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CONSTRUCTION OF MICROSATELLITE LINKAGE MAP AND DETECTION OF SEGREGATION DISTORTION IN *INDICA* RICE (*ORYZA SATIVA* L.)

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Abstract

High density molecular linkage map is a prerequisite to identify the quantitative traits loci. Construction of linkage maps, using mapping populations derived from two major subspecies*: indica and japonica* in rice, have been reported. However, limited work was conducted in *indica* subspecies. Present studies focused to construct a molecular map based on *indica* × *indica* (Shaheen Basmati × Pokkali) derived F_2 mapping population. The detected microsatellite polymorphism was only 50.64% between parental cultivars. A total of 107 microsatellite markers were employed to amplify 108 loci, distributed throughout the genome. The marker data of 190 individuals was recorded for map construction. Most of the markers were found co-dominant, where as eight markers exhibited dominance in favor of Shaheen Basmati alleles and four in favor of Pokkali alleles. Detected segregation distortion was 7.47%, which was significantly low than previous studies. MapMaker was used to construct the linkage groups. The map spanned 1753.9 cM (Kosambi function) with microsatellite markers on 12 rice chromosomes and an average distance of 16.2 cM between markers. This map contained a greater and least percentage of markers on linkage group 3 and 12, respectively. Maximum common interval size (MCIS) analysis revealed that present map (*indica* × *indica*) covered the rice genome 98% of previous investigation (*japonica* × *indica*). Because of diverse background of parental cultivars, low segregation distortion and high genome coverage, revealed that molecular linkage map would be used as marker framework to investigate the genetics of important agronomic traits in *indica* rice.

Introduction

Rice is the second most widely cultivated cereal in the world, after wheat, and is arguably the world's most important food. *Japonica* and *Indica* are most important types of *Oryza sativa*. The characteristics and forms of these two types of rice are entirely different. Most of the rice produced in Southern Asia, including India, Pakistan, Malaysia, Thailand, Vietnam and Southern China is *Indica* rice. A rapid progress has been made towards the development of molecular marker technologies in their application in linkage mapping, molecular dissection of complex agronomic traits and marker assisted breeding (Ali *et al*., 2010; Rabbani *et al*., 2010; Yamagishi *et al.,* (2010); Hammadi *et al*., 2011). Application of molecular marker technology greatly enhanced the efficiency and accuracy of breeding process.

Selection of an appropriate population and the type of genetic markers are of prime importance to construct a linkage map. While constructing a linkage map the technical criteria (rapidity, simplicity, robustness, transferability and cost of marker technology) and methodological criteria (efficiency, rapid checking of advanced generation/ segregating population plants for some specific chromosomal regions etc.) are of principal importance. Development of microsatellite markers, which is based on simple sequence repeat (SSR), have been reported in rice by Panaud *et al*., (1996) and Temnykh *et al*., (2000, 2001). The characteristics of these markers like detection of simple sequence length polymorphism (SSLP), technically simple procedure, requirement of small amount of DNA, relatively low cost, rapid turn-around time and high power of genetic resolution displaced the restriction fragment length polymorphism (RFLPs) for genetic studies. Therefore, effective utilization of microsatellite markers has been reported for genetic variation studies (Shah *et al.,* 2013; Bakht *et al.,* 2013; Turi *et al.,* 2012), germplasm conservation and characterization, identification of quantitative trait loci, comparisons of genomes and genetic diversity studies in crop plants (Temnykh *et al*., 2000; Koyama *et al*., 2001; Rabbani *et al*., 2010; Yamagishi *et al.,* (2010); Dixit *et al*., 2012; Sadia *et al*., 2010; Septiningsih *et al*., 2012).

Several maps have been constructed so far to characterize the genetic background of agronomic traits in rice. Different types of mapping populations were employed e.g. recombinant inbred lines (Koyama *et al*., 2001), F_1 doubled haploid population (Temnykh *et al*., 2001) and segregating populations (Wu *et al*., 2010; Septiningsih *et al*. 2012) have been used for the construction of linkage maps in rice. These populations were, often, derived from the crosses possessing the genetic backgrounds of both *indica* and *japonica* for discernible allelic differences. However, construction of molecular linkage maps in closely related cultivars (*indica* × *indica* or *japonica* × *japonica*) is far more interesting and useful to rice breeders (Koyama *et al*., 2001). Therefore, a molecular linkage map was constructed using microsatellite markers

and F_2 population derived from a cross between salt tolerant *indica* rice cultivars, Pokkali, and an *indica* fine rice cultivar, Shaheen Basmati.

Materials and Methods

Construction of mapping population: Pokkali and Shaheen Basmati were used as parental lines to construct a F_2 mapping population. Pokkali was used as donor in the cross. True hybrids seedlings were selected by microsatellite assay (Fig. 1) and grown in the pots to get F_2 seeds. For the construction of molecular linkage map, the DNA of 190 F_2 individual plants were extracted.

Plant DNA extraction: DNA extraction of parental lines and mapping population was carried out as described by Ikeda *et al*. (2001) because of its simplicity, convenience and efficiency. "A few leaf pieces (about 5 mm in size) of young rice seedlings (three weeks old) of F_2 population were taken in 1.5ml microtube. After adding 200µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) in microtubes, the leaf pieces were grinded thoroughly. The microtubes were placed in boiling water for 15 minutes. After while, an addition of 800 µl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), the microtube contents were mixed well and centrifuged at 14000 rpm for three minutes. The supernatant was collected and used as template DNA for PCR".

PCR amplification: PCR was performed in 50 µl reaction containing 5 µl of extracted DNA, 0.2 µM of each primer, 100 µM of each dNTP (dATP, dCTP, dGTP and dTTP), 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Tritionx-100, and 1 unit *Taq* DNA polymerase (TOYOBO, Japan). Amplifications were carried out in a Thermal Cycler MP (TAKARA) and PTC100 Programmable Thermal Controller (MJ Research Inc., USA) as follows:

94ºC for 5 minutes followed by 35 cycles of 94ºC for 1 minute, 55ºC for 1 minute, 72ºC for 2 minutes and ending with 5 minutes at 72ºC for the final extension.

Selection of molecular marker: Yamagishi *et al.,* (2010) constructed a linkage map using restriction fragment length polymorphic (RFLP) markers and randomly amplified polymorphic DNA (RAPD) markers in rice. However, microsatellite markers were used because of their unique sequences flanking microsatellite repeats as reported by Panaud *et al*., (1996), Temnykh *et al*., (2000, 2001) and McCouch *et al*., (2002). These markers were designed to generate well-matched primers, from 17 to 22 nucleotides long, with a G C contents around 50% (melting temperature approximately 60˚C), a low frequency of primer dimmers and preferably G or C rich at 3′ end. Primers were selected to produce a PCR product in the range of 80-320 bps.

Non-radioactive detection of microsatellite alleles: The amplified products of respective microsatellite regions were electrophoreses in 4% polyacrylamide denaturing gel with 0.5X TBE buffer. The visualization of microsatellite banding patterns was carried out by non-radioactive silver staining method as prescribed by Panaud *et al*., (1996).

Parental polymorphism survey and marker data of mapping population: Six *indica* rice parental lines (Pokkali, Nona Bokra, Shaheen Basmati, Pak 221, IR 9 and KS282) were included to make seven parental combinations for initial selection of appropriate parental lines through parental polymorphic survey. Eleven microsatellite loci were selected randomly, distributed throughout the rice genome, for parental polymorphic survey. The results are described in Table 2.

Further, parental polymorphism survey was conducted between Pokkali and Shaheen Basmati (Fig. 2). Polymorphic microsatellites were selected (based on low polymorphic length, chromosomal location and were relatively easy to score) and used for the genotyping of mapping population to obtain marker data to construct the linkage map covering the entire genome. The Fig. 3 describes the segregation pattern of RM 220 (Chromosome 1) in 190 individuals of mapping population.

Analysis of segregation pattern of microsatellite markers and detection of segregation distortion: For each segregating marker, a chi-square analysis was performed to check the deviation from the expected segregation ratio in the mapping population (1:2:1 for co-dominant markers and 3:1 for dominant markers in F_2 population). Segregation distortion is the deviation of observed genotypic frequencies from expected frequencies at specific locus in segregating population/mapping population. Segregation distortion is a problem comes across in mapping studies often (Wu *et al*., 2010). The segregation pattern of microsatellite markers and detected segregation distortion in mapping population is summarized in Table 3.

Linkage analyses and map construction: The molecular linkage map was constructed by multipoint analysis (Lander & Green, 1987), using the program MapMaker v. 2.0 (Lander *et al*., 1987), with the LOD threshold fixed at 3.5 and based on the marker data of F_2 population. Map distances between the microsatellite loci were presented in centiMorgan (cM), using the Kosambi function (Kosambi, 1944). Marker order of microsatellites was followed after Temnykh *et al*., (2001) and McCouch *et al*., (2002). Framework of microsatellites was constructed first. The markers with no information were then integrated into the framework map by using "compare" command in MapMaker program and information of physical location available on Gramene (http://www.gramene.org) and Rice Genome Research Project (http://rgp.dna.affrc.go.jp/). Twelve linkage groups are shown in Fig. 4.

Comparison of molecular maps: A high-density genetic linkage map with informative markers is essential for plant genome analyses, such as gene mapping, identification of quantitative trait loci, map-based cloning, and physical map construction. In order to examine the size of each linkage group, maximum common interval size (MCIS) is computed between the two maps (*indica* × *indica* and *japonica* × *indica*) and described in Table 3.

Fig. 1. Marker assay of F_1 plants of Shaheen Basmati × Pokkali cross by amplifying the microsatellite locus RM263 on chromosome 5. Pokkali (P) and Shaheen Basmati (S) exhibited 170 bases and 160 bases, respectively. True hybrid seedlings exhibited both bands (170bp and 160bp), whereas self seedlings (2 and 9) showed a single band (160bp).

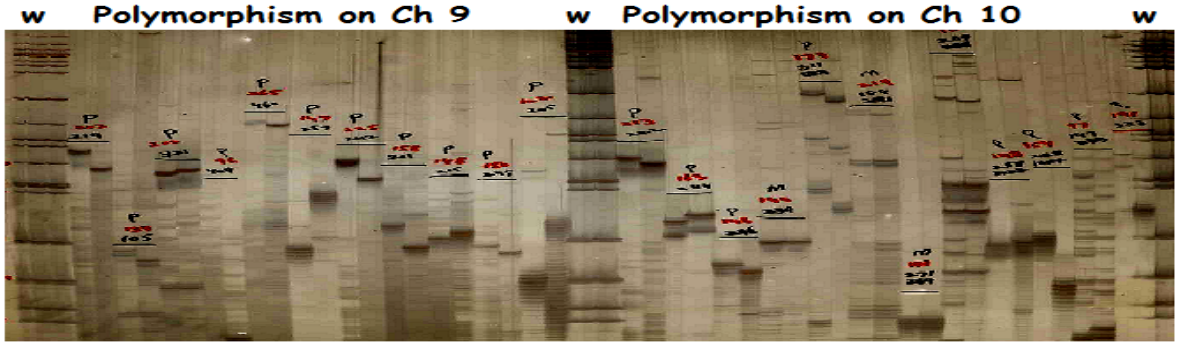

Fig. 2. Screening to identify the polymorphic microsatellite loci between salt tolerant *indica* cultivar, Pokkali, and *indica* fine rice cultivar, Shaheen Basmati on chromosome 9 and chromosome 10 (left and right, respectively).

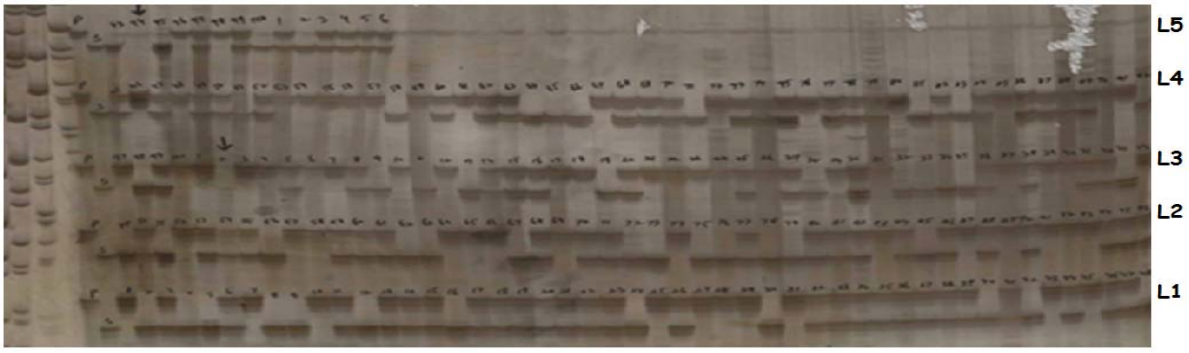

Fig. 3. Silver-stained polyacrylamide gel electrophoresis showing the segregation patterns of F_2 population derived from a cross between salt tolerant *indica* cultivar, Pokkali, and *indica* fine rice cultivar, Shaheen Basmati, at microsatellite locus RM 220 located on chromosome 1. Pokkali and Shaheen Basmati exhibited a weight of 125 and 118 bases, respectively. However, 190 F_2 individual plants showed segregation within the same range. For marker data, 1 and 3 (for parental cultivars) and 2 (hybrid plants) were used. Numbers of gel loadings (L1, L2, L3, L4, L5) are shown on right.

Results and Discussion

Highly saturated molecular maps were reported by Causse *et al*., (1994) and Harushima *et al*., (1998), which provided the foundation for molecular genetic analysis in rice. However, these were mainly constructed with RFLP markers. The development of microsatellite markers and microsatellite framework maps permitted to analyze agronomical traits much easier because microsatellite markers can detect high level of polymorphism and their detection procedure is technically simple and safe (Panaud *et al*., 1996; Temnykh *et al*., 2000, 2001; McCouch *et al*., 2002; Wu *et al*., 2010; Dixit *et al*., 2012). These maps have been constructed based on mapping populations in the background of *japonica* and *indica* rice cultivars. Koyama *et al*., (2001) described the importance of construction of molecular linkage map in closely related cultivars (*indica* × *indica* or *japonica* × *japonica*). Bing *et al*., (2006) constructed a linkage map using a mapping population derived from *japonica* × *japonica* cross. Therefore, in present studies we employed a mapping population derived from *indica* × *indica* cross. An appropriate parental combination is of prime importance in construction of a molecular map. Parental cultivars should exhibit a high polymorphism and there must not be any crossing incompatibility. Parental combinations were made on the basis of their extreme differences for morph-physiological traits. Cultivars Pokkali and Nona Bokra were included as donor parent because of their salt tolerance potential. To avoid the limitations described by Koyama *et al*., (2001), a polymorphism survey was conducted to screen the parental combinations. Seven parental combinations were used to assess the polymorphism. For polymorphism assay, 11 microsatellite markers, located on different chromosomal

regions and distributed well throughout the genome, were selected randomly and employed. Shaheen Basmati and Pokkali exhibited maximum polymorphism (90.9%) among these parental combinations (Table 1). The F_1 individuals, derived from a cross between Shaheen Basmati and Pokkali, were confirmed to be true hybrid using a polymorphic microsatellite locus RM 263 (Fig. 1). Pokkali and Shaheen Basmati exhibited a difference of 10 bases on this microsatellite locus. Two seedlings (2, 9) were found self crossed and therefore eradicated. F_1 true hybrid seedlings were grown in glass house to develop F_2 population. A total of 190 F_2 individual seedlings were used to extract the DNA for marker data.

A parental survey was conducted to identify polymorphic microsatellite markers between the two parental lines to find at least one polymorphic microsatellite marker per 20 cM throughout the genome. Out of 304 microsatellites markers, 157 (51.64%) exhibited good amplified polymorphic band pattern. Parental survey to identify the polymorphic microsatellites has been shown in Fig. 2. However, 107 pairs of microsatellite markers were employed to map the F_2 mapping population based on low polymorphic length, chromosomal location and were relatively easy to score. Amplified products of RM 4 revealed multiple loci, on linkage group 11 and 12 in present mapping studies. Thus, 107 microsatellite markers mapped 108 loci. Similar results have also been reported regarding RM 4 (Temnykh *et al*., 2000, 2001). Low marker polymorphism could be due to *indica* × *indica* parental combination. Koyama *et al*. (2001) and Septiningsih *et al*. (2012) constructed linkage maps using *indica* mapping populations and reported the low polymorphism rate between the parental cultivars. Septiningsih *et al*. (2012) reported 115 polymorphic and reliable SSR markers out of 1,074 (10.5%). Similar results were obtained when linkage map was constructed using a *japonica / japonica* mapping population (Bing *et al*., 2006).

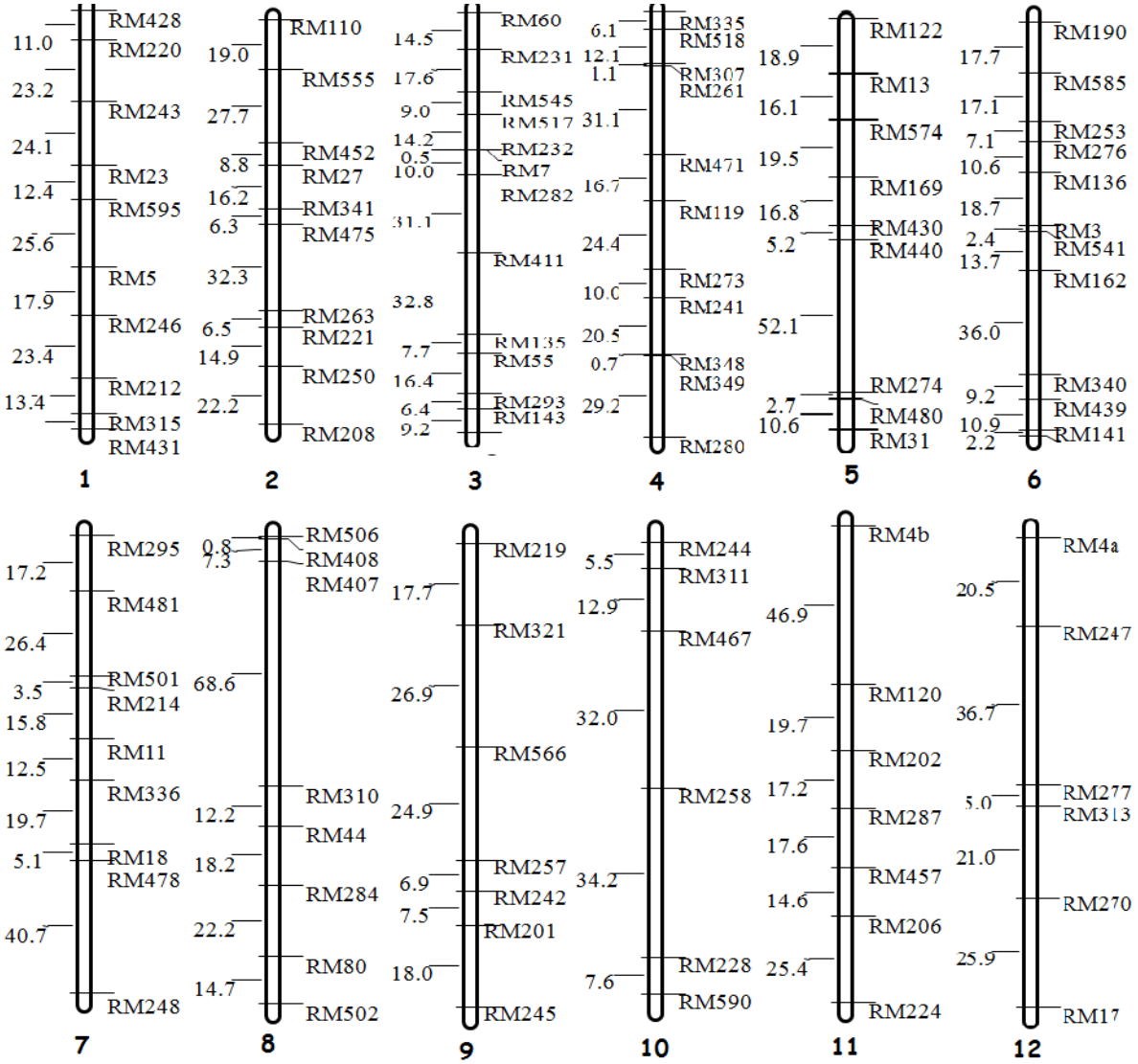

Fig. 4. A molecular linkage map construction by microsatellite markers using F_2 population derived from a cross between a salt tolerant *indica* cultivar, Pokkali, and a fine rice *indica* cultivar, Shaheen Basmati. Map distances between the microsatellite loci, in centiMorgan (cM), and microsatellite markers are presented on left and right of each chromosome, respectively, using the Kosambi function. Numbers (1-12) represent the chromosomes.

Table 1. Polymorphism survey for the selection of an appropriate parental combination.

Marker	Ch No.	Parental combinations[a]						
		1	2	3	4	5	6	7
RM 1	1	P[b]	M	P	M	P	P	P
RM 3	6	M[c]	M	P	P	P	P	M
RM 6	2	P	P	P	P	P	P	P
RM 7	3	P	P	M	P	P	M	M
RM 14	1	P	P	P	M	P	P	M
RM 82	3	P	P	M	P	P	P	P
RM 201	9	M	P	M	M	P	P	M
RM 202	11	P	P	P	P	P	P	P
RM 210	8	P	P	P	P	M	P	P
RM 273	4	M	M	M	M	M	P	M
RM 328	9	M	M	M	M	M	P	M
Polymorphism (%)		**63.6**	**63.6**	**54.5**	**54.5**	**72.7**	**90.9**	**45.5**

[a]Parental combinations, [b]Polymorphic, [c]Monomorphic

1= Pak 221 & Nona Bokra, 2= IR 9 (NIAB) & Nona Bokra, 3= Nona Bokra & Pokkali, 4= Pak 221 & Pokkali, 5= IR 9 (NIAB) & Pokkali, 6= Shaheen Basmati & Pokkali, 7= KS 282 & Pokkali

Table 2. Summary of microsatellite marker segregation and segregation distortion in mapping population.

Chromosome	0.05 p[1]	0.01 p	0.05	0.001 p	0.01 p	0.001	Total
1	8	0		1		1	10
2	8	1		0		1	10
3	13	0		0		0	13
4	11	0		0		0	11
5	9	0		0		0	9
6	12	0		0		0	12
7	8	1		0		0	9
8	8	0		0		0	8
9	7	0		0		0	7
10	6	0		0		0	6
11	6	1		0		0	7
12	4	0		0		2	6
Total	100	3		1		4	108
Frequency (%)	**92.59**	**2.78**		**0.93**		**3.70**	**100**

[1]The p-value was estimated by $\chi 2$-test with df = 1 for Mendelian segregation ratio in an F_2 population, 1:2:1 for homozygotes of the female parent: heterozygotes: homozygotes of the male parent

The summary of segregation pattern of 107 microsatellite markers at 108 loci is described in Table 2. Most of the markers were found co-dominant, where as eight (RM243, RM315, RM261, RM31, RM501, RM258, RM270, RM313) and four (RM5, RM541, RM310, RM202) exhibited dominance in favor of Shaheen Basmati and Pokkali alleles, respectively. Chi square test was applied to assess the deviation of microsatellite at particular locus from the normal segregation pattern. The segregation pattern at locus RM 220 is shown in Fig. 3. A difference of 7 bases was observed between Pokkali and Shaheen Basmati. The observed segregation ratio at locus RM 220 located on chromosome 1 did not show deviation from the expected ratio (1:2:1). A total of 8 markers (RM428, RM212, RM110, RM277, RM313 and RM208, RM501 and RM120) deviated from the expected segregation pattern. Among them 6 markers gave skewing in favor of Pokkali alleles and the rest of them in favor of Shaheen Basmati alleles. The segregation distortion detected in present studies is 7.47 %. Three markers (RM208, RM501 and RM120) exhibited significant deviation and five (RM428, RM212, RM110, RM277 and RM313) highly significant deviation from normal distribution pattern (Table 2). Microsatllite markers exhibited segregation distortion on chromosomes 1, 2, 3, 7, 11 and 12 (Fig. 4). Xu *et al*. (1997) reported the average deviation from the normal distribution in 14 interspecific mapping populations was 25.3%, where all mapping populations included the *japonica* and *indica* parental lines. Wu *et al*. (2010) mapped two populations, derived from *japonica* and *indica* crosses, and reported 23.9% and 27.1% segregation distortion in those populations. Whereas, when a linkage map was constructed using a population derived from *japonica* parents a 33.1% segregation distortion was reported (Bing *et al*., 2006). The genetic bases of segregation distortion

correspond to gametophytic genes, zygotic genes and hybrid breakdown genes. Gametophytic genes are revealed by reciprocal crosses of F_1 progenies and pollen fertility. Fifteen gametophytic genes, 34 sterility genes, and five hybrid breakdown genes have been reported and mapped by linkage analysis of classic morphological and molecular markers (Jiang *et al*., 2008). Harushima *et al*., (2001, 2002) reported that several gametophytic and zygotic barriers were uncovered from four inter-subspecific crosses in rice. The sterility and low germination ability of pollen and pollen killers could also be reproductive barriers and may relate to segregation distortion (Hu *et al*., 2006). Poor seed fertilities of F_1 were common in inter-subspecific crosses, *japonica* × *indica* , and fertilities were found lower in the F_1. Even though uncovering gametophytic genes by using F_2 populations is unrealistic, the gametophytic genes leading to segregation distortion in the F_2 populations could be inferred by comparative linkage and physical mapping of closely linked markers (Wu *et al*., 2010). The markers associated with these four gametophytic barriers showed favoring homozygotes in two F_2 population derived from *indica* × *japonica* crosses (Xu *et al*., 1997). In present studies, results revealed that a significant low segregation distortion (7.47%) was recorded in *indica* mapping population but a high segregation distortion (31.1%) was reported in *japonica* rice (Bing *et al*., 2006). It reflected that genetic basis of segregation distortion may need to be revisited.

Several linkage maps, using microsatellite markers, have already been reported in rice (Temnykh *et al*., 2000; McCouch *et al*., 2002). Since most of the markers employed in this study already have the map information like the linkage groups corresponding to the chromosomes and the marker order etc. These were used as framework markers for map construction. Markers, having no information in the previous framework maps, were also integrated into the framework of the present map. The most appropriate marker order for each linkage group was defined by "COMPARE" command of MapMaker program. The physical locations and order of the markers in each linkage group were also confirmed according to the rice genome information of GRAMENE (http://www.gramene.org). The constructed linkage map between Pokkali and Shaheen Basmati has been shown in Fig. 4. The total map size and the average interval distance between markers were 1753.9 cM and 16.2 cM, respectively. Except linkage group 1 and 9, several gaps of 30 or more than 30 cM were observed (Fig. 4) and could not be filled because of high monomorphism between the parental cultivars for all the microsatellite loci located in those chromosomal regions. The different biotypes, parental combinations and population structure would be the possible reasons because Temnykh *et al*., (2001) mapped a doubled haploid population derived from a F_1 cross between IR64 (*indica* cultivar) and Azucena (*javanica* cultivar) for the construction of molecular map, however present studies were carried out using a F_2 population derived from a cross between salt tolerant *indica* cultivar, Pokkali, and an *indica* fine rice cultivar, Shaheen Basmati. Similarly, Koyama *et al*. (2001) used a population derived from a multiple cross of *indica* cultivars, however, mapping of several chromosomes could not be carried out. Bing *et al*., (2006) constructed map using a *japonica* population but unable to cover even the linkage groups. Whereas, Liu *et al*., (2008) reported a linkage map using a *japonica* / *indica* mapping population and mapped a total size of 1,016.3 cM and average genetic distance between two markers 9.3 cM but could not produce a saturated linkage map with microsatellite markers. Wei-ming *et al*., (2000) reported a molecular map constructed on ALFP and RFLP marker data in *indica* rice and covered a length of 1435.8 cM but many chromosomal regions could not be covered. Therefore, it may need to insert several other microsatellites to construct a more saturated linkage map for precise quantitative trait loci.

Table 3. Comparison of chromosomal map length between *indica* x *indica* and *japonica* x *indica* mapping populations using MCIS (maximum common interval size).

Chromosome number/ linkage group	Maximum common interval size (MCIS)			
	Interval of microsatellite markers	Present map (A) Mapping population (*indica* × *indica*)	Map*(B) Mapping population (*japonica* × *indica*)	A/B
1	RM 428-RM431	166.7	196.5	0.85
2	RM110-RM208	163.9	203.4	0.81
3	RM60-RM227	179.4	231.0	0.78
4	RM335-RM280	162.9	155.8	1.05
5	RM122-RM31	150.9	141.8	1.06
6	RM190-RM494	157.6	149.7	1.05
7	RM295-RM248	149.9	116.6	1.29
8	RM506-RM502	152.0	128.6	1.18
9	RM219-RM245	108.9	114.7	0.95
10	RM244-RM590	98.2	118.3	0.83
11	RM4B-RM224	148.4	125.6	1.18
12	RM4A-RM17	115.1	112.7	1.02
Total		**1753.9**	**1794.7**	**0.98**

*Molecular map constructed by Temnykh *et al*., (2001)

The previous linkage map reported by Temnykh *et al*., (2001) was used to examine the present mapping. Therefore, to examine the size of each linkage group, maximum common interval size (MCIS) between the maps was also computed (Table 3). The recombination frequencies or the interval distances were also compared between the present study and that of Temnykh *et al*., (2001) to understand the marker order and location in each linkage group. The interval distances were approximately the same with some exceptions. The maximum differences between the interval distances ranged from -23 to 19cM. In linkage group 4, the interval distance between micsatellite loci, RM349 and RM280, was 5.5cM in the framework map. However, the interval distance between then was 29.2cM in present study with a difference of -23.7cM. The interval distance between RM541 and RM162 was 32.8cM in linkage group 6 as reported previously. Whereas, the interval distance between then was 13.7cM, with a difference of 19.1cM from the previous report. These differences in the interval distances could be due to the nature and structure of mapping populations. The linkage group sizes of present mapping were compared with that of Temnykh *et al.,* (2001). MCIS was used for this comparison and described in Table 3. This presents a better parameter than total linkage group size, because it eliminates errors due to the lack genome coverage at chromosomal ends. It is reflected that most of the linkage groups exhibited similar size. However, linkage groups 2, 3 and 10 showed a lower mean recombination rates. This was due to the small numbers of markers used in these linkage groups because of low polymorphism between Pokkali and Shaheem Basmati.

Conclusion

Present studies were carried out using a mapping population (F_2) derived from a cross between Pokkali, a salt tolerant *indica* cultivar, and Shaheen Basmati, a fine rice *indica* cultivar. The molecular linkage map was constructed based on marker data of 107 microsatellites, distributed throughout the rice genome. A total map size 1753.9 cM is reported by mapping 12 linkage groups. The segregation distortion exhibited by microsatellite markers was recorded significantly low, either in interspecific mapping populations (*japonica* × *indica*) or intraspecific mapping populations of *japonica* or *indica* type. Moreover, present map covers 98% genomic coverage in *indica* rice compared to previous studies in interspecific mapping populations.

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