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Phenotyping and microsatellite marker analysis of CSR10 (salt tolerant *indica*) × HBC19 (Taraori Basmati) F_6 lines obtained using single seed descent method

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Abstract

A population in F_6 generation comprising of 201 lines derived from the cross between CSR10 (salt tolerant, *indica*) and Taraori Basmati (HBC19) using single seed decent method, was evaluated for variation in agronomic and Basmati grain quality traits and for microsatellite allelic profile in relation to the parental rice varieties. Substantial variability was observed for plant height, productive tillers per plant, 1000-grain weight, yield per plant, kernel length (L) and breadth (B) and L/B ratio. The grain yield per plant showed positive correlation with number of productive tillers per plant and 1000-grain weight. Kernel length and breadth respectively had positive and negative correlations with L/B ratio. The path coefficient analysis recorded that number of productive tillers per plant, plant height and 1000-grain weight were the main contributors towards grain yield. A total of 20 randomly selected F_6 plants were subjected to SSR marker analysis using 15 SSR markers covering all the chromosomes. The F_6 plants had an allele from either of the two parental lines (homozygous condition) or alleles from both the parental rice varieties (heterozygous condition). At three SSR loci new/recombinant alleles were observed, which indicate the active recombination between genomes of two rice varieties. SSR allelic profile based two dimensional principal component analyses demonstrated high level of diversity between CSR10 and HBC19 with the 20 CSR10 × HBC19 F_6 plants interspersed between them. SSR analysis also showed an average homozygosity of 93.3% in F_6 lines, which is close to expected value of 98%.

Key words: Basmati, microsatellite, phenotyping, rice, recombinant inbred lines (RILs), *Oryza sativa* L.

Introduction

India is a home for rich diversity of aromatic and other quality rice types [1]. Of these, Basmati rice varieties with exquisite aroma, superfine grain characteristics and excellent cooking (extra elongation, soft and flaky texture) qualities have gained greater attention due to greater preference in domestic and international markets, increasing export and premium prices. Basmati rice has its origin in the foothills of Himalayas, is the result of centuries of selection and cultivation by farmers and is traditionally grown in north-western states of Indian sub-continent [2]. Traditional Basmati rice varieties have several undesirable agronomic traits including tall plant stature, long crop duration, sensitivity to photoperiod, and poor response to fertilizer application resulting in to low yield potentials, which is less than half compared to present-day *indica* or *japonica* rice varieties [3]. In rice, abundant germplasm exists for resistance against biotic and abiotic stresses and most of the agronomic, grain and nutritional quality traits, which are yet to be characterized and utilized effectively. Breeding efforts have been made to genetically improve Basmati rice by crossing with novel high yielding *indica* rice varieties but the progress has been limited due to higher degree of divergence between Basmati and *indica* rice varieties, hybrid sterility and polygenic nature of aroma and grain/cooking quality traits [3, 4]. Several semi-dwarf, high yielding Basmati rice varieties have been developed but these cross-bred varieties fall short of the quality features of traditional Basmati rice varieties, which is reflected in price differences between traditional and cross-bred Basmati varieties [3].

Molecular markers have been used for a variety of applications including DNA fingerprinting, varietal identification and diversity analysis, phylogenetic

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analysis, marker assisted breeding and map based cloning of genes in rice [5, 6]. Rice genome sequencing and comparison of sequence databases of *indica* and *japonica* rice genomes have provided an almost unlimited number of DNA markers (SSR, SNP, InDel, etc) for high-resolution genetic analysis [7]. Thousands of SSR markers have been mapped and developed as molecular markers; microsatellite markers being co-dominant are found suitable for evaluating genetic diversity and relationships among closely related plant accessions [8]. Mapping populations such as recombinant inbred lines (RILs), doubled haploids (DHLs), backcross/F_2/F_3 families or inbred lines (BILs) and chromosome segment substitution lines involving several diverse parental rice lines have been produced and used to construct molecular marker maps and identify genes or QTLs for several traits including yield and yield components and grain quality traits [9-12].

Marker-assisted selection can greatly improve the efficiency and precision of the breeding process especially in case of Basmati rice where you need to keep all the Basmati grain quality components together while introgressing desirable trait(s) from a donor (*indica*, *japonica* or wild) rice variety. Though some progress has been made towards the identification of molecular markers linked to the genes/QTLs for grain quality traits including aroma [13, 14] and kernel elongation [15], but to the best of our knowledge, appropriate populations for the linkage mapping of Basmati rice traits have not been developed so far. In this paper, we report the structure of a F_6 population developed from a cross between CSR10 (salt-tolerant, *indica*) and HBC19 (Taraori Basmati) using single seed descent method with respect to various agronomic and grain quality traits. Microsatellite marker analysis has been carried on the 20 randomly selected F_6 plants to assess the genetic constitution and level of homozygosity achieved.

Materials and methods

A total of 201 CSR10 x HBC19 F_6 lines and parental rice varieties were grown in Augmented Design during the kharif season at CCSHAU Rice Research Station, Kaul (Kaithal), using single-seed descent method. CSR10 (selection from CSR1/Jaya) has been developed by CSSRI, Karnal (India) and recommended for cultivation in saline soils [16]. HBC19 (a pure line selection from Taraori Basmati) is a commercially important premium traditional Basmati (TB) rice variety. Each progeny/line was planted in a single row of 2.5 m length. Seedlings were transplanted with plant to plant spacing of 15 cm and row to row spacing of 20 cm. All recommended agronomic practices were followed for raising a good crop. Twenty plants were randomly selected from the population of CSR10 × HBC19 F_6 lines and used for SSR analysis.

Field evaluation of CSR10 × HBC19 F_6 generation

From every line five plants were randomly selected and observations were recorded on plant height (cm), effective tillers per plant, grain yield/plant (g), 1000-grain weight (g), length of grain, breadth of grain, length-breadth ratio and aroma. Statistical methods used in this study included mean, coefficient of variation (GCV, PCV) as described by Burton [17], heritability (in broad sense) [18], genetics advance expressed as percentage of mean [19], correlation coefficient analysis [19] and path-coefficient analysis [20]. Aroma was detected by soaking the seeds in 1.7% KOH solution for 4-5 minutes [21].

Molecular marker analysis

A total of 20 plants were randomly selected from the CSR10xHBC19 F_6 population for microsatellite marker analysis. Genomic DNA was isolated from one month-old plant leaf samples (~100 mg each) using CTAB method [22]. The DNA was spooled out, washed twice with 70% ethanol and dissolved in TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) containing 25 μg/ml RNase-A, incubated at 37°C for 30 min and extracted with chloroform:*iso*-amyl alcohol (24:1 v/v). DNA was re-precipitated and dissolved in TE buffer. DNA was checked for its quality and quantity by 1% agarose gel electrophoresis using a standard containing 100 ng/μl genomic λ DNA.

Forty SSR markers (obtained from Research Genetics, Huntsville, AL, U.S.A.) were evaluated for polymorphism between CSR10 and HBC19 (data not shown) and fifteen of these showing polymorphism and clear discrete banding profile were used for analysis of CSR10 x HBC19 F_6 plants essentially as described by Jain *et al*. [23]. The original sources and repeat motifs for these markers can be found in RiceGenes database (http://www.gramene.org/microsat/RM_primers.html). The PCR reaction was conducted in a reaction volume of 20 μl containing 1× PCR buffer, 100 μM dNTPs, 0.4 μM of each primer, 1.2 mM $MgCl_2$, 1 unit Taq DNA polymerase and 50 ng template DNA. PCR amplification was performed with initial denaturation at 94°C for 5 min followed by 35 cycles of 94°Cfor 1 min, 55°C for 1 min, 72°C for 2 min and final extension at 72°C for 7 min before cooling at 4°C. Amplification products were denatured and resolved on 4% denatured

polyacrylamide gel, as described by Saini *et al.* [24]. The size (in nucleotides base pairs) of the most intensely amplified band of stutter for each microsatellite marker was determined based on its migration relative to molecular weight size marker (10 bp DNA ladder from Gibco BRL, Md.). The frequency of polymorphism between different lines of rice was calculated based on presence (taken as 1) or absence (taken as 0) of bands. The 0/1 matrix was used to calculate similarity genetic distance using 'Simqual' sub-program of software NTSYS-PC [25]. The resultant distance matrix was employed to construct two-dimensional PCA scaling using Jaccard similarity coefficient data (NTSYS-PC).

Results and discussion

Genetic variation among the CSR10 x HBC19 F6 lines

Field evaluation of CSR10 × HBC19 F_6 population showed significant variation among 201 lines for all the characters including plant height, grain yield per plant, effective number of tillers per plant, 1000-grain weight, and kernel length and breadth (Table 1). Among grain yield components, higher range was observed for grain yield per plant followed by 1000-grain weight, plant height and productive tillers per plant showing that these traits are responsible for variation in grain yield in various lines. The average plant height of CSR10 × HBC19 F_6 lines varied between 64 to 143 cm while the plant height of HBC19 and CSR10 were recorded as 67.7 cm and 136 cm, respectively. The F_6 population also showed wide variation for effective number of tillers per plant, which ranged from 5.3 to 25.3. A number of F_6 lines had significant lower number of effective tillers compared to the two parental rice varieties. Grain yield per plant ranged from 2.1 g to 86.8 g in CSR10 x HBC19 F_6 population in comparison to a grain yield of 23.5 g and 37.9 g in CSR10 and HBC19, respectively. The F_6 line number 102 had more than two-fold yield compared to the high-yielding parent, HBC19. In CSR10 × HBC19 F_6 lines, 1000-grain weight ranged from 6.0 to 53.9 g and a number of lines showed higher 1000 grain weight in comparison to HBC19. Kernel length and kernel breadth varied between 5.0-9.0 mm and 1.3-3.0 mm, respectively. The line number 8, 42 and 173 had significantly higher kernel length than HBC19. Out of 201 F_6 lines, 60 lines had Basmati aroma.

Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for grain yield parameters (Table 1), which is logical because of the fact that phenotypic coefficient of variation also includes the environmental components. The high GCV and PCV values for grain yield and its components were observed for grain yield per plant followed by productive tillers per plant, 1000-grain weight, kernel length and kernel breadth, indicating that greater amount of variability among the genotypes was genetic. Lowest PCV and GCV estimates were observed for plant height followed by L/B ratio and kernel breadth (Table 1).

Heritability (broad sense) estimates were high for plant height (97.0), yield per plant (95.8), 1000 grain weight (93.8) and L/B ratio (89.5) and moderate for number of tillers per plant (41.1) and kernel breadth (39.9) and low for kernel length (5.3) (Table 1). Characters having high heritability values could be improved directly through selection since these characters are relatively less influenced by environment and there would be greater correspondence between

Table 1. Mean, range, phenotypic and genotypic coefficients of variation, heritability and genetic advance for various traits in CSR10 x HBC19 F_6 population derived using single seed descent method

Characters	CSR10	HBC19	CSR10 x HBC19 F_6 population					
			Mean±SE	Range	PCV	GCV	Herita-bility	Genetic advance (% mean)
Plant height (cm)	67.7±0.9	136.7±3.3	105.8±1.24	64 -143.3	17.0	16.8	97.0	43.5
Productive tillers/ plant	20.00±1.5	25.00±4.6	11.6±0.24	5.3-25.3	47.1	30.2	41.1	51.1
Yield/plant (g)	23.5±2.6	37.9±1.8	10.5±0.50	2.1-86.8	69.3	67.8	95.8	175.1
1000 grain weight (g)	19.1±5.0	24.0±1.0	25.0±0.72	6.0-107.7	42.5	41.2	93.8	105.4
Kernel length (mm)	6.3±0.3	8.3±0.3	7.0±0.05	5.0-9.0	46.5	10.7	5.3	6.5
Kernel breadth (mm)	2.8±0.3	1.4±0.6	2.4±0.02	1.3-3.0	22.7	14.3	39.9	23.9
L/B ratio	2.3	6.0	3.0±0.04	2.0-5.4	20.8	19.7	89.5	49.2

phenotypic and breeding values. The high heritability estimates would be helpful for breeding superior genotypes on the basis of phenotypic performance of quantitative character. In this cross, high heritability with high genetic advance values was observed for grain yield per plant, 1000-grain weight, L/B ratio and plant height. This indicates the ample scope for improving above-mentioned traits through simple selection.

Phenotypic correlation coefficient analysis was carried to assess the association between various traits in CSR10 × HBC19 F_6 population as shown in Table 2. Yield per plant had positive correlation with number of productive tillers per plant (0.279; p = 0.01) and 1000-grain weight (0.148; p = 0.05). While kernel breadth had positive correlation with 1000-grain weight (0.200; p = 0.01), kernel length showed a positive correlation with L/B ratio (0.335; p = 0.01). Plant height had a negative correlation with number of productive tiller per plant (-0.212; p = 0.01) and kernel breadth (-0.829; p = 0.01) had a negative correlation with L/B ratio. Correlation between the different traits could be due to linkage or pleiotropy. Linkage-based correlation can be modified through recombination, but it may not be easy to modify correlation arising due to pleiotropy or developmental causes. The inclusion of various component characters in a selection scheme is obviously not practicable and under these situations, knowledge with respect to the association of various traits with grain yield would be an immense help in formulating an effective and efficient selection and screening program. Correlation between various traits was worked out to assess the association at genotypic as well as phenotypic levels. In most cases, the magnitude of correlation coefficient at genotypic level was higher than inherent association between different traits. In CSR10 × HBC19 F_6 population, correlation coefficient analysis between grain yield and its component traits indicated that grain yield has a positive and significant correlation with number of productive tillers/plant and 1000-grain weight, 1000-grain weight has a positive correlation with kernel breadth and kernel length has a positive correlation with L/B ratio.

An assessment of direct and indirect effects of component traits on grain yield per plant in F_6 population was determined by path coefficient analysis as shown in Table 3. The data shows that number of productive tillers per plant had highest positive direct effect on grain

Table 2. Correlation between various traits in CSR10 × HBC19 F_6 population as determined by phenotypic correlation coefficient

Characters	Plant height	Productive tillers/plant	Yield/ plant	1000 grain weight	Kernel length	Kernel breadth	L/B ratio
Plant height		–0.212**	0.116	–0.018	–0.019	0.070	0.001
Productive tillers/plant			0.279**	–0.008	0.030	–0.064	0.095
Yield/plant				0.148*	-0.016	0.067	0.003
1000 grain weight					0.066	0.200**	-0.058
Kernel length						–0.112	0.335**
Kernel breadth							–0.829**
L/B ratio							-

Table 3. Direct (diagonal) and indirect effects of component traits on grain yield/plant in F_6 population CSR10 × HBC19 as determined by path coefficient analysis

Characters	Plant height	Productive tillers/plant	1000 grain wt.	Kernel length	Kernel breadth	'r' with grain yield/plant
Plant height	0.1828	–0.0413	–0.0033	0.0053	0.0127	0.116
Productive tillers/plant	–0.0691	0.3056	–0.025	0.0183	–0.0147	0.279**
1000 grain weight	–0.0025	–0.0011	0.1421	0.0195	0.0284	0.148*
Kernel length	0.0006	0.013	0.0029	0.0211	–0.0033	–0.016
Kernel breadth	0.0033	–0.0022	0.0093	–0.0568	0.0467	0.067

Residual effect = 0.9345

Table 4. Allelic profile of 20 randomly selected CSR10 × HBC19 F_6 plants and parental rice varieties at 15 SSR loci

SSR markers	Chr. location	Parental genotypes		CSR10 x HBC19 F_6 plants Percent distribution of alleles*																					
		HBC19	CSR10	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	% distribution of alleles*
RM25	8	131	146	131	146	131	146	146	131	146	146	146	146	131	146	131	146	146	146	131	146	146	131	35:65:0:0
RM38	8	260	250	260	260	260/ 250	250	250	250	260	260/ 250	250	250	250	260	250	260/ 250	250	250	250	250	250	250	20:65:15:0
RM72	8	176	161	176	161	176	161	176	176	176	176	161	176	176	176	176	165	161	176/ 161	161	161	176	176	30:60:5:5
RM80	8	125	144	125	144	144	144	144	144/ 125	125	125	125	144	144	125	125	144	125	125	125	125	144	144	50:45:5:0
RM144	11	275	236	236	236	275	236	275	275	275	275	275	275	275	275/ 236	275	275/ 236	275	275/ 236	236	236	236	275	55:30:15:0
RM180	7	108	202	108	202	202	202	202	202	108	202/ 108	108	202	108	202	202	202	202	108	108	202	202	108	35:60:5:0
RM208	2	164	178	178	178	164	178	164	164	178/ 164	178	164	178	164	164	178	164	178	178	178	178/ 164	178	164	40:50:10:0
RM230	8	254	246	254	254	254	246	246	246	254	246	254	254	246	254	254	254	254	254	246	246	254	246	60:40:0:0
RM241	4	135	104	104	104	104	104	104	104	104	135	135/ 104	104	104	104	135/ 104	135	135	135	135	104	104	135	30:60:10:0
RM247	12	175	136	136	136	175	145	136	175	136	136	175	175	175	175	136	175	136	136	136	175	136	175	50:45:0:5
RM249	5	125	150	150/ 125	125	135	150/ 125	125	150/ 125	150/ 125	150	150/ 125	150/ 125	150	150	150	150	150	125	125	125	150	150	40:25:30:5
RM251	3	145	125	125	145	145	125	125	145	125	125	145	145	145	125	145	125	145	125	125	125	145	125	45:55:0:0
RM335	4	129	148	129	148	129	148	148	129	129	129	129	148	148	129	129	129	148	129	148	148	148	148	50:50:0:0
RM339	8	186	150	186	150	150	150	150	150	186/ 150	150	186	150	150	150	186	150	150	150	150	150	150	150	15:80:5:0
RM340	6	166	105	105	166	166	166	166	105	105	105	166	166	105	105	105	105	105	105	166	166	166	105	45:55:0:0
% Homo-zygosity**		100	100	93.3	100	93.3	93.3	100	86.6	80.0	86.6	86.6	93.3	100	93.3	93.3	86.6	100	86.6	100	93.3	100	100	40:52.3::6.7:1* 93.3**

*Ratio of the CSR10 × HBC19 F_6 plants with alleles from HBC19, CSR10, both the parents (CSR10 as well as HBC19) and new/recombinant alleles.
**Percent Homozygosity = No. of loci homozygous alleles/total no. of loci.

yield per plant (0.3056) followed by plant height (0.1828) and 1000-grain weight (0.1421). Regarding indirect effects, it was observed that all characters contributed to grain yield per plant through direct effect, no character was contributed to grain yield per plant significantly through indirect effect. The overall picture show that significant positive correlation was due to direct effects not by indirect effects.

Microsatellite marker analysis

Microsatellite marker analysis was carried out on twenty randomly selected CSR10 × HBC19 F_6 plants and two parental rice varieties using 15 polymorphic markers to assess allelic distribution and percent homozygosity achieved so far (Table 4). The 20 CSR10 × HBC19 F_6 plants had an allele from either of the two parental lines (homozygous condition) or alleles from both the parental rice varieties (heterozygous condition). Some of the plants had higher number of CSR10- or HBC19- specific alleles. At three SSR loci (RM72, RM247 and RM249), three of the 20 F_6 plants (3, 4 and 14) showed the presence of new or recombinants alleles different than those present in the two parental rice varieties. Occurrence of such new or recombinant alleles may have resulted from crossing-over. Some of the microsatellite loci have been reported to be the hot spots, where mutations occur up to 100 times more frequently than the normal mutation rate [6].

Percent homozygosity (percent loci having homogygous alleles) varied between 80-100% in F_6 plants with an average homozygosity of 93.3%. This value is close to the expected value (expected homozygosity = $2(2^{n-1})/2^{n+1}$ where, n = number of generation of self-pollination) of 98.4% in F_6 generation. SSR profile/diversity data were used to determine genetic relationships among 20 CSR10 × HBC19 F_6 plants and parental rice varieties. The two-dimensional PCA [25] generated using SSR allelic diversity data showed interspersing of 20 F_6 plants between the two diverse parental genotypes (Fig. 1). Notably, some of the aromatic F_6 plants were close to CSR10. Agronomical, grain quality and molecular data on CSR10 × HBC19 F_6 population clearly indicates that this population shows large variation for a number of grain yield/quality traits and can be used for linkage mapping of these traits once complete homozygosity is achieved in next two generations. The CSR10 x HBC19 population also displayed wide variation for salt tolerance attributes in F_3 generation [26]. SSR allelic data (Table 4) also shows preponderance of CSR over HBC19 specific alleles in 20 randomly selected F_6 plants indicating the occurrence of segregation and distortion, which has also been reported earlier in a wide range of plant species including rice [27].

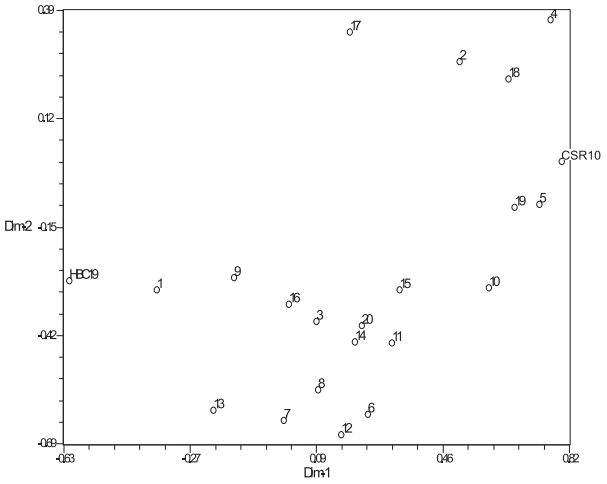

Fig. 1. Two-dimensional PCA (Principal Component Analysis) scaling of 20 CSR10 x HBC19 F_6 genotypes using Jaccard similarity coefficient data for 15 SSR markers

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