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Molecular diversity and segregation distortion measured by SSR markers in a new plant type based recombinant inbred line population of rice

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Abstract

A mapping population of 310 F_7 recombinant inbred lines (RILs) developed by crossing a new plant type derivative, Pusa1266 with Jaya was used for construction of linkage map and dissection of its genome for heterozygosity and segregation distortion by using SSR (Simple Sequence Repeat) markers. A set of 162 polymorphic markers between parents were utilized to genotype entire RIL population. Twenty three markers (14.20%) showed significant segregation distortion from expected 1:1 segregation ratio. A linkage map was constructed with 126 markers on 12 chromosomes representing total map length of 2023.1 cM with an average marker interval of 19.64 cM. Out of 310 RILs, 7 lines (2.3%) were highly skewed towards Pusa1266 and 6 lines (1.9%) towards Jaya. Heterozygosity was absent at 42 marker loci, moderate at 37 marker loci and high at 47 marker loci. The regions with higher average rate of recombination were observed on long arm of chromosome 3, 5, 6, 7, 8 and 10. The population had an average genome content of 46.7% of Pusa1266 and 49.3% of Jaya. The Pusa1266 × Jaya RIL population was found to be a good mapping population characterized by almost equal average genomic content of both parents, relatively low frequencies of heterozygosity and low percentage of skewed markers will facilitate molecular mapping of QTLs for the agronomic traits segregating in this population.

Key words: RILs, SSR markers, segregation distortion, genome content, heterozygosity, rice

Introduction

DNA markers arise from different classes of DNA mutations such as deletion, base substitution, rearrangements or errors in replication of tandemly repeated DNA. Among various DNA based markers, the microsatellite markers are particularly preferred in crop plants, as these are PCR based, genetically co-dominant, robust, reproducible, hypervariable, informative and relatively easy to use [1]. The construction of molecular map in rice paved the way for mapping rice genes on to specific locations on rice chromosomes. A number of workers have mapped micro satellite markers in rice [2-8]. The availability of rice genomic sequence database has offered new opportunities for high-resolution genetic analysis. A total of 18,828 Class I di-, tri- and tetra-nucleotide SSRs, representing 47 distinct motif families, were identified and annotated on the rice genome [9]. The sequence information and map positions of SSRs are available at <www.gramene.org>.

Construction of genetic linkage map is the primary step for molecular mapping of chromosomal regions influencing quantitative traits. However, the precision of mapping is greatly influenced by degree of segregation distortion and the extent of residual heterozygosity in a mapping population.

In an attempt to break the genetic ceiling to yield in rice, New Plant Types (NPT) from *indica* × *japonica* were conceptualized to increase both biomass (up to 23 tons/ha) and harvest index (up to 0.55) by developing a plant with attributes like moderate tillering ability, no unproductive tillers, more grains per panicle (200-250), semi dwarf stature with sturdy stems, dark green thick erect leaves and thicker and deeper roots [10]. Development of Recombinant Inbred Line (RIL) populations from genotypes derived from *indica* ×

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japonica would be useful in identifying chromosomal regions influencing new plant type traits. A RIL consisting of 310 lines (F_7) has developed by crossing cv.Pusa1266, a new plant type derivative from *indica* × *japonica* with cv.Jaya, an *indica* variety [11].

The present study was undertaken with a view to constructing a molecular genetic linkage map using this RIL and SSR markers and to estimate heterozygosity, segregation distortion and relative genome composition of RIL population for further utilization of this population for QTL mapping and fine mapping.

Materials and methods

The plant material consisted of the 310-line RIL population mentioned above. DNA was isolated by CTAB (Cetyl- Tetra Methyl Ammonium Bromide) method [12] and quantified by using gel electrophoresis in 0.8% agarose gel in 0.5 X TAE buffer along with known concentrations of genomic DNA as standard. The PCR reactions were carried out in 96-well PCR plates obtained from Axygen Scientific Inc., Union city CA, USA. The master mix consisted of 25 ng of genomic DNA, 0.2 U of *Taq* DNA polymerase, 1x PCR assay buffer with 1.5 mM $MgCl_2$, 12 ng (1.8 picomole) each of forward and reverse primer and 200 µM of dNTP mix in a 10 µl reaction volume. The reaction mix was prepared on ice and the PCR plate was immediately loaded in the thermal cycler (Eppendorf, Biometra or Applied Biosystems USA) for PCR using conditions of (1) initial denaturation at 94°C for 5 min; (2) 35 cycles of 94°C for 1 min, 55-60°C (depending on marker) for 1 min; 72°C for 2 min; (3) final extension at 72°C for 5 min. The PCR products were separated in 3% MetaPhor® agarose gel.

A set of 1063 markers comprising 521 Rice Microsatellite (RM), 139 Genic Non Coding Microsatellite (GNMS), 388 Hypervariable Simple Sequence Repeat (HvSSR) and 15 gene based markers were used for studying polymorphism between Pusa1266 and Jaya. The primer sequences for RM series markers were obtained from the gramene SSR marker web resource (www.gramene.org), GNMS markers from Parida *et al.* [13] and HvSSR markers from Harvinder *et al.* [14]. A set of 15 gene based primers were designed using the sequences of cloned genes for yield and yield related traits. The genotyping of the RILs was carried out with 162 polymorphic markers, providing genome wide coverage. The Banding pattern of each marker was recorded for each genotype. SSR allele size was determined depending on the position of band relative to the ladder.

The population χ^2 test was performed to test the deviation of the segregation pattern at each marker locus from the expected Mendelian segregation ratio of 1:1 in the RIL population. The markers showing significant deviation from the expected ratio (i.e., distorted segregation) were not used for linkage map construction. MAPMAKER/EXP software version 3.0 [15] was used to construct the genetic linkage map. A LOD score of 3.0 and a maximum distance of 47.5 cM were used to establish linkage between two markers. After determination of linkage groups and the correct linear arrangement of marker loci along the chromosomes, recombination frequencies between pairs of marker loci were estimated by using the Kosambi mapping function for conversion of recombination frequencies into genetic distances (in cM). The open-source software GGT 2.0 [16] available at site http://www.plantbreeding.wur.nl/UK/software_ggt.html was used to visualize the chromosomal maps of different RILs, for calculation of parental genome content of RILs, sorting of RILs based on parental genome and heterozygosity content. Cluster analysis of 310 RILs was done using UPGMA method based on Nei's genetic distance. The dendrogram was obtained by Tree view software and represented in the form of a curvogram.

Results and discussion

Out of the 1063 SSR markers used for polymorphism survey, 350 markers were found to be polymorphic between parental lines Pusa1266 and Jaya. The number of polymorphic markers per chromosome ranged from 63 markers on chromosome 3 to 17 markers on chromosome 12. Among the 12 chromosomes, chromosome 3 showed the highest percentage of polymorphism (48.09%) followed by chromosome 4 (43.48%) and it was minimum in chromosome 5 (23.81%). Out of the 350 polymorphic markers, 162 markers providing genome wide coverage were used for genotyping the entire RIL population (Table 1). Among 162 markers, the maximum (22) were present on chromosome 3 followed by 18 markers on chromosome 7 and the minimum number of markers (7) were present on chromosome 10. PCR product sizes of these markers ranged from 100 to 370 base pairs.

Based on χ^2 test, a total of 23 markers (14.20%) out of the 162 showed segregation distortion. Of these 23 markers, 7 were skewed towards Pusa1266 and 16 were skewed towards Jaya. The number of markers that

Table 1. Chromosome wise list of total number of markers screened, number of polymorphic markers identified, per cent polymorphism, number of markers used for genotypic RILs and name of markers

Chro No	Total markers tested	No. of poly markers	% Poly-morphism	No of markers genotyped	Polymorphic markers
1	126	34	26.98	16	RM495, RM1282, RM220, RGNMS114, RGNMS3879, RM493, RGNMS190, RM24, RM129, RGNMS239, RM237, RM302, RM212, RM5389, RM5310, RM6141
2	113	43	38.05	13	RM3732, RM7144, RM324, RM1178, RM1211, RM561, RM262, RM106, RGNMS813, RGNMS3876, RGNMS867, RM213, RM482
3	131	63	48.09	22	RGNMS1289, RM569, RM5474, RM489, RM3766, RM218, RM157A, RM1256, RGNMS3875, RGNMS3880, RM7, RGNMS1140, GS3, RM15283, RM251, RM3698, RM16, RM6266, RM168, RM55, RM570, RGNMS1135
4	69	30	43.48	14	RM551, RM16569, RM16553, RM307, RM401, RM273, RM241, HV4-21, RGNMS1539, RM3276, RM5709, RM1112, RM315
5	84	20	23.81	9	RM1182, RM159, RGNMS1776, RM413, RGNMS1826, RM18222, HV5-39, RGNMS1958, HV5-70
6	102	26	25.49	14	RM6273, RM190, RM204, RGNMS2200, RGNMS2220, RGNMS3878, RGNMS2238, RM3827, RGNMS2303, RM528, RM439, HV6-74, RM412, RM94
7	67	28	41.79	18	HV7-02, RM5344, RM5711, RM7121, RGNMS2453, RM21345, RM5499, RM214, HV7-31, RM70, RM1135, RM432, RM560, RM336, RM1132, RM505, RM429, RM3555
8	93	24	25.81	11	RM408, RM152, RM25, RM544, RGNMS2765, RGNMS2766, RGNMS2800, RGNMS2825, HV8-47, RGNMS2921, RM447
9	67	21	31.34	13	RM285, RM5688, RM219, RM6920, RGNMS3037, RM524, RM24233, RM257, RM6570, RM242, RM278, RM160, RM201
10	70	20	28.57	7	RM274, RM222, RM5348, RGNMS3250, HV10-29, RM171, RM591
11	71	24	33.80	14	RGNMS3575, RM1812, HV11-13, RM202, RM26499, RM7226, RM209, RM21, RM206, RGNMS3577, RGNMS3600, RGNMS3235, RM144, RGNMS3603
12	70	17	24.29	11	RM19, HV12-11, RGNMS3725, HV12-2, RM1261, RGNMS3766, RGNMS3781, HV12-41, RM3331, RM1103, RM17
	1063	350	32.93	162	

showed deviation from the expected 1:1 ratio per chromosome ranged from a minimum of one marker on the chromosomes 3, 4, 9 and 12 to a maximum of eight markers on chromosome 6. None of the markers on the chromosome 1, 2 and 11showed significant deviation from expected 1:1 ratio. In the present study, one segregation distorted region (SDR) was found on chromosome 6, which was skewed towards Jaya. The SDRs in the present study revealed that 17 markers in these regions on chromosome 3, 4, 5, 6 and 7 were the same regions where gametopytic or sterility loci (ga/S) have been reported [17]. On chromosome 6 markers with distorted segregation ratio were distributed all along length of chromosome including the ga/S loci. On Chromosome 4, six markers with distorted segregation were in the region where no such loci were reported earlier. The probable reason for higher (23) marker segregation distortion might be because of the RIL population was derived from the cross *indica* × *japonica.* Xu *et al.* [17] observed higher number of distorted markers in RIL population than five different types of mapping populations derived from a crosses involving indica/japonica genotypes. Segregation distortion of markers in RIL populations developed from *indica* x

japonica crosses has been reported earlier [18-21]. It is generally believed that differential gametophytic selection is responsible for segregation distortion in rice. For constructing linkage map, the 23 markers showing segregation distortion and the 13 markers showing insignificant linkages and high map distances with adjacent markers were removed from the data set.

The linkage map was developed with 126 markers distributed among 12 linkage groups spanning a total length of 2856.8 cM with an average interval of 25.06 cM (Table 2, Fig. 1a, b). The maximum number of markers (20) were present on chromosome 3 followed by chromosome 1 (15) and the minimum number of markers (5) were on chromosome 10. The map length was maximum (383.8 cM) on chromosome 3 followed by chromosome 1 (291.5 cM) and chromosome 9 had minimum map length (127.2 cM) and average marker interval (12.72 cM). The linkage map has eleven regions showing gaps on eight chromosomes. Ignoring these eleven gaps on eight chromosomes, the total map length was estimated as 2023.1 cM with an average marker interval of 19.64 cM. The linkage map obtained in the present study was largely in agreement with the other published maps in terms of the order of loci.

No linear relationship was found between physical and genetic distance because the rate of recombination varied along length of chromosome and from chromosome to chromosome. The average physical length per cM was estimated to be 169.97 Kb for this population. The average length per cM varied among 12 chromosomes from a minimum of 68.91 Kb on chromosome 8 to 315.69 Kb on chromosome 4. The regions with higher average rate of recombination were observed on long arm of chromosome 3, 5, 6, 7, 8 and 10. The recombination hot spots were also found in along chromosome length dispersed on all chromosomes. Genetically close markers may actually be far apart in terms of base pairs (or vice versa) due to differences in the frequency of recombination along the length of a chromosome [22]. Similarly, Amarawathi *et al*. [23] reported that two markers each on chromosome 4 and 11 were genetically unlinked but they were physically separated by only 1.2-1.3 MB. Discrepancy could exist between the physical and genetic distances as reported earlier in other crops like wheat [24] and barley [25].

Table 2. Chromosome wise number of markers, map length and average marker interval

Chromosome	No. of markers	Map length (cM)	Avg. marker interval (cM)
1	15	291.5	20.82
2	10	219.0	24.33
3	20	383.8	20.20
4	12	266.1	24.19
5	7	239.6	39.93
6	6	219.8	43.96
7	12	267.6	24.33
8	7	277.8	46.30
9	11	127.2	12.72
10	5	200.1	50.03
11	12	163.5	14.86
12	9	200.8	25.10
Total	126	2856.8	25.06

The average heterozygosity of the population was 0.9%, which is less than the expected heterozygosity in F_7 generation. Among 310 RILs, 102 RILs (32.90%) were completely homozygous whereas, 120 RILs (38.7%) were found to have <1.0% heterozygous loci (Table 3). Maximum heterozygosity (9.1%) was observed in RIL no. 51 followed by RIL no. 168 and 276 having 8.8% heterozygosity each. Heterozygosity genome content was maximum (1.7%) on chromosome 3 and minimum (0.1%) on chromosome 5. The percent heterozygosity among 126 markers ranged from 0-6.4%. Heterozygosity was absent at 42 (33.33%) marker loci and a moderate heterozygosity (<1.0%) was observed in 37 (29.37%) marker loci. Forty-seven (37.2%) marker loci showed > 1.0% heterozygosity of which, 14 (11.0%) loci were found to have higher proportion of heterozygosity than expected content in F_7 generation showing the stabilization of the population.

A total of 207 (66.8%) out of 310 RILs were not skewed towards any parent. These RILs had the Pusa1266/Jaya genome content ratio of 0.7-1.5 (Fig. 2). Moderate skewness was observed in 90 (29%) RILs of which, 69 were skewed towards Jaya (22.22%) with Pusa1266/Jaya genome ratio of 0.4-0.7. Almost equal number of RILs were highly skewed towards Pusa1266 (7 RILs, 2.3%) with Pusa1266/Jaya genome ratio of >2.0 and towards Jaya (6 RILs, 1.9%) with P/J ratio of <0.4. Similar observations on skewness of RILs and % hetetozygosity was observed by Liu *et al*. [26] in a 269 RIL population developed from Kaybonnet *low-phytic acid* mutant and Zhe733. In this population, 61.6% of 255 RILs were had no significant similarity with either

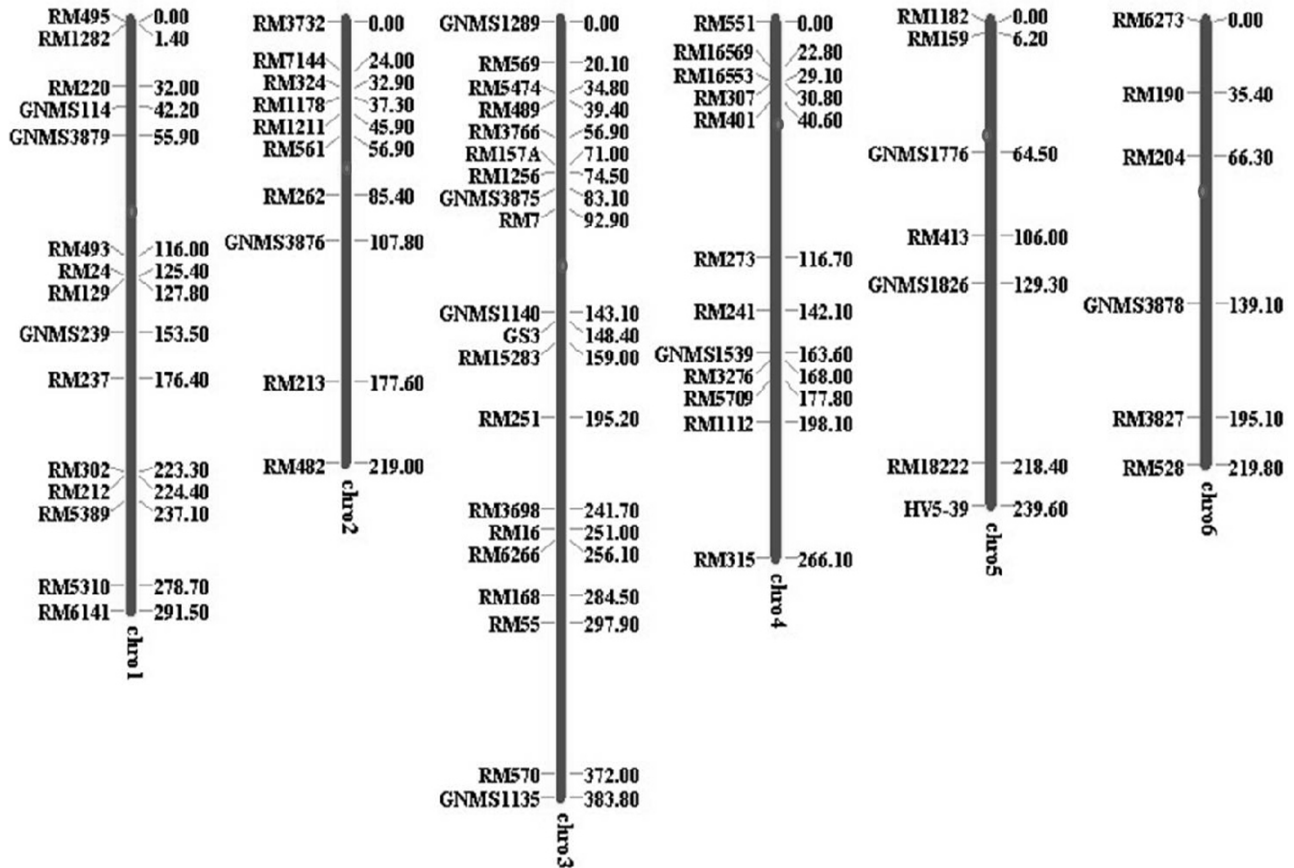

Fig. 1a. Molecular genetic linkage map of Pusa1266 x Jaya RIL population based on 126 marker data (Chromosome 1-6)

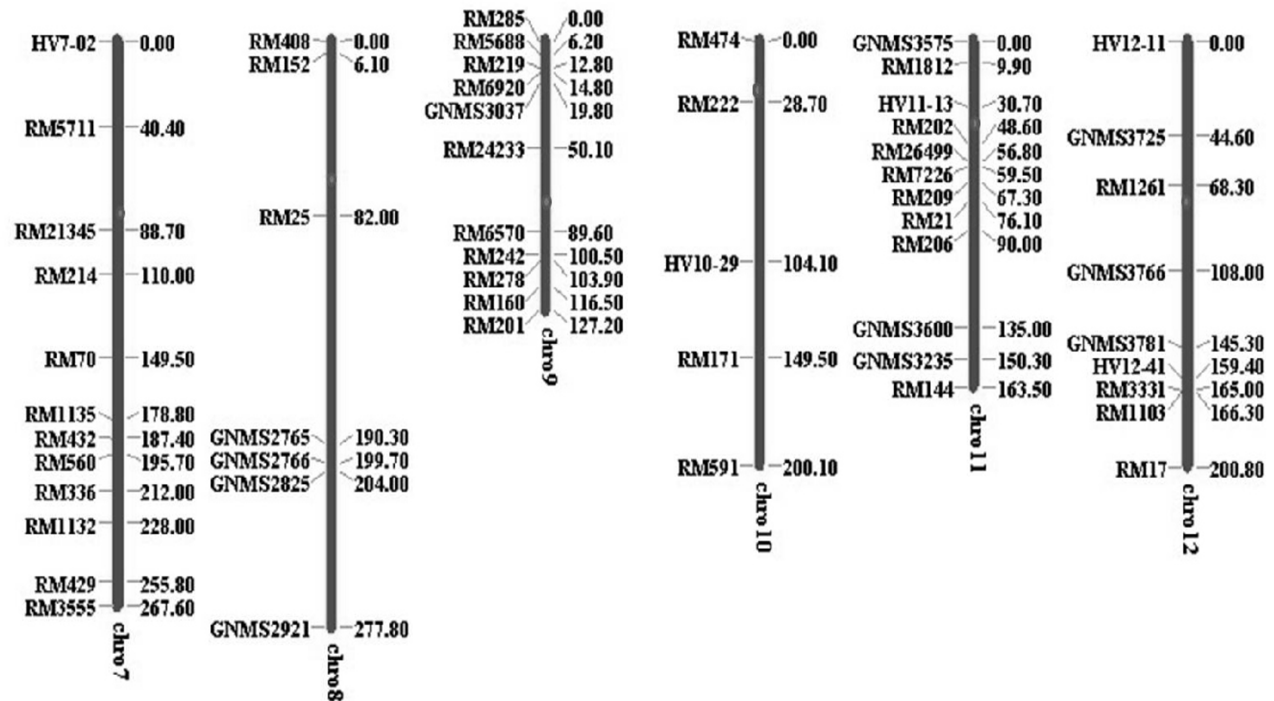

Fig. 1b. Molecular genetic linkage map of Pusa1266 x Jaya RIL population based on 126 marker data (Chromosome 7-12)

Table 3. Percent heterozygosity at marker loci in Pusa1266 and Jaya RIL population

S.No.	% of heterozygosity	No of Markers	% of Markers	No of RILs	% of RILs
1	0.0	42	33.33	102	32.90
2	0.1-0.5	14	11.11	71	22.90
3	0.6-1.0	23	18.26	49	15.80
4	1.1-1.5	8	6.35	34	10.97
5	1.6-2.0	14	11.11	22	7.10
6	2.1-3.0	11	8.74	12	3.87
7	3.1-4.0	7	5.50	9	2.90
8	> 4.1	7	5.50	11	3.54

Average % heterozygosity at marker loci 1.1; Average % heterozygous genome content in RILs 0.9

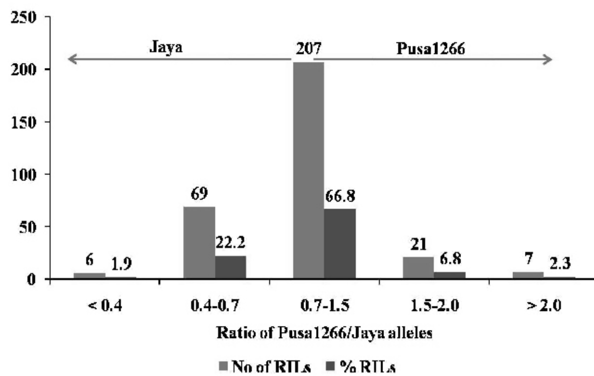

Fig. 2. Graph showing Skewness of RILs in Pusa1266 x Jaya population

of the parents and had an average heterozygosity of 1.3%. The missing data of the population ranged from a minimum of 0.9% on chromosome 1 to 6.4% on chromosome 6 with a mean of 3.1% which is considered to be negligible for QTL mapping purposes. In the present study, non parental alleles were not found at any marker loci of 310 RILs, showing the purity of the mapping population. Cluster analysis of 310 lines revealed continuous variation without formation of any sub groups within population. The continuity in distribution of RIL population indicates complete reshuffling of genome through multiple cycles of meiosis.

In the present study, the Pusa1266 × Jaya RIL population was found to be a good mapping population characterized by almost equal genomic content of both parents, relatively low frequencies of heterozygosity, absence of non-parental alleles, low percentage of skewed markers and skewed RILs will facilitate molecular mapping of QTLs for the agronomic traits segregating in this population.

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References

1. **Powell W., Machray G. and Provan J.** 1996. Polymorphism revealed by simple sequence repeats. Trends in Pl. Sci., **1**: 215-222.
2. **Wu K. S. and Tanksley S. D.** 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet., **241**: 225-235.
3. **Akagi H., Yokozeki Y., Inagaki A., Nakamura A. and Fujimura T.** 1996. A co dominant DNA marker closely linked to the rice nuclear restorer gene, Rf-1, identified with inter-SSR fingerprinting. Genome, **39**: 1205-1209.
4. **Panaud O., Chen X. and McCouch S. R.** 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol. Gen. Genet., **252**: 597-607.
5. **Chen X., Temnykh S. and Xu Y.** 1997. Development of a microsatellite framework map providing genome wide coverage in rice (*Oryza sativa* L.). Theor. Appl. Genet., **95**: 553–567.
6. **Temnykh S., Park W. D., Ayres N., Carinhour S., Hauck N., Lipovic L., Cho Y. G., Ishii T. and McCouch S. R.** 2000. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet., **100**: 698-712.
7. **McCouch S. R., Teytelman L., Xu Y., Lobos K. B., Clare K., Walton M., Maghirang B. F. R., Li Z., Xing Y., Zhang Q., Kono I., Yano M., Fjellstrom R.,**

DeClerck G., Schneider D., Cartinhour S., Ware D. and Stein L. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res., **9**: 199-207.

8. **Wu J., Maehara T., Shimokawa T., Yamamoto S., Harada C., Takazaki Y., Ono N., Mukai Y., Koike K., Yazaki J., Fuji F., Shomura A., Ando T., Kono I., Waki K., Yamamoto K., Yano M., Matsumoto T. and Sasaki T.** 2002. A comprehensive rice transcript map containing 6591 expressed sequence tag sites. Pl. Cell, **14**: 525–535.
9. **IRGSP.** 2005. The map-based sequence of the rice Genome. Nature, **436**: 793-800.
10. **Khush G. S.** 2000. New plant type of rice for increasing the genetic yield potential. Pp 99-108. Rice breeding and Genetics, Research priorities and challenges, Oxford and IBH publishing company, New Delhi.
11. **Balram M., Richa S., Sweta A., Nagarajan M., Singh N. K. and Singh A. K.** 2007. Development and characterization of a recombinant inbred line (RIL) mapping population in rice (*Oryza sativa* L.). Pusa Agri Sci., **29**: 16-21.
12. **Murray M. G. and Thompson W. F.** 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res., **8**: 4321-4325.
13. **Parida S. K., Dalal V., Singh A. K., Singh N. K. and Mohapatra T.** 2009. Genic non-coding microsatellites in the rice genome: characterization, marker design and use in assessing genetic and evolutionary relationships among domesticated groups. BMC Gen., doi:10.1186/1471-2164-10-140.
14. **Harvinder S., Deshmukh R. K., Singh A., Singh A. K., Gaikwad K., Sharma T. R., Mohapatra T. and Singh N. K.** 2010. Hypervariable SSR markers suitable for rice genotyping using agarose gels. Mol. Breed., **25**: 359-364.
15. **Lander E. S., Green P., Abrahamson J., Barlow A. and Daley M.** 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, **1**: 174-181.
16. **Berloo R. V.** 2008. GGT2.0: Versatile Software for visualization and analysis of genetic data. Jour. of Herid., **99**: 232-236.
17. **Xu Y., Zhu L., Xiao J., Huang N. and McCouch S.** 1997. Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol. and Gen. Genet., **253**: 535-545.
18. **Wang D., Zhu J. Li Z. and Paterson A. H.** 1999. User Manual for QTLMapper Version 1.0: A Computer Software for Mapping Quantitative Trait Loci (QTLs) with Main Effects, Epistatic Effects and QTL x Environment Interactions, 1-58.
19. **He P., Li J. Z., Zheng X. W., Shen L. S., Lu C. F., Chen Y. and Zhu L. H.** 2001. Comparison of molecular linkage maps and agronomic trait loci between DH and RIL populations derived from the same rice cross. Crop Sci., **41**: 1240-1246.
20. **Andaya V. C. and Mackill D. J.** 2003. QTLs conferring cold tolerance at the booting stage of rice using recombinant inbred lines from a *japonica* x *indica* cross. Theo. Appl. Genet., **106**: 1084-1090.
21. **Nandakumar N., Tyagi N. K., Balram M., Chikkappa K., Aprajita G., Mohapatra T., Prabhu K. V. and Singh A. K.** 2008. Molecular Analysis of Transgene (*psy*) Inheritance in a Golden Rice Line Developed in the Genetic Background of IR64. J. Pl. Biochem. and Biotech., **17**: 127-132.
22. **King J., Armstead I. P., Donnison I. S., Thomas H. M., Jones R. N., Kearsey M. J., Roberts L. A., Thomas A., Morgan W. G. and King I. P.** 2002. Physical and Genetic Mapping in the Grasses Lolium perenne and Festuca pratensis. Genetics, **161**: 315-324.
23. **Amarawathi Y., Singh R., Singh A. K., Singh V. P., Mohapatra T., Sharma T. R. and Singh N. K.** 2008. Molecular mapping of quantitative trait loci for basmati quality traits in rice (*Oryza sativa* L.). Mol. Breed., **21**: 49-65.
24. **Gill K. S., Gill B. S., Endo T. R. and Boyko E. V.** 1996. Identification and high density mapping of gene rich regions in chromosome group 5 of wheat. Genetics, **143**: 1001-1012.
25. **Kunzel G., Korzun L. and Meister A.** 2000. Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics, **154**: 397-412.
26. **Liu G., Bernhardt J. L., Jia M. H., Wamishe Y. A. and Jia Y.** 2008. Molecular characterization of the recombinant inbred line population derived from a *japonica-indica* rice cross. Euphytica, **159**: 73-82.