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Research Article

Estimation of *indica*-tropical *japonica* genome proportion in wide compatible restorer lines derived through inter sub specific hybridization and molecular diversity analysis among rice genotypes

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Abstract
The present investigation was carried out to estimate *indica*-tropical *japonica* genome proportion in two newly developed wide compatible restorer lines (RP6367 and RP6368) derived through *indica*-tropical *japonica* crosses and to study the molecular diversity among 12 rice genotypes namely, five promising *indica* restorers (RPHR1005, RPHR1096, IBL57, DR714-1-2 and Akshayadhan), two maintainers (IR58025B and APMS6B), two newly identified wide compatible restorer lines (RP6367and RP6368), two typical tropical *japonica* lines (IRGC66651 and IRGC66577) and one typical *indica* genotype (Nagina22) using 50 SSR markers and 45 InDELs. Out of the 95 markers, 54 were found to be polymorphic. The genotypic data of 54 polymorphic markers was used to estimate *indica*-tropical *japonica* genome proportion in derived lines and molecular diversity among 12 genotypes. The percentage of tropical *japonica* genome proportion in derived lines RP6367 and RP6368 was found to be 50% and 55.55% respectively. Our results suggest that the proportion of tropical *japonica* in hybrid rice parental lines is the key to select the best parents for efficient utilization of the heterosis present between *indica* and tropical *japonica* subspecies. Out of the 54 markers, 19 markers recorded PIC value above 0.5 and can be considered as highly informative and useful to study molecular genetic diversity. In this study, the SSRs primers showed higher PIC values compared to InDels. It is concluded that the use of highly polymorphic molecular markers detected in this study gives a better understanding of genetic relationship among closely related rice genotypes.

Keywords: Hybrid Rice, Genome proportion, Molecular diversity, SSRs, InDels.

INTRODUCTION

To meet the demands of ever increasing global population, rice breeders have to produce at least 40% more than that of the current production with limited arable land resources. To steadily increase the rice production, the approach of combining morphological improvement and heterosis utilization is the best practice (Yuan, 1994). Accordingly, large scale adoption of hybrid rice technology by farming community is one of the potential approaches

to bridge the productivity gap. Yet, in India so far, a total of 134 hybrids have been developed and released (AICRIP, Crop Improvement Report, 2022). Since the release of first hybrid rice 25 years ago, the total hybrid rice cultivation has reached only 3.5 million ha (6.8%) out of 44 million hectares (AICRIP, Crop Improvement Report, 2020). Majority of consumers prefer non-sticky cooked rice in India, but most of the early hybrids shows stickiness. Lack of enough compatible restorers is also a major constraint. Nowadays breeders mainly concentrate on wide compatible restorers to make hybrid rice economical and commercially viable (Matsuo, 1952). Rice is classified into three types namely *indica, japonica* and *javanica* based on geographical origin. Limited progress has been acheived because of the semi-sterility of F_{1s} in *indica-japonica* crosses. Few varieties of tropical *japonica* having good compatibility, showed normal fertility in F_1. Recently hybrid rice breeders made research efforts concentrated on breeding parental lines from *indica*-tropical *japonica* crosses to raise heterosis level and increase the genetic diversity among parents (Hari Prasad *et al*., 2018). The released hybrids are inter-varietal (*indica/indica*) in nature, which does not show a much significant gain of yield advantage over high yielding varieties. The level of yield advantage could be ranked as *indica*/temperate *japonica*>*indica*/tropical *japonica*> temperate *japonica*/ tropical *japonica*> *indica*/*indica*>*japonica*/*japonica* (Yuan *et al.,* 1997). However, in hybrid rice production there is a serious issue of hybrid semi sterility while utilizing inter sub specific heterosis. Ikehashi and Araki (1986) discovered wide compatibility (WC) genes, this paved the way for the use of *indica-japonica* heterosis in rice (Yuan, 1994). The tropical *japonica* lines, when crossed with semi dwarf *indica* rice, exhibited stronger heterosis for yield than *indica*/*indica* hybrids provided that either of the parental lines possessed the WCG (Bharaj *et al.,* 1994). Currently SSR markers linked to *Rf* genes are used for screening of major fertility restoration genes as they are co-dominant, highly robust and polymorphic. The use of molecular markers linked to *Rf* genes that restore fertility can increase decision effectiveness, save time, and avoid issues associated with phenotype-based screening (Prasanna *et al*., 2022). In the present study, the major objective was to estimate the *indica*-tropical *japonica* genome proportions (XU Xu-ming *et al*., 2009) in two newly developed wide compatible restorer lines RP6367 and RP6368. This study may help to understand the mechanism of differentiation present between *indica* and tropical *japonica* sub species which helps to select suitable parents for hybridization and improve the fertility status in inter sub specific hybrids. Based on the frequency of *indica* and tropical *japonica* genome segments, it can be possible to select most fit genotypes for hybridization.

MATERIALS AND METHODS

The present study was carried out during *Rabi*-2019 at Marker Assisted Selection (MAS) Laboratory, Hybrid Rice Section, ICAR-IIRR, Rajendranagar, Hyderabad.

Plant material: Twelve genotypes of rice consisting of five restorer lines (RPHR1005, RPHR1096, IBL57, DR714-1-2 and Akshayadhan), two maintainer lines (IR58025B and APMS6B), two newly identified wide compatible restorer lines (RP6367 and RP6368), two tropical *japonica* lines (IRGC66651 and IRGC66577) and one typical *indica* genotype (Nagina22) were used in the study. RP6367 was developed from a cross between promising *indica* restorer RPHR1096 and a tropical *japonica* line IRGC 66755. RP6368 was developed from a cross between promising *indica* restorer IBL-57 and a tropical *japonica* line IRGC 66651. These two were identified with three gene combination for restoration ability (*Rf4/Rf3*) and wide compatibility (*S5n*).

Genotyping

DNA isolation, Selection of molecular markers and PCR amplification: Genomic DNA was isolated from leaves of 15-days old seedlings of 12 genotypes following the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Dellaporta *et al.,* 1983). Forty-five InDel markers suggested by Lu *et al.* (2009) and a panel of 50 SSR markers having extensive genome coverage used by International Rice Research Institute (IRRI), Philippines under Generation Challenge Programme (GCP) were used to assess the genome proportion of *indica-tropical japonica* segments in derived lines and to study their molecular diversity. The PCR protocols and amplicon separation were carried out according Lakshmi *et al.* (2021).

Screening for fertility restorer genes (Rf3 and Rf4) and wide compatibility gene (*S5n*): For *Rf3,* fertility restorer genes using candidate gene-based marker DRRM-*Rf3*-10 (chr.1) (Balaji *et al.,* 2012) and a functional marker RMS-SF21-5 (chr.1) (Pranathi *et al.,* 2016) were used. Whereas for *Rf4,* a gene linked marker RM6100 (chr.10) (Singh *et al*., 2005) and a functional marker RMS-PPR9-1 (chr.10) (Pranathi *et al.,* 2016) were used. *S5n*-InDel marker were used for wide compatible (*S5n*) gene (Sundaram *et al.,* 2010).

Data Analysis**:** Only the clear and unambiguous amplified bands were scored manually for polymorphic markers using 100 bp DNA ladder. The genotypic data of polymorphic markers for 12 genotypes was used for analysis of genome proportion in the derived lines (RP6367 and RP6368). The allele amplified by typical tropical *japonica* lines (IRGC66651 and IRGC66755) was taken as a reference allele size (A) for each marker, based on this reference allele size, the genotypes which were amplified similarly were considered as tropical *japonica* alleles and remaining other alleles were considered as *indica* alleles and named as B,C,D,E,F, (999 is considered as missing band) respectively. The proportion of A allele among total number of amplified alleles was calculated and was considered as tropical *japonica* genome proportion of derived lines.

Percentage of Tropical *japonica* genome in derived lines = Total no. of A ÷ Total no. of polymorphic markers × 100.

For molecular diversity analysis, the genotypic data based on allele size was converted into binary data. Presence of particular size allele for each marker was indicated as 1 and absence as 0 and the same was followed for all alleles at each locus for 54 polymorphic markers. The PIC index can be used to evaluate the level of gene variation, the locus was considered of high diversity when PIC > 0.5; low diversity when PIC < 0.25; and the locus was of intermediate diversity, when PIC was between 0.25 and 0.5 (Botstein *et al.,* 1980).

$$PIC=1- \Sigma Pi2- \Sigma\Sigma Pi2Pj2.$$

Molecular diversity parameters were calculated using software POWERMARKER Ver 3.25 (Liu and Muse, 2005). Distance based clustering was performed by DAR win software ver 6.0.010 (Perrier and Jacquemoud Collet 2006). To determine the clustering pattern dissimilarity matrix was used, based on Unweighted Pair Group Method with Arithmetic Average (UPGMA) and Neighbour - joining method.

RESULTS AND DISCUSSION

In the present study, the major objective was to estimate the *indica* and tropical *japonica* genome proportions in derived lines RP6367and RP6368 and molecular diversity.

Molecular screening for fertility restorer genes and wide compatibility gene: For *Rf3* gene, both the markers (DRRM-RF3-10 and RMS-SF21-5) showed presence of *Rf3* in eight genotypes. For *Rf4* gene, out of the 12 genotypes, eight and seven genotypes were found positive for RM6100 and RMS-PPR9-1 markers respectively. For wide compatibility gene, out of 12 screened genotypes, six genotypes showed the presence of *S5* neutral allele. The genotypes which were identified to possess *Rf3, Rf4* and *S5* neutral allele could be utilized in inter sub specific hybridization to exploit higher level of heterosis. The present study clearly demonstrated the utility of primer *S5*-InDel in identifying wide compatible genotypes possessing *S5* neutral allele. Out of the12 genotypes, the restorer lines RPHR1005, DR714-1-2R are male parents for DRRH3 and DRRH2 hybrids respectively, which showed the presence of both *Rf3* and *Rf4*. A tropical japonica line (IRGC66755) was observed with the presence of both the (*Rf3* and *Rf4*) fertility restorer genes. These three lines were confirmed by two *Rf3* specific candidate gene specific markers and two *Rf4* specific markers. Two other restorer lines RPHR1096 and IBL57 were observed with the presence of *Rf4* and *S5n* gene. Akshyadhan, a restorer line released as a variety by CVRC was having *Rf4* and *S5n* gene combination. Out of two B lines, one B line (IR58025B) was observed with the absence of both the genes and this was confirmed by four markers and the other B line (APMS6B) was observed to have *Rf3* gene and this was confirmed by two *Rf3* candidate gene-based markers. This may be because of seed contamination. Nagina 22 was having *Rf3* and *S5n* gene combination. For Nagina 22, gene linked (RM6100) and functional marker (RMS-PPR9-1) for *Rf4* gene showed differential behavior. In the present study, the results showed RP6367 and RP6368 as WC genotypes (presence of *Rf3*, *Rf4* and *S5n* genes). IRGC66651 showed the presence of *Rf3* genes and absence of *Rf4* and *S5n* genes, while IRGC66577 showed the presence of both *Rf3* and *Rf4* genes and absence of *S5n* gene **(Fig. 1).** The success of hybrid rice is depending upon the fertility restoration and spikelet fertility due to interaction of *Rf* genes and CMS cytoplasm. To develop high yielding heterotic hybrids, primary step is to identify restorer which can restore the fertility of cytoplasmic male sterility lines (Virmani *et al.,* 1986). The line RP6367 derived from indica restorer (RPHR1096 with *Rf4* and *S5n* genes) and tropical japonica line (IRGC66755 with *Rf3* and *Rf4*) had the restorer genes *Rf3*, *Rf4* and *S5n*. Another RP6368 line derived from IBL57 (*Rf4* and *S5n* genes) and IRGC66651 (Tropical Japonica line with *Rf3* gene) had the restorer genes *Rf3*, *Rf4* and *S5n*. The fertility restorer gene *Rf4* and WC gene *S5n* transferred from indica restorers (female parents) while *Rf3* genes transferred from tropical japonica genotypes in the derived lines RP6367 and RP6368. These wide compatible restorer lines (RP6367and RP6368) would help in overcoming sterility problem in inter sub specific hybrids. In the present study, one tropical *japonica* line (IRGC66755) was identified with both the restorer genes and this line can be used as a resource to diversify the restorer gene pool and increase the heterosis levels.

Estimation of genome proportion in derived lines: 50 SSR markers recommended by IRRI under generation challenge programme and 45 InDel markers (Lu *et al.,* 2009) were used to assess the genome proportion of *indica* and tropical *japonica* genome segments in two derived lines (RP6367 and RP6368). Out of 95 markers, 54 were found to be polymorphic (32 SSRs and 22Indels were polymorphic) and 41 were monomorphic. The percentage of tropical *japonica* genome in derived line RP6367 (*Indica*-tropical *japonica* Derived line) was 50 % and RP6368 (*Indica*-tropical *japonica* Derived line) was 55.55%. The genome proportion in derived lines was more in RP6368 than RP6367 and other genotypes *viz*., IR58025B, Akshayadhan, DR714-1-2 has more tropical *japonica* genome percentage compared to other genotypes and least in RPHR1005 **(Table 1).**

Xu *et al.* (2009) analysed *indica japonica* differentiation in 18 parents and 38 derived lines using intron length polymorphism (ILP) markers along with Cheng's index and stated that this differentiation could give better explanation for the higher heterosis of *indica-japonica* hybrids. Zhao *et al.*(2009) developed sub species specific intron length polymorphic markers to differentiate *Oryza*

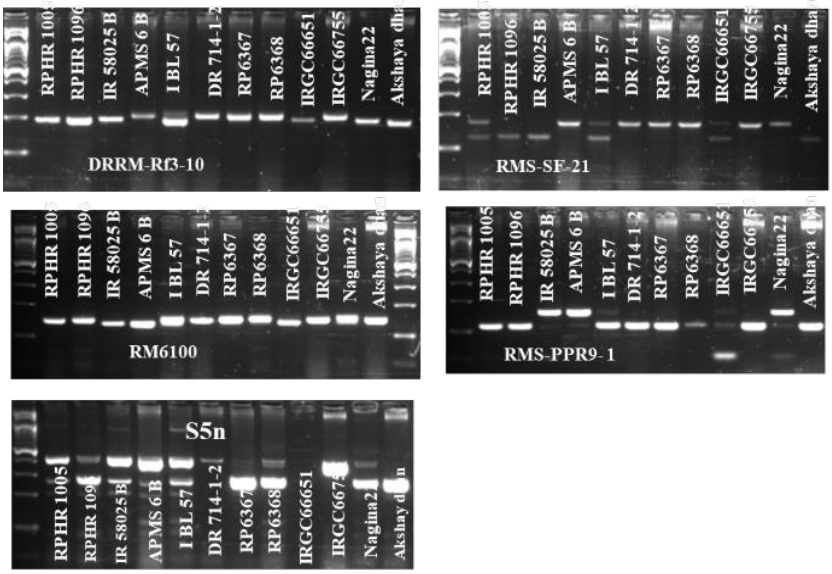

Fig.1. Molecular screening of 12 genotypes for *Rf3*, *Rf4* and *S5n* genes using reported candidate gene specific markers

rufipogon from *Oryza sativa* by comparing the *indica* and *japonica* cultivars. Here the primary objective was to estimate the *indica*-tropical *japonica* genome proportions in derived lines, RP6367 and RP6368. No 100% pure *indica* or tropical *japonica* type was detected, because of the result of recombination or loss of different genes, or mutual genetic introgression between *indica* and *japonica* during the course of natural or artificial selection (Xu *et al.,* 2009). In the present study, 50% and 55.55% of tropical *japonica* genome was found in the *indica*-tropical *japonica* derived lines RP6367 and RP6368 respectively. Our results suggest that the proportion of tropical *japonica* genome in the derived parental lines is the key to overcome the semi sterility problem which is often encountered between different subspecies crosses (*Indica* x *Japonica* or *Indica* x Tropical *Japonica*). Further test crossing of newly bred restorer lines with CMS lines is suggested to know the level of heterosis. Further study with more number of genotypes helps in selection of most suitable genotypes (intermediate parents derived from *indica* x tropical *japonica*/ *Japonica*) for hybrid development. When tropical *japonica* lines crossed with *indica*, the derived lines consist of wide compatible genes which are suitable for *tropical* conditions and to overcome the semi sterility problem, exhibiting stronger heterosis. Based on the genome proportions of *indica,* tropical *japonica* and derived lines we can describe the cause of high heterosis of inter sub specific hybrids and helps in

Table 1. The percentage of tropical *japonica* (IRGC66651 and IRGC66755) genome in derived lines (RP6367 and RP6368)

Samples	Tropical *japonica* allele (A) (%)	Other *indica* alleles B (%)	C (%)	D (%)	E (%)	F (%)	G (%)	999 missing	Total
RPHR1005	35.18	14.81	27.77	14.81	3.70	–	1.85	1.85	99.97
RPHR1096	38.88	12.96	37.03	7.40	3.70	–	–	–	99.97
IR58025B	51.85	9.25	22.22	3.70	9.25	1.85	–	1.85	99.97
APMS6B	42.59	22.22	18.51	12.96	–	–	–	3.70	99.97
IBL57	46.29	14.81	22.22	9.25	1.85	1.85	–	3.70	99.97
DR714-1-2R	50	14.81	20.37	11.11	3.70	–	–	–	99.98
RP6367	**50**	16.66	18.51	7.40	3.70	1.85	1.85	_	99.97
RP6368	**55.55**	20.37	11.11	7.40	5.55	–	–	_	99.98
Nagina22	38.88	22.22	22.22	11.11	1.85	3.70	–	–	99.98
Akshaydhan	50	18.51	20.37	7.40	3.70	–	–	–	99.98

selection of suitable parents, which facilities the use of beneficial genes (Xu *et al.,* 2009). Out of 50 SSRs, 32 (64%) markers were found to be polymorphic. In case of InDels, out of 45, 22 (49%) markers were found to be polymorphic. SSRs amplified 20 "A" alleles (TJ allele) in RP6367 (*Indica-tropical japonica* Derived line) and 21 "A" alleles in RP6368 (*Indica Japonica* Derived line) whereas, InDels amplified 10 in each derived line. The number of polymorphic markers and the amplified "A" alleles were more in SSRs as compared to InDels. SSRs could amplify more sub species specific alleles than InDels. In the present study SSRs showed a better response compared to InDels.

Molecular diversity analysis: A total of 194 alleles amplified across 12 genotypes by 54 polymorphic markers. The number of alleles ranged from 2 (RM507, RM484, RM178, RM489, R10M30, R12M43, R10M10, R6M14, R4M30 and R5M13,) to 7 (RM 144 and OSR13) with an average value of 3.59 per locus. The highest numbers of alleles were observed in RM144 and OSR13 (Seven alleles each). The value of major allele frequency ranged from 0.25 (RM237 and RM144) to 0.91 (RM489 and R10M30) with a mean value of 0.58. On an average, 58% of the 12 genotypes shared a common major allele at any given loci. Gene diversity ranged from 0.15 (RM489 and R10M30) to 0.83 (RM144) with an average value of 0.53. Heterozygosity was ranged from 0.00 to 0.33 (R2M37) with a mean 0.029.76% of the markers (41) showed no heterozygosity. The Polymorphism information content (PIC) value is reflection of allelic diversity and frequency among the genotypes and also varied from one locus to another. In the present study, PIC values ranged from 0.14 (RM498 and R10M30) to 0.81 (RM144) with an average PIC value of 0.47. The SSR loci RM489 on chromosome 3 and RM144 on chromosome 11 showed lowest (0.14) and highest (0.81) PIC values respectively **(Table 2).**

Results obtained in genetic diversity studies of genotypes with SSR markers showed that considerable amount of genetic diversity exist among these genotypes of rice. A wide range of genetic diversity was observed among 12 rice genotypes evaluated using SSR and InDels markers. The number of alleles per locus ranged from 2 to 7 with an average of 3.59 per locus. These results are in accordance with previously reported values of 4.44 alleles per locus (range 2-8) by Sruthi *et al*., (2020) reported. The reason behind the wide variation in the number of alleles detected was due to the different sets of germplasm, number of genotypes, number and distribution of SSR loci and method of gel electrophoresis done in different studies. The major allele frequency ranged from 25 to 91%. On an average, 58% of the genotypes shared a common major allele at any given loci. Heterozygosity analysis has displayed the extent of heterozygous individuals present among 12 populations. Having high level of heterozygosity at any of SSR locus is potentially meaningful because increase in heterozygosity levels would indicate that the plant population likely has a substantial amount of adaptive genetic variation to escape the effects of a control agent that limits the development and maintenance of plants (Maranho *et al.,* 2014), compared to plant populations showing a lower level of heterozygosity. In some cases, variation in heterozygosity and allele frequencies is due to different mutational properties of a marker. High PIC value of a locus indicates higher number of alleles per locus detected. In the present study PIC values ranged from 0.14 (RM498 and R10M30) to 0.81 (RM144) with an average PIC value of 0.47. Markers with PIC values of 0.5 or higher as demonstrated in this study are greatly informative for genetic studies and useful as a marker at a specific locus (Dewoody *et al*., 1995). Markers with PIC values of 0.5 or above are considered highly useful in distinguishing the genotypes (Akkaya and BuyukunalBal, 2004). Hence in the present study, out of 54 markers, 19 markers recorded PIC value higher than 0.5, which can be considered as highly informative and useful to study molecular genetic diversity. The SSRs showed high PIC values compared to InDels primers. These results indicated that better levels of polymorphisms were detected in these rice genotypes. The genotypes under this study were clearly distinguishable from each other when grouping was carried out using SSR and InDel markers. Markers with high PIC value are very useful for estimating relationship between genotypes. Marker with low PIC value on the other hand can be used to analyse chromosome region of special interest. Both the SSRs and INDELs markers are suitable for genotype identification, as they have high polymorphic information content. These markers are ideal for distinguishing between genotypes that are genetically more similar.

Molecular genetic diversity pattern by cluster analysis:It resolved 12 genotypes into 5 clusters. Cluster 1 contain three genotypes *viz*., RPHR1005, RPHR1096 and IR58025B. Out of three genotypes, the two genotypes RPHR1005, and RPHR1096 are *indica* restorer lines placed close to each other and IR58025B is a maintainer line placed away from these two genotypes. The cluster 2 consisted of two genotypes APMS6B and IBL57 belonging to maintainer line and *indica* restorer lines respectively, placed closely. The cluster 3 consisted of two genotypes DR714-1-2R and RP6367 which are *indica* restorer and *indica*-tropical *japonica* derived restorer line respectively. The cluster 4 included two genotypes RP6368 and Nagina-22 of *indica*-tropical *japonica* derived restorer line and typical *indica* respectively. RP6368 is a derived line of *indica* and tropical *japonica* so this cluster shows similarity between these two genotypes. The cluster 5 consists of three genotypes Akshyadhan, IRGC66651 and IRGC66755. Two tropical *japonica* genotypes IRGC66651 and IRGC66755 both clustered together while variety Akshyadhan and a restorer line placed away from tropical *japonica* genotypes in the same cluster **(Fig. 2).**

Table 2. Details of polymorphic markers along with molecular diversity parameters

S.No.	Marker	Chr. No	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
1	RM495	1	0.3333	4.0000	0.7083	0.0000	0.6519
2	RM1	1	0.6667	3.0000	0.5000	0.0000	0.4491
3	RM259	1	0.3333	5.0000	0.7639	0.0000	0.7260
4	RM237	1	0.2500	5.0000	0.7778	0.0000	0.7409
5	RM431	1	0.5000	3.0000	0.5694	0.0000	0.4768
6	RM514	3	0.5833	4.0000	0.5833	0.0000	0.5295
7	RM507	5	0.8333	2.0000	0.2778	0.0000	0.2392
8	RM334	5	0.5000	4.0000	0.6528	0.0000	0.5994
9	RM447	8	0.7500	3.0000	0.4028	0.0000	0.3633
10	RM316	9	0.5000	3.0000	0.5694	0.0000	0.4768
11	RM215	9	0.6667	3.0000	0.4861	0.0000	0.4235
12	RM474	10	0.7500	3.0000	0.4028	0.0000	0.3633
13	RM484	10	0.6667	2.0000	0.4444	0.0000	0.3457
14	RM552	11	0.4167	4.0000	0.6806	0.0000	0.6218
15	RM536	11	0.4167	5.0000	0.7222	0.0000	0.6800
16	RM178	5	0.5000	2.0000	0.5000	0.0000	0.3750
17	RM283	1	0.6667	3.0000	0.4861	0.0000	0.4235
18	RM154	2	0.6667	4.0000	0.5139	0.0000	0.4760
19	RM125	7	0.7083	3.0000	0.4479	0.0833	0.3968
20	RM105	9	0.5000	3.0000	0.5694	0.0000	0.4768
21	RM510	6	0.6667	3.0000	0.4861	0.0000	0.4235
22	RM171	10	0.4167	6.0000	0.7500	0.0000	0.7193
23	RM307	4	0.5833	6.0000	0.6146	0.0833	0.5827
24	RM287	11	0.6667	4.0000	0.5139	0.0000	0.4760
25	RM19	12	0.4167	5.0000	0.6701	0.0833	0.6113
26	RM489	3	0.9167	2.0000	0.1528	0.0000	0.1411
27	RM55	3	0.7500	3.0000	0.4028	0.0000	0.3633
28	RM455	7	0.6667	4.0000	0.5139	0.0000	0.4760
29	RM144	11	0.2500	7.0000	0.8333	0.0000	0.8119
30	OSR13	3	0.3750	7.0000	0.7813	0.0833	0.7549
31	RM152	8	0.6667	4.0000	0.5139	0.0000	0.4760
32	RM25	8	0.3333	5.0000	0.7639	0.0000	0.7260
33	R10M30	10	0.9167	2.0000	0.1528	0.0000	0.1411
34	R11M40	11	0.4583	4.0000	0.6354	0.0833	0.5665
35	R12M33	12	0.5833	3.0000	0.5694	0.0000	0.5045
36	R12M43	12	0.8333	2.0000	0.2778	0.0000	0.2392
37	R8M33	8	0.7083	4.0000	0.4514	0.1667	0.4040
38	R8M46	8	0.6667	3.0000	0.5000	0.1667	0.4491
39	R9M30	9	0.5000	4.0000	0.5729	0.0833	0.4832
40	R10M10	10	0.5000	2.0000	0.5000	0.0000	0.3750
41	R6M14	6	0.7500	2.0000	0.3750	0.0000	0.3047
42	R1M37	1	0.8750	3.0000	0.2257	0.0833	0.2124
43	R2M37	2	0.5417	4.0000	0.6215	0.3333	0.5686
44	R2M50	2	0.5000	3.0000	0.6111	0.0000	0.5355
45	R3M53	3	0.3333	4.0000	0.7083	0.0000	0.6519
46	R4M13	4	0.5833	3.0000	0.5417	0.0000	0.4598
47	R4M17	4	0.4167	5.0000	0.7222	0.0000	0.6800
48	R4M30	4	0.5833	2.0000	0.4861	0.0000	0.3680
49	R5M13	5	0.7500	2.0000	0.3750	0.0000	0.3047
50	R4M50	4	0.7500	3.0000	0.4028	0.0000	0.3633
51	R1M7	1	0.7083	5.0000	0.4722	0.1667	0.4457
52	R1M20	1	0.7083	3.0000	0.4479	0.0833	0.3968
53	R7M7	7	0.5000	3.0000	0.5694	0.0000	0.4768
54	R12M27	12	0.5000	4.0000	0.6424	0.0833	0.5827
	Mean		0.5849	3.5926	0.5355	0.0293	0.4798

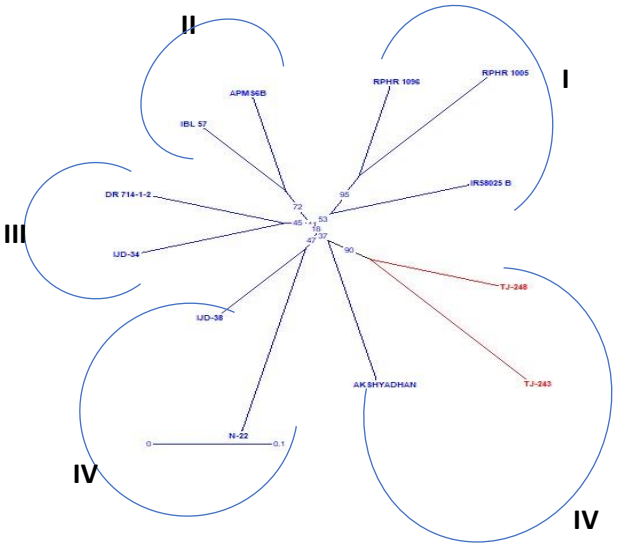

Fig. 2. Molecular clustering pattern based on DARwin Software: Radial representation of Neighbour-joining tree of 12 genotypes. Note: Tropical *japonica* genotypes IRGC66651 and IRGC66755 were coded as TJ243 and TJ248 respectively and RP6367 and RP6368 were coded as IJD34 and IJD38 respectively

For cluster analysis and the identification of *indica-japonica* differentiation, SSR and InDel primers were used to find the allelic loci in the tested materials. The magnitude of heterosis for yield and other agronomic traits in hybrids depends to some extent on genetic distance between parents. The cluster analysis based on genetic distance classified 12 genotypes into 5 groups, and the results showed that *indica* stable restorer lines (RPHR1005 and RPHR1096) placed together and having no divergence among them and IR58025B is a maintainer line maintained a divergence from these two genotypes. The two derived wide compatible lines (RP6367 and RP6368) formed greater divergence from their parents (RPHR1096 and IRGC66755, IBL57, IRGC66651), because the parents belong to two different sub-species. When the percent of recombinant frequency is more subsequently the divergence increases. The set of markers used in the present study could able to differentiate the 12 genotypes.

Molecular genetic distance matrix: Genetic distance ranged from 0.206 between (APMS6B and IBL57) to 0.46 (Nagina22 and IRGC66651). The highest genetic distance was observed between Nagina22 and IRGC66651 (0.467), between IRGC66651 and RPHR1005 (0.446) and between Nagina22 and RPHR1095 (0.420). The

Table 3. Molecular genetic distance matrix

Units	RPHR 1005	RPHR 1095	IR 58025 B	APMS 6 B	IBL 57	DR 714-1-2	RP 6367	RP 6368	IRGC 66651	IRGC 66755	Nagina 22	AKSHYA DHAN
RPHR 1095	0.253											
IR 58025 B	0.326	0.258										
APMS 6 B	0.353	0.295	0.251									
IBL 57	0.394	0.298	0.291	0.206								
DR 714-1-2	**0.405**	0.295	0.263	0.321	0.242							
RP6367	0.363	0.306	0.316	0.316	0.253	0.238						
RP6368	0.326	0.321	0.288	0.228	0.308	0.251	0.257					
IRGC66651	**0.446**	0.379	0.380	0.398	0.392	0.396	0.379	**0.405**				
IRGC66755	**0.411**	0.383	0.289	0.337	0.309	0.295	0.347	0.257	0.296			
Nagina22	**0.416**	**0.420**	0.368	0.374	0.399	**0.404**	0.373	0.305	**0.467**	0.358		
AKSHYA DHAN	0.363	0.337	0.337	0.363	0.309	0.280	0.342	0.294	0.385	0.306	0.394	

genetic distance between Nagina 22 andIRGC66651 represents the distance between typical indica and typical tropical japonica genotypes. The least distance was observed between APMS6B and IBL57 is 0.206 and the highest mean distance was observed between Nagina22 and IRGC66651 is 0.467 **(Table 3)**.

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