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Original Article

Genetic Analysis of SSR Markers in F_2 Reciprocal Populations of the Rice genotypes, Horkuch and IR29 show high segregation distortion

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ABSTRACT: A total of 58 simple sequence repeat (SSR) markers were tested for genotyping and constructing a genetic linkage map of a reciprocal F_2 populations of a salt tolerant rice landrace, Horkuch and high-yielding but sensitive IR29. The IR29♀ (IH) population was 200, while the Horkuch♀ (HI) one, 100. In the F_2 population, 24 (~73%) and 39 (78%) markers showed significant segregation distortion ($P<0.05$) in the IH and HI populations respectively. 14 and 10 markers were skewed for the female, IR29 and male Horkuch, respectively in the IH population. On the other hand, 19 markers each were skewed either toward the female parent Horkuch or male parent IR29 with one for the heterozygote in the HI population. Nuclear and cytoplasmic effects were detected for the 25 common markers used in the 2 populations. The segregation distortion of 16 (64%) and 9 (36%) markers were found to have nuclear and cytoplasmic effects respectively. Gametic and zygotic selections analyzed by allele frequency and F_2 genotype frequency distribution showed that the segregation distortion of 8, 5 and 22 markers were influenced by respectively by zygotic, gametic or both selections simultaneously. 15 out of 50 marker loci of the HI population were mapped on 7 linkage group covering a total length of 462.857 cM on the rice genome (average intervals of 30.86 cM between adjacent markers). No linkage group was found on chromosome 2, 4, 6, 8 and 11.

KEYWORDS: Horkuch, SSR markers, Segregation distortion, Genetic linkage map

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INTRODUCTION

Segregation distortion (SD) is a prevalent phenomenon caused by the deviation of the observed genotypic frequency from expected Mendelian segregation ratios within a segregating population. Segregation distortion is being increasingly recognized as a potent evolutionary force that is influenced by many factors, for example, mapping population, marker types, genetic transmission, gametic and zygotic selections, non-homologous recombination, gene transfer, transposable elements, environmental agents and so on[1-3].

In plants, Mangelsdorf and Jones[4] first reported the phenomenon of segregation distortion in maize and subsequently segregation distortion studies were conducted in many other plant species including wheat[5-7], sorghum[8], barley[9-12], tomato[13] and tobacco[14].

In rice, segregation distortion was also reported many times[15,16] involving F_2[17,18], reciprocal F_2[3,19,20], backcross progenies[19], Recombination Inbred Lines (RIL)[21] and doubled haploid[3] populations from intra and interspecific crosses.

Segregation distortion can be detected by wide range of markers including morphological markers, enzyme

markers and molecular markers[22,23]. Molecular markers are preferred over other markers as these are less prone to the influence of phenotype and more convenient for analysis of segregation distortion. Among molecular markers, Simple Sequence Repeats (SSR) markers are of particular interest for analyzing SD in all major crops and have also been extensively used for genome mapping both in plants and animals. Assigning SSR loci to linkage groups has been reported in many plant species including maize[24], rice[25-27], barley[28], soybean[29], chickpea[30,31] and oilseed rape[32]. Moreover, the development of high density molecular linkage maps can be used to survey the whole genome for loci showing distorted segregation[33,34].

In the present study, two reciprocal F_2 populations developed from IR29 (*indica*) and salt tolerant Bangladeshi rice variety Horkuch (*aromatic*)[35] were genetically analyzed with polymorphic SSR markers. We first constructed a rice genetic linkage map of the F_2 populations using SSR markers, then assessed the frequency of distorted marker alleles, followed by identification of chromosomal regions consistently associated with segregation distortion in rice and discussion of the potential factors involved in the latter. Influence of nuclear genetic or cytoplasmic factors on SD rates was detected from the distortion patterns of DNA markers in the reciprocal F_2 populations. Information of the loci, genetic elements and other factors responsible for SD in rice are important for the selection of breeding cultivars, and could also aid the development of molecular breeding programs.

MATERIALS AND METHODS

Plant materials

F_1 hybrids derived from reciprocal crosses between Horkuch (HK) and IR29 and two F_2 populations generated from the reciprocal F_1 hybrids were grown at the Plant Biotechnology Laboratory net house, University of Dhaka. In our study, 200 and 100 individuals of the F_2 generations derived from the crosses of IR29♀ × HK (IH) and HK♀ × IR29 (HI) respectively were genotyped.

DNA extraction

Genomic DNA was isolated from (0.5–1.0 g) pooled leaf tissue of an F_2 individual using the modified CTAB method[36] followed by quantification using the nanodrop spectrophotometer (Nanodrop 1000). The quality of the DNA was checked in 0.8% agarose gel in TAE (Tris acetate EDTA) buffer, pH 8.0.

SSR markers and PCR amplification for identification of polymorphic SSR

A total of 58 polymorphic markers were selected for genotyping of the IH and HI population. Chromosome 1, 5 and 12 have 8, 6 and 12 markers respectively whereas chromosome 3, 4, 7, 8, 9, 10 and 11 have 4 markers each. Chromosome 2 and 3 have 2 and 3 markers respectively. These markers were synthesized and obtained commercially from IDT-1st BASE, Singapore.

Polymerase chain reaction (PCR) was conducted with a final concentration of 2.3 mM $MgCl_2$, 0.1 mM dNTPs, 0.3 µM of each primer (forward and reverse primers) and 1 unit of recombinant Taq polymerase (Invitrogen). After initial denaturation at 95°C for 5 mins, DNA amplification was carried out by 35 cycles of denaturation at 95°C for 1 min, 1 min of annealing at 55-60°C (depending on primer Tm), 1 min of extension at 72°C with a final extension step at 72°C for 10 min.

Amplified PCR products were electrophoresed on non-denaturing 10% polyacrylamide gel electrophoresis in 1×TBE buffer. After EtBr staining the gels were visualized with alpha imager gel documentation system.

Genotyping and analysis of segregation distortion of markers

Based on the results of non-denaturing 10% polyacrylamide gel electrophoresis, the band type of per F_2 line similar to Horkuch was recorded as "A", similar to IR29 was recorded as "B", Heterozygotes were recorded as "H", indistinct or lack of band was recorded as "-".

For each locus in this research, the observed segregations were tested against the theoretical expected Mendelian ratio (1:2:1) in F_2 populations. Chi-square test was used to calculate the segregation distortion for each of the microsatellite loci with probability levels ($p = 0.05$ and $p = 0.01$) and the reason for segregation distortion was analyzed. Additionally, segregation distortion region (SDR) was detected if three or more closely linked markers distorted significantly in the F_2 population. The most deviated marker in an SDR was considered the most likely location of a distorting factor.

Analysis of genetic distortion factors

The significant extent of the distorted markers, allele frequency homogeneity ($p=q$) and the distribution of genotype frequencies (p^2:2pq:q^2) of distorted markers were computed using Chi-square test with probability levels ($p = 0.05$ and $p = 0.01$) in order to determine the gametic and zygotic selections of SD[17].

Construction of molecular genetic map

JoinMap 4.0[37] was used to construct the genetic map using the Kosambi mapping function. Kosambi mapping function was used to calculate the genetic distances between the markers and convert recombination frequencies into map distances (centimorgan, cM). The maximum recombination rate was set to 0.40 and LOD threshold of 3.0 was chosen. Mapchart 2.3 software[38] was used to draw the map using the map distances and loci obtained from JoinMap.

RESULTS

Chromosomal distribution of the SSR markers

A total of 58 polymorphic markers were used for genotyping of the reciprocal F_2 population that were distributed throughout 12 chromosomes (Figure 1).

Among these 58 markers, 25 markers were common for both the HI and IH populations. Additionally, 25 and 8 markers were used for genotyping of HI and IH population respectively.

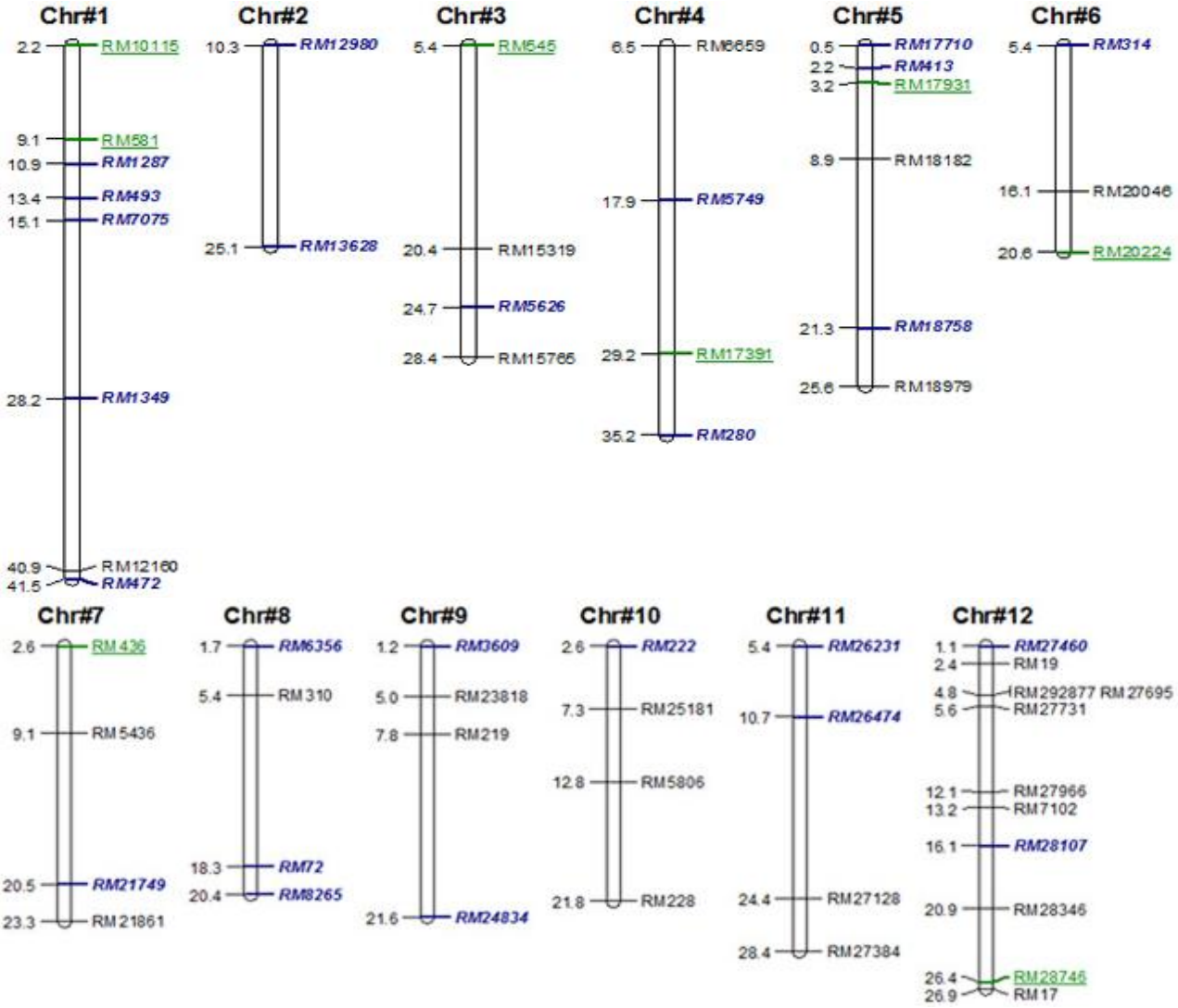

Figure 1. Physical location of markers on chromosomes (Mbp). Blue color = Common markers, Green color = Markers used in IH population, Black color = Markers used in HI population

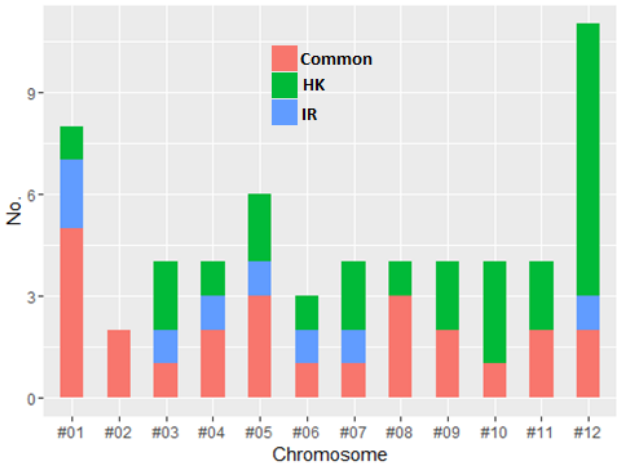

Figure 2. Chromosome-wise distribution of markers. Common = common markers for both IH and HI populations, HK = markers for only HI population, IR = markers for only IH population.

Chromosome 1 and 12 have 8 and 11 markers respectively whereas other chromosomes have 2-6 markers each. Chromosome-wise distribution of markers was illustrated in Figure 2.

Chromosomal distribution of the distorted markers

Genotyping of reciprocal F_2 populations (HI and IH) of Horkuch and IR 29 was conducted using SSR markers that were distributed throughout 12 chromosomes.

Segregation distortion of the F_2 populations was identified by the pattern of observed DNA markers after PCR and separation in gels. Among the 50 markers used to analyze the HI population, only 11 fitted the expected Mendelian

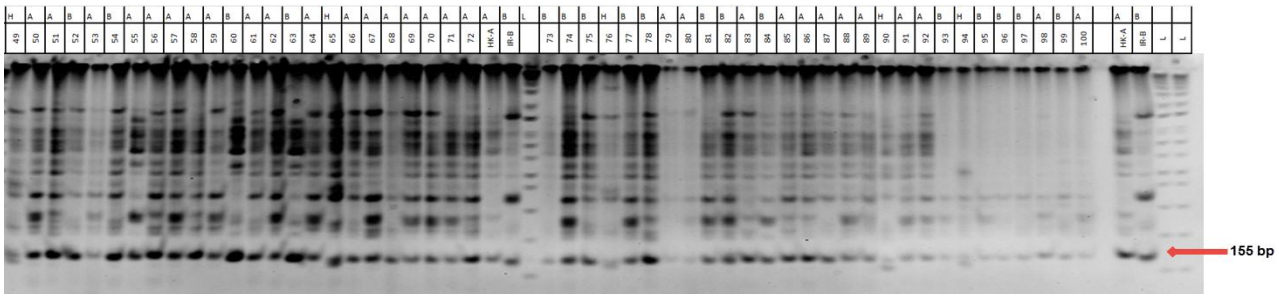

Figure 3. Representative gel showing the polymorphic bands in F_2 population of HI (genotyped with RM7075). L = Ladder, A = Horkuch, B = IR29, H = Heterozygotes, Each number represents on individual

segregation, whereas 4 (8%) significantly deviated from it (P<0.05),and 35 (70%) had extremely significant deviation (P<0.01). In case of the IH cross, where a total of 33 polymorphic markers were used, 9 markers (27.3%) were found to fit the expected Mendelian segregation, whereas 4 (12%) and 20 (60%) markers showed significant (P<0.05) and extremely significant deviation (P<0.01) respectively (Table 1).

Among the 25 markers common for both IH and HI, 24 markers were found to be distorted in both the populations, or at least in one of them. Only RM6356 did not show any segregation distortion.

All the markers on chromosome 4 and 11 showed significant deviation in both populations. All the markers except RM413 on chromosome 5 deviated significantly. However, RM413 showed significant distortion in the IH population. Both the markers on chromosome 2 were distorted significantly in HI population but none in IH population. In the HI population, seven out of nine markers on chromosome 12 were detected to have extremely significant deviation (P<0.01) (Table 1).

In the HI population, 19 each of the markers deviated either towards the female parent, Horkuch, or the male parent IR29, whereas only RM5749 was skewed towards the heterozygote.

On the other hand, among 24 distorted markers analyzed in the IH population, 14 and 10 markers skewed towards female parent, IR29 and male parent Horkuch, respectively but none towards heterozygotes (Table 1).

Table 1: Chi-square test for segregation distortion of markers in population.

		HK × IR29 (HI)					IR29 × HK (IH)				
Chr. No.	**Locus**	**A**	**h**	**B**	**χ^2**	**Favored genotype**	**A**	**h**	**b**	**χ^2**	**Favored genotype**
1	RM12160	67	29	3	99.73**	HK					
1	RM10115						155	11	2	405.56**	HK
1	RM1287	26	26	47	31.22**	IR	50	87	9	28.4**	HK
1	RM1349	18	38	26	2		33	72	55	7.65*	IR
1	RM472	37	29	31	16.42**	HK	89	0	75	166.39**	IR
1	RM493	21	42	35	6*	IR	27	86	54	8.88*	IR
1	RM7075	65	7	28	101.34**	HK	35	77	53	4.66	
1	RM581						98	0	64	176.27**	HK
2	RM12980	47	11	42	61.34**	HK	48	84	35	2.03	
2	RM13628	91	5	2	240.67**	HK	42	70	54	5.81	
3	RM15319	19	54	23	1.83						
3	RM15765	41	2	43	78.28**	IR					
3	RM5626	35	11	54	68.06**	IR	45	6	113	197.27**	IR
3	RM545						22	31	92	115.1**	IR
4	RM17391	5	50	43	29.51**	IR					-
4	RM280	23	18	56	60.81**	IR	71	6	89	146.77**	IR
4	RM5749	13	60	25	7.88*	Hybrid	22	80	49	10.19**	IR
4	RM6659	24	27	47	30.55**	IR					
4	RM17931						57	73	34	8.43*	HK
5	RM17710	15	46	35	8.5*	IR	37	32	86	84.41**	IR
5	RM18182	47	2	46	87.19**	HK					
5	RM18758	76	1	23	152.22**	HK	128	0	37	265.38**	HK
5	RM18979	30	2	61	105.84**	IR					
5	RM413	27	41	31	3.24		42	70	55	6.39*	IR
6	RM20224						44	78	43	0.5	
6	RM20046	27	45	27	0.82						
6	RM314	31	11	58	75.42**	IR	37	75	43	0.63	
7	RM21749	17	47	36	7.58*	IR	26	57	48	9.6**	IR

7	RM21861	59	3	31	98.25**	HK					
7	RM436						89	0	61	160.45**	HK
7	RM5436	17	49	34	5.82						
8	RM310	27	28	45	25.84**	IR					
8	RM6356	29	40	24	2.35		39	83	46	0.61	
8	RM72	45	10	45	64**	HK	47	3	115	209.27**	IR
8	RM8265	52	7	39	75.45**	HK	75	0	86	162.5**	IR
9	RM219	19	65	5	23.29**	HK					
9	RM23818	26	34	26	3.77						
9	RM24834	43	29	27	22.15**	HK	127	2	25	281.22**	HK
9	RM3609	69	9	20	114.31**	HK	14	28	126	224**	IR
10	RM222	45	34	21	21.76**	HK	50	74	30	5.43	
10	RM228	36	10	52	67.31**	IR					
10	RM25181	34	44	21	4.64						
10	RM5806	27	56	16	4.15						
11	RM26231	18	28	53	43.42**	IR	64	25	73	78.43**	IR
11	RM26474	52	0	48	100.32**	HK	100	0	67	180.04**	HK
11	RM27128	77	21	2	146.14**	HK					
11	RM27384	41	5	52	81.49**	IR					
12	RM17	20	10	70	114**	IR					
12	RM19	30	47	23	1.34						
12	RM27460	29	29	42	21.02**	IR	35	93	39	2.35	
12	RM27731	66	5	28	109.18**	HK					
12	RM27966	85	9	6	192.06**	HK					
12	RM28107	53	17	26	55.23**	HK	79	72	17	49.19**	HK
12	RM28346	31	43	24	2.47						
12	RM292877	10	35	45	31.67**	IR					
12	RM27695						31	57	21	2.06	
12	RM28746						74	17	65	96.45**	HK
12	RM7102	21	9	70	115.26**	IR					

*Note: * at 0.05 significant level; ** at 0.01 significant level. HK = Horkuch, IR = IR29, Hybrid = F_2 specific heterozygote, a = Horkuch like genotype, b = IR29 like genotype, h = heterozygote*

Nuclear and cytoplasmic effects on SD in reciprocal F_2 populations

Similar distortion in both reciprocal F_2 populations indicates that nuclear genetic factors are responsible for SD whereas if the markers are distorted in only one of the reciprocal F_2 populations, a cytoplasmic effect can be inferred. Out of the 25 common markers, 16 markers were deviated from the expected Mendelian segregation ratio in both reciprocal F_2 populations indicating effects of nuclear genetic factors on SD (Table 2).

Eight markers were distorted in only one of the two reciprocal F_2 populations indicating a cytoplasmic effect on SD. Among these 8 markers, two markers (RM1349 and RM413) showed SD only in the IH population whereas six markers (RM12980, RM13628, RM27460, RM222, RM7075 and RM314) showed SD only in the HI population.

For these 8 markers, favored marker genotypes also differed between the IH and HI populations. Both markers in IH population favored the IR29 genotype but in the HI population 3 out of 6 markers favored the Horkuch genotype, while the remaining 2 markers (RM314 and RM27460) favored the IR29 genotype (Table 2).

Table 2: Nuclear and cytoplasmic effects on SD.

HK X IR29 (HI)						IR29 X HK (IH)					
Locus	**a**	**h**	**b**	**χ^2**	**Favored genotype**	**A**	**h**	**b**	**χ^2**	**Favored genotype**	**Nuclear/cytoplasmic effects**
RM1287	26	26	47	31.22**	IR	50	87	9	28.4**	HK	Nuclear
RM472	37	29	31	16.42**	HK	89	0	75	166.39**	IR	Nuclear
RM493	21	42	35	6*	IR	27	86	54	8.88*	IR	Nuclear
RM5626	35	11	54	68.06**	IR	45	6	113	197.27**	IR	Nuclear
RM280	23	18	56	60.81**	IR	71	6	89	146.77**	IR	Nuclear
RM5749	13	60	25	7.88*	Hybrid	22	80	49	10.19**	IR	Nuclear
RM17710	15	46	35	8.5*	IR	37	32	86	84.41**	IR	Nuclear
RM18758	76	1	23	152.22**	HK	128	0	37	265.38**	HK	Nuclear
RM21749	17	47	36	7.58*	IR	26	57	48	9.6**	IR	Nuclear

RM72	45	10	45	64**	HK	47	3	115	209.27**	IR	Nuclear
RM8265	52	7	39	75.45**	HK	75	0	86	162.5**	IR	Nuclear
RM24834	43	29	27	22.15**	HK	127	2	25	281.22**	HK	Nuclear
RM3609	69	9	20	114.31**	HK	14	28	126	224**	IR	Nuclear
RM26231	18	28	53	43.42**	IR	64	25	73	78.43**	IR	Nuclear
RM26474	52	0	48	100.32**	HK	100	0	67	180.04**	HK	Nuclear
RM28107	53	17	26	55.23**	HK	79	72	17	49.19**	HK	Nuclear
RM1349	18	38	26	2	-	33	72	55	7.65*	IR	Cytoplasmic
RM413	27	41	31	3.24	-	42	70	55	6.39*	IR	Cytoplasmic
RM7075	65	7	28	101.34**	HK	35	77	53	4.66	-	Cytoplasmic
RM12980	47	11	42	61.34**	HK	48	84	35	2.03	-	Cytoplasmic
RM13628	91	5	2	240.67**	HK	42	70	54	5.81	-	Cytoplasmic
RM314	31	11	58	75.42**	IR	37	75	43	0.63	-	Cytoplasmic
RM27460	29	29	42	21.02**	IR	35	93	39	2.35	-	Cytoplasmic
RM222	45	34	21	21.76**	HK	50	74	30	5.43	-	Cytoplasmic

*Note: * at 0.05 significant level; ** at 0.01 significant level. HK = Horkuch, IR = IR29, Hybrid = F_2 specific heterozygote, a = Horkuch like genotype, b = IR29 like genotype, h = heterozygote*

The genetic distortion factors

For the HI population, the extent of distorted markers was analyzed by Chi-square test. Chi-square test of allele frequency homogeneity (p=q) and the distribution of genotype frequencies (p^2:2pq:q^2)was conducted to infer whether there were gametic or zygotic selections[17].

Among 39 distorted markers, 27 markers (69%) deviated significantly toward allele frequency homogeneity (H = I) and 32 markers (82%) toward F_2 distribution (H^2:2HI:I^2) in the HI population (Table 3).

Table 3: Analysis of allele frequency of distorted markers in HI population.

Marker	Allele frequency		χ^2	
	H	I	Allele frequency homogeneity (H = I)	F_2 distribution (H^2: 2HI:I^2)
RM493	0.438	0.563	3.00	1.19
RM1287	**0.394**	**0.606**	**8.91****	**20.05****
RM472	0.531	0.469	0.74	15.50**
RM7075	**0.685**	**0.315**	**27.38****	**70.19****
RM12160	0.823	0.177	82.75**	0.00
RM12980	0.525	0.475	0.50	60.75**
RM13628	**0.954**	**0.046**	**161.65****	**17.10****
RM5626	**0.405**	**0.595**	**7.22****	**59.56****
RM15765	0.488	0.512	0.09	78.18**
RM280	**0.330**	**0.670**	**22.45****	**32.66****
RM5749	0.443	0.557	2.49	6.22*
RM17391	0.306	0.694	29.47**	3.96
RM6659	**0.403**	**0.597**	**6.97****	**14.64****
RM17710	0.396	0.604	8.33**	0.00
RM18758	**0.765**	**0.235**	**56.18****	**94.52****
RM18182	0.505	0.495	0.02	87.17**
RM18979	**0.333**	**0.667**	**20.67****	**84.22****
RM314	**0.365**	**0.635**	**14.58****	**58.17****
RM21749	0.405	0.595	7.22**	0.06
RM21861	**0.651**	**0.349**	**16.86****	**80.27****
RM72	0.500	0.500	0.00	64.00**
RM8265	0.566	0.434	3.45	71.57**
RM310	**0.410**	**0.590**	**6.48***	**17.75****
RM3609	**0.750**	**0.250**	**49.00****	**55.88****
RM24834	**0.581**	**0.419**	**5.17***	**15.72****
RM219	**0.585**	**0.415**	**5.11***	**23.93****
RM222	**0.620**	**0.380**	**11.52****	**7.75***

RM228	**0.418**	**0.582**	**5.22***	**61.21****
RM26231	**0.323**	**0.677**	**24.75****	**12.37****
RM26474	0.520	0.480	0.32	100.00**
RM27384	0.444	0.556	2.47	78.79**
RM27128	0.875	0.125	112.50**	0.16
RM27460	0.435	0.565	3.38	16.81**
RM28107	**0.641**	**0.359**	**15.19****	**36.36****
RM17	**0.250**	**0.750**	**50.00****	**53.78****
RM27731	**0.692**	**0.308**	**29.17****	**76.93****
RM7102	**0.255**	**0.745**	**48.02****	**58.24****
RM27966	**0.895**	**0.105**	**124.82****	**27.16****
RM292877	0.306	0.694	27.22**	0.63

*Note: * at 0.05 significant level; ** at 0.01 significant level. Bold = significant distortion in both allele frequency homogeneity and F_2 genotype frequency distribution*

23 out of the 27 markers deviating toward allele frequency showed extremely significant distortion (P<0.01) whereas the remaining 4 markers showed significant distortion (P<0.05).

In case of F_2 distribution, 30 markers out of the 32 showed extremely significant distortion (P<0.01). OnlyRM5749 and RM222 showed significant distortion (P<0.05).

In case of 39 distorted markers, both allele frequency and F_2 genotype frequency distribution of 22showed significant segregation distortion indicating that their segregation was influenced by both gametic and zygotic selections simultaneously in HI population.

Allele frequencies of RM472, RM12980, RM5749, RM72, RM18182, RM8265, RM26474, RM27384 and RM27460 did not show significant distortion, but their F_2 genotype frequencies were distorted significantly. So it was inferred that their segregation were influenced by zygotic selection only.

Conversely, RM12160, RM17391, RM21749, RM27128 and RM292877 distorted significantly in allele frequency, but their F_2 genotype frequencies did not show any significant distortion. These results suggested that the gametic selection had greater influence on the segregations. Beside these segregations, allele frequency and F_2 genotype frequency of RM493 did not show any significant distortion but deviated toward IR29 genotype (Figure 4).

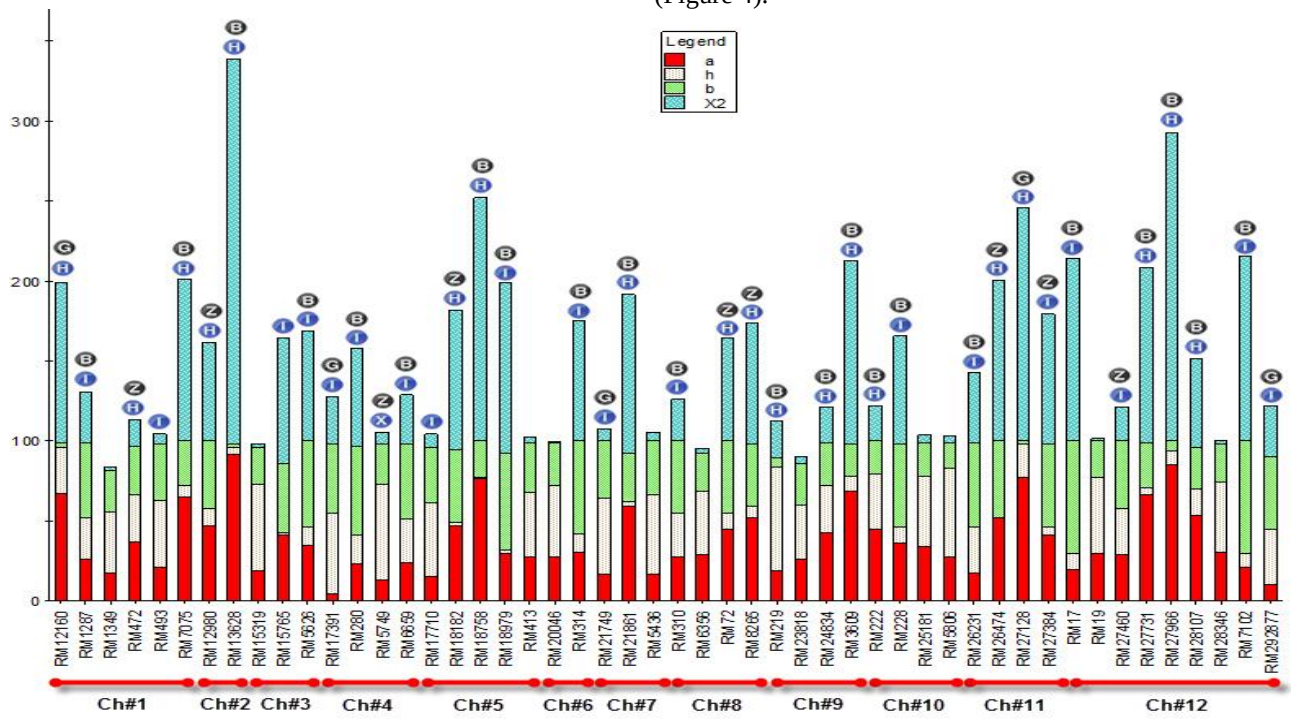

Figure 4. Locus genotype frequency, χ^2, genotype skewness and genetic distortion factors for the HI population.

Skewness towards genotype is represented by blue circle and genetic distortion factors as black circle over the bar. (H) = Skewed toward Horkuch, (I) = Skewed toward IR29, (X) = Skewed toward Heterozygote, (B) = both gametic and zygotic selections, (G) = gametic selection, (Z) = zygotic selection and (N) = No selection

In the IH population, analysis of allele frequency of distorted markers showed that 15 out of 24 markers (62.5%) distorted significantly (P<0.05) toward both allele frequency homogeneity (H = I) and F_2 distribution (H^2: 2HI:I^2), RM17710 towards none and remaining 8 markers toward either allele frequency homogeneity or F_2 distribution (Table 4). Each of 4 markers distorted toward allele frequency homogeneity and F_2 distribution (Figure 5).

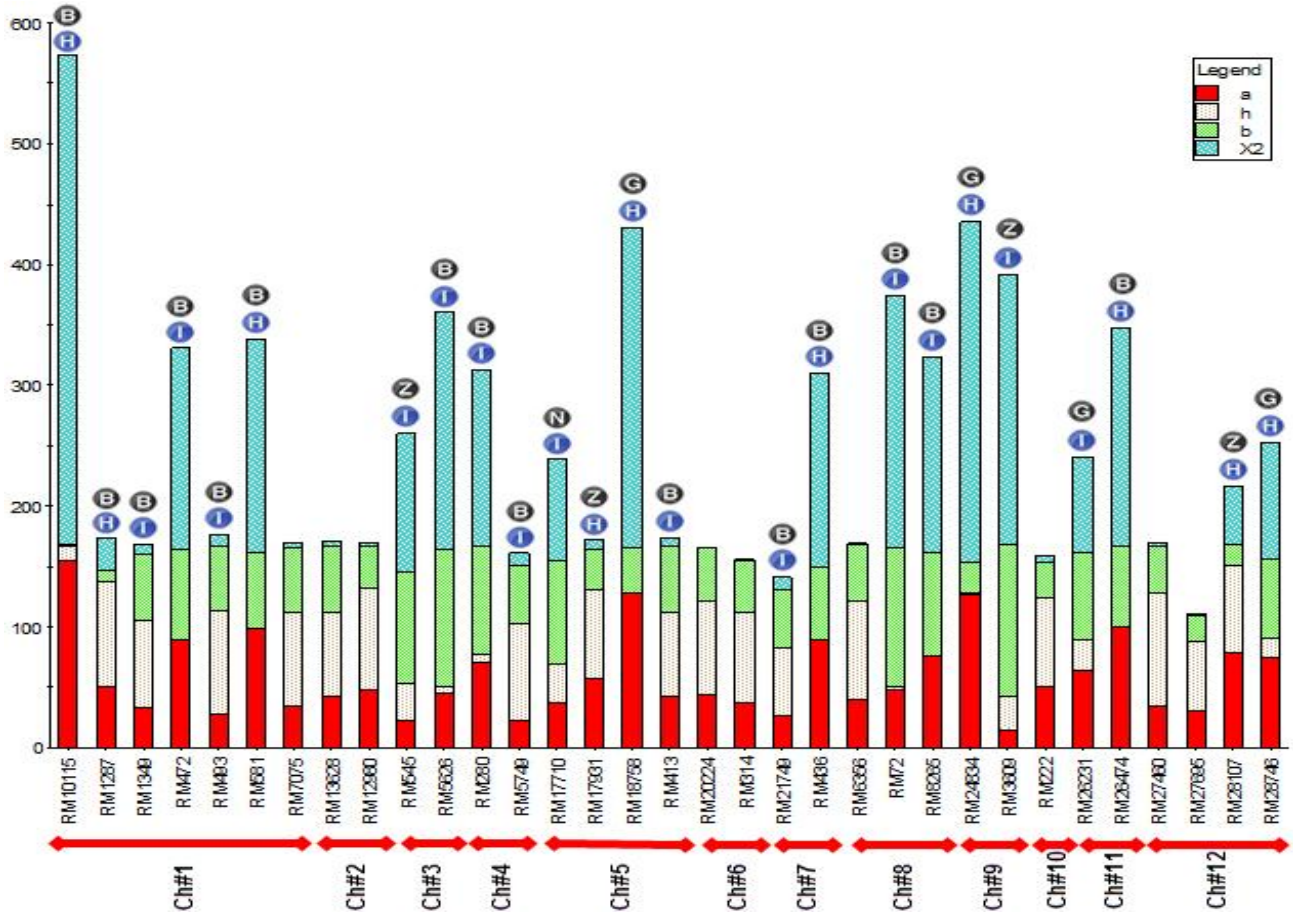

Figure 5. Locus genotype frequency, χ^2, skewness and genetic distortion factors for the IH population.

Skewness towards genotype is represented by blue circle and genetic distortion factors as black circle over the bar. (H) = Skewed toward Horkuch, (I) = Skewed toward IR29, (B) = both gametic and zygotic selections, (G) = gametic selection, (Z) = zygotic selection and (N) = No selection

Table 4. Analysis of allele frequency of distorted markers in IH population

Marker	Allele frequency		χ^2	
	H	I	Allele frequency homogeneity (H = I)	F_2 distribution (H^2: 2HI:I^2)
RM1287	**0.373**	**0.627**	**18.75****	**110.06****
RM1349	**0.378**	**0.622**	**19.01****	**11.58****
RM17710	0.516	0.484	0.32	1.90
RM18758	0.888	0.112	198.59**	2.63
RM21749	**0.382**	**0.618**	**14.67****	**6.56***
RM24834	0.906	0.094	202.92**	0.36
RM26231	0.620	0.380	18.78**	0.30
RM26474	**0.799**	**0.201**	**119.76****	**10.52****
RM280	**0.696**	**0.304**	**50.90****	**11.79****
RM28107	0.521	0.479	0.58	106.79**
RM3609	0.458	0.542	2.33	43.78**
RM413	**0.416**	**0.584**	**9.39****	**17.34****

RM472	**0.771**	**0.229**	**96.60****	**14.41****
RM493	**0.323**	**0.677**	**41.69****	**11.38****
RM5626	**0.619**	**0.381**	**18.55****	**34.80****
RM5749	**0.308**	**0.692**	**44.56****	**8.60***
RM72	**0.633**	**0.367**	**23.47****	**41.36****
RM8265	**0.733**	**0.267**	**69.88****	**21.38****
RM10115	**0.929**	**0.071**	**246.86****	**139.20****
RM17931	0.451	0.549	3.12	55.43**
RM28746	0.683	0.317	41.65**	0.23
RM436	**0.797**	**0.203**	**105.61****	**9.77****
RM545	0.469	0.531	1.12	10.88**
RM581	**0.802**	**0.198**	**118.57****	**9.82****

*Note: * at 0.05 significant level; ** at 0.01 significant level. Bold = significant distortion in both allele frequency homogeneity and F_2 genotype frequency distribution*

Construction of linkage map

Genetic linkage map was constructed for HI population using 50 polymorphic SSR markers. 15 out of 50 markers loci were located in the 7 linkage group, and mapped on 7 of 12 chromosomes spanning a total length of 462.857 cM of rice genome, with the average intervals of 30.86 cM between adjacent markers. No linkage group is mapped on chromosome 2, 4, 6, 8 and 11 (Figure 6). The IH population was not used to construct the genetic linkage map due to the lack of markers covering all the 12 chromosomes. Only 33 markers were used for genotyping of IH population as a result number of linkage group and markers in linkage group were very low.

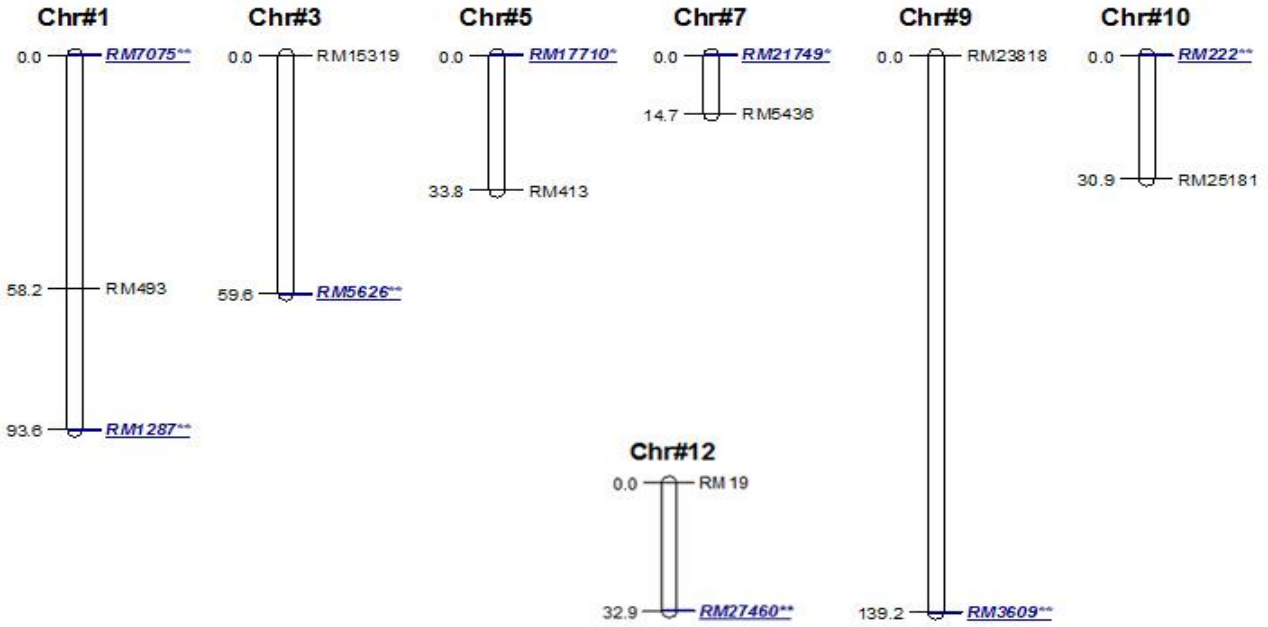

Figure 6. Genetic linkage map of HI F_2 population and the distribution of markers with segregation distortion in the map. The numbers on the left are the genetic distances in centiMorgans (cM) between markers. Marker names are on the right. Blue colored underlined markers = distorted markers. * and ** indicate significance levels of distorted segregation at 5% and 1%, respectively.

DISCUSSION

Segregation distortion is a common phenomenon in segregated populations derived from crosses between divergent parents in rice[39]. F_2 populations are used to construct genetic linkage maps in plants irrespective of self-pollination or cross-pollination as it is easy to establish in a short time[17]. In our study, we used F_2 reciprocal populations of Horkuch and IR29 to construct a genetic linkage map and to study SD, since we could not establish a linkage map with all the 12 chromosomes. IR29 is a salt sensitive *indica* rice variety whereas Horkuch is a salt tolerant rice variety of coastal region of Bangladesh that was reported to cluster with salt tolerant and aromatic rice varieties[40].

Higher segregation distortion ratio in interspecific population than that in intraspecific population has been reported in many plants[41-43]. There are different reports

about the amount of segregation distortion in rice, between *indica* and *japonica* subspecies. One report observed 43.7% and 40.2% segregation distortion in F_2 reciprocal crosses between *indica* and *japonica* varieties, respectively. Others have reported segregation distortion ranges from 12-35%in*indica* and *japonica* F_2crosses and reciprocal populations. We observed a much higher segregation distortion of markers in Horkuch and IR29 reciprocal F_2 populations that was73%and 78% respectively for IH and HI populations. These results indicated that IR29 (*indica)* and Horkuch (*aromatic*) used in this study may be more divergent than *indica* and *japonica*. However, the exact comparative genetic distance among the *indica* rice IR29, *aromatic* Horkuch and *japonica* rice needs to be determined.

Cytoplasmic and genetic factors are two of the major influencing factors causing of segregation distortion. Genetic factors include pollen tube competition, lethal pollen, preferential fertilization, sterility and chromosome translocation. The first three types are defined as gametic selection[23]. Several genetic factors have been identified in plant species such as maize[44], rice [17,45], soybean[46,47].

Segregation distortion was directly or indirectly associated with the effect of cytoplasm. The effect of cytoplasmic factors on the transmission of nuclear marker genes in barley[9] and effects of cytoplasmic factors on SD in rice[19] have been reported. Based on the results of our study, it can be inferred that SD was influenced by both nuclear and cytoplasmic factors in reciprocal F_2 populations.

Effect of gametophytic and zygotic factors on SD has been reported in rice. Zygotic selection causing SD is explained by those markers which are distorted in both female-and male segregating populations. The gametophytic (female and male gamete function) and zygotic selection as mechanisms underlying SD can be inferred from the distorted marker patterns[19]. In our study, SD of 22 and 15 markers were found to be influenced by gametic and zygotic selections simultaneously in the HI and IH population whereas 13 markers by either zygotic or gametic selection. So it can be concluded that gametic and zygotic selections had big effects on SD in the F_2 reciprocal populations.

Construction of linkage maps are often complicated in the presence of SD as it is known to affect recombination frequency and thus the construction of genetic linkage map. JoinMap 4.0 employed in this study, is a popular program for establishing genetic maps. Although we ignored the effect of segregation distortion on linkage analysis, the program used only 15 out of 50 markers for linkage analysis. Some programs, for example, MapManager and Mapdisto can handle segregation distortion by providing options for calculating linkage distances of distorted markers, and several algorithms were developed to adjust recombination frequency in such cases[48,49]. LOD $\geq$ 3.0 was used in linkage map construction using SSR data. LOD $\geq$ 3.0 is considered as the presence of a linkage between the two loci.7 linkage groups were mapped on 7 chromosomes but none on the other 5 chromosomes. Number of markers were few for those chromosomes with no linkage group and the lack of markers might be a reason for the missing linkage groups. No segregation distortion region (SDR) was detected in the map as no significant distortion in three or more closely linked markers. Analysis and identification of loci and their genetic and cytoplasmic effects responsible for SD in our study could lead to understanding of the underlying mechanisms. Moreover, if the salt tolerance determinants of Horkuch are to be put into *indica* backgrounds for breeding salt tolerant rice, a bridging rice cultivar, intermediate in genetic distance between *indica* and *aromatic* varieties may be required.

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