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IDENTIFICATION OF QTLS FOR SPIKELETS PER PANICLE IN RICE (*ORYZA SATIVA* L.)

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Abstract

Improvement of rice grain yield is an important goal in rice breeding. Yield is determined by its related traits such as spikelet fertility, 1,000 grain weight, and the number of spikelets per panicle. With the help of 48 primers using for QTLs identify in cross between Swarna × IR86931 B-6 F_3 population under irrigated and rain out shelter I and rain out shelter II condition and 38 primers using for QTLs identify in cross between MTU1010 × IR86931 B-6 F_3 population under irrigated condition during wet season 2012-2013 at Research cum Instructional Farm, Department of Genetics and Plant Breeding, College of Agriculture, I.G.K.V., Raipur, Chhattisgarh, India. Overall in Swarna × IR86931-B-6 F_3 population, a total of 35 QTLs were identified for number of filled spikelet and other yield and yield contributing traits in irrigated, rain out shelter I and II condition the LOD value for the QTLs ranged from 3.0 to 23.0. However, for QTLs were detected for filled spikelets on chromosome 3, 4 and whereas, only one QTL was identified on chromosome 4 for total number of spikelet. In MTU1010 × IR86931-B-6 F_3 population, 16 QTLs were identified with the LOD value ranging from 3.0 to 6.0 for total number of spikelets, a total of 5 QTLs were identified in irrigated condition using the composite interval mapping analysis hence, we can targeted to improve rice yield by marker aided selection.

Key words : Rice, QTL, SSR, HvSSR, spikelet fertility, spikelets per panicle.

Introduction

Food shortage is becoming a serious global problem because the rate of world population growth currently exceeds the rate of increase in food production. To meet the food growing demands, it is necessary to improve rice grain yield because rice (*Oryza sativa* L.) is a staple food in many countries (Liu *et al*, 2013). Rice (*Oryza sativa* L.) is one of the most important food crops worldwide belonging to the family *Graminae* and subfamily *Oryzoidea,* the staple food for one third of the world's population and occupies almost one-fifth of the total land area covered under cereals. Most of the world's rice is cultivated and consumed in Asia, Rice, one of the most important food crops for over half of the world's population accounts for around 23 % of the global calorie intake (Bernier *et al.*, 2008). Grain yield is one of the most valuable traits in crop production. Pursuing high grain yield is one of the most important goals in rice production. Grain yield is frequently replaced with grain yield per plant in quantitative trait locus mapping owing to control of field experimental scale (Hittalmani *et al*, 2003). Rice yield is a complex trait that exhibits a low heritability (Xiong, 1992), which creates a challenge to study it directly. Alternatively, yield components like 1,000-grain weight, the number of spikelets per panicle and spikelet fertility, directly contribute to yield. Although these yield related traits are also inherited quantitatively, they have comparatively higher heritability (Xing *et al*., 2001). Therefore, it is more feasible to focus on yield related traits rather than yield itself when dissected the genetic basis of yield. Grain yield per plant is determined by its three components, panicles per plant, 1,000-grain weight, and spikelets per panicle. Among the three components, SPP frequently makes the greatest contribution to grain yield and has received the most attention in genetic analysis. However, this trait is inherited in a quantitative manner and typically controlled by a number of major and minor QTL and is also affected by environment, which present a challenge in characterizing SPP. With QTL analysis based on molecular markers, SPP has been studied, and several of QTLs have been reported (Yu *et al*., 1997; Zhuang *et al*., 1997; Xing *et al*., 2002).

Table 1 : Characteristic features of parent.

S. no.	Parent	Pedigree	Salient features
1.	MTU-1010	Krishnaveni × IR64	Semi-dwarf,grains- long slender, white, resistant to blast and tolerant to BPH
2.	IR86931-B-6	Nagina22	Drought resistant, maturity 120 days, height 110-120 cm
3.	SWARNA	Vasishtha × Mashuri	Drought susceptibility, maturity 140 days, height 90-100 cm

Materials and Methods

The planting materials was 60 F_3 progenies of a cross between MTU1010 × IR86931-B-6 (derived from Nagina 22) and 45 F_3 progenies of a cross between SWARNA × IR86931-B-6 were evaluated in the field during wet season 2012-2013. Each F_3 progeny had 10 plants/lines. The characteristic features of parents included in present study are presented in table 1. The field trials for F_3 generation were conducted under irrigated condition. The fields selected for the study were upland in topology with good drainage and percolation rate and had sandy loam soil. The plant material was sown in raised bed nursery on 18th June, 2012 and transplanted after 15 days of sowing under puddled irrigated field condition and after 25 days separate the tiller and transplanted in rain out shelter condition. Under irrigated condition, normal package of practices was followed. The F_3 population namely 45 lines SWARNA × IR86931-B-6 and 60 lines MTU1010 × IR86931-B-6 were used for molecular studies. DNA was isolated by Miniprep method (Doyle and Doyle, 1978). For molecular work, the DNA was isolated from the leaves taken from F_3 progenies under irrigated condition. A total of 250 SSR and 60 HvSSR primers were used to detect parental polymorphism of these, 48 polymorphic primers used for making the genotypic data. In selected lines, composite interval mapping analysis using the win QTL cartographer software version 2.5 was used to estimate linkage between marker and trait.

Results and Discussion

QTL analysis

Total genomic DNA was extracted from 45 lines of rice (Swarna × IR86931-B-6) and 60 lines of (MTU1010 × IR86931-B-6) along with the parents using Doyle and Doyle (1978) method. Fresh and healthy leaves were used for extraction of DNA. The quantification was further done by using Nano Drop Spectroscopy (2000 C). The quantity of the samples range from 250-1500 ng/µl. DNA samples were then diluted with sterile water, such that the final concentration of DNA became 40 ng/µl. After standardization of the PCR protocol for SSR and HvSSR assay, it was used for all subsequent studies. The DNA of selected lines along with the parents was subjected to PCR based simple sequence repeat (SSR) technique to generate genotypic data using rice SSR and HvSSR primers.

Parental polymorphism analysis using HvSSR and SSR primers

A total of 310 SSR and HvSSR primers were used in this study for amplification of genomic DNA of mapping population through PCR. Out of 60 HvSSR and 250 SSR primers, 30 HvSSR and 100 SSR primers showed parental polymorphism between Swarna, MTU1010, and IR86931-B-6. The level of polymorphism was lower then that observed for mapping parents in studies by Bernier *et al*. (2007). The bands observed were designated as A, B, H and O, where A represents female parent like allele, B represent male parent like allele, H represents heterozygous and O represents other type allele. RM153 marker exhibited (heterozygosity 17% in the population with 35% female and 46% male allele), RM489 marker exhibited (heterozygosity 4% in the population with 78% female and 15% male allele) and HvSSR03-40 marker exhibited low percentage of heterozygosity 0% in the population (with 44.44% female and 55.55% male allele) The gel pictures for primer RM153, RM489, HvSSR 03-40, RM152 and used the 50bp ladder.

Identification of QTLs

The genotypic data of the whole segregating population was developed to detect the association of QTLs with traits. Bernier *et al*. (2008) also report to used selective genotyping for QTL detection. Test for QTL association with traits was performed by composite interval mapping approach. For the composite interval mapping analysis was followed to find out the significant association between traits and the markers. The map developed by Singh *et al*. (2009), Causse *et al*. (1994), McCouch *et al*. (2002) and the International Rice Genomic Sequencing project (2005) helped to identify relative position of markers on chromosome. The location and position of different SSR and HvSSR primer used in the study are depicted in (table 2) and molecular linkage map with position of QTLs for all traits of various condition presented in (figs. 3, 4, 5 and 6). Overall, the nine traits under irrigated and 5 traits under rain out shelter I and II conditions, 51 QTLs were identified with varied LOD values in 45 F_3 segregating population of cross Swarna x IR86931-B-6 and MTU1010 x IR86931-B-6 in *kharif*

Table 2 : List of marker used for development genotypic data in Swarna × IR86931- B-6 F_3 population with their location of chromosome.

S.no.	Markers (Swarna × IR86931-B-6)	Chr. No.	Position cM	Markers (MTU1010 × IR86931-B-3)	Chr. No.	Position cM
1.	RM1	1	29.7	RM1	1	29.7
2.	RM283	1	31.4	RM11943	1	37.85
3.	RM12146	1	40.71	RM12146	1	40.71
4.	RM9	1	92.4	RM490	1	51
5.	RM212	1	133.5	RM9	1	92.4
6.	RM174	2	33.8	RM486	1	153.5
7.	RM341	2	82.7	RM109	2	0
8.	RM221	2	143.7	HvSSR2-12	2	4.5
9.	RM530	2	158	HvSSR2-27	2	7.72
10.	HVSSR3-09	3	15.36	RM279	2	17.3
11.	RM489	3	29.2	RM174	2	33.8
12.	RM545	3	35.3	RM530	2	158
13.	RM232	3	76.7	RM231	3	15.7
14.	RM282	3	100.6	RM232	3	76.7
15.	RM411	3	127.9	RM16	3	131.5
16.	RM135	3	157.3	RM55	3	168.2
17.	RM55	3	168.2	RM468	3	202.3
18.	RM168	3	171.2	RM3471	4	16.7
19.	HVSSR4-35	4	22.88	RM335	4	21.5
20.	RM518	4	25.5	RM451	4	115.5
21.	HVSSR4-42	4	28.5	RM153	5	0.5
22.	RM261	4	35.4	HvSSR5-13	5	3.11
23.	RM273	4	74.5	HvSSR5-48	5	19.82
24.	RM119	4	76.1	HvSSR5-65	5	27.22
25.	RM451	4	115.5	RM169	5	34.7
26.	RM153	5	0.5	RM164	5	91.4
27.	HVSSR5-13	5	3.11	RM190	6	7.4
28.	RM413	5	26.7	RM225	6	26.2
29.	RM440	5	92.7	RM340	6	135
30.	RM459	5	93.7	RM481	7	3.2
31.	RM508	6	0	RM152	8	9.4
32.	RM549	6	42.7	RM25	8	52.2
33.	RM228	6	87.6	RM444	9	3.3
34.	RM340	6	135.5	RM553	9	76.7
35.	RM481	7	3.2	RM278	9	77.5
36.	RM125	7	24.8	RM228	10	130.3
37.	RM25	8	52.2	RM21	11	85.7
38.	RM434	8	57.7	RM206	11	104.2
39.	RM152	8	9.4	RM309	12	74.5
40.	RM316	9	1.8			
41.	RM105	9	32.1			
42.	RM278	9	77.5			
43.	RM222	10	5.5			
44.	RM184	10	41.6			
45.	RM21	11	85.7			
46.	RM1233	11	112.9			
47.	RM19	12	20.9			
48.	RM235	12	103.1			

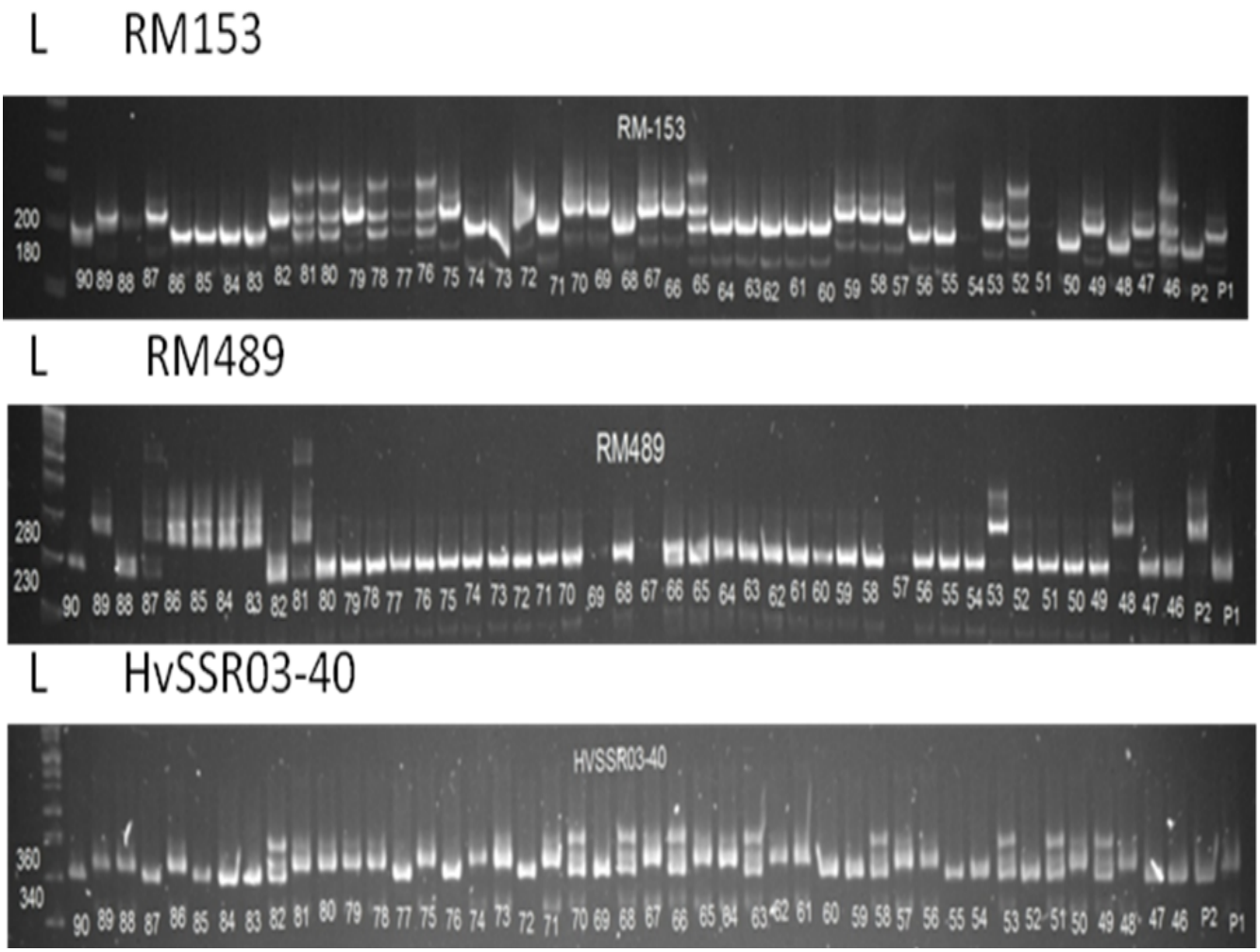

Fig. 1 : Banding pattern of F_3 lines in Swarna × IR86931-B-6 usingh rice microsatellite SSR [F_3 Segregating population, P1 – Swarna, P2 – IR 86931-B-6, L- ladder (50bp)].

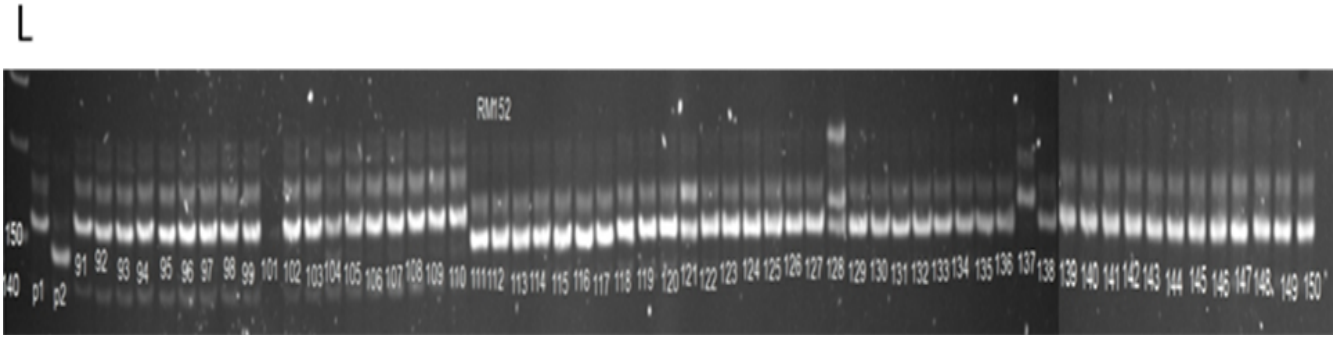

Fig. 2 : Banding pattern of F_3 lines in MTU1010 x IR86931-B-6 using rice microsatellite RM152 primer. [F_3 Segregating population, P1 – MTU1010, P2 – IR 86931-B-6, L- ladder (50bp)].

2012-2013. In the fourty five F_3 progenies of Swarna × IR86931-B-6 under irrigated condition, rain out shelter I and rain out shelter II a total of thirty five QTLs were identified for various traits. For number of filled spikelets from QTLs were identified two each in irrigated and rain out shelter I conditions at chromosome 4 (LOD = 3.4) and 12 (LOD=3.0) and one QTL at chromosome 3(LOD=4.5) and another QTL at chromosome 12 (LOD=3.2) the QTLs identified for number of filled spikelets at chromosome 12 under irrigated and rain out shelter I conditions showed same marker RM19 (20.9cM) - RM235 (103.1cM). Takai *et al*. (2005) reported two QTLs detected on chromosomes 8 and 12 were strongly associated with increased filling percentage

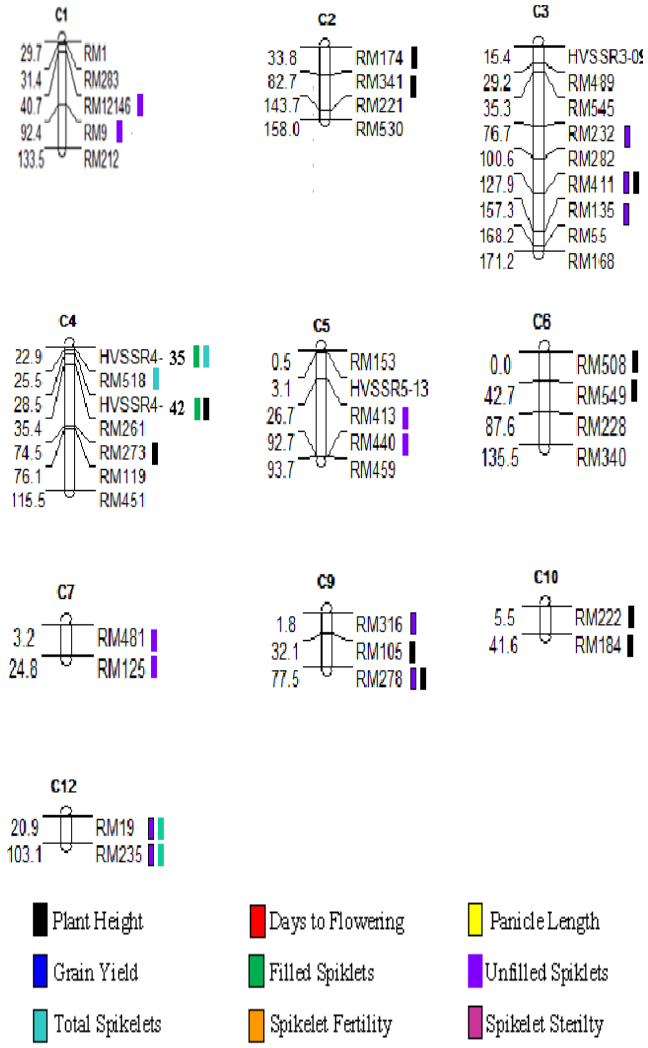

Fig. 3 : Molecular linkage map showing for all nine traits of (Swarna x IR86931-B-6) Irrigated condition.

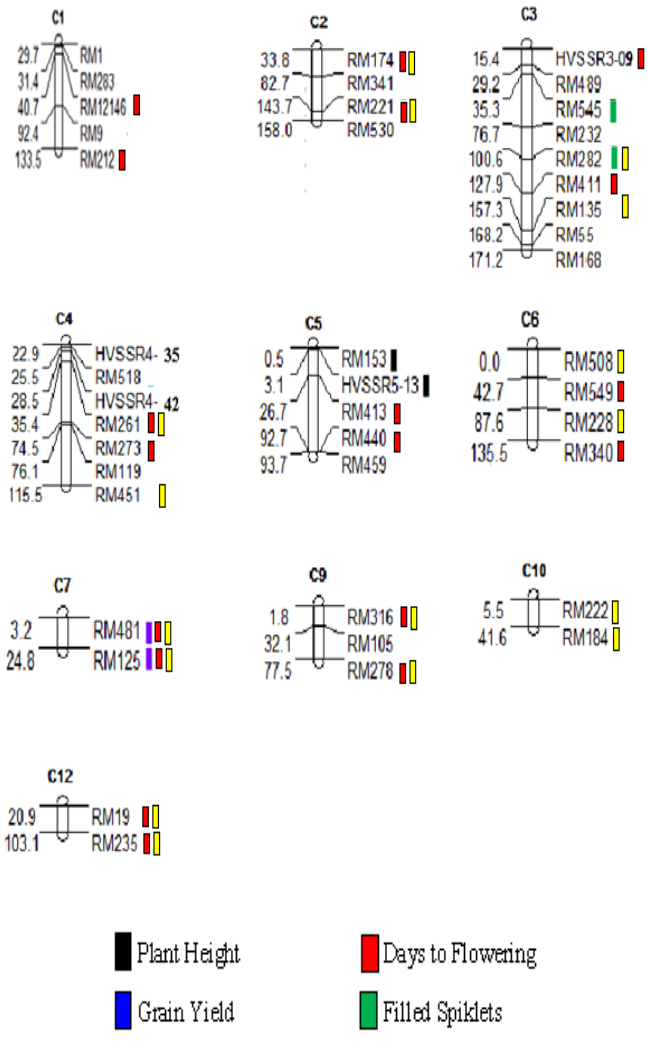

Fig. 4 : Molecular linkage map showing for all five traits of (Swarna x IR86931-B-6) rain out shelter I condition.

per panicle. Six QTLs were identified in irrigated conditions for number of unfilled spikelets on chromosome 12 (LOD=9.0), chromosome 9,7,5,3(LOD=8.0) and chromosome 1 (LOD= 5.0). on chromosome 12, same as for number of filled spikelets the markers were RM19(20.9cM) and RM235(103.1cM) and for chromosome 9, 316(1.8cM), RM105(32.1cM) and RM278(77.5cM), for chromosome 7 RM481(3.2cM) and RM125(24.8cM), for chromosome 5 RM 413(26.7cM) and RM440(92.7cM), for chromosome 3 RM232 (76.7cM) to RM135(157.3cM). Only, one QTL was identified for total number of spikelets on chromosome 4 in irrigated condition with the LOD of 3.0 with the marker HvSSR04-35(22.9cM) and RM518 (25.5cM).

For the grain yield two QTLs were recorded QTL acquired one on chromosome 12 (RM19(20.9cM)) with the LOD of 3.2 in irrigated condition and one on chromosome 1 (RM1(29.7cM)-RM12146(40.71cM)) with the LOD of 3.4 in rain out shelter II condition and also Bernier *et al.* (2007) has also identified QTL (qtl 12.1) on chromosome 12. Fu *et al.* (2010) reported QTLs for grain yield on chromosome 1, 2, 8 and 12. Xing *et al.* (2010) reported QTLs for grain yield on chromosome 1, 5, 6 and 7. In the sixty F_3 progenies of MTU1010 X IR86931-B-6 under irrigated condition, a total of sixteen QTLs were identified for various traits. The number of unfilled spikelets eight QTLs were recorded in irrigated condition. QTL required on the chromosome 1 (RM1(29.7cM)-RM486(153.5cM)), on chromosome 8 (RM152(9.4cM)-RM25(52.2cM)) with the LOD of 3.5 and chromosome 4 (RM3471(16.7cM)-RM451 (115.5cM)), on chromosome 5 (HvSSR05-48(19.82cM)-RM164(91.4cM)), on chromosome (RM553(76.7cM)-RM278(77.5cM)) with the LOD of 5.0 and one on chromosome 3 (RM232(76.7cM)-RM55(168.2cM)) with the LOD of 3.2 and on chromosome 6 (RM190(7.4cM)-

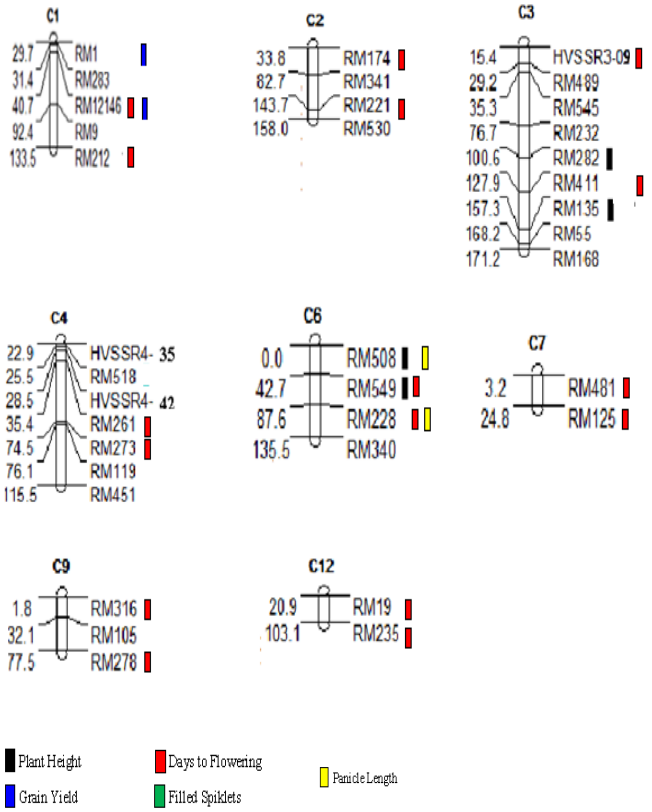

Fig. 5 : Molecular linkage map showing for all five traits of (Swarna × IR IR86931-B-6) Rainout Shelter II condition.

RM340(135cM)) with the LOD of 3.0. For the number of total spikelets five QTLs were recorded, QTL acquired on chromosome 4 (RM3471(16.7cM)-RM451(115.5cM)), on chromosome 3 (RM232(76.7cM)-RM16(131.5cM)) with the LOD of 4.0 and one on chromosome 5 (RM153(0.5cM)-RM169(34.7cM)) with the LOD of 4.5 and on chromosome 2 (HvSSR02-27(7.72cM)-RM174 (33.8cM)) with the LOD of 6.0. For the spikelets fertility and spikelets sterility percentage QTL on chromosome 2 (HvSSR02-27(7.72cM)-RM174 (33.8cM)) with the LOD of 3.2. For the grain yield one QTL recorded on chromosome 3 (RM231(15.7cM)-RM232(76.7cM)) with the LOD of 3.1.

Conclusion

In this study, we demonstrated that Swarna × IR86931-B-6 F_3 population, a total of 48 QTLs were identified for spikelets for panicle and other yield and yield contributing traits in irrigated, rain out shelter I and II condition the LOD value the QTLs ranged from 3.0 to 23.0. however, for QTLs were detected for filled spikelets on chromosome 3, 4 and whereas, only one QTL was identified on chromosome 4 for total number of spikelets, other than MTU1010 × IR86931-B-6 F_3 population, 16 QTLs were identified with the LOD value ranging from

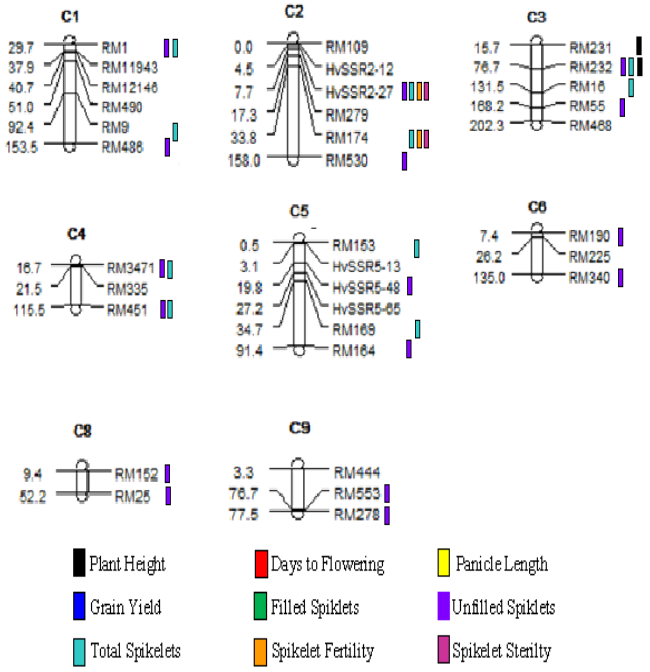

Fig. 6 : Molecular linkage map showing for all nine traits of (MTU1010 × IR86931-B-6) irrigated condition.

3.0 to 6.0 for total number of spikelets, a total of 5 QTLs were identified in irrigated condition using the composite interval mapping analysis. Based on the finding that the MTU-1010, IR86931-B-6 and SWARNA rice parent for the spikelets per panicle was beneficial in the indica cultivar backgrounds could be valuable for improving rice yields.

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