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Genetic mapping for grain quality and yield-attributed traits in Basmati rice using SSR-based genetic map

MADHVI SHARMA[1]*,†, SUNIL S GANGURDE[2,†], ROMESH K SALGOTRA[1], BUPESH KUMAR[3], ANIL K SINGH[1] and MANISH K PANDEY[2]*

[1]*School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu 180 009, India*

[2]*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad 502 324, India*

[3]*Division of Plant Breeding and Genetics, She-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu 180 009, India*

**Corresponding authors (Emails, madhvisharma413@gmail.com; m.pandey@cgiar.org)*

[†]Madhvi Sharma and Sunil S Gangurde have contributed equally to this work.

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Rice grain shape and nutritional quality traits have high economic value for commercial production of rice and largely determine the market price, besides influencing the global food demand for high-quality rice. Detection, mapping and exploitation of quantitative trait loci (QTL) associated with kernel elongation and grain quality in Basmati rice is considered as an efficient strategy for improving the kernel elongation and grain quality trait in rice varieties. Genetic information in rice for most of these traits is scanty and needed interventions through the use of molecular markers. A recombinant inbred lines (RIL) population consisting of 130 lines generated from the cross involving Basmati 370, a superior quality Basmati variety and Pusa Basmati 1121, a Basmati derived variety were used to map the QTLs for 9 important grain quality and yield related traits. Correlation studies showed that various components of yield show a significant positive relationship with grain yield. A genetic map was constructed using 70 polymorphic simple sequence repeat (SSR) markers spanning a genetic distance of 689.3 cM distributed over 12 rice chromosomes. Significant variation was observed and showed transgressive segregation for grain quality traits in RIL population. A total of 20 QTLs were identified associated with nine yield and quality traits. Epistatic interactions were also identified for grain quality related traits indicating complex genetic nature inheritance. Therefore, the identified QTLs and flanking marker information could be utilized in the marker-assisted selection to improve kernel elongation and nutritional grain quality traits in rice varieties.

Keywords. Genetic mapping; grain length; QTL; Basmati; RIL; SSR.

1. Introduction

Rice is one of the major staple food crops of more than 3.5 billion people across the globe. The production and consumption of global rice accounted for almost 90% in Asian countries; mainly, China and India alone contribute about 55% (Kong *et al*. 2015). Among the cereals, rice provides up to 20% of their regular calorie intake for millions of global population. Rice (*Oryza sativa* L.) is a self-pollinated crop belongs to the family Poaceae. It is a diploid cereal (2n=12) with genome size of 430 Mb. The genus *Oryza* comprises of two cultivated and twenty two wild species. The cultivated species are *Oryza sativa* (Asian rice) which is grown

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worldwide and the *Oryza glaberrima* (African rice), which remain confined to the parts of West Africa. Across the world, there are more than 40,000 different varieties of the Asian rice. In order to ensure nutritional food security, the projected rice production must be increased to 852 million tons by 2035 (Brar *et al*. 2018).

With the intensifications of diverse food demands and living standards of global populations, rice grain appearance and quality have become a primary concern for rice breeders. Therefore, there is an urgent need to increase the grain yield along with desirable grain nutritional quality traits in rice (Sreenivasulu *et al*. 2015; Sun *et al*. 2018). Grain quality has been recognized into two classes: (i) grain appearance (ii) cooking and eating qualities (Juliano and Villareal 1993). Most of the grain quality traits are controlled by quantitative trait loci (QTLs) as inferred from continuous phenotypic variation in the segregating progeny of intervarietal crosses (Amarawathi *et al*. 2008).Grain size is an important trait in rice breeding, serving as a factor in determining rice yield. Linear elongation of kernel on cooking is one of the major characteristics of fine rice (Sood *et al*. 1979). Lengthwise expansion without increase in girth is considered as a high desirable trait in high quality rice (Sood *et al*. 1983). The amylose content (AC) of rice is generally categorized into five classes; waxy (0–2%), very low (3–9%), low (10–19%), intermediate (20–24%) and high amylose (above 24%) (Cheng *et al*. 2012). Previous studies have found that AC is governed mainly by an allelic series of genes at one locus and by one or several modifier genes with minor effects. However, the AC inheritance pattern is complex due to cytoplasmic effects, epistasis and environmental effects. Amylose synthesis is catalyzed by granule-bound starch synthase (GBSS) encoded by the *Wx* locus (Zhang *et al*. 2005). Genetics of rice quality has also been studied in various genetic backgrounds using molecular markers (Bradbury *et al*. 2005a, b; Wanchana *et al*. 2005; Wan *et al*. 2006; Chen *et al*. 2006).

With the development of molecular biology and establishment of various genetic linkage maps, QTLs for grain quality of rice have been widely reported (Zhang *et al*. 2011, 2012a, b, 2013). He *et al*. (2003) detected 12 QTLs related to cooked rice grain size on chromosomes 2, 3, 4, 5, 6, 7 and 11 using a RILs population derived from Zhenshan 97 × Minghui 63. With a doubled haploid (DH) population derived from ZYQ8× JX17, Zhang *et al*. (2004a, b) identified three QTLs for rice elongation character. Shen *et al*. (2011) identified 14 QTLs for cooked grain characters using the same DH population. Both of Zhang *et al*. (2004a, b) and Shen *et al*. (2011) detected QTLs related to cooking traits in the regions near two known quality-effecting genes, namely *Wx* and *ALK*.

Li *et al*. (2003) identified QTLs for rice cooking traits including soaked and boiled grain size, grain expansion, elongation rate and other indices using a population of 132 RILs derived from the cross PA64s × 93–11 based on the high-density SNP based genetic map. These studies indicate that the grain size is a polygene controlled quantitative trait, resulting in substantial variation in the size and shape of the grains. Many genes influencing grain size have been cloned from rice cultivars through quantitative trait locus (QTL) analysis. Thus far, GS3 (Fan *et al*. 2006; Takano-Kai *et al*. 2009), qGL3 (Zhang *et al*. 2012a, b), GW6a (Song *et al*. 2015), TGW6 (Ishimaru *et al*. 2013), and GL7/GW7/SLG7 (Wang *et al*. 2015a, 2015b; Zhou *et al*. 2015) have been isolated as genes that increase grain length. Lanceras *et al*. (2000) found four QTLs for AC on chromosomes three, four, six and seven. These QTLs accounted for 80% of phenotypic variation observed in AC. Two QTLs on chromosome 6 and one on chromosome 7 were detected for GC, which accounted for 57% of phenotypic variation.

In the present study, quantitative trait loci (QTLs) for grain quality and yield-attributed traits were detected in a RIL population (Basmati 370 (female or recipient) × Pusa Basmati 1121 (male or donor)). A genetic map was constructed using SSR markers and phenotypic data along with genotypic data and genetic map information was used for identification of QTLs for grain quality traits. For detecting QTLs for kernel elongation rarely very-long-grain-length genotype and short-grain-length counterpart had been used which restricted trait variability and availability of robust QTL. The parental lines used for QTL analysis for kernel elongation were selected by screening for the longest grain size lines. Although many genes were cloned and identified, the molecular mechanisms underlying grain size determination and kernel elongation are not well understood, because most of the genes are not involved in the same signal transduction pathways. To clarify these mechanisms, it is necessary to identify and classify additional genes that contribute to grain size and kernel elongation. Keeping in view the global importance and relatively less attended traits of kernel elongation, the present research study was undertaken to dissect the genetic basis of kernel elongation and grain quality traits in rice.

2. Materials and methods

2.1 *Plant material and development of RIL population*

A F_7 recombinant inbred lines (RIL) population was developed by crossing Basmati 370 and Pusa Basmati1121.The F_1 obtained were further confirmed using SSR markers and 170 F_2 plants were produced. These F_2 plants were advanced using single seed descent (SSD) method to stabilize the lines to develop a RIL population of size 130. Pusa Basmati 1121 is a pioneering Basmati rice variety with Basmati quality attributes introgressed from conventional Basmati varieties including Basmati 370 and Type 3. It has extra-long slender milled grains (9.00 mm), a soothing aroma, and a cooked kernel elongation ratio of 2.5 with a cooked kernel length of up to 22 mm. (Singh *et al*. 2018) whereas, Basmati 370 is a rice variety that has a special aroma and flavor, along with fine quality long grain, cultivated in the R.S.Pura and adjoining villages in Jammu region. The 130 RILs along with parents were planted in season of 2017 and 2018 at Research Farm School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu in Randomized Block Design (RBD) (figure 1).

2.2 *Phenotyping of grain quality and yield-attributed traits*

Mature F_6 and F_7 grains of RILs along parents in replications were dried at room temperature (37°C) for one month before testing. Replicated data generated during the two consecutive *kharif* season 2017 and 2018 were phenotype for 15 agronomic traits, Plant height, Days to 50% flowering, Days to maturity, Panicle length, yield per plant 1000-grain weight, spikelet fertility, no. of effective tillers per plant, number of grains per panicle, kernel elongation, kernel elongation ratio, grain length, grain breadth, length breadth ratio, Amylose content (AC). All the agronomic traits were measured following DUS guidelines (Prakash 2007).

Kernel elongation was measured after cooking the rice by ordinary scale followed by the Dela Cruz and Khush (2000) protocol. 2 gm dehusked grains were soaked in 30 ml of distilled water for 30 min in a test tube. The samples were then placed in a water bath at 98°C for 10 min followed by cooling to room temperature. The cooked rice was transferred to petridish lined with filter paper.

Amylose content was estimated using procedure followed by Juliano (1971) with slight modification. Paddy grains were cleaned, dried and dehusked for estimation of amylose content. The dehusked grains were grinded in motor pestle and made to powder. 100 mg of rice powder was taken to which 1 ml of ethanol is added, shake well. 100 ml of 1N NaOH was added to it and was kept overnight. Next day the mixture was filtered through Whatman filter paper and 2.5 ml extract was taken from it. Three drops of phenolphthalein indicator was added and pink color developed. 0.1 N HCl drop by drop was added till the pink color just disappeared. 1 ml iodine reagent was added to it and the final volume of the mixture was made 50 ml by adding distilled water. The color developed was recorded at 590 nm using a UV spectrometer.

2.3 *Phenotypic data analysis*

All the basic statistical parameters for different traits were analyzed using SPSS software (v.20.0). Analysis of variance (ANOVA) was identified using R package software and correlation estimation was carried out using ICIM mapping software (v4.0) (Wang *et al*. 2012).

2.4 *High-quality genomic DNA isolation and genotyping using SSR markers*

Plant genomic DNA from 130 RILs and the two parents was extracted from fresh and tender leaf tissue using CTAB method (Doyle and Doyle 1990) with slight modifications (supplementary information 1). The DNA quality and quantity were checked on 0.8% agarose and Peq-Lab Nanodrop respectively.

276 simple sequence repeat (SSR) markers (supplementary table 1) were screened for parental polymorphism survey of which 70 polymorphic markers well distributed on 12 rice chromosomes were selected for genotyping RIL population. DNA amplification in PCR tubes for 10 µl reaction mixtures included 2 µl of template DNA (50 ng/µl), 0.5 µl of forward and reverse primers each of 10 pmol concentration,0.1 µl of Taq polymerase (5 U/µl), 2.0 µL of 5X PCR buffer with 0.8 µl $MgCl_2$ (0.25 mM), 0.25 µl of 10 mM each dNTPs and 3.85 µl of nuclease free ddH_2O The PCR conditions were set at the initial denaturation step of 5 min, followed by a loop of 35 cycles each consisting of denaturation (at 94°C for 30 s), annealing (55°C–58°C) for 30 s and

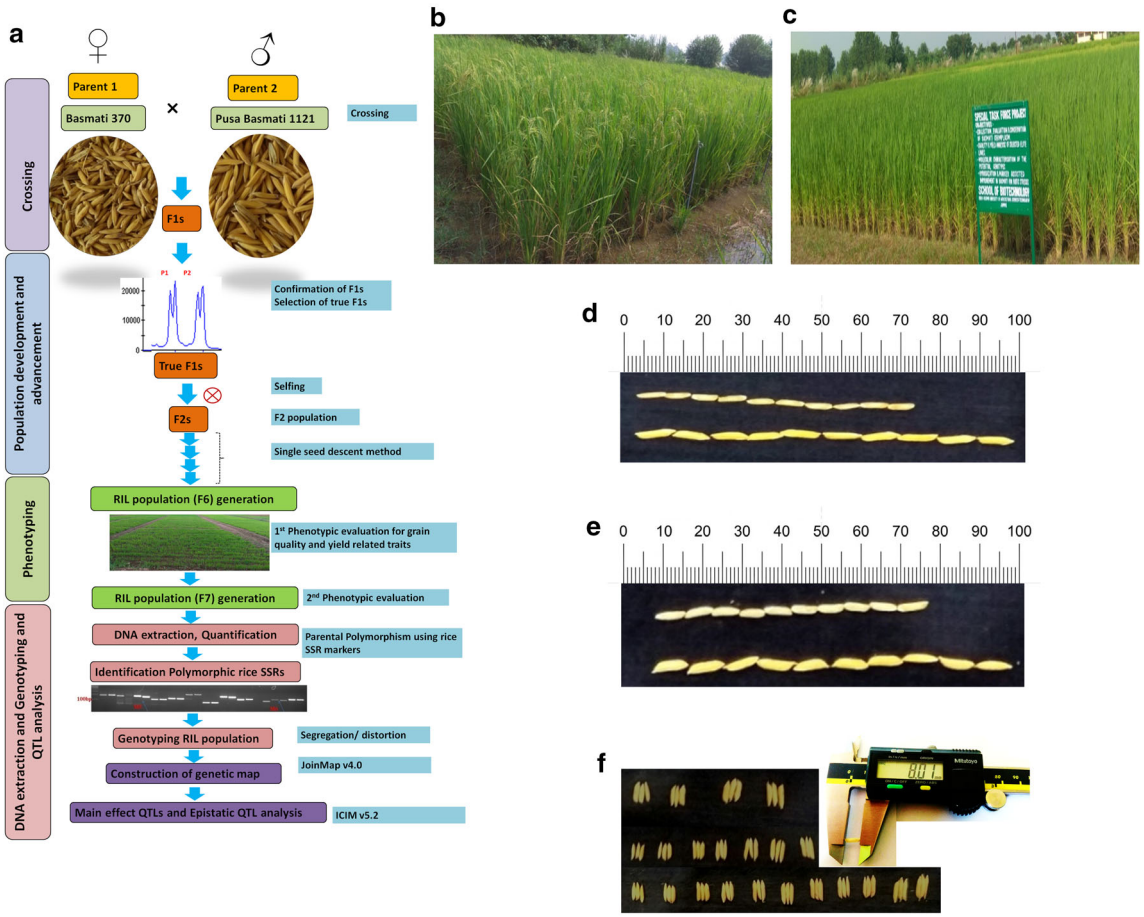

Figure 1. (**a**) Development of genetic mapping population Basmati 370 × Pusa Basmati 1121 using single seed descent method (SSD), phenotyping and genotyping, (**b**) planting in rainy season 2017, (**c**) planting in rainy season 2018, (**d**) grain length and width of Pusa Basmati 1121, (**e**) grain length and width of Pusa Basmati 1121 × Basmati 370, (**f**) variation of seed size in recombinant inbred lines.

extension at 72°C for 30 s. The final extension was performed at 72°C for 7 min. After PCR amplification, the PCR product for each reaction was separated on 3% agarose gel with ethidium bromide (EtBr) added to the gel to visualize the bands under UV trans-illuminator. Using above PCR conditions the genotyping data on 130 RILs and 2 parents was generated using 70 polymorphic SSR markers. The amplified fragments of RILs were scored as Basmati 370 (scored as 'A') type or Pusa Basmati 1121 (scored as 'B') and heterozygous ('H'), and missing (not amplified) (scored as '-'). The genotyping data obtained for each marker was recorded chromosome wise in an excel sheet and used for construction of genetic map.

2.5 *Construction of genetic linkage map*

A genetic map was constructed using genotypic data of 70 polymorphic SSR markers in Join Map version 4.0 (Vanaja and Babu 2006). The grouping and ordering of markers were carried out using regression mapping algorithm. Kosambi's mapping function was used for converting the recombination frequency into map distance in centiMorgan (cM). Markers at the same locus (with 0 cM interval) were counted with zero recombination frequency. The markers were ordered in 12 linkage groups by applying the independent LOD scores with LOD threshold ranging from 3 to 10 with minimum recombination frequency threshold of 50 %.

2.6 *Main effect and epistatic (Q × Q) analysis*

Main effect QTLs for kernel elongation and grain quality traits were identified using inclusive composite interval mapping-additive (ICIM-ADD) implemented in ICIM software (v4.0). In ICIM, the *p* value for entering variables (PIN) and removing variables (POUT) were set at 0.001 nd 0.002 and the Scanning

step was 1.0 cM. The QTLs were assumed present when the logarithm of the odds (LOD) scores was ≥3.0 based on the 1000 permutations during QTL analysis in ICIM. The additive and the dominant effects and the phenotypic variance explained by each QTL at the maximum LOD score were also estimated in ICIM-ADD method. Similarly, epistatic ($Q \times Q$) QTLs were also identified using ICIM-EPI implemented in ICIM mapping softwareversion 4.0 (Wang *et al*. 2012).The SSR-based genetic map information and phenotyping data was used for epistatic QTL analysis for grain quality traits. ICIM for epistatic QTLs with additive (two-dimensional scanning, ICIM-EPI) method with 5cM step and 0.001 probability mapping parameters in stepwise regression were employed in epistatic QTL analysis.

3. Results

3.1 *Phenotypic variation for grain quality and yield-attributed traits in RIL population*

Frequency distribution plots were constructed for grain quality traits by using phenotypic data of 130 RILs along with parents and shown normal distribution and huge variation in RIL population during the year 2017 and 2018 based on their bell shaped curves suggesting the polygenic nature of the inheritance of the traits (figure 2). Except for AC, significant positive correlation was observed between other traits. The strong positive correlation was observed between KER and KE (r=0.56) followed by ET and PH (r=0.26), LBR and PL (r=0.25), KE and GL (=0.22) and GB and PH(r=0.22).The significant positive correlation with medium value was observed between DM and PH (r=0.17), followed by GL and PL (r=0.15) and SF and PH (r=0.15) during the year 2017. However during the year 2018, significant positive correlation with medium value was observed between AC and PL (r=0.18), followed by KE and PL (r=0.16), KER and PL (r=0.16) and KE and PL (r=0.15). The minimum significant positive correlation value was observed between KE and ET (r=0.005), KE and GPP (r=0.03), KER and GPP (r=0.02) and GB and GW (r=0.01) (tables 1 and 2).

One hundred and thirty F_7 RILs from cross between Basmati 370 and Pusa Basmati 1121 along with parents were analyzed for Analysis of Variance (ANOVA) for various traits (table 3). The result indicated that all the traits showed highly significant variation at 5 percent level. It reflected the large variability among tested genotypes which can be further utilized in the rice improvement program. Genetic variability components like genotypic and phenotypic variance along with heritability were estimated to determine the heritable potential of the concerned traits as well the effect of environment over them. GCV and PCV estimates were classified as low (0–10 percent), moderate (10–29 percent) and high (>20percent) (Johnson *et al.* 1955), which suggests the possibility of improving this trait through selection. Heritability is a good index of

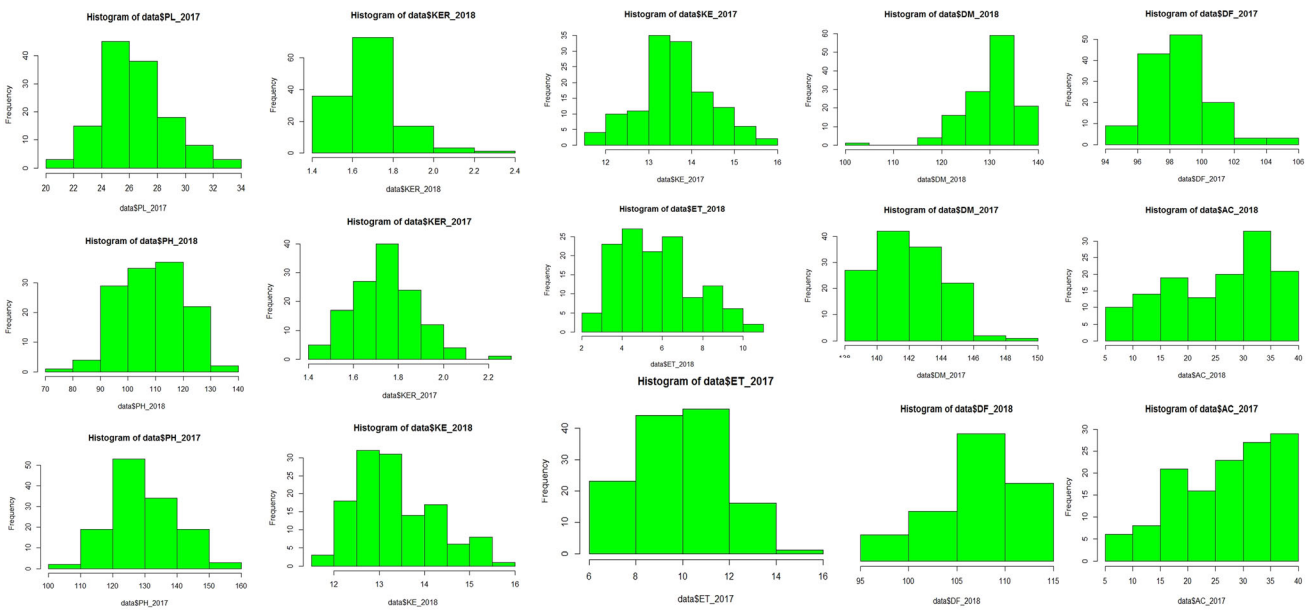

Figure 2. Frequency distribution plots for grain elongation and yield related traits in both seasons (2017 and 2018).

Table 1. Estimation of correlation coefficients between grain quality and yield-associated traits in Basmati 370 and Pusa Basmati 1121 and RIL population during the year 2017

Characters	PH	PL	ET	GL	DF	DM	AC	KE	KER
PH	1.000								
PL	0.100*	1.000							
ET	0.264*	−0.015*	1.000						
GL	0.026*	0.159*	0.101*	1.000					
DF	−0.058*	0.075*	−0.070*	0.033*	1.000				
DM	0.175*	−0.043*	0.078*	0.129*	0.058*	1.000			
AC	0.014*	0.005*	0.015*	−0.109*	0.054*	−0.1*	1.000		
KE	0.140*	0.068*	0.080*	0.224*	0.034*	0.041*	0.096*	1.000	
KER	0.084*	−0.084*	−0.014*	−0.671*	0.001*	0.089*	0.164*	0.56*	1.000

PH: Plant height; PL: Panicle length; ET: No. of effective tillers per plant; DF: Days to 50% flowering; DM: Days to maturity; AC: Amylose content; KE: Kernel elongation; KER: Kernel elongation ratio; GL: Grain length.

* Significant at 5% level of significance.

Table 2. Estimation of correlation coefficients between grain quality and yield-associated traits in Basmati 370 and Pusa Basmati 1121 and RIL population during the year 2018

Characters	PH	PL	ET	GL	DF	DM	AC	KE	KER
PH	1.000								
PL	0.33*	1.000							
ET	0.32*	0.21*	1.000						
GL	−0.058*	−0.044*	−0.0038	1.000					
DF	−0.268*	−0.021*	0.051*	−0.095*	1.000				
DM	−0.103*	−0.198*	−0.091*	−0.036*	−0.150*	1.000			
AC	−0.128*	0.185*	−0.166*	−0.113*	−0.056*	−0.048*	1.000		
KE	0.116*	0.168*	0.005*	0.150*	0.027*	−0.037*	0.087*	1.000	
KER	0.139*	0.163*	−0.007*	−0.592*	0.097*	−0.003*	0.146*	0.70*	1.000

* Significant at 5% level of significance; PH: Plant height; PL: Panicle length; ET: No. of effective tillers per plant; DF: Days to 50% flowering; DM: Days to maturity; AC: Amylose content; KE: Kernel elongation; KER: Kernel elongation ratio; GL: Grain length.

Table 3. Combined ANOVA of 130 RILs of Basmati 370 and Pusa Basmati 1121 for grain quality and yield-associated traits during the years, 2017 and 2018

Source of variation	Df	Plant height (cm)	Days to 50 % flowering (no.)	Days to maturity (no.)	Panicle length (cm)	Kernel elongation (mm)	Kernel elongation ratio (mm)	Grain length (L) (mm)	Amylose content %	Effective tillers (no.)
Replication	2	0.19*	0.07*	604.57*	579.97*	0.07*	1092.37*	0.7	0.07*	0.03*
Genotypes	129	172.76*	15.68*	23.25*	8.76*	1.17*	0.03*	0.35*	162.14*	6.05*
Error	258	15.9	8.37	18.73	1.16	0.23	0.01	0.08	0.53	4.33

* *Significant at 5% level of significance. * Significant at 1% level of significance; Df: Degree of freedom.

transmission of characters from parents to its progeny. The estimates of heritability help the plant breeder in selection of elite genotypes from diverse genetic population. High broad sense heritability was estimated for panicle length (99%), grain length (75%), plant height (67%), kernel elongation ratio (63%) and kernel elongation (60%) suggesting these traits are under high genetic control (table 4).

3.2 *Genotyping of the parents along with RIL population and construction of genetic map*

A total of 276 SSR markers were tested for polymorphism between parents and finally 70 markers were found polymorphic between parents Basmati 370 and Pusa Basmati 1121.All the 130 RILs were genotyped using 70 SSR markers and there was still some residual heterozygosity present in the RILs, probably due to insufficient number of self- pollination cycles at F_7 generation. SSR markers with normal segregation showed that on an average the RILs had achieved homozygosity for more than 98.95% of these SSR loci.70 SSR markers were uniformly distributed over all the 12 chromosomes and highest numbers of SSRs were observed on *Os*2. The genotyping data generated using 70 SSR markers on 130 RILs was used for construction of genetic linkage map. Chi-square test (x^2) was used to study the distortion among the markers. A genetic map was constructed with 44 SSR loci mapped in a length of 689.3 cM on 12 linkage groups. Highest number (11 SSRs) of loci were mapped on chromosome Os2 in a distance of 145.4 cM. Minimum two SSRs were mapped on each chromosome *Os*8, *Os*9, *Os*10, *Os*11 and *Os*12. Three SSR loci were mapped on each chromosome *Os*4, *Os*5, *Os*6 and *Os*7. On chromosome *Os*1 and *Os*3, 5 and 6 SSR loci were mapped. In such way a sparse genetic map was developed and used for QTL analysis (table 5; figure 3).

Table 5. Summary of SSR-based genetic map developed using RIL population (Basmati 370 × Pusa Basmati 1121)

Linkage group	Mapped loci	Map distance (cM)	Map density (cM/loci)
Os1	6	50.2	8.4
Os2	11	145.4	13.2
Os3	5	63.7	12.7
Os4	3	73.3	24.4
Os5	3	87.1	29.0
Os6	3	74.1	24.7
Os7	3	42.9	14.3
Os8	2	40.1	20.0
Os9	2	40.1	20.0
Os10	2	8.2	4.1
Os11	2	41.0	20.5
Os12	2	23.3	11.6
Total	44	689.4	15.6

Os: *Oryza sativa.*

3.3 *QTLs identified for grain quality and yield attributing traits*

The main focus of the present study was to identify QTLs for grain quality and yield-attributed traits using a stabilized RIL population.QTL analysis was performed using inclusive composite interval mapping additive (ICIM-ADD) method implemented in software ICIM (Meng *et al.* 2015). A total of 20 QTLs for grain

Table 4. Variability among 130 RILs of Basmati 370× Pusa Basmati 1121 based on individual and pooled data from 2017 and 2018 *kharif* seasons

			GCV %			PCV %				
Trait	Mean	Range	2017	2018	Pooled	2017	2018	Pooled	H^2 (%)	GAM (%)
AC	26.02	29.97	10.53	37.07	28.12	19.19	37.41	28.26	99	26.02
DF	102.91	10.67	0.94	3.09	1.51	3.26	5.41	3.19	22	109.92
DM	136.37	20.17	0.72	2.23	0.89	2.18	6.56	3.29	7	136.37
ET	7.96	7.17	3.94	26.9	9.5	11.02	42.28	27.8	11	7.96
KE	13.52	2.91	5.55	5.86	4.14	7.17	8.01	5.46	57	13.52
KER	1.76	0.54	7.47	6.79	4.82	9.43	10.46	6.99	47	1.76
PH	119.03	39.05	7.89	9.79	6.07	18.39	12.23	6.93	76	119.04
PL	25.05	9.28	9.15	7.97	6.35	9.18	11.99	7.67	68	25.05
GL	7.7	2	6.53	4.66	3.9	7.56	5.46	5.3	54	7.71

PH: Plant height; PL: Panicle length; ET: No. of effective tillers per plant; DF: Days to 50% flowering; DM: Days to maturity; AC: Amylose content; KE: Kernel elongation; KER: Kernel elongation ratio; GL: Grain length, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation H^2: Heritability, GAM: Genetic advance mean.

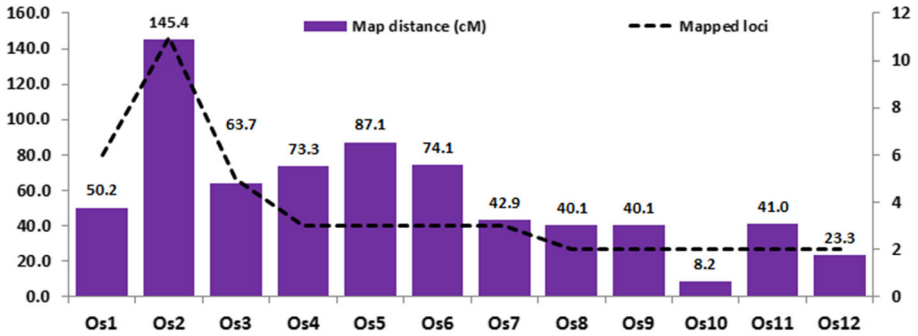

Figure 3. Summary of SSR-based genetic linkage map developed using RIL population Basmati 370 × Pusa Basmati 1121.

quality and yield-attributed traits in two seasons were identified using ICIM-ADD on 12 rice chromosomes.

3.4 *Major effect QTLs identified for yield-attributed traits*

Two QTLs, *qPH-Os1.1* and *qPH-Os1.2* were identified for plant height on *Os*1 chromosome explaining 15.2 and 13.6% phenotypic variance explained (PVE) respectively with a LOD 3.4 for both QTLs. Only one QTL (*qDM-Os2.1*) was found to be associated with days to maturity located on chromosome *Os*2 at 35 cM with 20.6% PVE and LOD 3.3 in year 2018. However, two QTLs were identified in season 2017, *qDM-Os2.2* and *qDM-Os2.3* on at 98 cM and 104 cM chromosome *Os*2 with 19.6 and 23.3% PVE and LOD of 8.7 and 5.8 respectively. One QTL (*qPL*) for panicle length was identified on chromosome *Os*7at 20.1 cM with 20.7% PVE and LOD score of 3.0 in 2018. Two QTLs (*qET-Os1.1* and *qET-Os1.2*) were detected for effective (productive) tillers; *qET-Os1.1* was identified on chromosome Os1 at 25.1 cM explaining 17.7% PVE with LOD 3.6. The other *qET-Os1.2* was also detected on chromosome *Os*1 with 26.9 % PVE and LOD of 4.7. In season 2017, two QTLs (*qET-Os6.3* and *qET-Os8.2*) identified on chromosome *Os*6 and *Os*8 explaining 12.6 and 29.6% PVE with LOD scores of 3.4 and 4.3, respectively (table 6; figure 4).

3.5 *Major effect QTLs identified for grain quality traits*

One QTL (*qKE-Os3.1*) was identified at 16 cM on chromosome *Os*3 with LOD of 5.6 explaining 47.6% PVE in season 2018. The other QTL (*qKE-Os3.2*) identified on chromosome Os*3* at a distance of 17.0 cM with LOD 6.2 explaining47.5 % PVE during season 2017. A consistent QTL for kernel elongation ratio (KER) was identified on chromosome *Os*5 at 21.2 cM explaining 15.6% PVE with LOD score of 4.2 in season 2017 and 2018. A major effect and consistent QTL for kernel elongation ratio (KER) was identified on chromosome *Os*5 at 61.2 cM explaining 15.6% PVE with LOD 4.2 in seasons 2017 and 2018.Three QTLs for amylose content (AC) were identified among them two consistent QTLs (*qAC-Os5.1* and *qAC-Os5.3*) were located on chromosome *Os*5 explaining 52.6 and 30.3% PVE with LOD scores 7.1 and 4.8 respectively. The third QTL (*qAC-Os8.2*) was identified on chromosome Os8 with LOD 4.3 and 33.0% PVE. The QTL on chromosome *Os*5 were identified as consistent QTLs in both seasons (table 6; figure 4).

Major epistatic QTLs were identified for effective tillers (ET) and amylose content (AC). Single epistatic QTL for ET was identified between the loci on chromosome 3 and 5 with 5.4 LOD and 62.0 PVE. Three epistatic QTLs were identified for amylose content, of these two major epistatic interactions were identified between chromosome 4 and 5 with LOD of 5.2 and 5.1 and 77.8 and 65.7% PVE respectively. The third epistatic QTL was identified between chromosome 4 and 6 with LOD of 5.0 and 60.8% PVE (table 7; figure 5).

4. Discussion

Basmati rice (*Oryza sativa* L.) is characterized by extra-long slender grains, distinct aroma and light texture (Nagaraju *et al.* 2002). Kernel length, L/B ratio and linear elongation of the kernel on cooking play a pivotal role in consumer preference as well as the commercial success of a variety, especially in quality rice. Therefore, improvements of these characters are of paramount importance in any breeding program. For commercial purposes, the grains of rice are classified according to the kernel length as short grain, medium grain, long grain and extra-long grain. There are very few investigations on inheritance of kernel length and

Table 6. List of QTLs associated with kernel elongation and various grain quality and yield-attributed traits identified during the year 2017–2018

Trait	Season	QTLs	Chr	Position (cM)	Left Marker	Right Marker	LOD	PVE (%)	Add
AC	2018	*qAC-Os5.1*	*Os5*	62	RM121	RM1232	7.1	52.6	−6.90
	2018	*qAC-Os8.2*	*Os8*	8	RM26063	RM3286	5.3	33.0	−4.90
	2018	*qAC-Os5.3*	*Os5*	16	RM5488_1	RM121	4.8	30.3	−5.20
	2017	*qAC-Os5.4*	*Os5*	62	RM121	RM1232	6.3	54.5	−6.20
	2017	*qAC- Os5.5*	*Os5*	17	RM5488_1	RM121	4.3	27.5	−4.40
DF	2017	*qDF-Os7.1*	*Os7*	7	RM3644	RM85	4.4	23.3	−1.20
DM	2018	*qDM-Os2.1*	*Os2*	35	RM249	RM289	3.3	20.6	−2.50
	2017	*qDM- Os2.2*	*Os2*	98	RM1328	RM7300	5.7	19.6	−1.10
	2017	*qDM- Os2.3*	*Os2*	104	RM7300	RM240	5.8	23.3	−1.10
ET	2018	*qET-Os1.1*	*Os1*	41	RM18A	RM286	3.6	17.7	−0.90
	2018	*qET- Os1.2*	*Os1*	46	RM286	RM6880	4.7	26.9	−1.10
	2017	*qET- Os6.3*	*Os6*	72	RM178	RM5488	3.4	12.6	−0.70
	2017	*qET- Os8.4*	*Os8*	6	RM26063	RM3286	4.3	29.1	0.90
KE	2018	*qKE-Os3.1*	*Os3*	16	RM1812	RM332	5.6	47.6	0.70
	2017	*qKE- Os3.2*	*Os3*	17	RM1812	RM332	6.2	47.5	0.70
KER	2018	*qKER-Os5.1*	*Os5*	62	RM121	RM1232	4.2	15.7	0.04
	2017	*qKER- Os5.2*	*Os5*	61	RM121	RM1232	5.2	15.6	0.03
PH	2018	*qPH- Os1.1*	*Os1*	42	RM18A	RM286	3.4	15.2	−5.00
	2018	*qPH- Os1.2*	*Os1*	45	RM286	RM6880	3.4	13.6	−4.70
PL	2018	*qPL-Os7.1*	*Os7*	35	RM85	RM545	3.0	20.7	−1.20

PH: Plant height; PL: Panicle length; ET: No. of effective tillers per plant; DF: Days to 50% flowering; DM: Days to maturity; AC: Amylose content; KE: Kernel elongation; KER: Kernel elongation ratio; LOD: Logarithm of Odds; PVE: Phenotypic variance explained; Add: Additive effect.

kernel elongation in Basmati rice. Kernel length and kernel elongation in rice are known to be genetically controlled (Bhattacharya 2011).The kernel elongation after cooking is one of the unique appearance quality traits of Basmati rice, known to be a complexly inherited quantitative trait governed by numerous genetic factors with small effects regarded as quantitative trait loci (QTL). Deborah *et al*. (2017) demonstrated that the combination of linkage and association mapping analysis can enhance the mapping efficiency and accuracy. The present study was undertaken with the objective of identifying QTLs for grain quality, agronomical and yield-attributed traits in Basmati rice. We have identified 20 QTLs governing economically important traits of Basmati rice employing Recombinant Inbred Line (RIL) mapping population derived from a cross between Basmati 370 and Pusa Basmati 1121.

In the past, several genes/QTLs governing quality traits were identified in indica and japonica sub-species of *Oryza sativa*. The major genes related to quality traits includes waxy gene for amylose content (AC) (Isshiki *et al*. 1998). Vemireddy *et al*. (2007) identified QTL for grain length after cooking (GLAC) on chromosome 12. Although the grain length after cooking is one of the unique quality traits of the Basmati rice, the genomic regions governing the trait are not yet identified. In non-Basmati rice, however, scattered reports of mapping QTL regions for this trait are available. Among them, initially, a QTL on chromosome 8 associated with cooked kernel elongation has been identified and concluded that this QTL was loosely linked to the fragrance gene (Ahn *et al*. 1993; Faruq and Zakaria 2013). Subsequently, three QTLs on chromosomes 2, 6 and 11 (Ge *et al*. 2005) and a single QTL on chromosome 3 (Li *et al*. 2004) and two QTLs each on chromosomes 2 and 6 (Tian *et al*. 2006) have been identified for this trait. Ahn *et al*. (1993) reported that one major QTL is located on chromosome 8. The evolution of chromosome number 8 in rice is of particular interest because genes for both aroma and GE have been mapped on to this chromosome (Jain *et al*. 2006). However, no QTL for GE was detected on chromosome 8 in the present study, even though the chromosome is fairly dense with evenly distributed markers. We have identified one QTL each for kernel elongation (KE), q*KE*-1 and q*KE*-2 on chromosome 3 during the year 2017 and 2018. However, previously, QTL for grain length after cooking (GLAC) on chromosome 12 was identified (Vemireddy *et al*. 2007) (supplementary table 2).

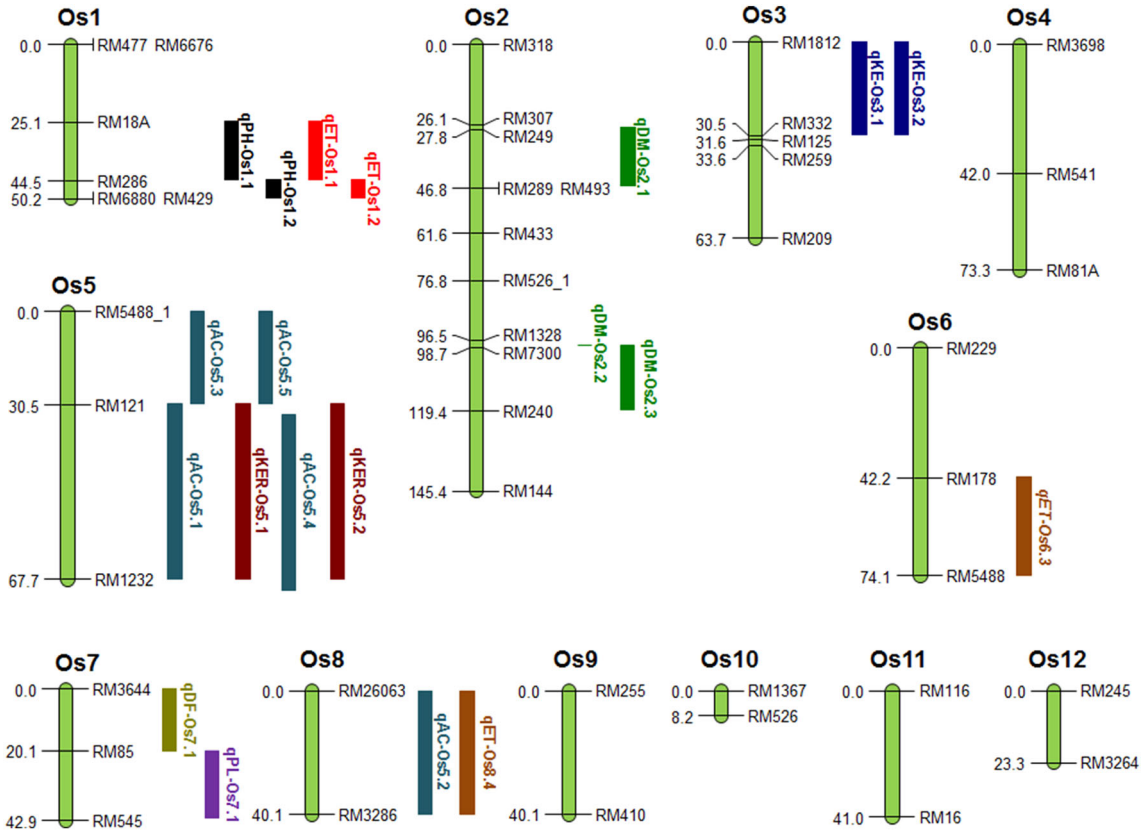

Figure 4. QTLs identified for grain quality traits with their positions (cM) and flanking markers on rice chromosomes. PH: Plant height; PL: panicle length; ET: No. of effective tillers per plant; GPP: Grains per panicle; GW: Grain weight; YPP: yield per plant; SF: Spikelet fertility; GL: Grain length; GB: Grain breadth; LBR: Length breadth ratio; DF: Days to 50% flowering; DM: Days to maturity; AC: Amylose content; KE: Kernel elongation; KER: Kernel elongation ratio; LOD: Logarithm of Odds; PVE: Phenotypic variance explained; Add: Additive effect.

Long slender grain is a defining characteristic of Basmati rice varieties. Based on the official notification of standards issued by the Ministry of Commerce, Government of India the minimum GL for Agrade Basmati rice is 7.0 mm. Pusa Basmati 1121 possesses extra-long slender milled grains (9.00 mm), while Basmati 370, the short-grain Basmati parent of the RILs, had comparatively shorter grains of 6.64 mm. The kernel elongation in the RILs ranged from 12.30 to 15.21 mm with a population mean of 13.52 mm. The two parental lines Basmati 370 and Pusa Basmati 1121 were quite different in their grain length and kernel elongation. Genotypes exhibited significant differences in mean performance for kernel elongation with an overall pooled mean of 13.40±0.89 mm. The smallest and the longest kernel elongation was found to be 11.53 mm and 15.62 mm, respectively in the year 2017. When analyzing their pooled values it has been found that the RIL81 had shortest kernel elongation (12.30 mm) on an average while RIL44 had the longest kernel elongation (15.21 mm). Between the parents, Basmati 370 had higher amylose content (23.56%), whereas the amylose content of Pusa Basmati 1121 was lower (19.21%) during the year 2017. From the pooled value it has been observed that the RIL103 had 24.5 per cent amylose content which was the highest among all the RILs. We identified one QTL each for kernel elongation ratio (KER), qKER-1 and qKER-2 on chromosome 5 during the year 2017 and 2018. Similar findings have been obtained by Vemireddy *et al.* (2007), where they identified a QTL for ER, elr11-1 on chromosome 11. Likewise, three more QTLs have been identified on chromosomes2, 4 and 12 with major QTL being qER-2 (Liu *et al* 2008). Cheng *et al*. 2014 identified Two QTLs for GE on chromosome 2, designated *qGE-2-1* and *qGE-2-2*. The *qGE-2-1* mapped to the interval RM53-RM174, with a LOD score of 3.80, and explained 23% of the phenotypic variance, whereas *qGE-2-2* was mapped to the marker interval RM525-RM6, with a LOD score of 3.04 and explained 10% of the phenotypic variance. One QTL for GE was previously reported on chromosome 2 in the marker

Table 7. List of epistatic QTLs identified for kernel elongation and various grain quality and yield-attributed traits

Trait	Chr1	Pos1	Left Marker1	Right Marker1	Chr2	Pos2	Left Marker2	Right Marker2	LOD	PVE (%)	Add1	Add2	AddbyAdd
ET_2018	3	45	RM259	RM209	5	60	RM121	RM1232	5.3	62.0	−0.3	0.5	−1.1
AC_2017	4	60	RM541	RM81A	5	45	RM121	RM1232	5.2	77.8	1.7	−6.5	2.6
AC_2017	4	50	RM541	RM81A	5	10	RM5488_1	RM121	5.1	65.7	2.5	−4.7	3.6
AC_2017	4	20	RM3698	RM541	6	60	RM178	RM5488	5.0	60.8	1.7	−2.8	5.7

ET: No. of effective tillers per plant; AC: Amylose content.

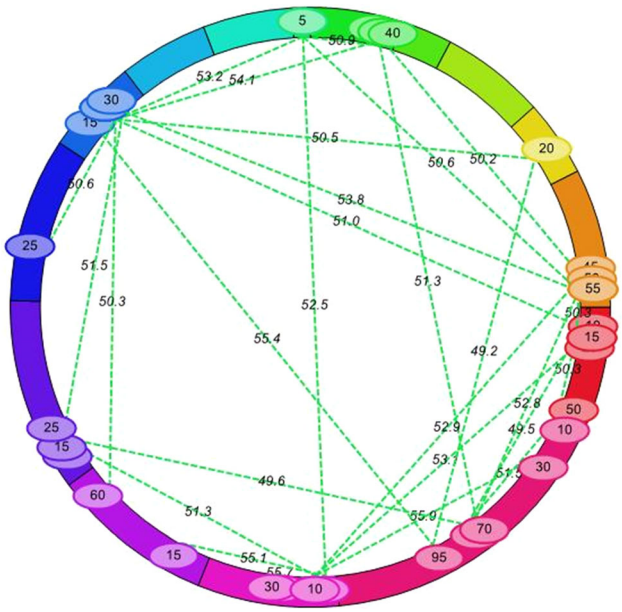

Figure 5. Epistatic interactions (Q × Q) identified for grain quality traits. The different colors on the circumference indicate 12 rice chromosomes and green links inside the circle are the interactions between the difference genomic regions controlling rice grain quality traits.

interval R2510-RM211 in the study conducted by Ge *et al*. (2005).

Previous reports suggested that the SSIIa is one of the important biosynthetic enzymes determining starch structure and its properties The SSIIa enzyme seems to have a role in the elongation of A and B1 amylopectin chains, and determines the ratio of two chain lengths, i.e., L-type (present in indicarices) and S-type (present in japonica rice) (Bradbury *et al*. 2005a; Nakamura 2002). However, in Basmati rice, being a separate group from indica and japonica rice, it would be interesting to understand the role as well as the structure of SSIIa. In the present study, we identified five QTLs for amylose content, qAC−1, qAC−2qAC−3and qAC−1qAC−2on chromosome 5 and 8 during the years 2017 and 2018 respectively. Although different amylose classes, viz., waxy (∼0 %), low (2–19 %), intermediate (20–25 %) and high (>25 %) are known to be associated with the variability in the waxy gene which encodes granule- bound starch synthase GBSSI) on chromosome 6, the waxy gene alone could not explain the global phenotypic variability of the trait due to the availability of subclasses within each major class prompting us to speculate the existence of the loci other. Vemireddy *et al.* (2007) identified one QTL for amylose content, qAC4.1 on chromosome 4.The QTLs identified in the present

study could possibly be used for further fine mapping and identification of the specific genes to develop functional markers for these traits. The findings in this study indicate that further analysis is required to exemplify the complete genetic basis of the large variation among the three studied traits in nature.

Estimates of heritability expressed as per cent of mean was high for most of the morphological characters whereas the genetic advance expressed as a per cent of mean showed mixed values among these genotypes. Panse (1957) stressed the importance of heritability in addition to mean performance and variability. The present study revealed moderate heritability for most of the morphological characters, viz. days to 50 per cent flowering, days to maturity, panicle length, number of effective tillers, kernel elongation, and kernel elongation ratio. However, genetic advance was reported to be low for days to 50% flowering, days to maturity, number of effective tillers, kernel elongation, kernel elongation ratio and moderate for panicle length. These findings are in accordance to early reports of different workers in rice breeding (Ashfaq *et al*. 2012; Dutta *et al*. 2013; Kumar *et al*. 2013; Luo *et al*. 2013; Qiang-ming *et al*. 2013; Singh *et al*. 2013).

5. Summary

The main focus of this study was to utilize the QTL-mapping approach to identify QTLs for various grain quality and yield-attributed traits in an F_7 population derived from the two parents (Basmati 370 and Pusa Basmati 1121) differing in grain elongation and other grain quality traits. The RIL population was grown in replicated trails at the farm of school of biotechnology, SKUAST-J, Jammu. The parents and the population were evaluated for mapping of QTLs associated to various agronomical traits. Analysis of variance showed significant differences in the parents for almost all the traits. For mapping of QTL's, parental polymorphism survey was done using 276 SSR rice markers spanning all the 12 linkage groups. Out of 276 primer pairs, 70 primers showed polymorphism between the two parents in 3.0% agarose gel. A set of 130 RILs, were analyzed for 70 polymorphic SSR markers and linkage map generated with 44 SSR markers, rest being unlinked. Among all the 70 SSR markers, three markers for plant height, two markers for panicle length, 3 markers for effective tillers per plant, two markers for days to maturity, five markers for amylose content, two markers for kernel elongation and two markers for kernel elongation ratio which showed significant association in all the analysis carried out using data taken under observation. For each trait, minimum LOD scores of 3.0 were used for the identification of putative QTL. One QTL for kernel elongation on chromosome 3, one QTL for kernel elongation ratio on chromosome 5, two QTLs for plant height on chromosome 1, one QTL for panicle length on chromosome 1, two QTLs for effective tillers per plant on chromosome 1, one QTL for days to maturity on chromosome 2, three QTLs for amylose content on chromosome 5 and 8. Total 20 QTLs were identified for various agronomical traits.

6. Conclusion

Grain quality and yield-attributed traits are vital for commercial rice production, and they also affect the dietary value of the grain. Huge variation ingrain quality traits in RIL population developed from Pusa Basmati 1121 and Basmati 370 was observed in the current study. Through ICIM-ADD methods, a total of twenty QTLs for 9 traits, namely, PH, ET, DM, AC, KER, PL, DF and KE, were identified on 6 chromosomes of rice. By comparison of the comprehensive literature survey and publically accessible Gramene database, 20 polymorphic markers were significantly associated with traits related to PH, ET, DM, AC, KER, PL, DF and KE. The 4 trait-associated QTLs are novel on chromosomes *Os1* and *Os5*. Therefore, these QTLs and validated SSR polymorphic markers could be used in future marker-assisted breeding program for improving grain quality traits in rice.

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