, RM335, RM272, RM576, RM1, RM297, RM3148, RM11687, RM11649, RM1152, RM493, RM12351, RM263, RM12939, RM341, RM426, RM6297, RM14302, RM14796, RM411, RM6314, RM16792, RM1359, RM17506, RM3866, RM119, RM17479, RM317, RM574, RM13, RM39, RM6054, RM19614, RM276, RM204, RM275, RM234, RM20884, RM445, RM22168, RM20847, RM22188, RM22143, RM3340, RM281, RM342, RM23683, RM5799, RM107, RM105, RM25653, RM228, RM6737, RM25651, RM25669, RM25670, RM6100, RM1108, RM171, RM7226, RM441, RM28561
26.	IR79156B/Swarna Sub1	RM495, RM335, RM272, RM11549, RM576, RM297, RM11748, RM11687, RM443, RM5, RM493, RM262, RM263, RM12696, RM12939, RM240, RM341, RM475, RM426, RM14234, RM6297, RM14302, RM14796, RM411, RM6314, RM16830, RM16852, RM16792, RM17506, RM17521, RM17479, RM317, RM231, RM13, RM31, RM39, RM6054, RM19614, RM276, RM275, RM19552, RM225, S5-t1, BF-S5, S5-InDel, RM234, RM10, RM21194, RM20884, RM5672, RM445, RM20827, RM3340, RM342, RM25, RM107, RM105, RM25653, RM228, RM6737, RM25669, RM5373, RM6100, RM1108, RM206, RM26384, RM7226, RM287
27.	IR79156B/ BF-16B	RM283, RM272, RM11549, RM9, RM472, RM576, RM1, RM3148, RM1152, RM493, RM7033, RM12435, RM12492, RM12624, RM263, RM341, RM6314, RM16852, RM16792, RM17506, RM17521, RM17479, RM317, RM574, RM13, RM39, RM19614, RM111, RM19552, RM234, RM445, RM20847, RM22143, RM3340, RM342, RM210, RM278, RM216, RM25653, RM228, RM258, RM6737, RM294A, RM26384, RM7003, RM28561
28.	IR79156B/ <i>Oryza meridionalis</i>	RM11734, RM104, RM335, RM11549, RM9, RM11558, RM11722, RM472, RM576, RM1, RM11573, RM165, RM297, RM11748, RM11687, RM11649, RM443, RM5800, RM5, RM493, RM7033, RM262, RM263, RM13992, RM240, RM322, RM450, RM475, RM426, RM14234, RM14285, RM6297, RM15573, RM6314, RM16830, RM16852, RM3742, RM17521, RM3866, RM119, RM1388, RM17479, RM241, RM17155, RM317, RM231, RM574, RM13, RM31, RM39, RM509, RM136, RM111, RM276, RM204, RM19552, RM50, RM225, RM234, RM560, RM21194, RM21242, RM20884, RM432, RM445, RM22081, RM22168, RM21183, RM22188, RM22143, RM22153, RM3340, RM23077, RM281, RM210, RM284, RM524, RM107, RM23958, RM23887, RM105, RM566, RM23805, RM160, RM278, RM216, RM25653, RM269, RM228, RM258, RM6737, RM25669, RM25670, RM294A, RM1108, RM171, RM184, RM26370, RM26464, RM7226, RM457, RM287, RM7003, RM28561, RM28585, RM28669
29.	IR79156B/ IR91-1591-3	RM11549, RM9, RM576, RM443, RM1152, RM5, RM493, RM12351, RM7033, RM12492, RM262, RM263, RM12696, RM12939, RM341, RM15573, RM16852, RM17479, RM317, RM231, RM574, RM13, RM6054, RM19614, RM432,

		RM20827, RM3340, RM23077, RM342, RM107, RM23887, RM25653, RM6737, RM25651, RM25670, RM6100, RM294A, RM206, RM7226, RM287, RM28561, RM28669
30.	PRR78/Dular	RM495, RM335, RM272, RM157B, RM9, RM7466, RM11722, RM1, RM11573, RM3148, RM11687, RM11629, RM443, RM1152, RM6436, RM128, RM5800, RM5, RM493, RM12351, RM7033, RM12492, RM12673, RM262, RM263, RM13992, RM12939, RM240, RM341, RM322, RM450, RM475, RM426, RM14234, RM14302, RM15573, RM411, RM16, RM564, RM6314, RM16830, RM16843, RM16852, RM16792, RM1359, RM17506, RM17521, RM3866, RM1388, RM6540, RM317, RM231, RM13, RM31, RM39, RM6054, RM19462, RM19521, RM136, RM276, RM204, RM19552, RM50, RM225, RM19592, RM234, RM20884, RM22081, RM20827, RM22143, RM23077, RM281, RM210, RM44, RM25, RM5970, RM524, RM107, RM23958, RM23887, RM105, RM160, RM278, RM216, RM25653, RM228, RM258, RM6737, RM25664, RM25651, RM25669, RM25670, RM5373, RM6100, RM294A, RM25103, RM1108, RM171, RM206, RM26384, RM7226, RM287, RM28561
31.	PRR78/Samba Mahsuri Sub1	RM335, RM272, RM11549, RM157B, RM9, RM7466, RM1360, RM576, RM3148, RM443, RM6436, RM5800, RM5, RM12435, RM12492, RM263, RM13992, RM12939, RM240, RM341, RM561, RM450, RM475, RM426, RM14302, RM15573, RM411, RM16, RM6314, RM16852, RM16792, RM17506, RM119, RM17479, RM231, RM13, RM31, RM39, RM6054, RM19614, RM111, RM276, RM225, RM19592, RM234, RM10, RM20884, RM432, RM20847, RM20827, RM342, RM210, RM25, RM524, RM23683, RM5799, RM107, RM160, RM278, RM216, RM25653, RM258, RM6737, RM25664, RM25669, RM25670, RM294A, RM1108, RM171, RM184, RM26384, RM7226, RM457, RM28669
32.	PRR78/ IR64 Sub1	RM495, RM11734, RM3632, RM335, RM9, RM11558, RM11722, RM1360, RM576, RM1, RM11573, RM297, RM11748, RM11687, RM11629, RM128, RM5800, RM493, RM12492, RM12646, RM262, RM263, RM12696, RM13992, RM12939, RM341, RM450, RM475, RM426, RM14285, RM14302, RM14810, RM15573, RM16852, RM3742, RM1359, RM17506, RM119, RM1388, RM6540, RM317, RM13, RM31, RM19521, RM19614, RM136, RM412, RM20705, RM276, RM141, RM19552, RM225, RM19592, RM3207, RM234, RM10, RM21194, RM21242, RM20884, RM432, RM5672, RM22081, RM22168, RM20847, RM22188, RM20827, RM22143, RM342, RM210, RM5970, RM524, RM23683, RM566, RM25653, RM228, RM6737, RM25669, RM25670, RM5373, RM6100, RM26370, RM26384, RM457, RM7203, RM28669
33.	PRR78/ BR-11 Sub1	RM495, RM104, RM335, RM9, RM11629, RM1152, RM6436, RM5, RM493, RM12351, RM262, RM263, RM13992, RM12939, RM240, RM341, RM450, RM475, RM426, RM14302, RM14796, RM16, RM6314, RM16852, RM16792, RM17506, RM17521, RM3335, RM1388, RM17479, RM231, RM574, RM13, RM31, RM19614, RM276, RM19592, RM234, RM21194, RM20884, RM432, RM445, RM3340, RM342, RM524, RM107, RM105, RM160, RM278, RM228, RM258, RM25669, RM25670, RM5373, RM6100, RM184, RM26384, RM7226, RM457, RM28561, RM28669
34.	PRR78/ TDK-1 Sub1	RM495, RM272, RM11549, RM9, RM7466, RM1360, RM576, RM3148, RM6436, RM5800, RM5, RM12351, RM12492, RM263, RM13992, RM12939, RM240, RM341, RM450, RM475, RM426, RM14796, RM411, RM16, RM6314, RM16852, RM16792, RM17506, RM17521, RM119, RM1388, RM13, RM32, RM31, RM39, RM6054, RM50, RM234, RM10, RM21194, RM20884, RM432, RM20847, RM20827, RM3340, RM23077, RM281, RM342, RM210, RM44, RM524, RM107, RM105, RM566, RM160, RM278, RM25653, RM228, RM258, RM6737, RM25664, RM25669, RM25670, RM171, RM184, RM206, RM26384, RM7226, RM287, RM28561, RM28669
35.	PRR78/ CR-1009 Sub1	RM495, RM11549, RM9, RM1, RM297, RM3148, RM11687, RM11649, RM1152, RM6436, RM5800, RM5, RM493, RM12351, RM263, RM13992, RM12939, RM240, RM341, RM322, RM450, RM426, RM6297, RM14302, RM14796, RM16, RM6314, RM16852, RM16792, RM1359, RM17506, RM3866, RM119, RM1388, RM231, RM574, RM13, RM31, RM39, RM19614, RM276, RM204, RM19592,

		RM234, RM10, RM20884, RM432, RM22168, RM20847, RM22188, RM20827, RM22143, RM3340, RM281, RM342, RM524, RM23683, RM5799, RM107, RM105, RM160, RM278, RM25653, RM228, RM258, RM25651, RM5373, RM6100, RM1108, RM171, RM184, RM26384, RM7226, RM457, RM441, RM28561, RM28669
36.	PRR78/Swarna Sub1	RM495, RM9, RM297, RM11748, RM11687, RM443, RM6436, RM5800, RM5, RM493, RM262, RM263, RM12696, RM13992, RM12939, RM240, RM341, RM322, RM450, RM475, RM426, RM14234, RM6297, RM14796, RM16, RM6314, RM16830, RM16852, RM16792, RM17506, RM17521, RM1388, RM13, RM19614, RM276, RM19552, RM225, S5-t1, BF-S5, S5-InDel, RM19592, RM234, RM21194, RM20884, RM432, RM5672, RM3340, RM342, RM25, RM524, RM107, RM105, RM160, RM278, RM228, RM258, RM6737, RM25670, RM5373, RM6100, RM1108, RM184, RM206, RM26384, RM7226, RM457, RM287, RM28669
37.	PRR78/ <i>Oryza meridionalis</i>	RM11734, RM104, RM272, RM9, RM11558, RM11722, RM472, RM1, RM11573, RM165, RM297, RM11748, RM11687, RM11649, RM443, RM6436, RM5800, RM5, RM493, RM7033, RM262, RM263, RM13992, RM240, RM341, RM450, RM475, RM14234, RM14285, RM6297, RM14302, RM15573, RM411, RM16, RM6314, RM16830, RM16852, RM3742, RM17521, RM3866, RM119, RM17479, RM241, RM17155, RM317, RM231, RM574, RM13, RM31, RM509, RM6054, RM136, RM111, RM276, RM204, RM275, RM19552, RM50, RM225, RM19592, RM234, RM10, RM560, RM21194, RM21242, RM20884, RM432, RM22081, RM22168, RM21183, RM22188, RM20827, RM22143, RM22153, RM3340, RM23077, RM281, RM210, RM284, RM23958, RM23887, RM105, RM566, RM23805, RM160, RM278, RM216, RM25653, RM269, RM228, RM258, RM6737, RM25670, RM5373, RM6100, RM294A, RM1108, RM171, RM26370, RM26384, RM26464, RM7226, RM287, RM7003, RM28561, RM28585, RM28669
38.	KMR3/Dular	RM335, RM157B, RM9, RM11722, RM1, RM11573, RM3148, RM11687, RM11629, RM443, RM128, RM5800, RM5, RM493, RM7033, RM12673, RM263, RM12939, RM240, RM341, RM322, RM426, RM14234, RM15573, RM411, RM564, RM16843, RM16852, RM16792, RM1359, RM3866, RM1388, RM6540, RM317, RM231, RM13, RM31, RM6054, RM19462, RM19521, RM136, RM276, RM204, RM19552, RM50, RM234, RM20884, RM22081, RM20827, RM23077, RM281, RM210, RM44, RM25, RM5970, RM23958, RM23887, RM105, RM216, RM25653, RM6737, RM25664, RM25669, RM25670, RM6100, RM294A, RM25103, RM171, RM206, RM26384, RM7226, RM287, RM28561
39.	KMR3/Samba Mahsuri Sub1	RM495, RM335, RM157B, RM9, RM297, RM3148, RM11687, RM11649, RM443, RM1152, RM5, RM12351, RM7033, RM12435, RM262, RM263, RM12696, RM341, RM561, RM426, RM15573, RM411, RM6314, RM16830, RM16852, RM16792, RM17521, RM119, RM1388, RM17479, RM317, RM231, RM13, RM31, RM6054, RM204, RM275, RM19552, RM225, RM234, RM20884, RM432, RM20847, RM20827, RM22143, RM23077, RM342, RM210, RM25, RM544, RM23683, RM5799, RM107, RM566, RM216, RM25653, RM228, RM258, RM6737, RM25664, RM25651, RM25669, RM25670, RM5373, RM6100, RM294A, RM171, RM206, RM26384
40.	KMR3/ IR64 Sub1	RM11734, RM3632, RM335, RM272, RM11549, RM9, RM7466, RM11558, RM11722, RM576, RM1, RM11573, RM297, RM11748, RM11687, RM11629, RM11649, RM1152, RM6436, RM128, RM5800, RM5, RM493, RM12351, RM7033, RM12646, RM262, RM263, RM13992, RM240, RM341, RM475, RM426, RM14285, RM14810, RM15573, RM16, RM6314, RM16830, RM16852, RM3742, RM17521, RM119, RM6540, RM17479, RM317, RM13, RM31, RM39, RM19521, RM19614, RM136, RM111, RM412, RM20705, RM276, RM141, RM204, RM275, RM19552, RM225, RM3207, RM234, RM21194, RM21242, RM20884, RM432, RM5672, RM22081, RM22168, RM20847, RM22188, RM20827, RM22143, RM23077, RM342, RM210, RM544, RM5970, RM23683, RM107, RM566, RM160, RM278, RM25653, RM228, RM258, RM6737, RM25651, RM25669, RM25670, RM5373, RM1108, RM184, RM206, RM26370, RM26384, RM7226, RM7203

41.	KMR3/ BR-11 Sub1	RM104, RM335, RM272, RM11549, RM9, RM7466, RM1360, RM576, RM297, RM11687, RM11629, RM11649, RM1152, RM5800, RM493, RM7033, RM12492, RM12696, RM426, RM14796, RM6314, RM16830, RM16852, RM16792, RM3335, RM317, RM231, RM574, RM13, RM31, RM39, RM111, RM204, RM275, RM19552, RM225, RM234, RM10, RM21194, RM20884, RM432, RM445, RM22143, RM3340, RM23077, RM342, RM544, RM107, RM105, RM566, RM258, RM25651, RM25669, RM25670, RM6100, RM1108, RM206, RM26384, RM28561
42.	KMR3/ TDK-1 Sub1	RM9, RM297, RM3148, RM11687, RM11649, RM1152, RM7033, RM262, RM263, RM12696, RM341, RM426, RM14302, RM14796, RM411, RM6314, RM16830, RM16852, RM16792, RM17521, RM119, RM17479, RM317, RM13, RM32, RM31, RM39, RM6054, RM19614, RM111, RM276, RM204, RM275, RM19552, RM50, RM225, RM19592, RM234, RM21194, RM20884, RM432, RM20847, RM20827, RM22143, RM3340, RM281, RM342, RM210, RM44, RM544, RM107, RM105, RM566, RM25653, RM6737, RM25664, RM25651, RM25669, RM25670, RM5373, RM6100, RM1108, RM171, RM206, RM26384, RM457, RM287, RM28561
43.	KMR3/ CR-1009 Sub1	RM272, RM9, RM7466, RM1360, RM576, RM1, RM3148, RM493, RM7033, RM12492, RM262, RM263, RM12696, RM341, RM322, RM475, RM426, RM6297, RM14302, RM14796, RM6314, RM16830, RM16852, RM16792, RM1359, RM17521, RM3866, RM119, RM17479, RM317, RM231, RM574, RM13, RM31, RM39, RM111, RM275, RM19552, RM225, RM234, RM20884, RM432, RM22168, RM20847, RM22188, RM20827, RM22143, RM3340, RM23077, RM281, RM342, RM544, RM23683, RM5799, RM105, RM566, RM25653, RM171, RM206, RM26384, RM441, RM28561
44.	KMR3/Swarna Sub1	RM272, RM11549, RM9, RM7466, RM1360, RM576, RM11748, RM11649, RM443, RM1152, RM5, RM493, RM12351, RM7033, RM12492, RM263, RM240, RM341, RM322, RM426, RM14234, RM6297, RM14302, RM14796, RM16852, RM16792, RM17479, RM317, RM13, RM39, RM111, RM276, RM204, RM275, RM19552, S5-t1, BF- S5, S5- InDel, RM234, RM10, RM21194, RM20884, RM432, RM5672, RM22143, RM3340, RM23077, RM342, RM25, RM544, RM105, RM566, RM228, RM6737, RM25651, RM25670, RM5373, RM206, RM26384, RM7226, RM287
45.	KMR3/ <i>Oryza meridionalis</i>	RM495, RM11734, RM104, RM11549, RM9, RM7466, RM11558, RM11722, RM472, RM1360, RM576, RM1, RM11573, RM165, RM297, RM11748, RM11687, RM443, RM1152, RM5800, RM5, RM493, RM12351, RM7033, RM12492, RM262, RM263, RM12696, RM13992, RM12939, RM240, RM341, RM450, RM14234, RM14285, RM6297, RM15573, RM411, RM16852, RM3742, RM17506, RM3866, RM119, RM1388, RM17479, RM241, RM17155, RM317, RM231, RM574, RM13, RM31, RM39, RM509, RM6054, RM19614, RM136, RM276, RM19552, RM50, RM234, RM560, RM21194, RM21242, RM20884, RM432, RM22081, RM22168, RM21183, RM22188, RM20827, RM22153, RM3340, RM23077, RM281, RM342, RM210, RM544, RM284, RM524, RM107, RM23958, RM23917, RM23887, RM105, RM566, RM160, RM278, RM216, RM25653, RM269, RM258, RM6737, RM25651, RM25670, RM6100, RM294A, RM171, RM184, RM206, RM26370, RM26384, RM26464, RM7226, RM457, RM287, RM7003, RM28561, RM28585, RM28669

