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Identification and mapping of QTLs for agronomic traits in recombinant inbred line population derived from *Japonica X Indica* sub-species in Rice (*Oryza sativa* L.)

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Abstract
Rice (*Oryza sativa* L.) is one of the most important crops in the world, especially Asian countries. Genetics of important traits in rice for yield improvement have always been a major breeding objective. Agronomic traits are inherited quantitatively, so quantitative trait loci (QTL) mapping for the potential use of molecular markers would be very helpful to plant breeders in developing improved rice varieties. In this investigation, a SSR linkage map of rice was constructed using 40 polymorphic simple sequence repeat (SSR) markers. The mapping population of 121 F_{11} families derived from the cross JNPT89x IR64 (*Japonica x Indica*) was used for QTL mapping of agronomic traits. As many as 21 QTLs were detected to be associated with agronomic characteristics; some of them are being reported for the first time. The identified QTLs on specific chromosome regions explaining high phenotypic variance could be considered to use in marker-assisted selection (MAS) programs.

Keywords: rice, agronomic traits, QTL mapping and marker-assisted selection

Introduction
Rice (*Oryza sativa* L.) is the staple food for almost half of the world's population. Rice accounts for 23% of the world's supply of calories; about 90% of this is produced and consumed in Asia. Rice breeders are attempting to develop improved rice cultivars with higher grain yield, increased diseased resistance, increased abiotic stress tolerance and suitable agronomic traits. These traits are mostly quantitative and are controlled by many genes, each of which has a relatively small effect on the overall phenotype. Molecular markers technology help rice breeders to identify segments of chromosomes contain useful quantitative trait loci (QTL) on the linkage map of particular attribute (Tanksley, 1993; Yano and Sasaki, 1997) [15, 9].
Although, agronomic traits are easy to score quantitatively but, from the breeding point of view, genetic advance through phenotypic selection is less due to low heritability and complex quantitative traits could be very helpful to breeders. 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) [1].
Based on the chromosomal locations of QTLs, it is found that some of the QTLs detected in different studies share the same locus on the chromosome suggesting the existence of a common locus for the differentiation among rice varieties. Therefore, QTL analysis allows a comprehensive analysis of the genetic relationship among morphological, agronomic and physiological traits.
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 their allele in a genetic background to break genetic barrier to yield. The objectives of the present study were to identify QTLs governing agronomic traits sub specific cross between JNPT 89 x IR64. These QTLs could be further fine mapped and used to transfer into high yielding genotypes of rice.

Material and Methods
The genetic material for this experiment was involved F_{11} one hundred twenty-one

Recombinant Inbred lines developed in the cross of JNPT 89 & IR 64. JNPT 89 derive from (*Japonica x Indica) cross and* IR64 (*Indica)* highly adopted high yielding *Indica* rice variety 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 was followed to raise a good crop.

Data on agronomic traits

After being transferred in the field, plants from each F_{11} families were assessed for agronomic traits including culm height was measured in centimetres from ground level to the start of the panicle of the main culm, plant height (PH) in cm from the soil surface to the tip of the tallest panicle, panicle length, tillers were counted for each randomly selected plants at the end of active tillering stage. panicle length from its base to the apex in cm, panicle weight plant^{-1} (g**)** total weight in grams of panicle was recorded after two days of sun drying number of filled grains , weight of thousand filled grains in g, were measured following the guidelines Standard Evaluation System for Rice (IRRI 1996) [4].

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 nanopore 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 ethidium bromide (10 µg/10 ml) for 40-45 min. Gel was visualized on UV-transilluminator and image was observed on the computer screen.

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 (Moncada *et al*., 2001: Septiningsih *et al.,* 2003 and Thomson *et al.* 2003) [10, 7, 8]. 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_{11} RIL population.

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 Interval Mapping (IM).

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) [6] 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).

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) [6]. Segregation distortion in the population was tested by the X^2 statistics. Such distorted segregations in mapping populations have been frequently reported earlier (Harushima *et al*. 2002; Xu *et al.* 1997) [13, 3].

Association of molecular markers with agro morphological traits and QTL analysis

Identification of QTLs associated with economically valuable phenotypes is of particular interest as it becomes the basis for developing efficient strategies for genomic-based plant improvement. In rice, 8646 QTLs have been identified as of October 2009 (http://www.gramene.org) [11].

Analysis of variance revealed significant differences ($P < 0.01$) between the two parental lines in all agronomic 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 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 found.

Twenty-one controlling agronomic traits were placed on the linkage map (Table 1, Fig. 1). One QTL q DTF 8-1 identified for days to 50% flowering was mapped on chromosome 8 (RM 25 – RM 447) near to RM 447 with 5.71 LOD and 64.90 % phenotypic variance which indicate major gene. Ten putative QTLs for total tillers per plant were mapped on chromosome 2, 3, 7, 8, 10 and 11. Among these five were major genes and other five were major QTLs. The QTL on chromosome 2 is qTT 2-1 (RM 279 – RM 341) have peak LOD 7.03 and phenotypic variance (54.28%). The three QTLs viz., qTT 3-1, qTT 3-2 and qTT 3-3 were identified, between the region RM 231 – RM 517, RM 517 – RM 251 and RM 251 – RM 16. The QTL qTT 3-2 had higher LOD score 5.01 and phenotypic variance 52.88%. The three QTLs, qTT11-1, qTT11-2 and qTT11-3 were also identified between the region between (RM 208 – RM 222, RM 552 – RM 287 and RM 287 – RM 224). The QTL qTT11-3 have higher LOD score 4.88 and phenotypic variance 56.10 %.Three putative QTLs viz., qFSN 2-1, qFSN 2-2, qFSN 2-3 were identified for filled spikelets per panicle on chromosome 2 with 4.62, 5.77 and 3.47 LOD and phenotypic variance 55.79%, 67.27% and 52.69%.

QTLs for grains per panicle reported on chromosomes 1, 2, 3, 4, 5, 6, 9, 11, and 12 (Brondani *et al.* 2002 [2]; Marri *et al.* 2005 [5]; M; Septiningsih *et al*. 2003; Thomson *et al.* 2003; Xiao *et al.* 1998). Six putative QTLs for total number of spikelets per panicle were located on chromosome 2, 3, 7 and 8. Three QTLs viz., qTNS2-1, qTNS 2-2 and qTNS2-3 identified on chromosome 2 the region between (RM 279 – RM 341, RM279-RM341 and RM 341 – RM 221). QTL

qTNS 2-2 (RM 279– RM 341) have higher LOD 4.46 and phenotypic variance 56.56%. One QTL viz., qTNS 3-1, qTNS7-1 and qTNS 8-1 were located on chromosome 3, 7 and 8 with 3.34, 3.45 and 3.66 LOD and phenotypic variance 38.95%, 32.72% and 38.15%. The QTL Loci for total number of spikelets per panicle, qTNS 3-1, qTNS 7-1 and qTNS 8-1 were increased 45.44, 41.77 and 45.04 number total spikelets per panicle respectively.

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. Overlapping of QTLs in these regions could be either due to linkage of genes or pleiotropy.

The identified QTLs on specific chromosome regions explaining high phenotypic variance could be considered to use in marker-assisted selection (MAS) programs. Additional research using same markers, genetic background and environment is needed for further dissection of QTL overlapping. The challenge would be to develop a variety with increased NP, GW, WTFG and NFG by pyramiding the positive alleles from different sources through MAS.

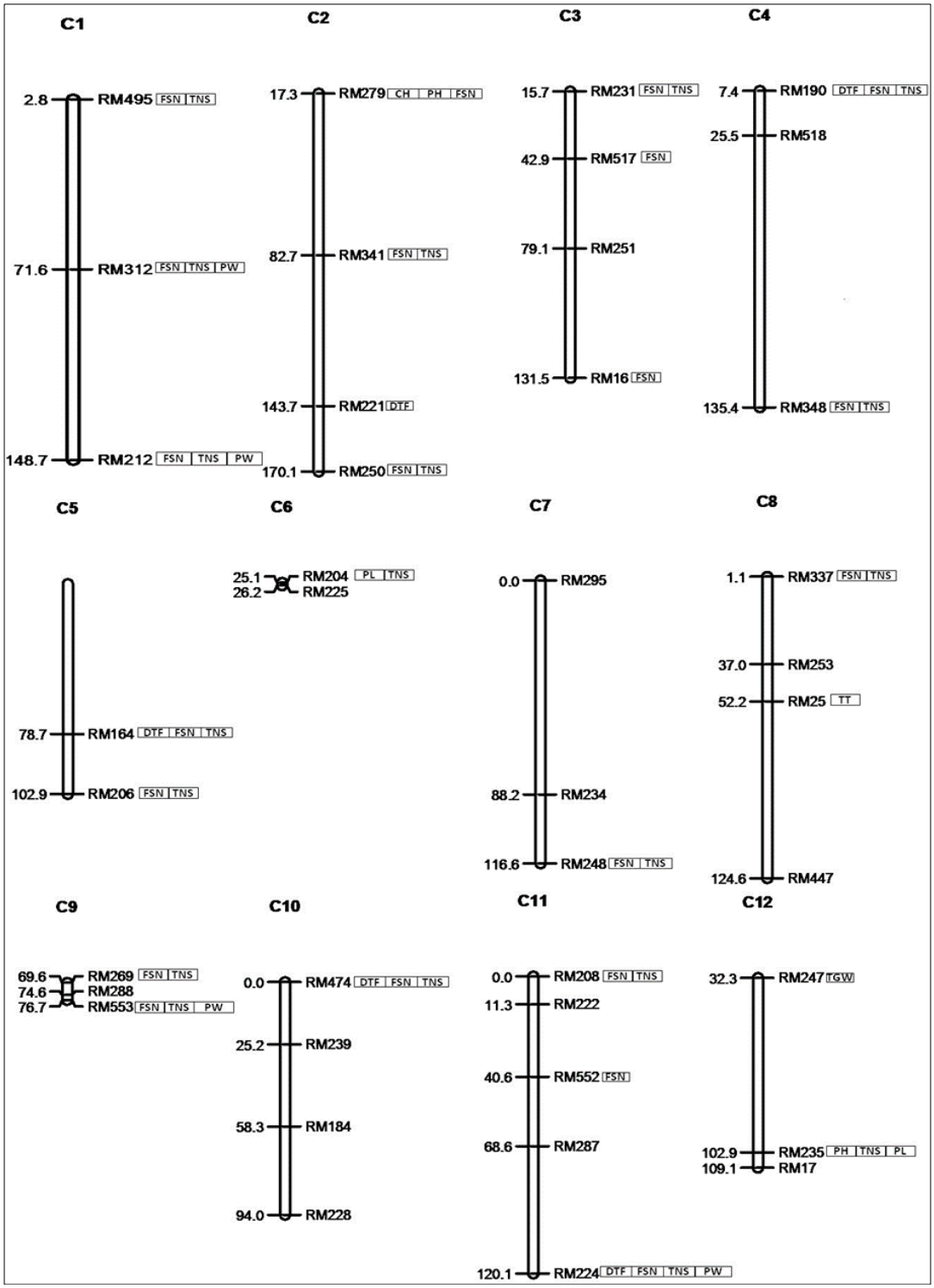

Fig 1: Mapping of QTLs DTF: days to 50% flowering, TT: total tillers per plant, FSN filled spikelets per panicle, TNS: total number of spikelets per panicle and HI: harvest index Significance threshold for interval mapping determined at LOD = 2.5

Table 1: QTLs identification for Agronomic traits in JNPT89/IR64 RIL population

QTLS	Marker interval	Chromosome	Position cM	Add effect	LOD	R^2 (%)
Days to fifty percent flowering						
q DTF 8-1	*RM25 - RM 447*	8	107.10	7.63	5.71	64.90
Total tillers per plant						
q TT 2-1	*RM279 - RM 341*	2	62.00	-2.07	7.03	54.28
q TT 3-1	*RM231 - RM 517*	3	18.00	-1.66	4.40	43.43
q TT 3-2	*RM517 - RM 251*	3	45.20	-1.83	5.01	52.88
q TT 3-3	*RM251 - RM 16*	3	79.40	-1.80	3.75	51.00
q TT 7-1	*RM234 - RM 248*	7	110.20	-1.76	6.33	48.45
q TT 8-1	*RM25 - RM 447*	8	115.10	-1.62	3.52	41.37
q TT 10-1	*RM474 - RM 239*	10	0.00	-1.33	3.47	27.36
q TT 11-1	*RM208 - RM 222*	11	4.00	-1.31	3.18	27.04
q TT 11-2	*RM552 - RM 287*	11	58.60	-1.89	3.85	55.19
q TT 11-3	*RM287 - RM 224*	11	86.60	-1.89	4.88	56.10
Fillled spikelets numbers per plant						
qFSN 2-1	*RM279 - RM 341*	2	58.00	38.13	4.62	55.79
qFSN 2-2	*RM341 - RM 221*	2	85.40	43.73	5.77	67.27
qFSN 2-3	*RM341 - RM 221*	2	130.40	41.34	3.47	52.69
Total number of spikelets per plant						
qTNS 2-1	*RM279 - RM 341*	2	58.00	54.90	3.67	51.61
qTNS 2-2	*RM279 - RM 341*	2	79.40	58.63	4.46	56.56
qTNS 2-3	*RM341- RM 221*	2	130.40	56.11	3.28	44.80
qTNS 3-1	*RM517- RM 251*	3	51.20	45.44	3.34	38.95
qTNS 7-1	*RM234 - RM 248*	7	112.20	41.77	3.45	32.72
qTNS 8-1	*RM25 - RM 447*	8	117.10	45.04	3.66	38.15
Harvest index						
qHI 8-1	*RM253 - RM 25*	8	43.90	11.98	2.34	43.87

DTF: days to 50% flowering, TT: total tillers per plant, FSN: filled spikelets per panicle, TNS: total number of spikelets per panicle and HI: harvest index

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