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Segregation analysis using SSR marker in rice (*Oryza sativa* L.)

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Abstract
Rice (*Oryza sativa* L.) is one of the major food crops, feeding more than half of the world's population. Major aim of rice breeding program is to enhance the yield potential by utilizing genetically diverse parent. The study was conducted in Kharif 2016 and Rabi 2016-17 at the Research and Instructional Farm, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh and Kharif 2017 at Research Farm of National Rice Research Institute (NRRI), Cuttack, Odisha (India). 10 rice parental genotypes, Chandrahasini, Samleshwari, Durgeshwari, IC-134022, IC-388728, IC-389860, IC-390376, IC-548384, Indira Barani Dhan1, IRHTN-105 and seven F3-F4 populations were taken for the study. Two hundred eighty-eight microsatellite primers (288 SSR and 38 gene based markers) were individually assayed on DNA of the parental genotypes (IC-548384, Chandrahasini, IC-390376, Samleshwari, IC-134022, Durgeshwari, and IC-388728, IC-389860) to reveal polymorphisms. Of these primers, 32 markers showed polymorphisms were utilized for trace the allelic contribution of the parental lines to their progeny; and drought and yield related specific genes. Total 32 segregating loci (95.65%) showed Mendelian pattern in segregation at p = 0.05 and p = 0.01, all are fit to a 1: 2: 1 ratio (Table 1). Distorted segregation ratios were found for markers RM-11846 ((IC-548384 X Chandrahasini) with $\chi 2$ = 6.456, (IC-390376 X Samleshwari) with $\chi 2$ = 10.75 & (IC-134022 × Durgeshwari) with $\chi 2$ = 6.78), RM-20773 ((IC-389860 X Samleshwari) with $\chi 2$ =6.087) and qDT-3850 (IC-548384 X Chandrahasini) with $\chi 2$ =12.432) at p<0.05 and p.01 probability level in the F3 population.

Keywords: Genes, microsatellite primers, molecular marker, polymorphisms, distorted segregation

Introduction
Rice is one of the leading food crop feeding more than half Global population has cultivated across a wide range of ecosystem e.g. irrigated, rainfed lowland, rainfed upland and aerobic ecosystem. Increasing population and decreasing agricultural lands etc. need a higher rice grain to feed entire population. Hybridization is the basic breeding to combine desirable traits to improve grain yield in rice (Selvi *et al*. 2015) [7]. Analysis of segregating population is foremost thing to know the combination of desirable traits which are more desirable and durable. Analysis of segregation pattern is more helpful to know the segregation distortion which gives the idea about the sterility and gametophytic genes. Segregation distortion depends on many factors e.g. mapping population, genetic transmission, gametic and zygotic selection, non-homologous recombination, gene transfer, transposable element and environment agents (Xu *et al*. 1997) [10].

No. of molecular markers are available e.g. morphological, isozymes, and DNA markers (Zamir and Tadmor, 1986) [11] for segregation study. In barley, RFLP (Heun *et al*., 1991), RAPD (Manninen, 2000) [3], AFLP (Qi *et al*., 1998) [6] are commonly used. As molecular markers are suitable for distortion segregation analysis as these are not influenced by environment, co-dominant in nature, highly polymorphic in nature, evenly distributed in the genome, efficient, less quantity of DNA is required, highly cost effective and transferability (Mason, 2015) [4].

SSR markers are effective for the analysis of genetic polymorphism (Forrester *et al*., 2020) [1], population structure analysis, gene mapping and tagging, linkage map construction, tracing marker-trait association, Marker Assisted Selection (MAS) and others. (Shivani *et al*., 2020) [8]. Marker segregation distortion were determined and compared with chi-square analysis and given genotypic class within a segregation population (Xu *et al*., 1997) [10]. The present study was concentrated on the presence of segregation distortion using SSR markers.

Material and Methods

Molecular analysis of breeding lines: The leaf samples of 306 lines of seven breeding population were collected from 15-20 days old seedlings grown at ICAR-NRRI, Cuttack during early hours (8am to 9am) and stored at -80oC for DNA isolation. Total genomic DNA was extracted from young leaves by modified CTAB method (Murray and Thompson, 1980) [5].

Data Analysis

Chi-square test: The F2, F3, populations were analysed independently for each trait to determine the mode of inheritance by following $\chi 2$ (chi-square) test as suggested by Fisher (1936) [12].

Result and Discussion

Level of polymorphism in parental genotypes

Two hundred eighty-eight microsatellite primers (288 SSR and 38 gene based markers) were individually assayed on DNA of the parental genotypes (IC-548384, Chandrahasini, IC-390376, Samleshwari, IC-134022, Durgeshwari, IC-388728, IC389860) to reveal polymorphisms. Of these primers, 32 markers showed polymorphisms were utilized for trace the allelic contribution of the parental lines to their progeny; and drought and yield related specific genes.

Segregation analysis

Presence or absence of each fragment was scored in the F3 population of six combinations. Total 32 segregating loci (95.65%) showed Mendelian pattern in segregation at p = 0.05 and p = 0.01, all are fit to a 1: 2: 1 ratio (Table 1; Fig.1). Distorted segregation ratios were found for markers RM-11846 (IC- 548384 × Chandrahasini with $\chi 2$ = 6.456, (IC-390376 × Samleshwari) with $\chi 2$ = 10.75 & IC134022 × Durgeshwari with $\chi 2$ = 6.78), RM-20773 (IC-389860 × Samleshwari with $\chi 2$ =6.087) and qDT-3850 (IC- 548384 × Chandrahasini with $\chi 2$ =12.432) at $p<0.05$ and $p<0.01$ probability level in the F3 population (Wang *et al*., 2005) [9]. Complete homogeneity for alleles of either parent, suggesting that F3 populations are true representatives of the normal gametic constitution in F2. It indicated that allelic contribution both parents in each F3 populations are equal, followed Mendelian fashion of inheritance (Sayed *et al*. 2002) [13]. Hence, derivatives generated are equally contributed by both parental genomes.

Table 1: Allelic contribution of parental lines to their F3 derivatives

Marker	Crosses	F_3 population			X^2
		$F_{a\ (0.375)}$	$F_{b\ (0.375)}$	$F_{h\ (0.25)}$	
RM10167	C1	0.48	0.44	0.08	0.156
	C2	0.64	0.28	0.08	1.112
	C3	0.68	0.27	0.05	2.016
	C4	0.68	0.28	0.04	0.002
	C5	0.52	0.42	0.06	0.118
	C6	0.28	0.66	0.06	0.248
RM11258	C1	0.38	0.52	0.10	0.092
	C2	0.28	0.64	0.08	1.058
	C4	0.34	0.60	0.06	0.020
	C5	0.52	0.42	0.06	0.149
	C6	0.50	0.42	0.08	3.011
RM-11846	C1	0.82	0.14	0.04	6.456*
	C2	0.50	0.44	0.06	0.750
	C3	0.93	0.02	0.05	10.759**
	C4	0.78	0.16	0.06	6.780*
	C5	0.58	0.38	0.04	0.360
	C6	0.56	0.40	0.04	4.002
RM-12469	C1	0.47	0.51	0.02	0.055
	C2	0.54	0.42	0.04	0.045
	C3	0.40	0.55	0.05	0.079
	C4	0.42	0.54	0.04	0.003
	C5	0.44	0.48	0.08	0.048
	C6	0.46	0.46	0.08	0.002
RM-14946	C1	0.57	0.33	0.10	0.072
	C2	0.72	0.26	0.02	4.118
	C3	0.61	0.34	0.05	1.084
	C5	0.28	0.64	0.08	1.459
RM-15855	C1	0.47	0.52	0.01	0.553
	C2	0.20	0.62	0.18	0.251
	C3	0.57	0.42	0.01	2.273
	C5	0.44	0.53	0.03	0.260
RM-17263	C1	0.26	0.70	0.04	2.460
	C3	0.34	0.59	0.07	0.062
	C5	0.42	0.56	0.02	0.054
RM16706	C1	0.44	0.36	0.10	0.392
	C5	0.56	0.40	0.04	0.073
RM-17349	C1	0.42	0.54	0.04	0.084
	C2	0.48	0.46	0.06	0.030

	C3	0.40	0.55	0.05	0.376
	C4	0.22	0.46	0.52	0.02
	C5	0.58	0.38	0.04	0.609
	C6	0.48	0.48	0.04	0.044
RM-18530	C1	0.54	0.44	0.02	0.644
	C4	0.36	0.56	0.08	0.353
	C5	0.46	0.35	0.19	0.178
RM-19101	C1	0.52	0.44	0.04	0.014
	C2	0.44	0.52	0.04	0.971
	C3	0.50	0.42	0.08	0.108
	C4	0.60	0.34	0.06	0.400
	C5	0.50	0.44	0.06	0.457
	C6	0.46	0.52	0.02	0.144
RM-20724	C1	0.36	0.56	0.08	0.542
	C5	0.44	0.48	0.08	1.298
RM-20773	C1	0.42	0.46	0.12	0.146
	C3	0.52	0.42	0.06	0.105
	C5	0.68	0.26	0.06	2.507
	C6	0.74	0.18	0.08	6.087*
RM-21024	C1	0.35	0.31	0.04	0.517
	C4	0.30	0.60	0.04	0.288
RM-21539	C1	0.46	0.44	0.04	0.128
	C2	0.66	0.26	0.04	1.161
	C3	0.54	0.37	0.12	0.234
	C4	0.42	0.50	0.04	0.130
	C6	0.44	0.32	0.12	0.236
RM-21842	C1	0.38	0.50	0.12	0.212
	C3	0.44	0.41	0.15	0.102
	C4	0.42	0.54	0.04	0.040
	C5	0.68	0.24	0.06	0.338
	C6	0.50	0.42	0.08	0.081
RM-22914	C1	0.44	0.34	0.22	0.510
	C4	0.60	0.34	0.06	0.420
	C5	0.34	0.62	0.04	0.350
	C6	0.54	0.38	0.00	0.251
RM-23937	C1	0.45	0.45	0.10	0.159
	C2	0.30	0.64	0.06	0.259
	C3	0.55	0.40	0.05	0.664
	C4	0.42	0.46	0.04	1.303
RM-24240	C1	0.69	0.29	0.02	0.337
	C2	0.24	0.64	0.12	0.736
	C5	0.36	0.48	0.16	0.140
RM-24414	C6	0.62	0.32	0.06	0.601
RM-24448	C1	0.45	0.42	0.13	0.520
	C2	0.46	0.52	0.02	0.274
	C3	0.52	0.40	0.08	0.617
	C5	0.58	0.38	0.04	0.200
RM-24495	C1	0.50	0.46	0.04	0.643
	C2	0.53	0.45	0.02	0.229
	C5	0.52	0.36	0.12	0.592
RM-25679	C2	0.50	0.34	0.16	0.884
	C3	0.49	0.43	0.08	0.575
	C4	0.48	0.42	0.10	0.733
	C5	0.50	0.40	0.10	0.137
RM-25735	C3	0.39	0.56	0.05	0.519
	C5	0.26	0.62	0.12	0.013
RM-26302	C1	0.42	0.54	0.04	0.331
	C4	0.36	0.54	0.10	0.436
	C5	0.70	0.26	0.04	3.305
	C6	0.52	0.41	0.07	0.326
RM-28767	C1	0.22	0.76	0.04	1.375
	C2	0.36	0.60	0.04	0.887
	C3	0.31	0.64	0.05	0.012
	C4	0.52	0.36	0.12	0.195
	C5	0.22	0.74	0.04	4.366
	C6	0.52	0.40	0.08	0.618
RM-279	C2	0.46	0.52	0.02	0.076

	C3	0.52	0.46	0.02	4.195
	C4	0.42	0.56	0.02	0.134
	C5	0.62	0.34	0.04	1.987
	C6	0.50	0.48	0.02	0.195
GS-3-SR-17	C1	0.64	0.34	0.02	0.015
	C2	0.47	0.49	0.04	0.147
	C5	0.52	0.44	0.04	0.080
GS-3-RGS-1	C5	0.36	0.56	0.08	1.299
GN1A	C1	0.68	0.30	0.02	0.291
QDT-16030	C7	0.32	0.54	0.14	0.332
QDT-3850	C7	0.94	0.00	0.06	12.432**
Mean		0.48	0.45	0.07	

Note: ∗- Significantly deviated at 0.05 ($\chi 2$ (t) = 5.99 for F_3, ∗∗-significantly deviated at 0.01 ($\chi 2$ (t) = 9.21 for F_3; (F_a) frequency of A allele, (F_b) frequency of B allele, (F_h) Frequency of heterozygote.

Note:
C1- IC- 548384 x Chandrahasini
C2- IC -390376 x Chandrahasini
C3- IC-390376 x Samleshwari
C4- IC-134022 x Durgeshwari
C5- IC-388728 x Chandrahasini
C6- IC-389860 x Samleshwari
C7- Indira Barani Dhan 1 x IRHTN-105

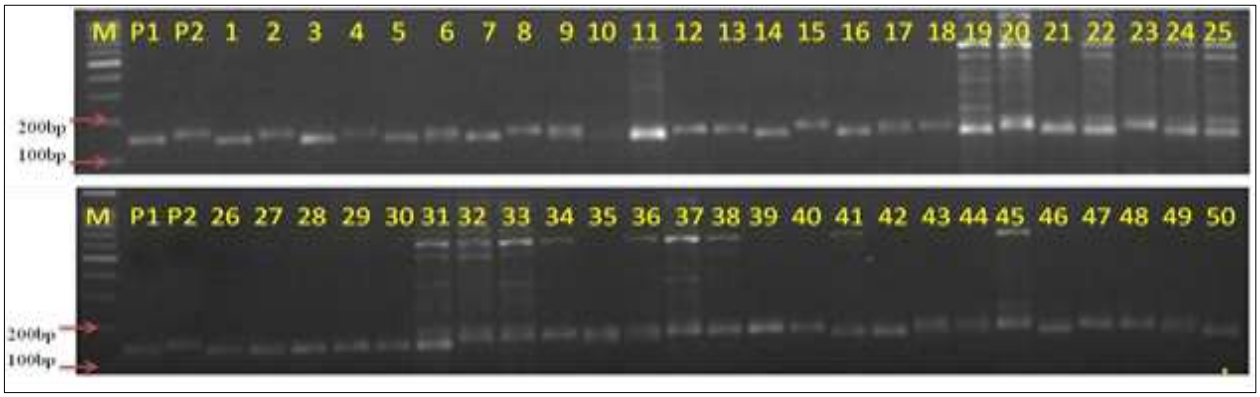

Fig 1: Banding pattern of RM28767 in C4 breeding lines (a & b), P1=IC-134022, P2 = Durgeshwari, lines-1-50, Marker (M) = 100 bp, amplicon range-150 bp-180 bp

Conclusions

Preferential sustainability of gametes of better parent in segregating generation found to be having substantial segregation distortion. Confirming the homozygosity, molecular analysis of the segregants is helpful to trace the allelic contribution of parental genotypes in derivatives. Molecular marker based assessment of allelic contribution/genetic gain in derivative population was carried out which shown substantial results. Total 32 segregating loci (95.65%) showed Mendelian pattern in segregation at p = 0.05 and p = 0.01, all are fit to a 1: 2: 1 ratio (Table 1). Distorted segregation ratios were found for markers RM-11846 ((IC-548384 X Chandrahasini) with $\chi 2$ = 6.456, (IC-390376 X Samleshwari) with $\chi 2$ = 10.75 & (IC-134022 × Durgeshwari) with $\chi 2$ = 6.78), RM-20773 ((IC-389860 X Samleshwari) with $\chi 2$ =6.087) and qDT-3850 (IC-548384 X Chandrahasini) with $\chi 2$ =12.432) at p<0.05 and p<0.01 probability level in the F3 population. Complete homogeneity for alleles of either parent, suggesting that F3 populations are true representatives of the normal gametic constitution in F2. It indicated that allelic contribution both parents in each F3 populations are equal, followed Mendelian fashion of inheritance. Hence, derivatives generated are equally contributed by both parental genomes.

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