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Identification of Yield Improving Quantitative Trait Loci Alleles from *Japonica* x *Indica* Sub-Species in Recombinant Inbred Lines Rice (*Oryza sativa* L.)

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A B S T R A C T

Rice recombinant inbred lines (RILs) of *Japonica* x *Indica* cross were grown in irrigated conditions for identification of quantitative trait loci (QTLs) for yield associated traits. One hundred twenty one RIL lines were evaluated in the field environment. 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. In total, nine QTLs were identified for yield attributing traits. The number of QTLs were one to six detected in different yield attributing traits, whereas phenotypic variance ranged from 27.27 % - 66.46 % among the yield and yield attributing traits. They were located on chromosome 3, 7, 10 and 11. Six QTLs were identified for total tillers per plant, two QTLs for total number of spikelets per panicle and one QTLs for number of filled spikelets per panicle. The Loci for number of filled grains per panicle, qFSN 11-1 were increased 39.15 numbers filled grains respectively. Moreover, phenotypically these characters have more association with each other. Hence these markers may be useful for marker assisted breeding programme.

Introduction

Rice is the staple food for most of the people in the world. With the increasing world population, rice yield is still a hot topic in rice breeding despite the increasing grain yield after the green revolution (Ashikari *et al.*, 2005; Song *et al.*, 2007). Yield needs to be continuously improved. Different breeding strategies such as introduction, selection, recombination breeding, and heterosis breeding have been used to increase the yield of rice. Yield is a complex trait governed by several quantitative trait loci and genes, and influenced by genetic context and environment. Traits such as plant height, days to flowering, number of tillers and panicle number, spikelet fertility, grain number, and grain weight which are themselves complex traits and contribute to overall yield have also been map identifying QTLs followed by fine mapping and gene discovery (Bai *et al.*, 2012). Many yield-related QTLs/genes have been mapped and cloned in rice in recent years. For example, Moc1 and IPA1 regulate tiller number (Jiao *et al.*, 2010; Li *et al.*, 2003), Gn1a, APO1, and DEP1 regulate grain number (Ashikari *et al.*, 2005; Huang *et al.*,

2009; Ikeda *et al.,* 2007). Ghd7, which encodes a CCT domain-containing transcription factor was reported to regulate rice grain yield, plant height, and heading date (Xue *et al.,* 2008). However, yield traits are quantitatively inherited and their genetic basis is thus explained only in part by these cloned genes. Hence, it is necessary to detect QTLs/genes associated with yield traits using more populations in order to understand their genetic bases well. However, the related subspecies of *oryza sativa* such as *japonica* carry many favourable alleles which can be used for improvement of India.

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 sub specific derivatives is useful for rice improvement. Identification of favourable alleles in *japonica/indica* will play way to marker assisted mobilization of theirs allele in a genetic background to break genetic barrier to yield.

The objectives of present study were to identification of QTLs and map genomic regions influencing yield attributing traits using phenotyping data from a recombinant inbred population generated by inter sub specific cross between JNPT 89 (*Japonica x Indica)* with IR 64 (*Indica)* highly adopted 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 yield and yield attributing traits. Bernier *et al.,* (2008) also report to used selective genotyping for QTL detection.

The RILs lines 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 days seeding of each genotype was 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 a raise a good crop. Observations were recorded on randomly selected five plants from each genotype line in each replication at maturity. These plants were harvested and threshed separately. The observations of yield and yield attributing characters were recorded as per standard procedure.

DNA preparation

Preparation of genomic DNA from the parents and RILs lines followed the mini prep method. The extracted DNA content was quantified and parental polymorphism studies done through 112 SSRs 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 ul of each forward and reverse primer, 0.5 ul Taq DNA polymerase. PCR Amplification was performed with the following steps: predenaturing at 94° for 4 min, followed by 35 cycles of 94° for 1min, 55℃ for 1min, and 72° for 2 min, and last step is 5min at 72℃.

Amplified products were analyzed using 5% polyacrylamide gel. Electrophores is done for 1hr at 199 volts. The gel along with the DNA sample then stained with Eithidium bromide (10 µg/10ml) for 40-45 mins. Gel was visualized on UV- transilluminator and image can be seen in computer.

SSR assay and linkage analysis

For the SSR assay, 40 SSR primer pairs of 112 micro satellite markers (SSRs) derived from Cornell SSR linkage map (McCouch *et al.,* 2002) tested on JNPT 89 and IR64, showed polymorphism between the parental DNAs. A total of 40 SSR primer pairs were analyzed for the population. QTLs were identified using QTL Cartographer 2.5 with a threshold LOD of 3 QTLs for yield and yield attributing traits were identified using Interval Mapping (IM).

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.

Results and Discussion

Analysis of variance revealed significant differences ($P < 0.01$) between the two parental lines in all yield 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 yield and yield attributing traits.

Trait variation in RILs

The two parents JNPT 89 and IR 64, showed highly significant differences in the yield attributing traits specially 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. An approximate normal distribution was observed for phenotypic performance of the traits. Transgressive segregation was observed for yield attributing traits (Table 1).

Phenotypic correlation

The grain yield had positive and significant correlation with panicle index (0.776**), harvest index (0.729**), biological yield per plant (0.298**), number of tillers per plant (0.254**), plant height (0.212**), culm height (0.198**), panicle weight per plant (0.156**), test weight (0.150**) and panicle length (0.106*), whereas it had negative significant association with days to maturity (-0.116*) and number of unfilled spikelets per panicle (-0.106*) (Table 3).

SSR polymorphisms

Polymorphism is recognizes as a measurement for genetic diversities between the breeding parents. In this study total 112 SSR markers were used to detect the polymorphism between the parents which are 40 SSRs to show polymorphism (35.7%). The results shows that the rate of polymorphism is lower than generated in the interspecific and inter sub specific crosses, which the polymorphism ranged from 59.6% -90% reported by some previous studies (Moncada *et al.,* 2001: Septiningsih *et al.,* 2003 and Thomson *et al.,* 2003). The reason for the low polymorphism might be explained that the parents used in this study have higher genetic similarities. The selected 44 polymorphic SSR markers were employed to genotype the F_{10} RIL population (Figs. 1 and 2).

Construction of framework map using SSR markers

A total of fourty four polymorphic SSR markers evenly distributed on the 12 chromosome 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, tested by the X^2 statistic. Such distorted

segregations in mapping populations have been frequently reported (Harushima *et al.*, 2002; Xu *et al.*, 1997).

Yield attributing traits and QTL analysis

In total nine QTLs were identified, using the QTL approach of simple interval mapping (SIM). The number of QTLs were one to six detected in different yield attributing traits, whereas phenotypic variance ranged from 27.27 % - 66.46 % among the yield and yield attributing traits.

The QTLs were mapped on chromosome 3, 7, 10 and 11 (Table 2).

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

Traits	Mean	Range Lowest	Range Highest	h^2 (Broad Sense)
Days to 50% flowering	95.22	72.50	114.50	98.89
Days to maturity	125.22	102.50	144.50	98.89
Culm Height	76.16	56.10	133.20	92.29
Panicle Length	25.73	21.00	31.20	56.16
Plant Height	101.89	81.80	160.10	91.12
Tillers/ Plant	6.62	2.90	12.75	89.42
Filled Grains/ Panicle	168.04	90.05	291.70	93.47
Un-filled Grains/ Panicle	50.98	5.51	236.50	96.54
Spikelets/ Panicle	219.44	121.31	452.40	97.39
Panicle weight/ plant	26.78	16.25	53.30	92.56
1000 Grain weight	24.77	18.80	31.45	94.90
Biological Yield/ plant	57.53	36.05	79.90	81.26
Grain yield/ plant	25.99	8.40	49.15	85.02
Panicle Index %	100.70	35.92	235.43	82.78
Harvest Index %	45.90	16.18	90.06	98.36

List of RILs showing transgressive segregants for yield attributing traits						
	Transgressive Segregants				Parental Value	
	Highest		Lowest			
	RIL Number	Value	RIL Number	Value	JNPT 89	IR64
TT	RIL14	12.75	RIL9	2.90	3.70	12.40
FSN	RIL64	291.70	RIL31	90.05	290.50	120.95
TNS	RIL91	452.40	RIL101	121.31	376.00	127.10

Table.2 QTLs for yield and yield attributing traits in JNPT89/IR64- RIL population identified by simple interval mapping (QTL Cartographer v. 2.5)

QTL	marker interval	Chromosome	Position cM	Add effect	LOD	R^2 (%)
Total tillers per plant						
q TT 3-1	*RM231 - RM 517*	3	18.01	-1.68	4.40	48.44
q TT 3-2	*RM517 - RM 251*	3	45.21	-1.82	5.01	57.40
q TT 7-1	*RM234- RM 248*	7	108.21	-1.73	6.37	51.26
q TT 10-1	*RM474 - RM 239*	10	0.01	-1.26	3.47	27.27
q TT 11-1	*RM208 - RM 222*	11	6.01	-1.30	3.30	29.26
q TT 11-2	*RM552 - RM 287*	11	58.61	-1.83	3.85	57.15
Fillled spikelets numbers per plant						
qFSN11-1	*RM552- RM 287*	11	54.61	39.15	3.20	66.46
Total number of spikelets per plant						
qTNS 3-1	*RM517- RM 251*	3	53.21	43.46	3.36	38.83
qTNS 7-1	*RM234- RM 248*	7	112.21	38.74	3.45	30.70

Table.3 Phenotypic correlation

Character	DTM	CH	PL	PH	NOT	NOFG	NOUS	NOS	PWPP	TGW	BYPP	PI%	HI%
DTM	**1.0000**	-0.2961**	0.0138	-0.2783**	-0.3709**	0.3992**	0.4134**	0.4692**	0.3963**	-0.0084	0.0005	-0.3389**	-0.1446**
CH		**1.0000**	0.114*	0.975**	0.0808	0.118*	-0.2116**	-0.0214	0.0256	0.0673	0.0600	0.158**	0.194**
PL			**1.0000**	0.3293**	-0.0220	0.1450**	-0.0209	0.0880	0.0219	0.1593**	-0.0933	0.0660	0.1323**
PH				**1.0000**	0.0719	0.1448**	-0.2058**	-0.0009	0.0292	0.0991	0.0364	0.1648**	0.2134**
NOT					**1.0000**	-0.5914**	-0.5105**	-0.6582**	-0.2134**	-0.0989	0.1038*	0.3484**	0.1651**
NOFG						**1.0000**	0.4315**	0.8802**	0.5379**	0.1380**	0.0060	-0.3269**	-0.0115
NOUS							**1.0000**	0.7992**	0.4978**	0.0195	0.0747	-0.3555**	-0.1974**
NOS								**1.0000**	0.6146**	0.0968	0.0404	-0.4046**	-0.1093*
PWPP									**1.0000**	0.0324	0.0784	-0.4443**	0.0693
TGW										**1.0000**	0.0439	0.0920	0.0899
BYPP											**1.0000**	0.1851**	-0.3520**
PI%												**1.0000**	0.6262**
HI%													**1.0000**
GYPP	**-0.1163****	**0.1980****	**0.1057***	**0.2115****	**0.2538****	**0.0068**	**-0.1058***	**-0.0535**	**0.1566****	**0.1503****	**0.2985****	**0.7764****	**0.7296****

DTM-Days to Maturity, CH- Culm Height, PL-Panicle Length, PH - Plant Height, NOT- Number of Total Tiller, NOFG-Number of Filled Grains, NOUS-Number of Unfilled Grains, NOS-Number of Spikelets Per Panicle, PWPP-Panicle Weight Per Plant, TGW-Total Grain Weight, BYPP-Biological Yield of Per Plant, PI-Panicle Index, HI- Harvest Index

Fig.1 Gel picture of parental polymorphism

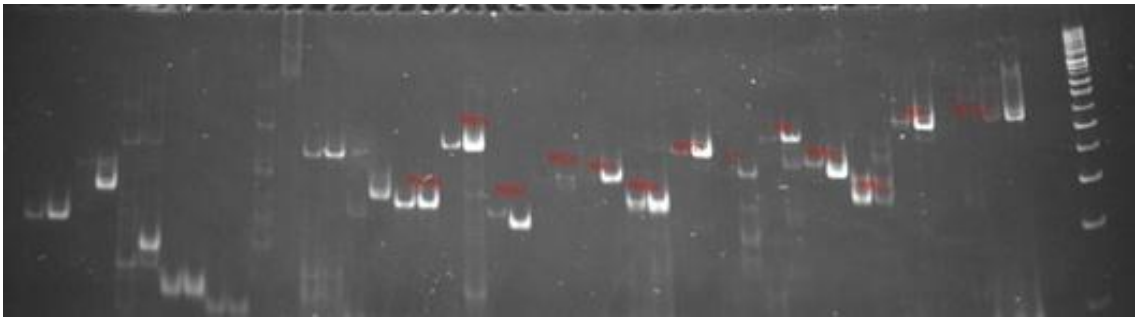


Fig.2 Gel picture of RM 348 used in RIL population

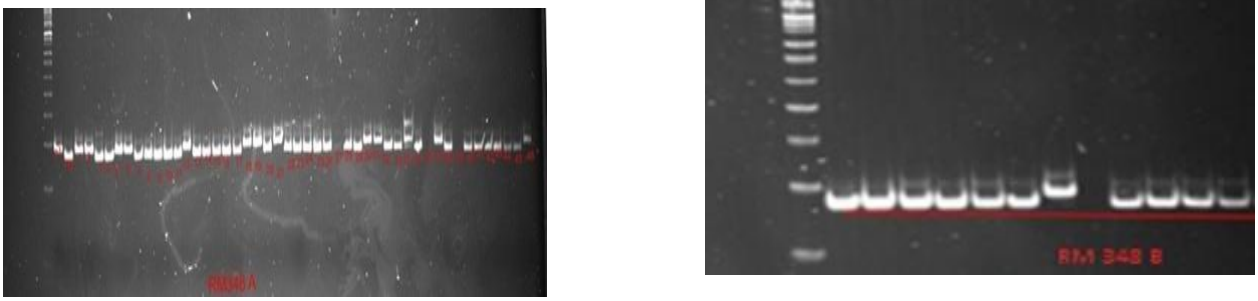
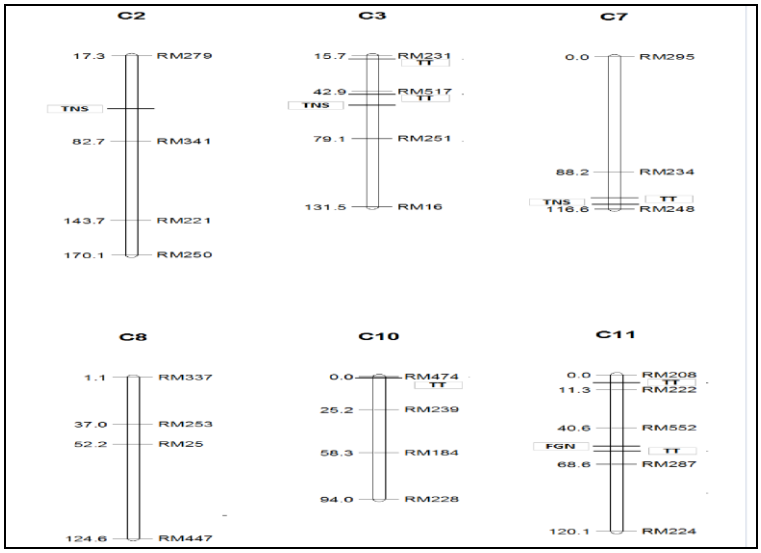


Fig.3 Molecular linkage map showing the position of filled spikelets per panicle (FSN), total number of spikelets per panicle (TNS) and panicle weight per plant (PWPP) QTLs identified in RIL population



In the RILs population of JNPT 89 x IR 64 for yield and yield attributing traits, six QTLs were identified for total tillers per plant followed by two QTLs for total number of spikelets per panicle and one QTLs for number of filled spikelets per panicle. Out of nine QTLs identified from which two QTLs had LOD score more than 5.0, while others seven QTLs had LOD score more than 3.0.

Total tillers per plant

Six QTLs were distributed on four chromosomes (3, 7, 10 and 11). The QTL on chromosome 7 (RM 234 – RM 248) has highest peak LOD 6.37 and phenotypic variance 51.26%. Two QTLs identified on chromosome 3, located on the region between RM 231 – RM 517 and RM 517 – RM 251. The region between RM 517 – RM 251 associated with higher LOD score (5.01) and phenotypic variance (57.40%) with its position more near to RM 517. One QTL was identified on chromosome 10 with 3.47 LOD and phenotypic variance 27.27%. Two QTLs identified on chromosome 11 (RM 208 – RM 222 and RM 552- RM 287). The additive effects of three QTLs were negative.

Filled spikelets per panicle

One putative QTLs (qFSN 11-1) were identified for filled spikelets per panicle on chromosome 11 with 3.20 LOD and phenotypic variance 66.46%. The Loci for number of filled grains per panicle, qFSN 11-1 were increased 39.15 numbers filled grains respectively.

Total number of spikelets per panicle

Two putative QTLs for total number of spikelets per panicle were located on chromosome 3 and 7 from which one QTL qTNS 3-1 and qTNS 7-1 located on chromosome 3 and 7 with 3.36 and 3.45 LOD and phenotypic variance 38.83% and 30.70% respectively. It was also observed that the QTLs for total number of spikelets per panicle viz., qTNS 3-1 and qTNS 7-1 were increased with 43.46 and 38.74 numbers respectively (Fig. 3).

QTLs for grains per panicle reported on chromosomes 1, 2, 3, 4, 5, 6, 9, 11, and 12 (Brondani *et al.,* 2002; Marri *et al.,* 2005; Moncada *et al.,* 2001; Septiningsih *et al.,* 2003a; Thomson *et al.,* 2003; Xiao *et al.,* 1998). Marker interval RM 517 and RM 251 showed association with total tillers per plant, total number of spikelets per panicle. 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.

This suggested that populations derived from diverse parents in both phenotype and genotype were more chance to detect QTL. The accessions with contrasting phenotype characters are recommended as parents for developing the mapping population in future. Yield is a complex trait with a low heritability, which suggested yield was controlled by many minor QTLs frequently interacting with environments; and the LOD threshold value claiming a QTL in this study was higher stringent, leading to a failed detection of a small effect QTL. Basically, even though as yield component, few QTLs can be located in the same region of yield QTL (Hittalmani *et al.,* 2003; Fu *et al.,* 2010). Another reason is that yield components are often negative corrected. If one QTL or two closely linked QTLs involved such negative correlated trait, the genetics effect of these gene or genomic region on yield would cancelled each other, leading to a failed detection of QTL for yield.

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