Indian Journal of Agricultural Sciences **92** (7): 853–6, July 2022/Article
https://doi.org/10.56093/ijas.v92i7.108577

Marker assisted selection and segregation distortion studies in rice (*Oryza sativa*)

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Received: 16 December 2020; Accepted: 02 March 2022

ABSTRACT

Rice (*Oryza sativa* L.) is an important staple food crop and is grown across different regions and moisture regimes across Asian countries. The present study was carried out during rainy (*kharif*) season 2017–19 for introgressing genes, raising F_1 plants, evaluation of F_2 plants and marker distortion studies at Marker Assisted Selection Laboratory, University of Agricultural sciences, Bangalore. True F_1 plants were confirmed using polymorphic SSR marker RM242 and selfed to develop F_2 plants. Marker assisted identification using 11 trait specific SSR markers linked to higher contributing alleles for drought, blast and bacterial leaf blight (BLB) resistance in F_2 population was derived from a japonica variety Moroberekan and indica variety IR 64. F_2 population exhibited highly significant segregation distortions for four (36.4%) out of 11 SSR markers. Out of four distorted markers, RM242 and RM235 were towards IR64 (male parent) and RM11943 and RM243 distorted towards heterozygote and Moroberekan (female parent), respectively. The allele frequency and distribution of genotype frequencies in the F_2 population were analyzed for SSR markers, to find out factors that attributed to segregation distortion. Three markers (RM11943, RM243 and RM235) showed distorted allele frequency, with normal genotype frequency and one showed distorted genotype frequency with normal allele frequency, which indicates that these markers were influenced at gametic and zygotic level, respectively.

Keywords: Contributing allele, Drought, Rice, Segregation distortion, SSR marker

Rice (*Oryza sativa* L.) is a principal crop that is grown across different ranges of moisture regime, viz. irrigated, rainfed lowland, rainfed upland and aerobic conditions. Out of the total 20.7 million ha of rainfed rice area in India, approximately 16.2 million ha is in eastern India (Singh and Singh 2000). The global reduction in rice production due to drought averages 18 million tonnes annually (O'Toole 2004). Rice blast and bacterial leaf blight (BLB) diseases caused by *Magnaporthe grisea* (Cooke) and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), are very harmful causing heavy yield loss in most rice growing regions of the world. Hybridization helps to combine contributing alleles of different desirable traits differing in the parents (Selvi *et al*. 2015). Since F_2 is a segregating population, it contains an array of individual genotypes with different combinations of alleles of desirable traits from both parents. Plants with several combinations of desirable alleles are more durable and such plants can be developed by hybridization and their traits can be confirmed using tightly linked markers. Segregation distortion is deviation of the observed genotypic frequencies from their expected values of Mendel's segregation ratio. Segregation distortion is influenced by several factors such as mapping population, genetic transmission, gametic and zygotic selection, non-homologous recombination, gene transfer, transposable element and environment agents (Xu *et al*. 1997, Knox and Ellis 2002). DNA markers are not influenced by environment and are more suitable for analysis of segregation distortion. SSR markers are also suitable for the analysis of segregation distortion as they are co-dominant markers. Allele frequency and the distribution of different genotype frequency are analyzed to test the existence of gametic or zygotic selection. In the present study, attempt has been made to select F_2 plants containing combination of contributing alleles of drought tolerance, blast and bacterial leaf blight resistance, and yield traits using linked SSR markers. This study also reports the presence of segregation distortion using SSR markers in F_2 japonica-indica rice introgression.

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MATERIALS AND METHODS

The cross between Moroberekan, a West African

japonica variety (tolerant to drought, resistant to blast and BLB and having high water use efficiency) and IR64, an indica high yielding variety was attempted during *kharif* 2017 and summer 2018. F_1 plants were raised in pot culture and evaluation of F_2 plants was done during *kharif* 2018 at University of Agricultural sciences, Bangalore. True F_1 plants were confirmed with the help of polymorphic SSR marker RM242 and were selfed to develop 250 F_2 populations. Several traits were observed for selection of a combination of desirable traits among the plants in F_2 population using linked SSR markers. Eighteen plant morphological traits were recorded (Table 1) in the F_2 population grown under low moisture stress aerobic condition. Scoring of leaf rolling and culm angle was made as per the Standard Evaluation System of Rice (IRRI 2002). Plant vigor of each F_2 genotype was computed by compound growth rate analysis of plant height taken at different intervals of time from 78 days after sowing up to stabilization of plant height. SSR markers tightly linked to drought tolerance traits, blast and BLB resistance were selected by referring available literature (Table 2).

DNA isolation and SSR marker analysis: Fresh leaves were collected from each F_2 plant and ground in liquid nitrogen. DNA was extracted from the ground tissue by the cetyltrimethylammonium bromide (CTAB) method (Cao and Oard 1997). For molecular marker analysis, a total of 50 trait specific SSR markers (blast and bacterial leaf blight resistance, drought related traits) were screened for polymorphism between parents and 29 polymorphic markers were found. Eleven polymorphic trait specific SSR markers were used to screen whole F_2 population. The PCR product was resolved on 3.5% agarose gel treated with ethidium bromide stain to visualize bands under UV light by using Gel Doc XR^+system.

Scoring and interpretation: Alleles were contributed by female parent Moroberekan and some traits by male parent IR64. Therefore, band type of F_2 plant similar to donor parent was recorded as '+' (positive) and similar to other parent as '-' (negative), F_2 special band (heterozygous) as '±' and missing band as '.'. Since alleles in homozygous condition are stable over the generations, only F_2 plants with homozygous alleles were selected. Number of homozygous alleles (+) for desired traits in each of 250 F_2 plants was recorded.

Statistical analysis: In this research, 11 trait specific SSR markers were screened on the F_2 population. Chi square test was employed to test the segregation distortion of SSR marker from normal Mendelian 1:2:1 ratio in F_2 population and also used to test marker allele frequency, homogeneity and marker genotype distribution to analyze the reason for segregation distortion.

RESULTS AND DISCUSSION

In this pedigree method of breeding, the aim was to combine different drought resistant traits such as root thickness and length, total root weight, dry root weight, stomatal conductance, leaf rolling, yield under drought stress, shoot biomass, and seedling vigor, blast and bacterial leaf blight resistance from Moroberekan parent and osmotic adjustment and high yielding semi-dwarf characters from an IR64 parent. True F_1 crosses were identified using the marker RM242 that showed high polymorphic allelic pattern among the parents. Since F_2 population is highly segregating, it consists of an array of genotypes with various combinations of desirable traits donated by both the parents. Phenotypic selection of plants for the above mentioned traits is labor intensive, expensive and time consuming, therefore molecular markers, especially trait specific SSR markers can be helpful in selecting plants with desired characters.

In F_2, plant number 11 contained 8 homozygous and 2 heterozygous contributing allele with the grain yield of 25.1 g/plant and plant number 169 and 144 had 7 each of homozygous and 2, 1 heterozygous contributing allele with the grain yield of 24.9 and 34.4 g/plant, respectively. Plant number 11 and 144 had contributing alleles of drought and disease resistance along with high yield under drought stress. Various combinations of contributing alleles of desired traits were observed in the F_2 population. Since heterozygous loci segregates in next generation, it needs to be screened further in F_3 family for homozygous loci. It was observed from the results that most of the plants with more number of contributing alleles were less yielding which otherwise were expected to give high yield under drought conditions. The probable reasons could be the larger environmental influence on the yield and the QTLs linked to the SSR markers in the study were contributing part of the total phenotypic expression. Therefore, in the next generation, it is suggested to go for selection of high yielding plants in the plant families with more number of contributing alleles to get the drought resistant high yielding genotypes. Since each plant in F_2 population was unique in its genotypic combination, we could not screen the same population for blast and bacterial leaf blight (BLB) resistance phenotype in the hotspots/sick plots. However, the F_2 population had been screened for blast and bacterial leaf blight by using closely linked SSR marker RM1003 and RM13 respectively. Hittalmani *et al.* (2000) also reported marker-assisted selection for blast resistance genes in F_2 population. It was observed that most of the plants carrying more number of contributing alleles also had both blast and BLB resistant alleles.

Disease resistance can be confirmed by phenotypic selection in the succeeding families growing in hotspots. Selection of plants with more number of homozygous contributing alleles for drought and disease resistance traits in F_2 population by using trait specific SSR marker was most appropriate. Phenotypic performance of F_2 genotypes would also be considered while selection (Table 1). Chi-square test performed on 11 co-dominant SSR markers revealed that 7 SSR markers fitted normal Mendelian segregation ratio 1:2:1, whereas 4 SSR markers (36.4%) were distorted from normal Mendelian segregation ratio (Table 2). Of the 4 distorted markers, RM242 distorted significantly towards male parent IR64 ($P<0.05$) and

RM11943, RM243 and RM235 were extremely deviated (P<0.01) towards heterozygote, Moroberekan (female) parent and IR64 (male) parent, respectively. Maker RM243, which deviated toward Moroberekan, displayed the highest extent of deviation among the distorted SSR markers. Distorted SSR markers were located on 1, 9 and 12th chromosomes. Genetic background of the biparents may be related to segregation distortion. In a study of Zhao *et al*. (2006) a total of 49 (33.1%) out of 148 markers showed segregation distortion in an F_2 population from a cross of japonica variety Nipponbare and indica variety Guangai 4. Peng *et al.* (2006) also used an RIL population from a cross of Aipei 6 (derived from Nipponbare and indica) and japonica variety Nipponbare to construct a linkage map, 58 (63%) of the 92 markers showed distorted segregation. The results indicated that segregation distortion was prone to happen in intersub-specific population. In this study, F_2 population derived from cross between japonica variety Moroberekan and indica variety IR64 exhibited segregation distortion of 4 (36.4%) out of 11 SSR markers, which was higher than the above reported. The significant extent of the 4 distorted markers was analyzed by using Chi-square test (Table 2) **(**Pham *et al.* 1990; Zhang *et al*. 1997; Tan Jun *et al*. 2004). Allele frequency homogeneity ($p^1=p^2$) and the distribution of different genotypes in F_2 population was checked in co-dominant SSR markers to ascertain whether there existed gametic and/or zygotic selection, which are major factors of segregation distortion. In the present study, RM242, RM243 and RM235 showed significant allele frequency distortion, but normal genotype frequency indicated that these markers were influenced at the gametic level. Remaining marker RM11943, showed significant genotype distortion but normal allele frequency indicated that this marker was influenced at zygotic level.

SSR markers are co-dominant in nature and hence could be used for the analysis of allele frequency and genotypic frequency simultaneously and to determine the reason for distorted segregation. Segregation distortion could be influenced by genetic factors like pollen tube competition, pollen lethal, preferential fertilization and selective elimination; the first three types were defined as gametic selection. From the segregation distortion in F_2 population in japonica and indica rice cross Nipponbare/GuangLuAi-4, Zhao *et al*. (2006) explained that gametic selection had great influence on the segregation distortion. Genetic factors of segregation distortion were mostly at the gametic level and Yan *et al*. (2003) and Zhao *et al*. (2006) drew the same conclusion in corn and rice, respectively. Zhao *et al*. (2006) observed in rice linkage map study that most of the distorted markers were clustered in special regions on chromosomes, which are termed as Segregation distortion regions (SRG). In rice, 11 gametophyte (ga) and 20 sterility genes (S) causing segregation distortion were mapped (Kinoshita 1993, He *et al*. 2001). Most of these genes were distributed on SRG and its neighbourhood. This supports that segregation distortion was mainly due to selection at gametic level. In our study, the causes of

Table 1 Genetic parameters for 18 quantitative traits in F_2 population (Moroberekan/IR64) rice cross evaluated under aerobic condition during *kharif* season 2019

Character	Mean	Minimum	Maximum	Standardized range
Leaf rolling	4.73	0	9	1.90
Plant height (cm)	92.57	54	120	0.71
Leaf width (mm)	9.87	6.4	25.2	1.90
Days to flowering (days)	120.86	97	144	0.39
Days to maturity (days)	149.39	113	168	0.37
Total tillers/plant	20.92	5	58	2.53
Productive tillers/ plant	13.51	2	41	2.89
Panicle weight (g)	3.28	0.5	8.82	2.54
Panicle length (cm)	24.34	14.5	32	0.72
Test weight (g)	2.91	1.03	4.5	1.19
Spikelet fertility (%)	73.76	11.39	100	1.20
Biomass yield (g)	86.23	9.42	371.44	4.20
Grain weight per plant	24.79	0.77	166.09	6.67
Harvest index	0.25	0.01	0.56	2.55
Spikelets/panicle	121.15	30	328	2.46
Plant vigor (%)	14.52	3.39	48.57	3.11
Panicle exertion (±)	5.32	1	1	1.50
Culm angle (degree)	2.02	1	7	2.98

Table 2 Chi-square test for segregation distortion of SSR markers screened on F_2 population of Moroberekan/IR64 rice

Marker	Genetic characteristic	Genotype			χ^2	Direction of distortion
		A/A	A/B	B/B		
RM263	Codominant	55	139	83	5.66	
RM242	Codominant	63	125	87	6.46*	IR64
RM80	Codominant	73	144	61	1.40	
RM11943	Codominant	61	169	48	14.17**	Heterozygous
RM13	Codominant	57	120	68	1.09	
RM248	Codominant	76	132	49	5.86	
RM276	Codominant	58	141	79	3.23	
RM1003	Codominant	75	141	62	1.27	
RM243	Codominant	89	120	43	17.37**	Moroberekan
RM21	Codominant	59	138	80	3.19	
RM235	Codominant	46	123	91	16.33**	IR64

segregation distortion were too complex, however, it still could be observed as the influence of *ga* or *S* genes.

ACKNOWLEDGEMENT

The financial support of DBT New Delhi and Pioneer Hi-Bred Research International is acknowledged.

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