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Identification of Quantitative Trait Loci for Panicle Associated Traits in Recombinant Inbred Line Population Derived from *Japonica X Indica* sub-species in Rice (*Oryza sativa* L.)

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Abstract

Rice recombinant inbred lines (RILs) of *Japonica* x *Indica* cross were grown in irrigated conditions for identification of quantitative trait loci (QTLs) for panicle associated traits. One hundred twenty one RIL lines were evaluated in the field environment with two replications. A total of one hundred twenty two SSR markers were used for polymorphism out of forty markers that showed parental polymorphism. Forty SSR markers were used to construct the genetic linkage map by employing single marker analysis. Maximum number of QTLs was identified for total number of spikelets per panicle and minimum for panicle weight per plant. Clustering of QTL for different traits at the same marker RM 224 and RM 312 was observed for total number of spikelets per panicle, filled spikelets per panicle and panicle weight per plant. This suggests that pleiotropism and or tight linkage of different traits could be possible for the congruence of several QTLs. Moreover, phenotypically these characters have more association with each other. Hence, these markers may be useful for marker assisted breeding programme.

Key words: Rice, Single marker analysis, Panicle traits, QTL identification.

Introduction

Rice is a staple food for more than half of the world's population including two billion Asians, who obtain 60-70% of their energy intake from rice and its derivatives. Rice is globally grown on about 154 million hectares annually with total production of 600 million tons. To meet the growing demand from human population which is expected to touch 9 billion by 2050, in a changing global climatic order, rice varieties with higher yield potential and greater yield stability need to be developed. One of the means of achieving the projected production demand is by integrating classical breeding techniques with modern biotechnological tools for rice improvement (1). In rice panicle length, grains per panicle and grain sterility are crucial determinants of grain yield together with number of panicles per plant (2). These traits are inherited in a quantitative manner and typically controlled by a plurality of major and minor QTLs. A key development in the field of complex trait analysis was the discovery of DNA based genetic markers, physical establishment of high density genetic maps and development of QTL mapping methodologies such as single marker analysis, interval mapping and multitrait mapping.

There are number of reports on mapping and introgression of QTLs from wild species in rice. However, the related subspecies of *Oryza sativa* such as *japonica* carry many useful alleles which can be used for improvement of *Indica*. It has been observed that derivatives of *indica/japonica* cross have higher yield vigour than either *indica/ indica* or *japonica/japonica* derivatives. Therefore,

identifying the chromosomal locations influencing yield and yield related traits in inter subspecific derivatives is useful for rice improvement. Identification of favourable alleles in *japonica/indica* will pave way to the marker assisted mobilization of their allele in a genetic background to break genetic barrier for higher yields. The objectives of the present study were to identify QTLs and map genomic regions influencing panicle associated traits using phenotyping data from a recombinant inbred population generated by inter subspecific cross between JNPT 89 (*Japonica x Indica*) with IR 64 which is highly adopted and a high yielding *indica* rice variety.

Materials and Methods

The material for this study consisted of F_{10} one hundred twenty one Recombinant Inbred lines developed in the cross of JNPT 89 & IR 64. Marker analysis selective genotyping method was used to detect the association of QTLs with panicle associated traits. The RILs along with parents were planted in an Alpha lattice design with two replications at Seed Breeding Farm, Department of Plant Breeding and Genetics, J.N.K.V.V., Jabalpur. Twenty-one-day seedlings of each genotype were planted in five rows of three meter length with 20 cm row spacing keeping single seedling per hill. Recommended package of practices were followed to raise a good crop. Observations were recorded on randomly selected five plants from each genotype, in each replication at maturity. These plants were harvested and thrashed separately. The data were recorded for panicle traits viz., panicle length, number of filled grains per panicle, total number of spikelets per panicle and panicle weight per plant and grain yield per plant.

DNA isolation *:* Preparation of genomic DNA from the parents and RILs followed the mini prep method. The extracted DNA content was quantified and parental polymorphism studies were carried out through 112 SSR primers. PCR mix for one reaction (volume 20 µl) contained 2 µl DNA, sterile and nanopure water 13.5 µl, 10x assay buffer, 1 µl dNTP, 0.5 µl of each forward and reverse primers, and 0.5 µl Taq DNA polymerase. PCR amplification was performed with the following steps: pre denaturing at 94! for 4 min, followed by 35 cycles of 94! for 1 min, 55! for 1 min and 72! for 2 min, and last step for 5 min at 72!. Amplified products were analysed using 5% polyacrylamide gel. Electrophores was carried out for 1 hr at 199 volts. The gel along with the DNA sample was stained with eithidium bromide (10 µg/10 ml) for 40-45 min. Gel was visualized on UV-transilluminator and image was observed on the computer screen.

SSR assay and linkage analysis :For the SSR assay, 40 SSR primer pairs of 112 microsatellite markers (SSRs) derived from Cornell SSR linkage map (3) was tested on JNPT 89 and IR64, which showed polymorphism between the parental DNAs. A total of 40 SSR primer pairs were analyzed for the population. Test for QTL association with traits was performed by single marker approach. The single marker analysis, t-test was followed to find out the significant association between traits and the markers. Single marker analysis, t-test was calculated for each of the phenotypic traits with all the marker classes. The potential relationship between the marker and trait was established considering the significance of the t-test. It was found that a single marker was related with many traits and a single trait related to many markers.

Data Scoring *:* The female parent band was scored as 'A' while male parent band was scored as 'B', the bands of individual RIL lines were scored either as A or B depending on its position like female and male parent, respectively. The bands other than A and B were termed as E. Test for QTL association with traits was performed by single marker approach. The single marker analysis, t-test was followed to find out the significant association between traits and the markers.

Results and Discussion

The performance of the RILs lines and their parents JNPT 89 and IR 64 in irrigated condition for panicle associated traits is tabulated in table 1. Analysis of variance revealed significant differences ($P < 0.01$) between the two parental

lines in all panicle related traits assessed in the current study. Therefore, it could be expected that the RILs population derived from the cross between the two parents would be suitable for mapping of the QTLs for panicle traits. An approximate normal distribution was observed for phenotypic performance of the traits. A wide variation in the performance of the RIL lines for all traits was observed. However, the performance of parents varied considerably for number of filled grains per panicle, total number of spikelets per panicle and panicle weight per plant while the magnitude of the variation was less in panicle length and grain yield per plant. Maximum variation was observed for total number of spikelets per panicle, while panicle length had the least.

Phenotypic correlation *:* Phenotypic correlations were conducted among the evaluated panicle related traits based on means. The traits with the highest positive significant correlations were found between number of filled spikelets per panicle and number of spikelets per panicle (0.8802) (Table-2).

SSR polymorphisms *:* Polymorphism is recognized as a measurement for genetic diversities between the breeding parents. In this study, a total of 112 SSR markers were used to detect the polymorphism between the parents with 40 SSR markers that showed polymorphism (35.7%). The results show that the rate of polymorphism is lower than generated in the interspecific and inter subspecific crosses, where the polymorphism ranged from 59.6%-90% as reported in some previous studies (4,5,6). The reason for the low polymorphism might be explained that the parents used in this study have higher genetic similarities. The selected 40 polymorphic SSR markers were employed to genotype the F_{10} RIL population (Fig. 1 and Fig. 2).

Table 1. List of RILs showing transgressive segregants for panicle associated traits

Trait	Transgressive Segregants				Parental Value	
	Highest		Lowest			
	RIL Number	Value	RIL Number	Value	JNPT 89	IR64
PL	RIL99	29.55	RIL60	21.20	28.30	25.25
FSN	RIL81	280.30	RIL31	94.05	301.50	126.95
TNS	RIL91	413.40	RIL101	128.61	395.00	135.10
PWPP	RIL91	49.75	RIL102	17.60	31.60	37.10
GYPP	RIL77	47.00	RIL3	9.50	34.85	31.12

Table 2. Estimation correlation coefficient between panicle associated traits in RILs

Character	PL	FSN	TNS	PWPP	GYPP
PL	**1.0000**	0.2049**	0.1693*	0.0804	0.0098
FSN		**1.0000**	0.8544**	0.4793**	-0.0691
TNS			**1.0000**	0.5968**	-0.1661*
PWPP				**1.0000**	0.0720
GYPP					**1.0000**

PL-Panicle Length, FSN- Filled Spikelets Number Per Panicle, TNS-Total Number of Spikelets Per Panicle, PWPP-Panicle Weight Per Plant, GYPP- Grain Yield Per Plant.

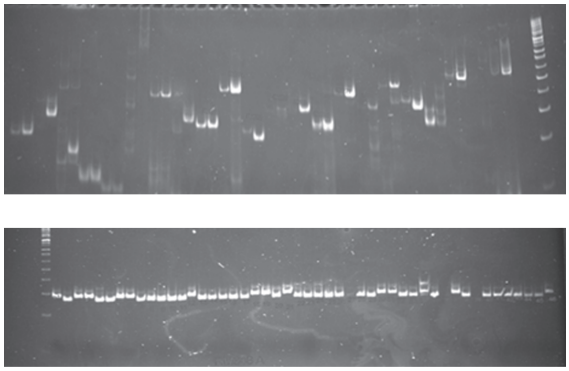

Fig. 1. Gel picture of parental polymorphism

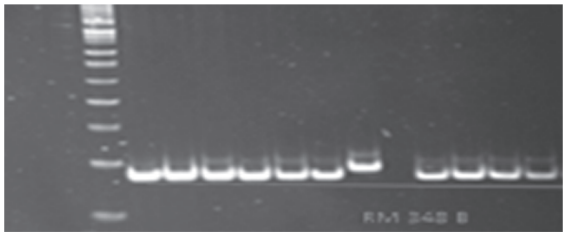

Fig 2. Gel picture of RM 348 used in RIL population

Construction of framework map using SSR markers *:* A total of forty four polymorphic SSR markers evenly distributed on the 12 chromosomes were used for construction of the linkage map with the RIL population. Map order was in agreement with that provided by McCouch *et al.* (2002). Segregation distortion in the population was tested by the X^2 statistics. Such distorted segregations in mapping populations have been frequently reported earlier (7,8).

Association of molecular markers with Panicle traits and QTL analysis : While maximum number of QTLs were identified for total number of spikelets (24), minimum were observed for panicle weight per plant (3) and none of the QTLs were identified for panicle length and grain yield per plants. The QTLs were identified on all the twelve chromosomes presented in Table 3 and Fig 3. Filled spikelets per panicle exhibited association with twenty markers viz., RM348, RM341, RM231, RM248, RM279, RM312, RM16, RM337, RM495, RM517, RM552, RM206, RM212, RM269, RM190, RM164, RM208, RM247, RM474 and RM250. All twelve chromosomes except chromosome number 6 showed association for filled spikelets per panicle (4, 5, 6, 9, 10). All chromosomes contained QTLs for total number of spikelets per panicle. Total number of spikelets per panicle showed association with RM235, RM348, RM341, RM231, RM248, RM224, RM312, RM16, RM337, RM495, RM206, RM212, RM269, RM204, RM553, RM221, RM190, RM164, RM208, RM247, RM474 and RM250. This result is in agreement with the finding of QTLs for total number spikelets per panicle on chromosome 1, 6 and 9 (11) and on chromosome 1, 2, 3, 9 and 12 by using an advanced backcross population between *Oryza rufipogon* (IRGC 105491) x Jefferson (6).

Panicle weight per plant had association with RM 312, RM 553 and RM 224 located on chromosome 1, 9 and 11 at locus 71.6 cM, 76.7 cM and 120.1 cM respectively. Pursuing high grain

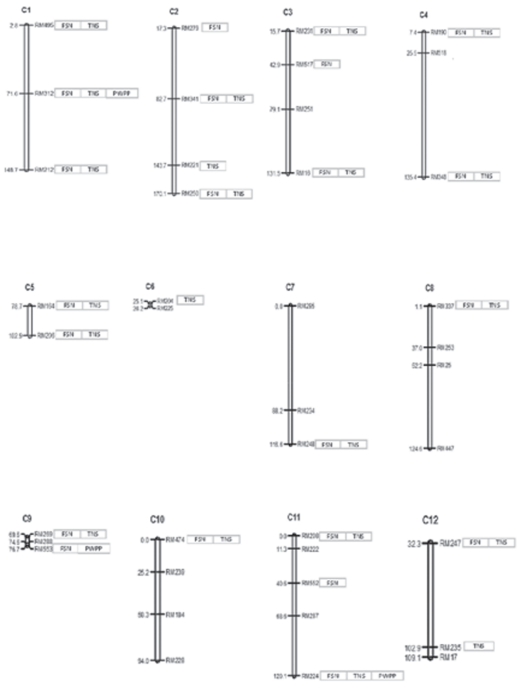

Fig. 3. Molecular linkage map showing the position of filled spikelets per panicle (FSN), total number of spikelets per panicle (TNS) and panicle weight per plant (PWPP) QTLs identified in RIL population.

Table 3. Single marker analysis (t-test) of SSR primer for RILs

S.No.	Markers	PL	FSN	TNS	PWPP	GYPP
1	RM235	1.79	1.93	2.06*	-0.28	0.05
2	RM348	0.66	4.86**	3.78**	0.22	-1.92
3	RM341	0.26	3.85**	3.34**	0.89	-0.62
4	RM231	1.04	3.25**	2.66**	1.27	-1.46
5	RM248	-0.55	2.66**	2.74**	1.27	-0.96
6	RM224	0.61	3.30	3.78**	2.92**	-1.48
7	RM279	0.42	2.69**	1.66	0.46	-1.01
8	RM312	1.10	3.55**	3.15**	2.44*	-0.16
9	RM16	-2.63	2.15*	2.13*	0.54	-1.63
10	RM337	0.08	2.17*	3.12**	0.97	-2.42
11	RM495	-0.09	2.86**	2.83**	-0.70	-1.91
12	RM517	-2.12	2.58**	1.87	1.60	-0.94
13	RM552	0.50	2.32*	1.36	0.37	0.05
14	RM206	-0.63	2.19*	2.37*	0.57	-1.17
15	RM212	0.45	4.10**	4.03**	1.92	-0.54
16	RM269	-1.08	2.11*	2.76**	0.27	-1.56
17	RM204	1.07	1.55	2.16*	-1.42	-0.88
18	RM190	1.34	3.88**	3.99**	1.36	-2.89
19	RM553	-0.75	0.91	2.46*	2.85**	-0.74
20	RM221	0.27	1.44	3.07**	1.15	-1.77
21	RM164	-1.89	2.09*	2.74**	0.69	-0.98
22	RM208	-1.05	3.38**	3.33**	0.33	-1.92
23	RM247	-0.37	2.10*	2.13*	-0.51	-1.30
24	RM474	1.56	3.01**	3.41**	-0.48	-3.62
25	RM250	1.28	3.15**	2.61**	0.82	-0.95

* = Significant at 0.05 probability level ** = Significant at 0.01 probability level

yield is one of the most important objectives in rice production. The genetic bases of number of filled spikelets per panicle and total number of spikelets per panicle have received much attention because of their importance in rice breeding. RM 224 and RM 312 showed association with filled spikelets per panicle and total number of spikelets per panicle and panicle weight per plant. The congruence of the QTL loci on the chromosome for various traits may be due to either linkage or pleiotropism. This signifies the plural selection efficiency by selecting markers closely associated with these traits. Since the direction of the additive effect of the QTL was also in the same direction, selection if exerted would be very effective. QTL markers could be fine mapped and made use of for detecting the complex traits like yield and its other contributing traits. In the present study, RIL population should be tested for detecting QTLs across different environments for the stability of QTL for yield and yield attributing traits. Location specific QTLs can be used for selection at specific locations and to develop better genotypes.

In the conclusion, the detection of new QTLs associated with panicle traits should be useful for rice yield improvement in the future. After validation of all tightly linked flanking markers for these QTLs, these markers could potentially be routinely used by breeders for marker assisted selection programs.

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