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Identification of QTLs for Yield and Associated Traits in F$_2$ Population of Rice

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ABSTRACT

Identification of quantitative trait loci (QTLs) controlling yield and yield-related traits in rice was performed in the F$_2$ mapping population derived from parental rice genotypes DHMAS and K343. A total of 30 QTLs governing nine different traits were identified using the composite interval mapping (CIM) method. Four QTLs were mapped for number of tillers per plant on chromosomes 1 (2 QTLs), 2 and 3; three QTLs for panicle number per plant on chromosomes 1 (2 QTLs) and 3; four QTLs for plant height on chromosomes 2, 4, 5 and 6; one QTL for spikelet density on chromosome 5; four QTLs for spikelet fertility percentage (SFP) on chromosomes 2, 3 and 5 (2 QTLs); two QTLs for grain length on chromosomes 1 and 8; three QTLs for grain width on chromosomes1, 3 and 8; three QTLs for 1000-grain weight (TGW) on chromosomes 1, 4 and 8 and six QTLs for yield per plant (YPP) on chromosomes 2 (3 QTLs), 4, 6 and 8. Most of the QTLs were detected on chromosome 2, so further studies on chromosome 2 could help unlock some new chapters of QTL for this cross of rice variety. Identified QTLs elucidating high phenotypic variance can be used for marker-assisted selection (MAS) breeding. Further, the exploitation of information regarding molecular markers tightly linked to QTLs governing these traits will facilitate future crop improvement strategies in rice.

KEYWORDS

Rice; QTL mapping; linkage map; SSR markers; composite interval mapping; marker-assisted selection

1 Introduction

Rice (*Oryza sativa* L.) belonging to the family Poaceae [1] is an edible starchy cereal grain. Around half of the world's population, comprising most of East and Southeast Asia, is wholly reliant on rice as a staple food crop, where 95 percent of the rice crop throughout the globe is consumed by humans. Most of the population residing in the developing countries in Asia predominantly depends on rice for the daily requirement of calories. To fulfill the demand by the vigorously growing population in Asia, significant efforts towards the development of high-yielding varieties are required. Conventional methods of crop improvement will fall short to meet the needs of the growing population, therefore advanced approaches like marker-assisted breeding (MAB) look promising to fulfill the gaps. For the efficient utilization of genetic resources through MAB, information about the genomic loci governing the trait is important. The mapping of Mendelian traits is relatively simpler than the complex quantitative traits like yield and yield-related traits. In this regard, mapping approaches like QTL mapping have proven to be efficient in identifying genomic intervals associated with the trait. Enormous genomic information is available for the rice crop allowing researchers to find genes and quantitative trait loci (QTLs) linked to a variety of agronomic, physiological, and morphological aspects. The QTL mapping approaches have been explored in rice to enhance the overall yield stability and potential of rice cultivars in diverse environments [2]. Efficient molecular marker genotyping and precise phenotyping are the two most important factors deciding the effectiveness of the QTL mapping. The advent and convenience of simple sequence repeats (SSRs) have made it possible for rice breeders to perform genotyping with over hundreds of markers covering the entire genome [3].

Yield is a highly complex trait having a high level of environmental effect and is also governed by many small-effect genes [4]. As compared to the yield, yield-related variable traits such as grain size, grains per panicle, thousand-grain weight, and panicle number per plant are relatively easy to study. Understanding of yield components also helps to better design high yielding rice varieties with ideal architecture [5]. Several QTL mappings studies have been performed to identify loci governing yield component traits. However, the genomic location of the identified QTLs depends on the allelic variation that existed in the parental lines. Grain yield, which is a major breeding objective, is controlled by a variety of variables. Seed-setting rate and grain weight are usually the main elements affecting rice grain production, whereas plant height and heading date have an indirect effect. Hundreds of yield-related QTLs have been found in rice so far, however, the assessment and use of yield-positive alleles are still limited [6]. Furthermore, more research into elite genetic resources from a wider spectrum is required. Considering this fact numerous studies have been performed for the same trait but using mapping populations developed using different parental lines.

The F_2 population has been utilized in several studies to uncover QTLs with additive and dominant effects across the whole rice genome, as well as to examine the genetic component of rice yield and its components. EP3, APO1, DEP2/EP2, DEP3, SRS3, and GIF1 are a few of the genes that have been successfully recognized and isolated by exploiting F_2 populations. In addition, several genes regulating important traits have been cloned through the QTL mapping. Recent reports signify that 20 QTLs that significantly influence paddy grain yield and its other elements, have been successfully replicated with NIL-F_2 generation, and 14 additional grain yield QTLs have been confirmed in NILs [7].

Considering the global significance of rice yield, the current research was conducted to identify QTLs for yield and yield-related traits. Total rice yield is majorly determined by various traits such as plant height (PH), number of tillers per plant (TPP), panicle number per plant (PPP), spikelet fertility percentage (SFP), spikelet density (SD), grain width (GW), grain length (GL), yield per plant (YPP) and 1000-grain weight (TGW). In the F_2 population derived from the two parental lines, K343 and DHMAS, 30 QTLs were discovered. Furthermore, SSR markers were also used to create a linkage map for all yield-related traits. The molecular markers linked with definitive QTL which are identified for different traits in the present

study can be useful for MAS. Chromosomal regions carrying important QTLs identified during the current investigation can be enriched with more markers for high-resolution mapping and reducing genetic background noise. Information about molecular markers tightly linked to the QTLs that control yield traits should greatly accelerate future rice breeding through the rapid generation of improved lines with desired trait improvement in any elite background.

2 Materials and Methods

Over a two-year period (2015–16 and 2016–17), the current study was conducted at the Research Farm and the Molecular Laboratory of Rice, School of Biotechnology, SKUAST-Jammu, India.

2.1 Plant Material and Cultivation

An F_2 population was created by crossing several rice lines K343 and DHMAS. K343 (a major non-basmati rice cultivar having medium height plant with medium/short & bold grains) is a popular rice cultivar and a high yielding variety of hill region of J&K. It usually takes 100 to 130 days to mature, with an average yield of 5.5–6.0 t/ha. The second parent was DHMAS, a long-grain variety developed at IRRI and CSK HPKV Palampur (HP) using doubled haploid marker-assisted selection (MAS). DHMAS usually takes 120 to 140 days to reach full maturity, because DHMAS is tall, it lodges in heavy soils, resulting in a poor yield. Hence, the cross of these contrasting traits was focused upon and used as a potent technique for detecting novel QTLs for this diverse cultivar combination. At Experimental Farm, parental lines and F_2 progenies were sown and transplanted in an augmented design-I (un-replicated design) during *Kharif* 2016. Rice seedlings were transplanted in five-meter-long rows with a planting density of 20 × 20 cm. For crop production, the recommended package and techniques were implemented. A total of 233 plants were saved and employed in genotypic and phenotypic assessment tests.

2.2 Phenotyping

All the observations were taken from a single plant in each row of the plot, which was tagged assigning a specific number to them. The variation of the yield and its component characters were evaluated using a one-way ANOVA, which revealed significance at the 5% level of significance. Pearson's correlation analysis with SPSS was used to obtain the Pearson's correlation coefficient (PCC) between the attributes (version 20). The parents and F_2 mapping population were examined, and data for phonological, morphological, yield and its component traits were recorded for various variables as shown in Table 1 using the DUS guidelines of DRR, Hyderabad [8].

To establish the heritable potency of the relevant genes as well as the influence of the environment on them, genetic variability components such as heritability in the broad sense (H^2), phenotypic and genotypic coefficients of variation (PCV and GCV), and genetic advance (GA) were calculated.

2.3 Genotyping

To evaluate the genotype of the F_2 population, the modified Doyle and Doyle, method [9,10] was used to carry out genomic DNA isolation. Using agarose gel electrophoresis and the Nanodrop device (spectrophotometer Nanodrop 2000), the quality and amount of genomic DNA were determined. DNA concentration was calculated by correlating the intensity of genomic DNA bands with that of known recognized standards and optical density (OD) of samples that were measured at 260 and 280 nm utilizing Peq-Lab Nanodrop. To dilute samples of DNA to a final concentration of 50 ng/L, Milli Q water was employed.

Table 1: Descriptive data for several agronomic parameters examined in an F_2 population resulting from a hybrid between K343 and DHMA rice genotypes

SL. No.	Traits	Mean ± SD	Range		CD value		CV value
			Max.	Min.	Test	Check	Value
1.	Plant Height (cm)	27.93 ± 2.79	36.00	24.00	8.11	3.82	9.99
2.	Days to Flowering	83.90 ± 7.08	91.00	65.00	8.85	4.17	8.43
3.	No. of Tillers/Plant	19.20 ± 2.64	28.00	15.00	1.44	0.68	13.73
4.	Duration of Grain Filling	111.83 ± 6.94	126.00	94.00	16.30	7.68	6.20
5.	Panicle No./Plant	19.18 ± 2.62	28.00	15.00	1.44	0.68	13.66
6.	Days to Maturity	126.61 ± 10.78	140.90	100	3.32	1.56	8.51
7.	Spikelet Fertility Percentage	86.80 ± 7.82	100.00	56.47	12.29	5.79	9.01
8.	Spikelet Density	5.07 ± 0.83	8.18	3.61	1.03	0.48	16.42
9.	Grain Width (mm)	2.35 ± 0.15	2.70	1.97	0.092	0.043	6.62
10.	Grain Length (mm)	4.77 ± 0.48	7.08	3.92	0.52	0.24	10.05
11.	Yield/Plant (g)	37.57 ± 5.90	50.12	29.07	0.90	0.42	15.70
12.	1000 Grain Weight	25.07 ± 2.12	31.71	20.11	1.09	0.51	8.48

2.4 SSR Genotyping and PCR Profile

A set of 450 SSRs covering the entire rice genome were selected. Double distilled, autoclaved and deionized water was used to dilute primers at a concentration of 10 pmol. To study polymorphism among F_2 population genotypes generated from K343 and DHMAS combination, 96 well thermal-cycler was used to carry out PCR amplification subjected to the thermal profile. The thermo-cycler was configured with a 5-min. denaturation stage, followed by 35 cycles of denaturation (at 94°C for 30 s), annealing (at 55°C for 30 s), and extension (at 72°C for 30 s). The last extension was done for 7 min at 72°C, after that, the PCR products were kept at 4°C. However, the identical reaction mixture lacking genomic DNA was used for each reaction, as a negative control. The SSR markers which showed parental polymorphism were used for genotyping. Based on polymorphism, out of 450 SSR markers, 53 markers were utilized for the PCR amplification of the genomic DNA of the 233 F_2 mapping population (Table 2). The fragment size (bp) matching the 100 bp molecular weight marker was used to score observable and reproducible alleles amplified for each SSR. PCR products produced in the anticipated size range of SSRs were used to score alleles for each SSR locus. When a PCR result failed to amplify, the data for the relevant genotype was considered null allele.

2.5 Genetic Linkage Map Construction

MAPMAKER/Exp v. 3.0-based program was used to compute the genetic linkage order and genetic distances of the 53 marker loci [11]. The electrophoresis findings were converted into scoreable data, and computer-based running software was used in accord with the instructions. GROUP command and LOD 3.0 were used to do linkage analysis and grouping and the rate of recombination was set to 50 cM. However, when the number of connected markers was lower than 8, COMPARE command was used to optimize them. The error detection rate was set at 1%, with triple error detection enabled. The "Kosambi" map program was used to translate the recombination rate into a genetic map (distance, cM). To select the relevant linkage groups, the found SSR markers [12] were used as anchor tags (Fig. 1).

**Table 2:** The F_2 population was genotyped using a selection of SSR markers [1]

S. No.	Marker	Sequence	T_m	Product size
1.	RM216	5' GCATGGCCGATGGTAAAG 3'		
		3' TGTATAAAACCACACGGCCA 5'	55	146
2.	RM13838	5' CCCAACTGCTAGGTTTCTGATCC 3'		
		3' ACTGTGTTACTGTGTGCCGTTGC 5'	55	129
3.	RM262	5' CATTCCGTCTCGGCTCAACT 3'		
		3' CAGAGCAAGGTGGCTTGC 5'	55	154
4.	RM5	5' TGCAACTTCTAGCTGCTCGA 3'		
		3' GCATCCGATCTTGATGGG 5'	55	113
5.	RM528	5' GGCATCCAATTTTACCCCTC 3'		
		3' CCGTAGGTTAAAATGGGGAC 5'	55	232
6.	RM6832	5' GTTGTAAATGCCTGAGTGC 3'		
		3' AAAGAGCTAAACCGCTAGG 5'	55	182
7.	RM223	5' GAGTGAGCTTGGGCTGAAAC 3'		
		3' GAAGGCAAGTCTTGGCACTG5'	55	165
8.	RM4A	5' TTGACGAGGTCAGCACTGAC 3'		
		3' AGGGTGTATCCGACTCATCG 5'	55	159
9.	RM7492	5' AGATGGTTGCCAAGAGCATG 3'		
		3' GTCACGTGGCGATTTAGGAG 5'	55	145
10.	RM517	5' GGCTTACTGGCTTCGATTTG 3'		
		3' CGTCTCCTTTGGTTAGTGCC 5'	55	266
11.	RM580	5' GATGAACTCGAATTTGCATCC 3'		
		3' CACTCCCATGTTTGGCTCC 5'	55	221
12.	RM5699	5' ATCGTTTCGCATATGTTT 3'		
		3' ATCGGTAAAAGATGAGCC 5'	55	167
13.	RM447	5' CCCTTGTGCTGTCTCCTCTC 3'		
		3' ACGGGCTTCTTCTCCTTCTC 5'	55	111
14.	RM471	5' ACGCACAAGCAGATGATGAG 3'		
		3' GGGAGAAGACGAATGTTTGC 5'	55	106
15.	RM202	5' CAGATTGGAGATGAAGTCCTCC 3'		
		3' CCAGCAAGCATGTCAATGTA 5'	55	189
16.	RM413	5' GGCGATTCTTGGATGAAGAG 3'		
		3' TCCCCACCAATCTTGTCTTC 5'	55	79
17.	RM169	5' TGGCTGGCTCCGTGGGTAGCTG 3'		
		3' TCCCGTTGCCGTTCATCCCTCC 5'	55	169
18.	RM80	5' TTGAAGGCGCTGAAGGAG 3'		
		3' CATCAACCTCGTCTTCACCG 5'	55	142

(Continued)

Table 2 (continued)

S. No.	Marker	Sequence	T_m	Product size
19.	RM101	5' GTGAATGGTCAAGTGACTTAGGTGGC 3'		
		3' ACACAACATGTTCCCTCCCATGC 5'	55	324
20.	RM13840	5' CGGTCTTTAGTAATGGTGCTTTGC 3'		
		3' GAGGCAGGTGTTTGTCGTCTAGC 5'	55	195
21.	RM25003	5' GATTGATCCGAGAGACAAATCC 3'		
		3' TCGATCAATAGTAGCAGCAGTAGG 5'	55	115
22.	RM3295	5' TCGTGTCATGCGATCGAC 3'		
		3' GCTTCGACTCGACCAAGATC 5'	55	92
23.	RM7	5' TTCGCCATGAAGTCTCTCG 3'		
		3' CCTCCCATCATTTCGTTGTT 5'	55	180
24.	RM208	5' TCTGCAAGCCTTGTCTGATG 3'		
		3' TAAGTCGATCATTGTGTGGACC 5'	55	173
25.	RM7102	5' TTGAGAGCGTTTTTAGGATG 3'		
		3' TCGGTTTACTTGGTTACTCG 5'	55	169
26.	RM149	5' GCTGACCAACGAACCTAGGCCG 3'		
		3' GTTGGAAGCCTTTCCTCGTAACACG 5'	55	233
27.	RM240	5' CCTTAATGGGTAGTGTGCAC 3'		
		3' TGTAACCATTCCTTCCATCC 5'	55	132
28.	RM1370	5' AAACGAGAACCAACCGACAC 3'		
		3' GGAGGGAGGAATGGGTACAC 5'	55	173
29.	RM3874	5' TGGGTGATCTTAGTTTGGCC 3'		
		3' AATGTGCCTGCACATGTCAC 5'	55	206
30.	RM232	5' CCGGTATCCTTCGATATTGC 3'		
		3' CCGACTTTTCCTCCTGACG 5'	55	158
31.	RM28048	5' TTCAGCCGATCCATTCAATTCC 3'		
		3' GCTATTGGCCGGAAAGTAGTTAGC 5'	55	93
32.	RM3	5' ACACTGTAGCGGCCACTG 3'		
		3' CCTCCACTGCTCCACATCTT 5'	55	145
33.	RM220	5' GGAAGGTAACTGTTTCCAAC 3'		
		3' GAAATGCTTCCCACATGTCT 5'	55	127
34.	RM110	5' TCGAAGCCATCCACCAACGAAG 3'		
		3' TCCGTACGCCGACGAGGTCGAG 5'	55	156
35.	RM204	5' GTGACTGACTTGGTCATAGGG 3'		
		3' GCTAGCCATGCTCTCGTACC 5'	55	169
36.	RM324	5' CTGATTCCACACACTTGTGC 3'		
		3' GATTCCACGTCAGGATCTTC 5'	55	175

(Continued)

Table 2 (continued)

S. No.	Marker	Sequence	T_m	Product size
37.	RM1211	5' TACAGTGGCGAAAGGAATAC 3'		
		3' CCATCACGCATGTTAGTTAG 5'	55	213
38.	RM218	5' TGGTCAAACCAAGGTCCTTC 3'		
		3' GACATACATTCTACCCCCGG 5'	55	148
39.	RM242	5' GGCCAACGTGTGTATGTCTC 3'		
		3' TATATGCCAAGACGGATGGG 5'	55	225
40.	RM167	5' GATCCAGCGTGAGGAACACGT 3'		
		3' AGTCCGACCACAAGGTGCGTTGTC 5'	55	128
41.	RM219	5' CGTCGGATGATGTAAAGCCT 3'		
		3' CATATCGGCATTCGCCTG 5'	55	202
42.	RM144	5' TGCCCTGGCGCAAATTTGATCC 3'		
		3'GCTAGAGGAGATCAGATGGTAGTGCATG 5'	55	237
43.	RM225	5' TGCCCATATGGTCTGGATG 3'		
		3' GAAAGTGGATCAGGAAGGC 5'	55	140
44.	RM227	5' ACCTTTCGTCATAAAGACGAG 3'		
		3' GATTGGAGAGAAAAGAAGCC 5'	57	106
45.	RM15838	5' CGATGTCATTCGGTAGAAACAAGC 3'		
		3' CCTAGTCAAGGCATGGTCAATCC 5'	57	262
46.	RM3524	5' CGGAGCTGGTCTAGCCATC 3'		
		3' GTCTCCGTCTTCCTCACTCG 5'	57	129
47.	RM1282	5' AAGCATGACAGCTGCAAGAC 3'		
		3' GGGGATGAAGGGTAATTTCG 5'	58	157
48.	RM7300	5' TCCGTATCCTAGTCGCGATC 3'		
		3' CGCCGTCATGACTCATACTC 5'	58	102
49.	RM168	3' TGCTGCTTGCCTGCTTCCTTT 3'		
		5' GAAACGAATCAATCCACGGC 5'	58	116
50.	RM231	5' CCAGATTATTTCCTGAGGTC 3'		
		3' CACTTGCATAGTTCTGCATTG 5'	58	182
51.	RM545	5' CAATGGCAGAGACCCAAAAG 3'		
		3' CTGGCATGTAACGACAGTGG 5'	58	226
52.	RM1178	5' CAGTGGGCGAGCATAGGAG 3'		
		3' ATCCTTTTCTCCCTCTCTCG 5'	58	112
53.	RM315	5' GAGGTACTTCCTCCGTTTCAC 3'		
		3' AGTCAGCTCACTGTGCAGTG 5'	58	133
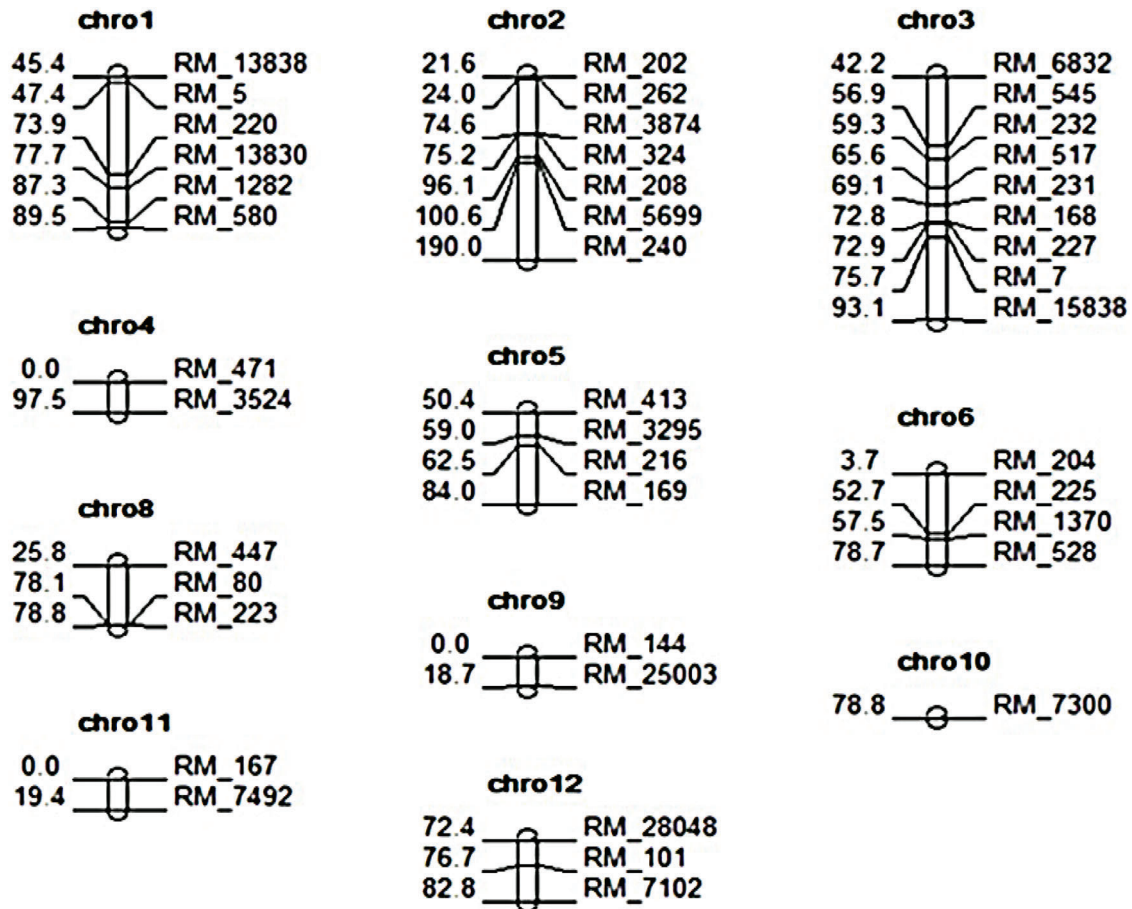
Figure 1: Genetic linkage map showing the position of polymorphic markers in rice chromosomes using MAPMAKER/EXP 3.0 program

2.6 QTL Identification

QTLs associated to yield and its component traits were detected and identified using Statistical software viz., QTLCARTOGRAPHER version 2.5 [13]. The number of genotypes, phenotypic values, completely constructed linkage map, and the yield & yield-related trait QTLs were evaluated using composite interval mapping (CIM). In multiple studies for each variable trait, minimum LOD values of 2.5/3.0 were employed to identify putative QTL. The threshold LOD scores for QTL identification were computed for each experiment using 1000 permutations at $P \leq 0.05$ [14]. When the significance level was set at 0.05, and the walk speed was set at 2 cM, the results were displayed in the appropriate genetic linkage map. LOD scores below the threshold for QTL LOD were only treated as informative QTL, whereas those with LOD values over a certain limit were regarded as definitive QTL. The putative sites of the QTL were obtained from the LR peak points in the linkage map. For each trait, the additive effect and the percentage of phenotypic variation (PVE%) were calculated. The detected QTLs were given names according to McCouch's naming system [15].

3 Results

3.1 Linkage Map Construction

Both K343 and DMAS have been genotyped using SSR molecular markers. The alleles present at two different positions in both the parental genotypes were scored as polymorphic. Table 2 lists the polymorphic

53 SSRs out of 450 primers that exhibited 11.77 percent polymorphism [1]. Based on polymorphic microsatellite markers, MAPMAKER/EXP 3.0 software resulted in forming seven linkage groups whereas five markers were designated as unlinked. The seven linkage groups were mapped for 11 chromosomes (chromosomes 1–12), but there were no connected markers on chromosome 7. Chromosomes 1, 2, 3, and 4 had 6, 7, 9, and 2 markers with map distances of 421.2, 582.1, 607.6 and 97.5 cM, respectively. Four markers were found on chromosomes 5 and 6, covering a map length of 255.9 and 192.6 cM, correspondingly. With 3 and 2 markers, chromosomes 8 and 9 had a map distance of 567.9 and 18.7 cM, respectively, however, with a single marker, chromosome 10 had a map length of 78.8 cM. There were 2 and 3 markers on chromosomes 11 and 12, respectively, with map distances of 19.4 and 231.9 cM, for a total of 3971.1 cM (Fig. 1) [1].

Table 3: A total of 30 QTLs identified for yield and yield related traits along with chromosome number, marker-interval of peak LOD, additive effect, peak LOD and the phenotypic variation explained (R^2)

Trait	Chromosome	No. of QTLs	QTL	LOD value	Marker interval	Position (cM)	Additive effect	PVE % (R^2)
Number of tillers per plant (TPP)	1	2	qTPP1.1	2.67	RM13838	30.5	−0.58	12
			qTPP1.2	3	RM13838	36.5	−0.57	14
	2	1	qTPP2	2.5	RM5699-RM240	123	0.12	57.2
	3	1	qTPP3	3.5	RM6832	12	−1.36	11
Panicle number per plant (PPP)	1	2	qPPP1.1	2.6	RM13838	30.5	−0.59	11.8
			qPPP1.2	3	RM13838	36.3	−0.58	14
	3	1	qPPP3	3.38	RM6832	10	−1.48	11.3
Plant height (PH)	2	1	qPH2	3.5	RM5699-RM240	125	2.83	55.3
	4	1	qPH4	7.9	RM471-RM3524	44	666	65.2
	5	1	qPH5	2.5	RM413	28.1	−4.16	7
	6	1	qPH6	3.1	RM204-RM225	22	−7.11	36.7
Spikelet density (SD)	5	1	qSD5	2.5	RM413	12.1	−0.27	4.4
Spikelet fertility percentage (SFP)	2	1	qSFP2	3.8	RM5699-RM240	129	−10.45	45.8
	3	1	qSFP3	2.8	RM6832	2	2.21	5
	5	2	qSFP5.1	3.7	RM413	10.6	1.88	15.2
			qSFP5.2	4.5	RM413	20.1	2.39	16
Grain length (GL)	1	1	qGL1	25.2	RM13838	40.3	−0.29	47.7
	8	1	qGL8	3	RM447	6	0.16	5.9
Grain width (GW)	1	1	qGW1	2.6	RM13838	41.9	0.028	3
	3	1	qGW3	4	RM6832	26.9	0.06	5.8
	8	1	qGW8	5.2	RM447-RM80	26	0.8	21

(Continued)

Table 3 (continued)

Trait	Chromosome	No. of QTLs	QTL	LOD value	Marker interval	Position (cM)	Additive effect	PVE % (R²)
1000-Grain weight (TGW)	1	1	*qTGW1*	10.2	RM13838	41.9	−0.98	16.8
	4	1	*qTGW4*	5.8	RM471-RM3524	28	2.7	37
	8	1	*qTGW8*	3.5	RM447-RM80	26	0.8	11
Yield per plant (YPP)	2	3	*qYPP2.1*	2.5	RM262-RM3874	74.5	−1.9	4.5
			qYPP2.2	4.9	RM5699-RM240	123	1.37	69
			qYPP2.3	3.8	RM5699-RM240	139	0.83	65
	4	1	*qYPP4*	7.2	RM471-RM3524	52	7.19	67
	6	1	*qYPP6*	3	RM204-RM225	26	−2	43
	8	1	*qYPP8*	3	RM447-RM80	26	−0.73	55

3.2 Detection of QTLs for Various Traits

In the present study, Composite interval mapping (CIM) using genotypic data, phenotypic and genetic linkage map recognized total of 30 QTLs for nine yield and yield contributing traits. There were 30 QTLs for yield and yield-related traits identified, with chromosomal number, marker-interval of peak LOD, additive effect, peak LOD value and the phenotypic variation explained (R^2) (Table 3). Pictorial representation of major QTLs for various traits found in this study are given in Fig. 2.

3.3 Number of Tillers Per Plant (TPP)

There were a total of four QTLs discovered for the number of tillers per plant, out of which 2 QTLs were detected at chromosome 1, with a flanking marker RM13838 on linkage map with 30.5 cM position named as *qTPP1.1* and at 36.3 cM named as *qTPP1.2*, respectively, one QTL, *qTPP2* was detected at chromosome 2 with 123 cM position between the peak marker interval RM5699-RM240 and one QTL was located at chromosome 3, at flanking marker RM6832 on linkage map with 10.0 cM position named as *qTPP3*. LOD value and phenotypic deviation described for QTL *qTPP1.1* was 2.67 and 12 percent, for QTL *qTPP1.2* was 3 and 14 percent, for QTL *qTPP2* was 2.5 and 57.2 percent, whereas, for QTL *qTPP3* was 3.5 and 11 percent, respectively. The peak is shown in Fig. 3.

3.4 Panicle Number Per Plant (PPP)

Three QTLs were identified for panicle number per plant out of which 2 QTLs were located at chromosome 1, at a flanking marker RM13838 on linkage map with 30.5 cM position named *qPPP1.1* with a LOD value 2.6 and 11.8 phenotypic variation explained percent and at 36.3 cM position with flanking marker RM13838, named *qPPP1.2* with LOD value 3.0 and phenotypic variation explained was

14 percent. Another QTL was located at chromosome 3, at flanking marker RM6832 with 10.0 cM position named *qPPP3*, the LOD value was 3.38 and 11.3 percent was the phenotypic variation explained. The peak is shown in Fig. 4.
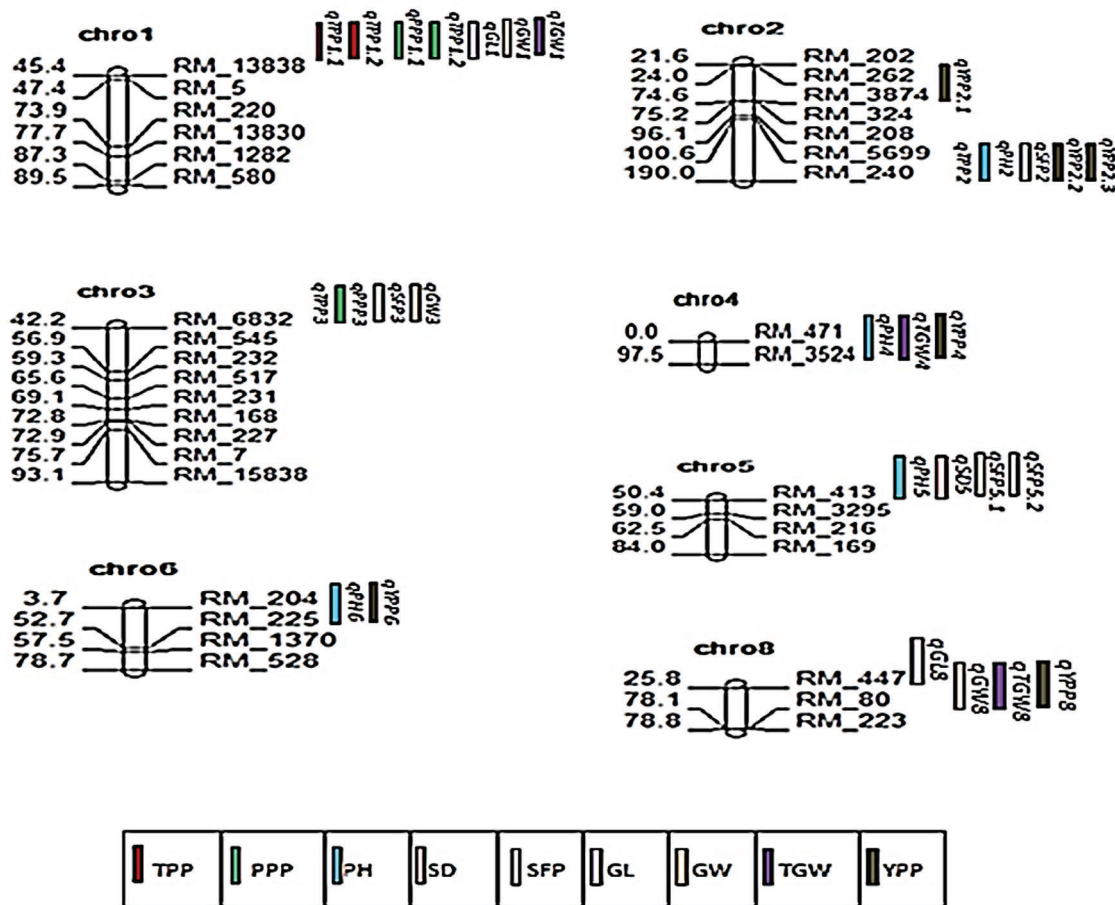

Figure 2: Rice chromosomes showing the location of major QTLs for yield and yield contributing traits
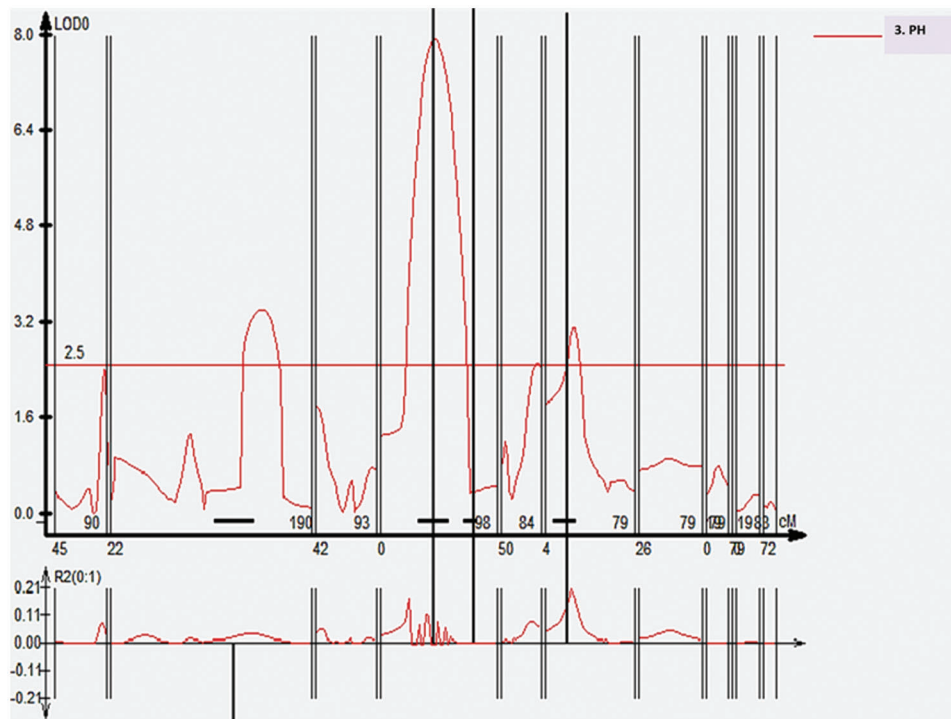
3.5 Plant Height (PH)

Four QTLs were detected for plant height. One QTL named *qPH2* at 125.0 cM on chromosome 2 between the peak marker intervals RM5699-RM240 was found with LOD value 3.5 and 55.3 percent phenotypic variation explained. Another QTL named as named as *qPH4* was detected with a LOD value 7.9 and 65.2 percent phenotypic variation explained on chromosome 4 between the peak marker intervals RM471-RM3524 at 44.0 cM position. One QTL, *qPH5* was detected at chromosome 5 at 28.1 cM position with RM413 flanking marker, LOD value 2.5 and 7.0 percent phenotypic variation explained was observed. Another QTL, *qPH6* was detected with LOD value 3.1 and 36.7 phenotypic variation explained percent on chromosome 6 with 22.0 cM position between the marker-interval of RM204-RM225. The peak is shown in Fig. 5.
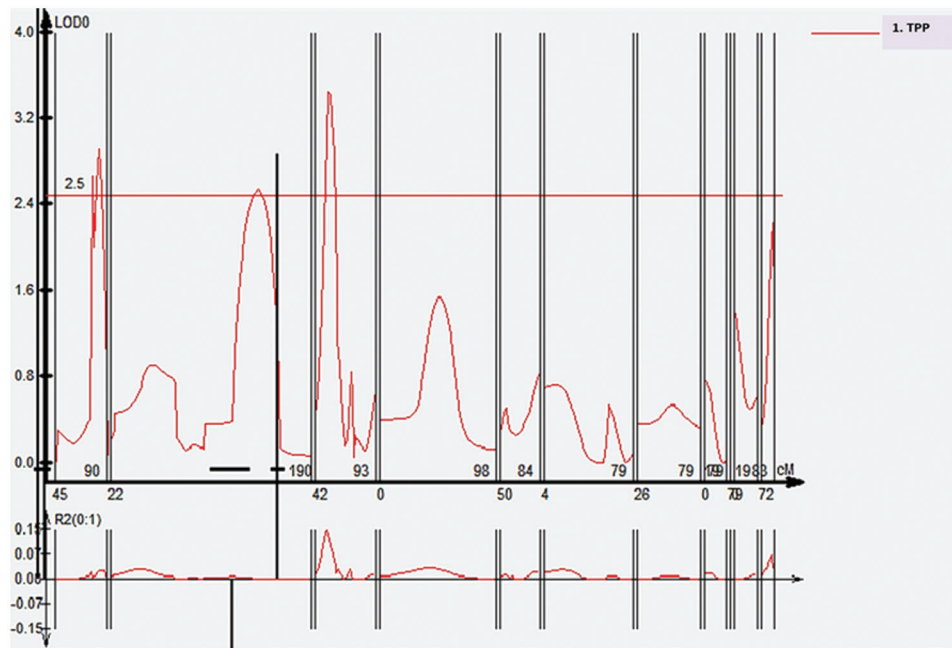
Figure 3: QTL likelihood plots indicating LOD scores for tiller per plant (TPP) using CIM using QTL cartographer version 2.5
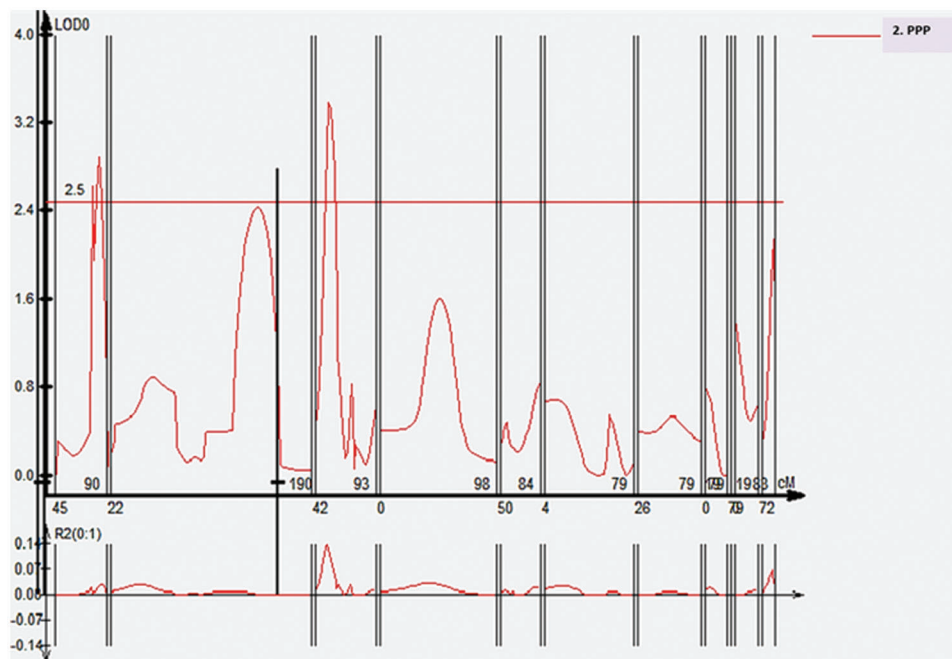
Figure 4: QTL likelihood plots indicating LOD scores for panicle number per plant (PPP) using CIM using QTL cartographer version 2.5

Figure 5: QTL likelihood plots indicating LOD scores for plant height (PH) using CIM using QTL cartographer version 2.5
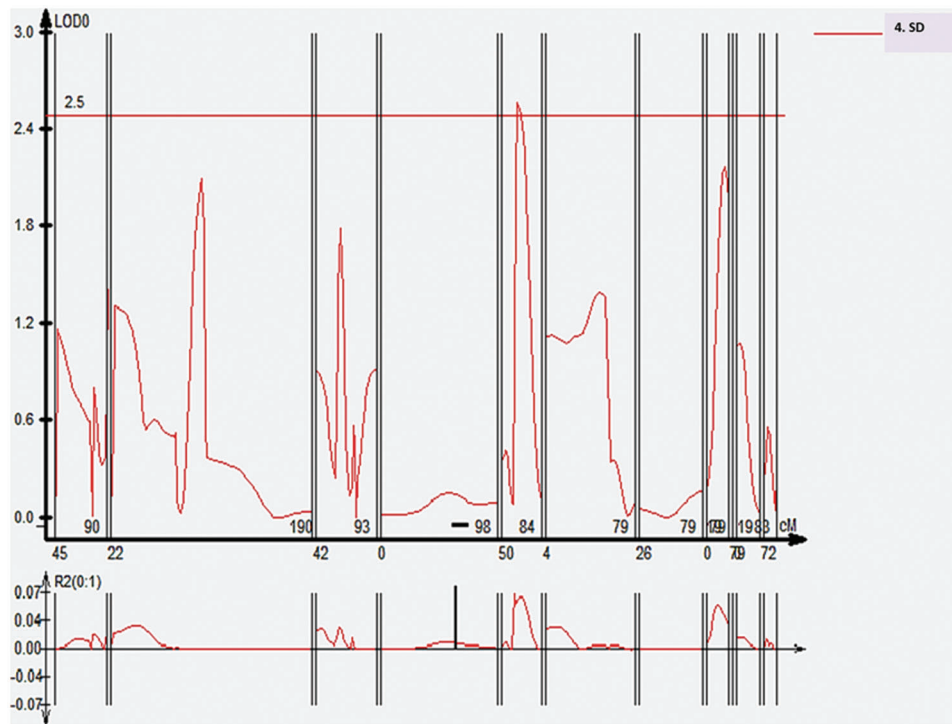
3.6 Spikelet Density (SD)

On chromosome 5, a substantial QTL qSD5 was discovered at 12.1 cM with a flanking marker RM413, and LOD value for this QTL was 2.5 and explained the phenotypic variability by 4.4%. The peak is shown in Fig. 6.

3.7 Spikelet Fertility Percentage (SFP)

Four QTLs namely *qSFP2*, *qSFP3*, *qSFP5.1* and *qSFP5.2* were located at 129.0, 2.0, 10.6 and 20.1 cM position at chromosome number 2, 3 and 5, respectively. QTL, *qSFP2* was found between the marker-interval RM5699-RM240 with 3.8 LOD value and 45.8 percent phenotypic variability explained. QTL, *qSFP3* was found with a flanking marker RM6832 with 2.8 LOD value and 5 percent phenotypic variability explained. Two QTLs were found on chromosome 5, *qSFP5.1* was found with a flanking marker RM413, with 3.7 LOD value and 15.2% phenotypic variability explained, another QTL, *qSFP5.2* was found with a RM413 as flanking marker, and LOD value for this QTL was 4.5 and described the phenotypic by 16% (Peak is shown in Fig. 7).
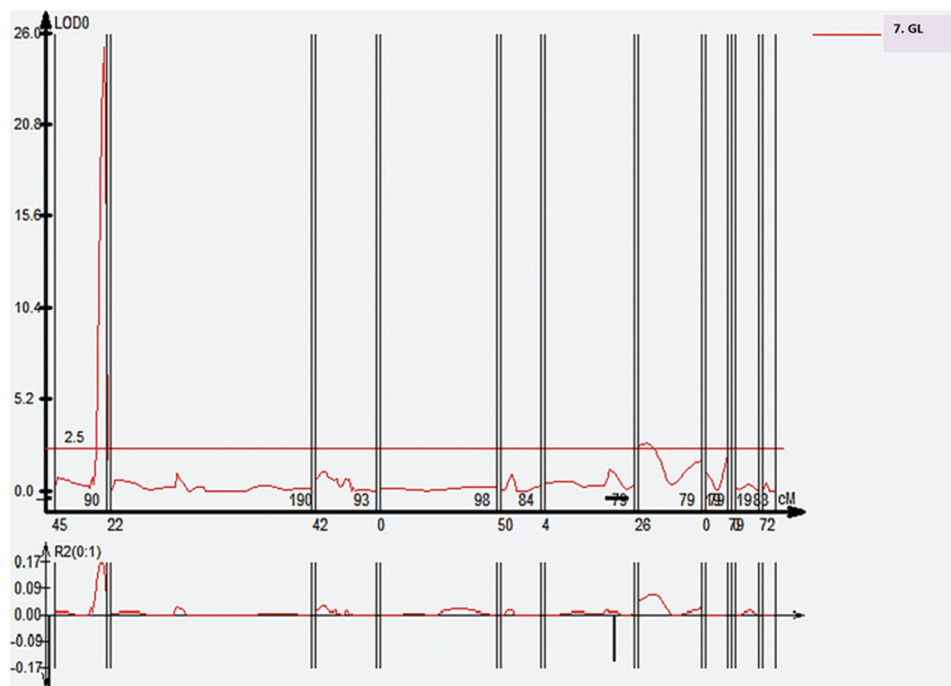
3.8 Grain Length (GL)

Two QTLs were identified for grain length at 40.3 and 6.0 cM distance at chromosomes 1 and 8, respectively. QTLs, *qGL1* and *qGL8* were found with the flaking marker RM13838 and RM447, with the LOD value 25.2 and 3, respectively. The phenotypic variability explained was 47.7 percent and 5.9 percent for *qGL1* and *qGL8*, respectively. The peak is shown in Fig. 8.

Figure 6: QTL likelihood plots indicating LOD scores for spikelet density (SD) using CIM using QTL cartographer version 2.5
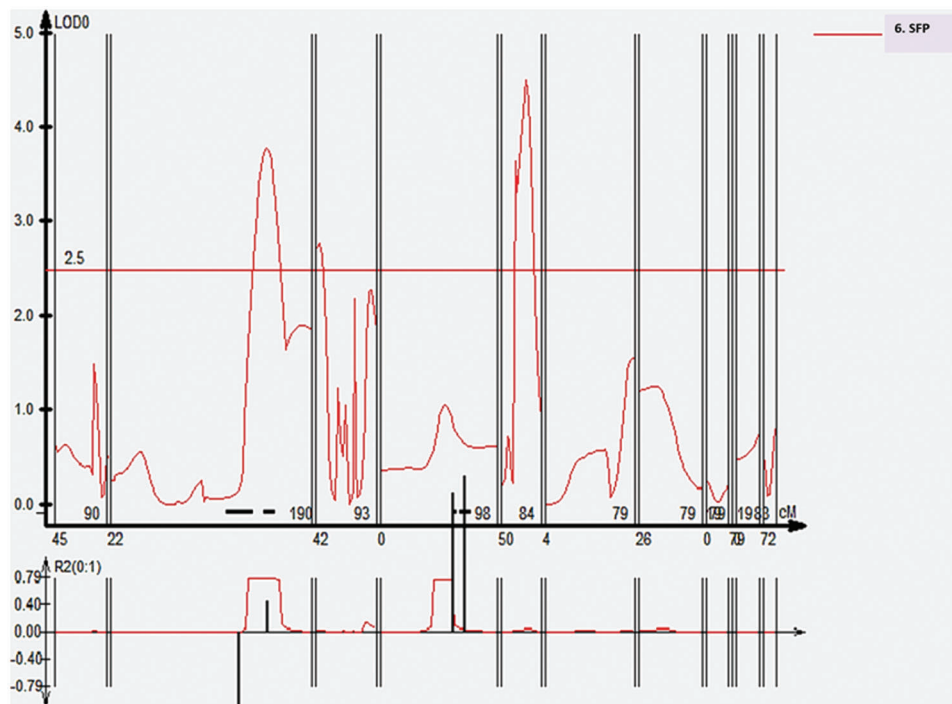
Figure 7: QTL likelihood plots indicating LOD scores for spikelet fertility (SFP) using CIM using QTL cartographer version 2.5

Figure 8: QTL likelihood plots indicating LOD scores for grain length (GL) using CIM using QTL cartographer version 2.5

3.9 Grain Width (GW)

In the case of grain width, three QTLs were discovered. QTL namely *qGW1* was located on chromosome 1 at flanking marker RM13838 on linkage map with 41.9 cM distance, QTL namely *qGW3* was located on chromosome number 3 at flanking marker RM6832 with 26.9 cM distance and QTL namely *qGW8* was located on chromosome 8 between the peak marker intervals RM447-RM80 with 26.0 cM distance. All 3 QTLs, *qGW1*, *qGW3* and *qGW8* had a LOD value 2.6, 4 and 5.2 also phenotypic variability explained was 3, 5.8 and 21 percent, respectively. The peak is shown in Fig. 9.

3.10 1000-Grain Weight (TGW)

Three QTLs namely *qTGW1*, *qTGW4* and *qTGW8* were identified for 1000-grain weight on chromosome numbers 1, 4 and 8 at 41.9, 28.0, 26.0 cM distance, respectively. *qTGW1* was located with the flaking marker RM13838, *qTGW4* was located between the peak marker interval RM471-RM3524 and was located between the peak marker interval RM447-RM80. The LOD value and the phenotypic variability explained for the 3 QTLs, *qTGW1*, *qTGW4* and *qTGW8* was 10.2, 5.8 and 3.5 and 16.8, 37 and 11.0 percent. The peak is shown in Fig. 10.

3.11 Yield Per Plant (YPP)

A total of six QTLs namely *qYPP2.1*, *qYPP2.2*, *qYPP2.3*, *qYPP4*, *qYPP6* and *qYPP8* were identified for yield per plant on chromosome number 2, 4, 6 and 8 at 74.5, 123.0, 139.0, 52.0, 26.0 and 26.0 cM distance, respectively. Three QTLs were detected on chromosome number 2, *qYPP2.1* was located between the peak marker-interval RM262-RM3874; the LOD with this QTL was 2.5 and described the phenotypic dissimilarity by 4.5%, *qYPP2.2* was located between the peak marker-interval RM5699-RM240; the LOD with this QTL was 4.9 and elucidated the phenotypic difference by 69.0%, and *qYPP2.3* was located between the peak marker-interval RM5699-RM240; the LOD value for this QTL was 3.8 and explained

the phenotypic variation by 65.0 percent. Another QTL *qYPP4* was located between the peak marker-interval RM471-RM3524; the LOD value for this QTL was 7.2 and explained the phenotypic variation by 67.0 percent. QTL *qYPP6* was located between the peak marker-interval RM204-RM225; the LOD value for this QTL was 3.0 and explained the phenotypic variation by 43 percent, whereas, QTL *qYPP8* was located between the peak marker interval RM447-RM80; the LOD with this QTL was 3.0% and 55.0% was the phenotypic variability explained. The peak is shown in Fig. 11.
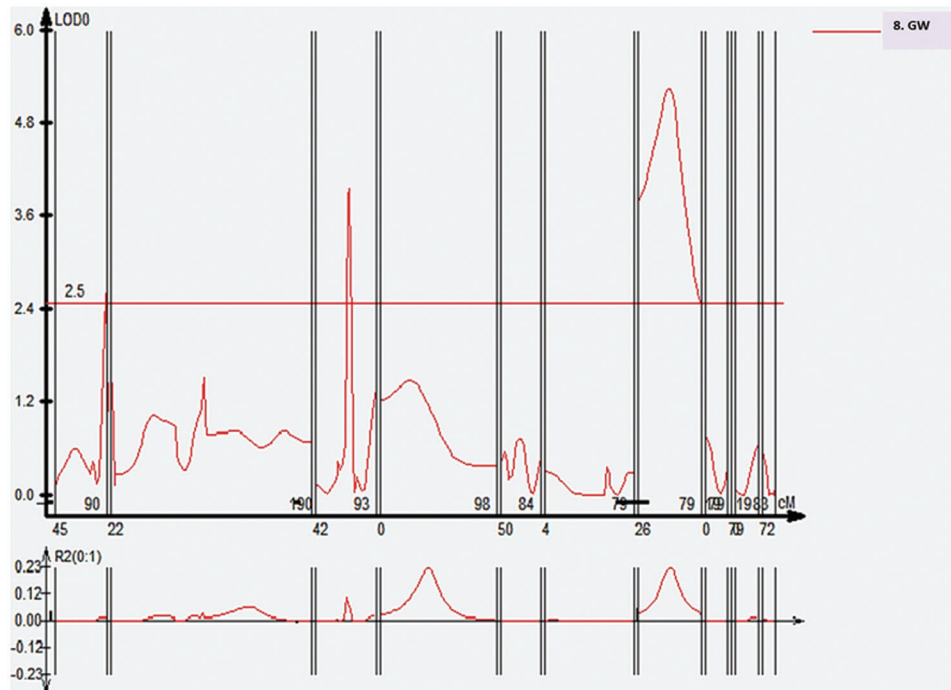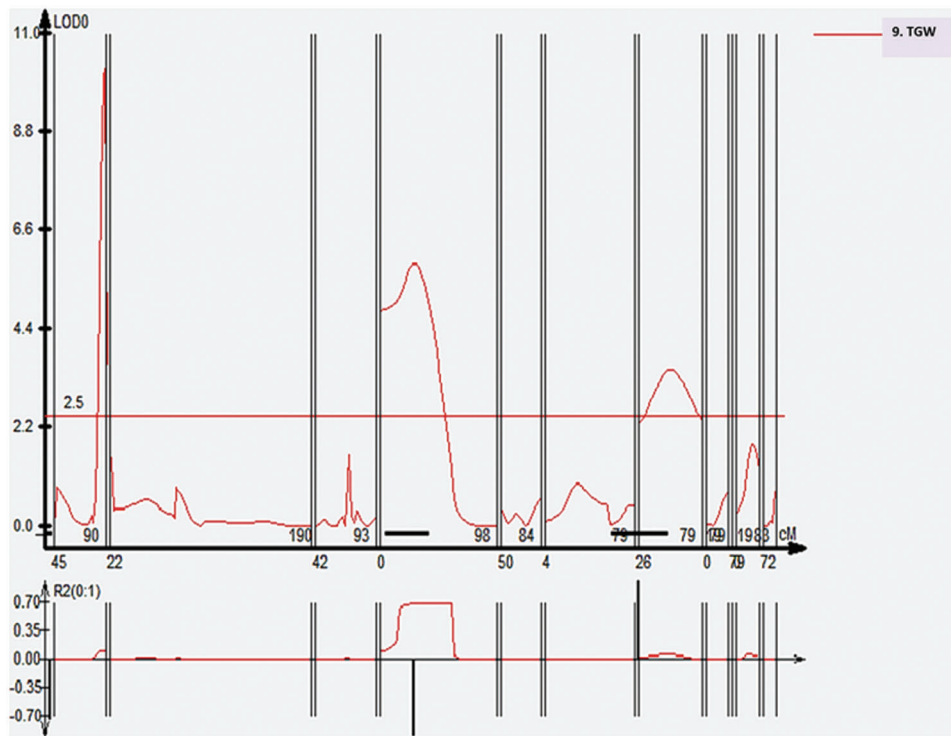

Figure 9: QTL likelihood plots indicating LOD scores for grain width (GW) using CIM using QTL cartographer version 2.5

4 Discussion

QTL analysis is based on the idea of identifying a relationship between phenotype, marker genotype, and population type, suggesting that the marker locus employed to partition the mapping population is connected to a QTL regulating the trait [16]. Both environmental and pleiotropic effects of genes for non-target traits influence the phenotypic expression of yield and its component traits. The total phenotypic variation is contributed by the number of QTLs but each QTL explains the part of the overall phenotypic deviation which is used to differentiate it as a minor or major QTL. A minor QTL explains less than 10% of total phenotypic variance, whereas a major QTL explains more than 10% of total phenotypic variation. The same standard has been used in many QTL studies in rice and other crops [17–19]. Furthermore, small and environment-specific QTLs are neglected during QTL analysis, although there are only a few "stable" QTLs that have been identified [20]. The findings were reviewed based on the above-mentioned studies, and the discovered QTLs were named using McCouch's nomenclature criteria [21].

Figure 10: QTL likelihood plots indicating LOD scores for 1000-grain weight (TGW) using CIM using QTL cartographer version 2.5
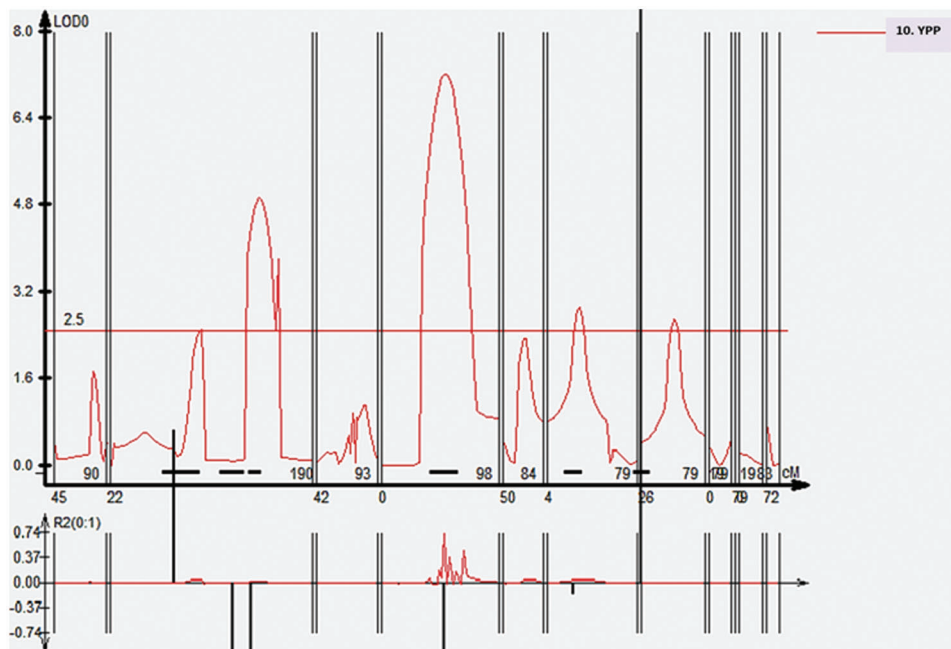
Figure 11: QTL likelihood plots indicating LOD scores for yield per plant (YPP) using CIM using QTL cartographer version 2.5

Out of the 30 QTLs found in this study, four significant QTLs for the number of tillers per plant were identified as phenotypic variation explained (PVE%) for all QTLs, i.e., *qTPP1.1*, *qTPP1.2*, *qTPP2*, *qTPP3* was above 10%, i.e., 12%, 14%, 57.2%, 11% respectively and LOD value ranged from 2.5–3.5 for all the QTLs, which are quite a significant QTLs and 3 major QTLs were found for panicle no per plant, PVE% observed was above 10%, i.e., 11.8, 14.0, 11.3 percent and LOD value was ranging from 2.6–3.38 for all the QTLs, similar LOD score was observed in F$_2$ population [22], but as per the reports of [23], heritability for both traits is low (0.65), similar results were shown by a study [24]. Among the 130 QTLs detected for grain yield and its attributing components using composite interval mapping, 36 were major effects QTLs and 8 QTLs were determined to be stable over chromosomal locations [25]. Chief response QTLs for flag leaf length (*qfll3.1*) with 46 percent phenotypic difference were identified 6 recognized QTLs (*qph3.1*, *qnt3.1*, *qnt3.2*, *qTGW-4*, *qTGW4–1*, *qPPP4–2*) were also confirmed and co-localized in 3 and 4 chromosomes along with QTLs genomic regions recognized in this study. There are 15 major and 23 minor effects QTLs in these genomic locations, covering over 300 genes. This research might help break down yield genetic obstacles for long-term food security. Three major and one minor QTLs namely *qPH2*, *qPH4*, *qPH5*, *qPH6* were detected for plant height as Phenotypic variation explained (PVE%) was 55.3, 65.2, 7.0, 36.7, respectively. The lowest LOD value observed was 2.5 for *qPH5*, followed by 3.1 for *qPH6* and 3.5 for *qPH2*, however, a very high LOD value of 7.9 was observed for *qPH4*. Similar results for chromosome 6 have been reported for plant height with the LOD value 3.5 and flaking marker RM204. Spikelet density showed a minor QTL *qSD5* located at 12.1 cM distance [26]. For this QTL, the LOD value utilized in the study was 2.5 [27], and the LOD value of 2.2 was used in another study and explained the phenotypic variability by 4.4 percent similar to PVE% (2013).

Although heritability for spikelet density was observed to be 0.68, still detection of minor QTLs may be due to the set of markers employed or the phenotypic appearance of such traits may be affected by both environmental effects and pleiotropic effects of genes for non-target traits. Spikelet fertility is another important trait. In the present investigation, three major & one minor QTLs namely *qSFP2*, *qSFP5.1*, *qSFP5.2*, and *qSFP3* respectively were identified for spikelet fertility percentage. The highest LOD value of 4.5 was observed in *qSFP5.2* same LOD value [28], followed by *qSFP2* and *qSFP5.1* with LOD values of 3.8 and 3.7; the same LOD value for spikelet fertility was reported in another study [29], lowest LOD value 2.8 was observed in *qSFP3*. Grain size can be specified by grain length, width and thickness is largely regulated by cell proliferation, expansion of maternal integument, and zygotic tissues [30].

Grain length and grain width are the important constituents of plant yield and are controlled by quantitative trait loci (QTLs) [31]. In the present study, one major QTL, *qGL1* for grain length was detected with a very high LOD value of 25.2 and significant PVE of 47.7%, and one minor QTL with a LOD value of 3.0% and 5.9% PVE was detected on chromosome 8 with 6.0 cM position. Similarly, for grain width, one major QTL, *qGW8* with a high LOD value of 5.2 was detected, this QTL showed 21% PVE at chromosome 8. Two minor QTLs, *qGW1* and *qGW3* were detected with LOD value 2.6 and 4.0, respectively. The same LOD value of 4.0 and PVE 24.5% for grain width in the F$_2$ population was reported in another study [22].

Three major QTLs namely *qTGW1*, *qTGW4*, and *qTGW8* were identified for 1000-grain weight. *qTGW1* showed the highest LOD value 10.2 with 16.8 PVE% followed by QTL *qTGW4* with 5.8 LOD value with PVE 37% and the lowest LOD value was observed in *qTGW8* 3.5 with PVE 11.0%. The LOD value and the phenotypic variability explained for the 3 major QTLs were seen as significant. A similar study has reported the LOD value of 3.2 and 10.0, also 18% PVE for 1000-grain weight in their studies which is quite close to the results mentioned above [28], hence exploring these QTLs further to the next generation can help identification of closely linked genes. Also, they reported that a major QTL, *qTGW8.1* s was located on chromosome 8 with a flaking marker RM80 which is related to the findings in the current research. A 37% PVE for 1000-grain weight was reported by another study [32].

Maximum numbers of QTLs were detected for the trait yield per plant; similar reports for yield were shown in a similar study [33]. A total of 6 QTLs were detected for YPP, out of which only minor QTL *qYPP2.1* was found rest four QTLs *qYPP2.2*, *qYPP2.3*, *qYPP4*, *qYPP6*, *qYPP8* were found to be the major QTLs. The lowest LOD value and PVE%, i.e., 2.5 and 4.5, respectively, were observed for *qYPP2.1*. The highest LOD value 7.2 and highest PVE% 69 were observed in *qYPP4* and *qYPP2.2*, at chromosome 4 and chromosome 2, respectively. Maximum numbers of QTLs for YPP were located at chromosome 2; a similar study also reported a QTL at chromosome 2 with 3.6 LOD values, which shows a similarity to the present study [34].

The variation explained by individual QTLs ranged from a low of 3.0% to a high of 69.0%, with the majority of them explaining the range of 10%–20% of the variation. One QTL of plant height shows a close linkage with one QTL of yield per plant & one QTL of 1000-grain weight as they were detected on the same chromosome number 4 and with 44, 28 and, 52 cM distance, respectively, and all these three QTLs were detected between the same marker-interval RM471-RM3524. Most of the QTLs were detected on chromosome 2, so further studies on chromosome 2 could help unlock some new chapters of QTL for this set of parents. The molecular markers linked with definitive QTL identified for different traits in the current research can be beneficial for MAS. The chromosomal regions carrying important QTL identified during the present study can be enriched with more markers by high-resolution mapping. Evidence about molecular markers tightly linked to QTL that govern these traits may accelerate crop improvement strategies in rice.

5 Conclusion

Any scientific advancement that focuses on increasing rice yields will have a significant impact on global food and nutrition security. Given the rapid growth of the global population, the global population is anticipated to reach nine billion by the middle of this century. Rice grain yield should be increased by 70%–100%, relative to the current levels, to feed the increasing global population [35,36]. The present investigation revealed that most of the studied traits were controlled by one or more major QTLs, elucidating a high percentage of phenotypic variance and several minor QTLs. The major QTLs identified can be used in rice improvement programs. The effect of the identified alleles on of the yield parameters of the rice population can be further studied for a multiple-environment experiments which may demonstrate that both genotypic and environmental variations play a substantial role in the observed variable in yield per plant [2]. Various genomic regions governed more than one trait, indicating linkage and/or pleiotropic effects. The significant correlations between studied traits can be explained by these genomic regions containing pleiotropic or tightly linked QTLs. The present study also revealed that all the major QTLs of a given trait are found in the same range of markers on a specific chromosome, which gives useful information that focusing and exploring those particular chromosome regions and the ignoring rest of the markers can lead to saving time and accelerate useful findings in the field of rice research. Thus, the information generated in this study will be useful in fine genetic mapping and identifying the genes behind significant robust QTLs, as well as transferring all favorable QTLs to a single genetic background to break down genetic barriers to yield for long-term food security.

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