

Phenotyping of agronomic and grain quality traits of recombinant inbred lines (RIL) derived from the cross CSR 10 x HBC 19

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ABSTRACT

A F_7 and F_8 population comprising of 231 recombinant inbred lines derived from the cross between CSR10 (salt tolerant indica) x HBC19 (Taraori Basmati) using single seed descent method, was evaluated for variation in agronomic and grain quality traits. Both generations showed significant variation among 231 lines for all the characters including plant height, productive tillers per plant, grain yield per plant, 1000 grain weight, hulling percentage, raw grain dimensions, cooked grain dimensions, elongation ratio, length breadth ratio, aroma, alkali value and amylose content. Heritability (broad sense) estimates were high for all the traits except thickness of raw grains. Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the metric traits indicating less of environmental impact. Correlation studies of yield components and various physicochemical traits in 231 RILs revealed correlation among all the metric traits. Transgressive segregation was also observed for almost all the traits in both the generations.

Key words: rice, variation, correlation, quality, segregation

INTRODUCTION

Basmati rice varieties with exquisite aroma, superfine grains and excellent cooking qualities have gained greater attention due to greater preferences in domestic and international markets (Ahuja *et al.*, 1995). Basmati rice has its origin in the foot hills of Himalayas and is the result of centuries of selection and cultivation by farmers (Khush, 2000). Some of them have more scent and elongation ratio than basmati but in overall score basmati surpasses all of them in cooking qualities and is most revered of all scented rices hence called the queen of rice (Ahuja *et al.*, 2008). Basmati has its own share of problems as tall plants are prone to lodging and non-responsive to fertilizers, photosensitive, sensitive to alkaline soil and is a poor combiner. Several high yielding basmati varieties have been developed and released for cultivation but most of these varieties fall short of one or more of grain quality parameters (Singh *et al.*, 2005). Efforts to sustain high productivity coupled with high quality had only limited success. Genetics of complex quality traits is not well understood, and finding a desirable segregant possessing all desirable components require screening on a very large scale.

Marker assisted selection can greatly improve the efficiency and precision of the breeding process especially in case of Basmati rice where one need to keep all the basmati grain quality components together while introgressing desirable trait(s) from a donor. In rice, permanent populations, such as double haploids (DH) and recombinant inbred lines (RIL) are used most often, since they can be replicated over locations and years and therefore are of immense value in mapping QTLs (Burr and Burr, 1991). RILs have advantages of providing a permanent DNA supply and phenotyping opportunities for many different studies. Allelic differences are limited in these populations, since only two alleles segregate at each polymorphic locus (You *et al.*, 2005). In the present investigation, recombinant inbred lines derived from the cross between a salt tolerant indica and an aromatic rice variety were studied for evaluation of variation in agronomic and grain quality traits.

MATERIALS AND METHODS

A total of 231 CSR10 x HBC19 F_7 and F_8 lines and parental rice varieties were grown in Augmented Design during wet season at CCS HAU Rice Research Station,

Kaul using single seed descent method for two successive seasons, which falls under semi-tropical region of north India. CSR10 which is a selection from CSR1/Jaya developed and released by CSSRI, Karnal (India) and recommended for cultivation in saline soil (Mishra *et al.*, 1992). HBC-19 (a pure line selection from Taraori Basmati) is a commercially important premium traditional basmati rice variety. Each progeny and lines were planted in a single row of 3.5 meter length. Seedlings were transplanted with plant to plant spacing of 15cm and row to row spacing of 20 cm.

Three sets of 5 plants each *i.e.* total of fifteen plants were randomly selected from each line and evaluated for four agronomical traits including plant height, effective no of tillers plants⁻¹, yield plant⁻¹ and 1000 grain weight. Mature grains of both generation RILs and parental lines were taken for raw and cooked grain dimensions.

Aroma of milled rice grains was determined by a sensory evaluation panel according to the method of (Sood and Siddiq, 1978) with minor modifications. Ten milled rice grains were placed in Petri plate containing 10ml of 1.7% KOH and incubated for 10 min. with lids on. The lids were then opened one by one and samples were than smelled and rated for aroma by a panel of three experts, where 0 was non-aromatic, 1 was mild aromatic and 2 was highly aromatic. Two blind checks, HBC19 (highly aromatic) and CSR10 (non-aromatic) were included with each batch of seven samples (Parent 1, Parent 2 and 5 RILs) analyzed by a sensory panel to increase the reliability of aroma rating. The alkali spreading value was determined by the method of Little *et al.*, 1958. Amylose content was estimated using the procedure of (Juliano, 1971) with minor modifications.

Statistical methods used in this study included mean, coefficient of variation (GCV, PCV) as described by Burton, 1952 and heritability. Correlation between the trait pairs were computed using formula of Johanson *et al.*, 1955.

RESULT AND DISCUSSION

Field evaluation of CSR10 x HBC19 F₇ and F₈ population showed significant variation among 231 lines for all the characters including plant height, productive tillers plant⁻¹, grain yield plant⁻¹, 1000-grain weight, hulling percentage, raw grain dimensions, cooked grain

dimensions, elongation ratio, length breadth (L/B) ratio, aroma, alkali value and amylose content. On the basis of KOH test out of 231 lines (including parents) 115 lines were non-aromatic, 105 were mild aromatic and 11 were highly aromatic. Similarly higher range was observed among almost all the agronomic and grain quality traits. (Table 1 and 2). Similar results were reported by other workers in rice by Mahala *et al.*, 2008 and Zhang *et al.*, 2010.

Correlation between different traits was worked out to asses the associations at genotypic and phenotypic levels among both generations. Yield per plant had highest PCV and GCV followed by 1000-grain weight, moderate for raw grain dimensions and cooked grain dimensions, amylose content, alkali value or elongation ratio and lowest PCV and GCV were observed for thickness of raw grain and hulling percentage (Table 1 and 2).

Heritability in a broad sense estimates were high for length of raw grain followed by plant height, length: breadth ratio, amylose content, alkali value, elongation ratio, length of cooked grain, yield per plant, tiller number, hulling percentage and moderate heritability was noticed for 1000-grain weight, breadth of raw grain and low for thickness of raw grains (Table 1, 2). Phenotypic correlation coefficient analysis was carried out to assess the association between various traits in F₇ and F₈ population as shown in (Table 3). Significant variation was reported for all the agronomic and grain quality traits among 231 lines of both population. Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the metric traits because of the fact that phenotypic coefficient of variation also includes the environmental components. High heritability was reported for all agronomically important traits. Characters having high heritability values could be improved directly through selection since these characters are relatively less influenced by environment and there would be great correspondence between 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 both generations correlation coefficient analysis between grain yield and component traits indicated that grain yield had a positive and significant

Table 1. Showing mean, range, phenotypic and genotypic coefficients of variation and heritability estimates for various traits in F₇ population.

Characters	CSR-10	HBC-19	CSR10xHBC19 F ₇ population		PCV	GCV	Heritability%
	Mean+ S.E	Mean+ S.E	Mean + S.E	Range			
Plant height (cm)	66.27 + 0.99	139.20 + 0.76	113.50 + 1.99	62.66 – 163	17.07	16.8	96
Productive tillers plant ⁻¹	25.00 + 0.12	18.33 + 0.24	14.39 + 1.12	7.66 – 32	29.84	26.6	79
Yield/plant (g)	24.73 + 0.38	19.20 + 0.45	13.03 + 1.38	2.03 - 40.1	46.35	42.56	84
1000 grain weight (g)	25.90 + 0.63	24.37 + 0.54	23.32 + 3.63	8.6 - 44.1	38.1	26.98	50
Hulling percentage	76.90 + 0.59	75.50 + 0.37	71.24 + 1.59	55.6- 82.8	8.33	7.37	78
LRG(mm)	5.34 + 0.06	7.71 + 0.09	6.57 + 0.06	5.06 - 8.6	10.24	10.09	97
BRG (mm)	2.55 + 0.15	1.73 + 0.09	2.20 + 0.15	1.59 - 2.74	11.72	11.81	49
TRG (mm)	1.75 + 0.07	1.50 + 0.06	1.72 + 0.07	1.51 - 1.97	9.41	5.38	32
L/B Ratio	2.09 + 0.05	4.45 + 0.04	3.05 + 0.05	1.88 - 4.44	17.26	16.94	96
LCG(mm)	8.00 + 0.30	15.61 + 0.37	10.13 + 0.30	6.94 - 15.54	15.54	14.67	89
BCG(mm)	3.30 + 0.16	2.17 + 0.12	2.68 + 0.16	1.99 - 3.4	16.44	12.52	58
Elongation Ratio	1.50 + 0.04	2.03 + 0.08	1.55 + 0.04	1.11 - 2.17	17.1	16.27	89
Alkali value	2.00 + 0.10	3.50 + 0.18	2.72 + 0.10	2 - 5.9	31.81	31.1	95
AC	27.57 + 0.03	21.43 + 0.07	19.98 + 0.03	14.07-27.73	16.32	16.32	96

LRG= Length of raw grain, BRG= Breadth of raw grain, TRG = Thickness of raw grain, L/B ratio= Length/ breadth ratio, LCG=Length of cooked grain, BCG= Breadth of cooked grain, AC = Amylose content

correlation with 1000-grain weight. Similar association was reported by (Jaydeep, 2009 and Mahala *et al.*, 2008). Positive and significant association of yield with plant height as observed in present study was also reported by (Wattoo *et al.*, 2010). No association of yield per plant was recorded with grain quality and physiochemical traits, which indicate that yield, could

be combined with different grain and physiochemical traits. 1000-grain weight showed positive and significant association with breadth of raw grain similarly reported by Zhang *et al.*, 2005 which reflects that the broad grain will have higher grain weight. Length of raw grain was positively and significantly associated with length of cooked grain and L/B ratio. This indicates that long

Table-2. Showing mean, range, phenotypic and genotypic coefficients of variation and heritability estimates for various traits in F₈ population

Character	CSR10	HBC19	CSR10x HBC19 F ₈ Population		PCV	GCV	Heritability
	Mean±SE	Mean±SE	Mean±SE	Range			
Plant height (cm)	66.2±0.58	139.4±0.51	114.3±1.0	63.8-162	17.53	17.34	99
Productive tillers plant ⁻¹	24.0±0.51	19.0±1.06	15.0±0.93	6.8-32.4	31.87	29.92	88
Yield per plant (gm)	24.7±0.39	19.6±0.32	14.2±0.58	4.3-41.26	40.86	40.23	88
1000 seeds weight (gm)	25.16±0.15	24.0±0.09	23.0±0.90	6.18-42.02	30.68	29.02	66
Hulling Percentage	77.56 + 0.55	74.70 + 0.35	72.23 + 1.55	56.2- 82.9	8.42	7.42	79
LRG (mm)	5.69±0.064	7.89±0.028	6.62±0.01	5.07-8.46	10.20	10.16	98
BRG(mm)	2.53±0.020	1.71±0.012	2.14±0.07	1.68-2.67	10.09	10.07	72
TRG (mm)	1.74±0.002	1.50±0.004	1.71±0.01	1.47-1.99	5.73	5.70	68
L/B ratio	2.19±0.028	4.59±0.045	3.11±0.03	2.1-4.29	15.37	15.24	98
LCG(mm)	8.00±0.071	15.58±0.037	10.65±0.07	7.72-15.18	13.56	13.02	94
BCG (mm)	3.28±0.037	2.20±0.032	2.78±0.032	2.18-4.18	11.44	11.19	75
Elongation ratio	1.49±0.022	1.98±0.006	1.62±0.01	1.10-2.18	14.70	14.65	92
Alkali value	2.10 + 0.12	3.52 + 0.19	2.74 + 0.10	2 - 5.9	32.11	33.11	96
AC	27.58 + 0.05	21.41 + 0.08	19.98 + 0.03	14.07-27.73	16.32	16.34	96

LRG= Length of raw grain, BRG= Breadth of raw grain, TRG = Thickness of raw grain, L/B ratio= Length/ breadth ratio, LCG=Length of cooked grain, BCG= Breadth of cooked grain, AC = Amylose content

Table 3. Phenotypic Correlation Coefficient between various morphological and grain quality traits in F₇ generation (above diagonal) and F₈ generation (below diagonal) derived lines from the cross CSR10 x HBC19 (Red +ve and blue -ve correlation)

Characters	Yield plant ⁻¹ (g)	1000 grain wt(g)	Plant height (cm)	Tiller plant ⁻¹	Hulling %	LRG (mm)	BRG (mm)	TRG (mm)	L/B Ratio	LCG (mm)	BCG (mm)	ER	Alkali value	AC
Yield plant ⁻¹ (g)	1	0.153*	0.149*	0.126	-0.022	0.046	0.121	0.028	-0.081	0.077	0.011	0.039	-0.117	0.023
1000 grain wt(g)	0.164*	1	0.092	-0.026	0.022	0.09	0.09	0.045	-0.139*	0.017	0.102	-0.043	0.116	0.031
Plant height(cm)	0.159*	0.089	1	0.089*	0.136*	-0.053	-0.011	-0.053	0.062	0.150*	0.038	0.087	-0.128	0.102
Tiller plant ⁻¹	0.128*	-0.028	0.099*	1	-0.012	-0.045	0.07	0.004	-0.036	0.067	0.008	0.031	-0.069	0.009
Hulling %	-0.031	0.031	0.165*	-0.022	1	-0.024	0.085	0.063	-0.067	0.180*	0.066	0.165*	-0.121	0.006
LRG(mm)	0.048	0.11	-0.064	-0.054	-0.032	1	0.079	0.025	0.666*	0.170*	-0.061*	0.456*	-0.077	0.004
BRG(mm)	0.138	0.13	-0.009	0.09	0.089	0.084	1	0.304*	-0.685*	-0.106	0.293*	-0.042	0.199*	0.012
TRG(mm)	0.035	0.054	-0.064	0.006	0.065	0.026	0.026	1	-0.258*	-0.078	0.078	-0.056	0.003	-0.036
L/BRatio	-0.078	-0.145*	0.064	-0.045	-0.074	0.675*	-0.284*	-0.225*	1	0.215*	-0.295*	0.029	0.225*	-0.022
LCG(mm)	0.079	0.025	0.154*	0.074	0.192*	0.184*	-0.089	-0.065	0.305*	1	-0.033	0.787*	0.006	0.026
BCG(mm)	0.021	0.122	0.042	0.009	0.06	-0.075*	0.087	0.059	-0.289*	-0.045	1	0.007	-0.132*	0.052
ER	0.041	-0.054	0.089	0.035	0.174*	0.476*	-0.065	0.031	0.035	0.789*	0.009	1	-0.044	-0.03
Alkali value	-0.201	0.132	-0.129	-0.074	-0.125	-0.079	0.006	0.006	0.229*	0.009	-0.145*	-0.049	1	-0.011
AC	0.043	0.035	0.105	0.007	0.009	0.009	-0.042	-0.025	-0.026	0.031	0.056	0.032	-0.021	1

significant at 5% level

grains tended to have high cooked grain length and are slender. Similar results were reported by (Li *et al.*, 1999, Rabiei *et al.*, 2004 and Chakraborty *et al.*, 2009). Grain length did not show any association with physicochemical traits like alkali value and amylose content as reported by Juliano, 1984. According to him grain length is not an index of chemical properties of grains and is possible to combine grain length with these characters. In F₇ population, elongation ratio showed positive association with L/B ratio, but no such association had been reported by other workers. L/B ratio in the present study showed negative association with width of the cooked grain meaning that broad kernels will have low L/B ratio after cooking. Similar results were reported by (Li *et al.*, 1999 and Rabiei *et al.*, 2004). It is concluded that 1000-grain weight was contributing towards grain yield but was negatively correlated with L/B ratio or grain fineness which in turn was associated with other cooking qualities. In breeding for quality rice's, high L/B ratio is required so a balance has to be maintained for selecting grain weight in breeding of quality rice's. It was also associated with alkali value, which is again an important physiochemical trait in cooking quality. Almost, similar pattern of correlation was observed among both populations.

A frequency distribution pattern of both populations indicate that the two parental varieties were highly diverse for all the traits. Frequency distribution for all the traits among RILs of both generations for agronomic and grain quality traits also show similar pattern. Normal distribution and wide range of variation of the investigated parameters indicated transgressive segregation for several traits and segregation in one direction in some traits indicating polygene and oligogenes control. The continuous pattern of distribution suggests quantitative inheritance, a result similar to that of Zhang *et al.*, 2010, who observed a wide range of variations for amylose content, gelatinization temperature and gel consistency in recombinants inbred lines of rice. Yu *et al.*, 1999 and Cao *et al.*, 2001 done QTL studies on important traits in rice, such as yield and yield-related traits, plant height and heading date have clearly shown the importance of epistatic interactions and Zhang *et al.*, 2005 reported the importance of pleiotropy and linkage for these quantitatively inherited traits. Hagiwara *et al.*, 2001,

analyzed the polygenic balance model for yield components in rice and revealed that transgressive segregation occurred due to hidden genetic variation concealed in the introgressed segment even though the phenotypic difference in the characteristics was minor between the parental lines. This leads to conclusion that adaptive traits might be created in part through the reconstruction of gene combinations in the flanking region using preexisting variation rather than newly derived mutations. Frequency distribution of both the populations showed transgressive variations and maximum traits had normal distribution indicating polygenic control. Thus on the basis of above results, it may be concluded that variation among lines of both generations found in this study offers opportunities to breed for yield attributes and grain quality in either direction in order to meet the specific requirements of QTL analysis and linkage mapping.

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