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QTL Hotspots Detected for Yield Contributing Traits in Rice (*Oryza sativa* L.) using Composite Interval Mapping Analysis

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QTL hotspots are the genomic regions influencing several traits by harboring important regulators. Therefore in the present study F_5 mapping population was used to map the novel genomic regions and genomic hotspots by composite interval mapping. In all 130 QTLs were identified for grain yield and its attributing traits. Out of 130 QTLs, 36 QTLs were major effects QTLs and 8 QTLs were found stable over the locations. We identified strong major effects QTL for flag leaf length (qFLL3.1) with 46% phenotypic variance. In this study 6 known QTLs (qph3.1, qnt3.1, qnt3.2, qTGW3-4, qTGW4-1, qPPP4-2) were also validated and co-localized in chromosome 3 and 4 along with currently identified QTLs genomic regions. These genomic regions consist, hotspots of 15 major and 23 minor effects QTLs, which encompasses >3000 genes. Selection for advantageous allele underlying major robust QTLs will be useful to break genetic barriers of yield to sustained food security.

Keywords: Environment, Markers, Quantitative traits, QTLs, Rice, Yield.

Rice (*Oryza sativa* L.) is one of the most important food crops worldwide and staple foods for more than half of the world's population including two billion Asians, and more than 70 percent Indians. It contributes 43 percent to the total food grain and 53 per cent to the cereal production and thus holds the key to sustain food sufficiency in the country (Siddiq *et al*. 2004). To meet the growing demand from human population which is expected to touch 9 billion by 2050, rice varieties with higher yield potential and greater yield stability need to be developed (Marathi *et al*. 2012). The major way to meet the projected production demand is to integrate the classical breeding techniques with modern biotechnological tools for rice improvement (Collard *et al*. 2008).

Yield and yield related traits are a complex in nature and governed by several minor genes, quantitative trait loci and affected by environmental factors. These traits also showed continuous variation in segregating populations. Rice varieties differ tremendously in the levels of grain yield, with immense variability in the combinations of component traits owing to the vast diversity of genetic constitutions. The inheritance of quantitative traits classically involves multiple genes, each having a small effect that is sensitive to environmental changes. These traits are known in general as having low heritability and thus have earned the reputation of being difficult to

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investigate. Presence of significant G × E interaction has been reported by comparing QTLs detected in multiple environments. The disappearance of QTLs detected in one environment in another has been considered a manifestation of G × E interaction and the detection of QTLs with consistent expression across environments is considered as stability indicator for the utilization of these QTLs in breeding program (Cho *et al*. 2007). However, the development of molecular marker, genome mapping, and QTL analysis technologies has greatly facilitated the investigation of genetic bases of quantitative traits for a single Mendelian genetic dissection study and can further clarify the genetic effects of their size and mode of action (Meng *et al*. 2012). This is important not only for an understanding of the genetic mechanism of agronomic traits in rice, but also for molecular marker assisted selection. In rice, researchers have constructed high-density genetic linkage maps based on restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) markers (McCouch *et al*. 2002). Mapping population's speciûcally designed for dissecting the genetic bases of yield traits via QTL mapping have been constructed, producing large amounts of data leading to the identiûcation of hundreds of QTLs for yield traits.

Based on the above, a study was planned to understand the genetic mechanism and molecular players of the traits associated with grain yield using molecular marker technology with objectives to map novel genomic regions and QTL hotspots influencing grain yield and its component traits by using the two locations phenotypic data of F_5 recombinant inbred population generated by new parental cross combination of Swarna and IR86931B-6 rice genotypes.

Experimental Materials and Methods

The experimental material for the present investigation comprised of 85 lines of F_5 population derived from Swarna (a high yielding Semi-dwarf widely adapted indica *variety*) with IR86931B-6 (Semi-tall, inter specific line derived from Nagina22).

Evaluation of F_5 Recombinant inbreed Lines

The F_5 RILs were evaluated with two replications in randomized block design under irrigated condition at two different locations in Raipur. The data for yield and yield contributing traits were recorded from three selected representative plants in all the genotypes in each location in replicates. The method adopted for recording observation for each of the fifteen characters is presented as: Seedling height (SH) at seedling stage was measured in cm from base to tip of leaf at 25 days after sowing (DAS). Total number of tillers per m^2 area (TT) were counted and recorded at maturity. Total number of Effective tillers (panicle bearing tillers) in per m^2 area (ET) were counted and recorded at the time of harvest. Plant height (PH) was measured in cm from base to tip of leaf on main tiller at harvest of the crop. Flag leaf length (FLL) of the main tiller was measured in cm at beginning of anthesis. Flag leaf width (FLW) of the main tiller was measured in cm at beginning of anthesis. Flag leaf area (FLA) was recorded by multiplication of FLL and FLW (FLA = FLL* FLW). Number of days taken from sowing to panicle emergence in 50 per cent of the population was recorded as days to fifty percent flowering (DFF). Panicle length was recorded as (PL) length of the primary panicle from the panicle base to tip was measured in centimeter and recorded. Panicle Weight (PW) was recorded as weights of 3 random panicles in grams at maturity. Total number of filled grains in each of 12 random panicles was counted and the mean was calculated and recorded as grain per panicle (GPP). Hundred grain weight (HGW) recorded as weight of 100 grains selected at random from each genotype in grams. Total number of filled and unfilled grains was counted from 12 randomly selected panicles and mean filled and unfilled grains per panicle were calculated to estimate the per cent fertile spikelets in a panicle. Harvest Index (HI) is the ratio between the grain yield and the total dry matter of the plant. Grain yield per m^2 (YLD), the weight of the dried (14% moisture) and cleaned grains from the plants grown in one $meter^2$ area were measured and expressed in grams.

Statistical analysis of the phenotypic data

Analysis of variance (ANOVA), test of significance of variance components were carried out as suggested by Panse and Sukhatme, (1967). Frequency distributions estimated and histograms were plotted for characters with the help of STATISTICA7 software (Sa 2007). All data was analyzed without any transformation.

The genetic parameters like genotypic

and phenotypic coefficients of variation (GCV and PCV), heritability in broad sense (h^2), expected genetic advance (GA), Coefficient correlation was calculated for all possible combination among the characters at genotypic, phenotypic and environmental levels were estimated with the help of SPAR 1 (Doshi and Gupta 1991). The significance of correlation coefficients was tested, against Fisher's table value (1936) for (g-2) degree of freedom at 5 % and 1 % level of significance, where g is the number of genotypes. The calculated (r) is then compared with table value of 'r' at 5% and 1% level of significance (Snedecor and Cochran 1967).

Genomic DNA isolation, polymorphism survey and genotyping of mapping population

For generating the genotypic data DNA was extracted from fresh leaf tissues as described by modified CTAB (Pervaiz *et al*. 2011) method with slight modifications, further quantification and diluted to final concentration of 50çg/ìl for PCR analysis. The polymerase chain reaction (PCR) was carried out in 96 well PCR plates obtained from

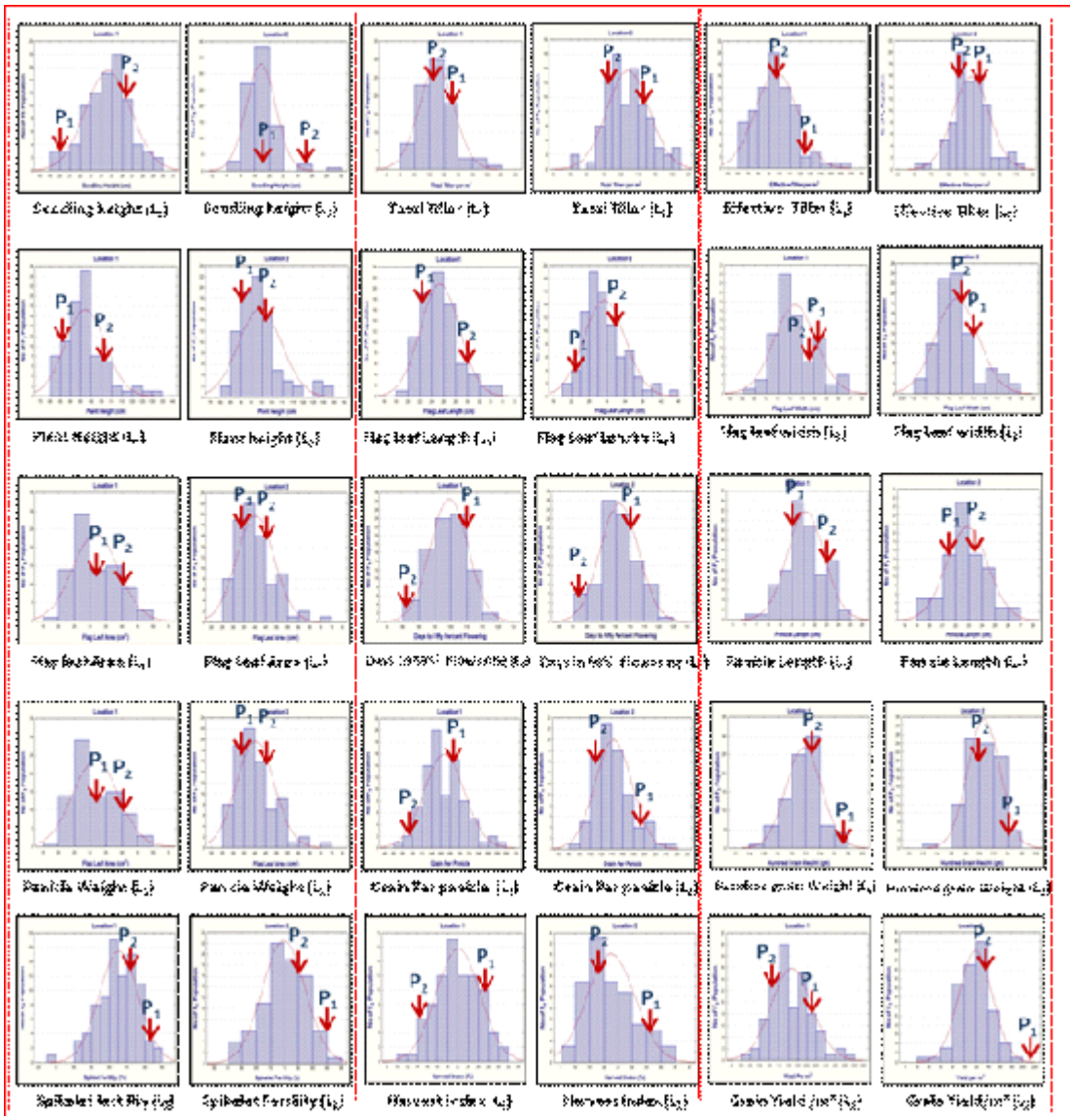


Fig. 1. Frequency Distribution of Yield and Yield Contributing Traits in Location1 and Location 2

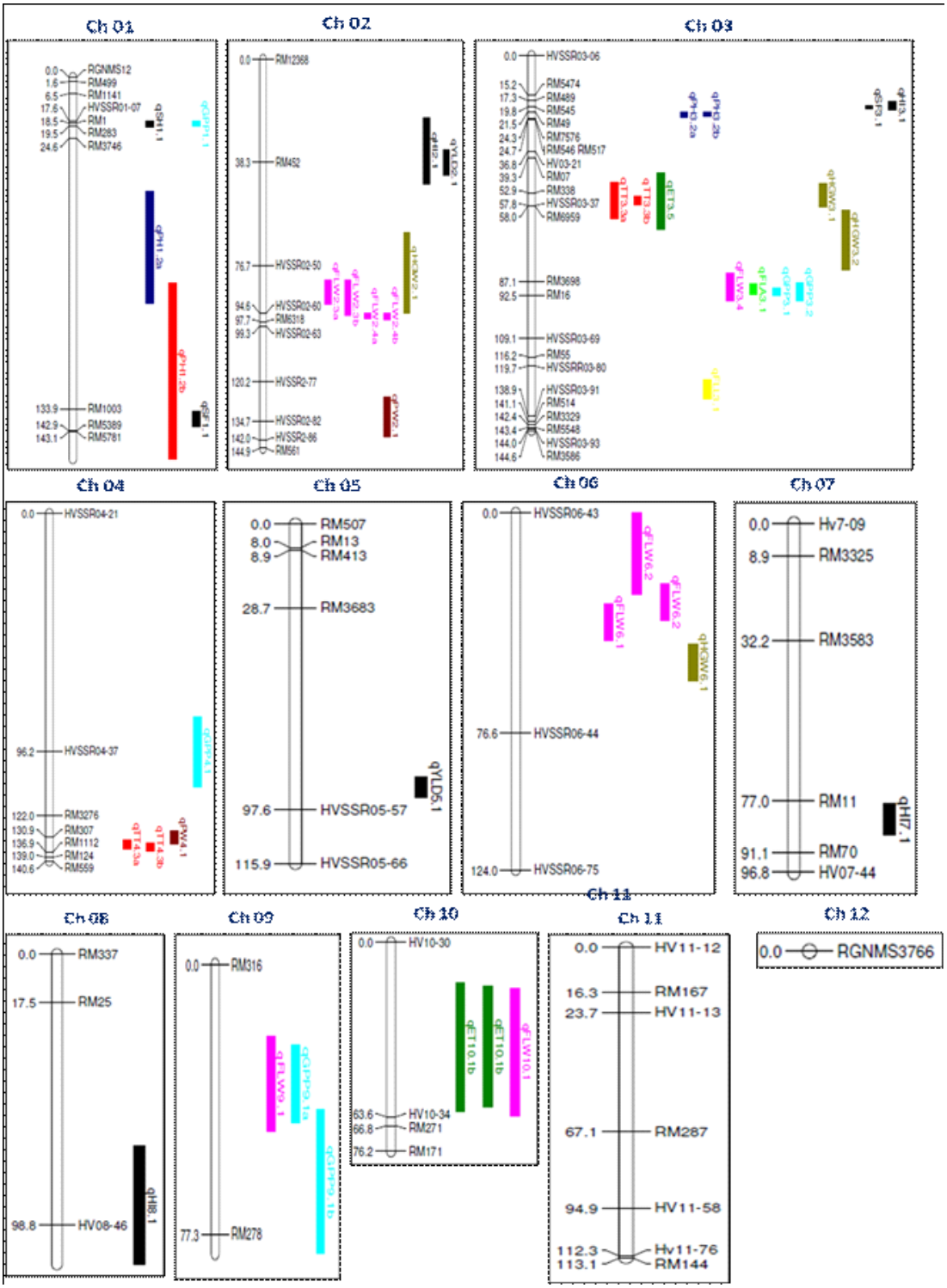
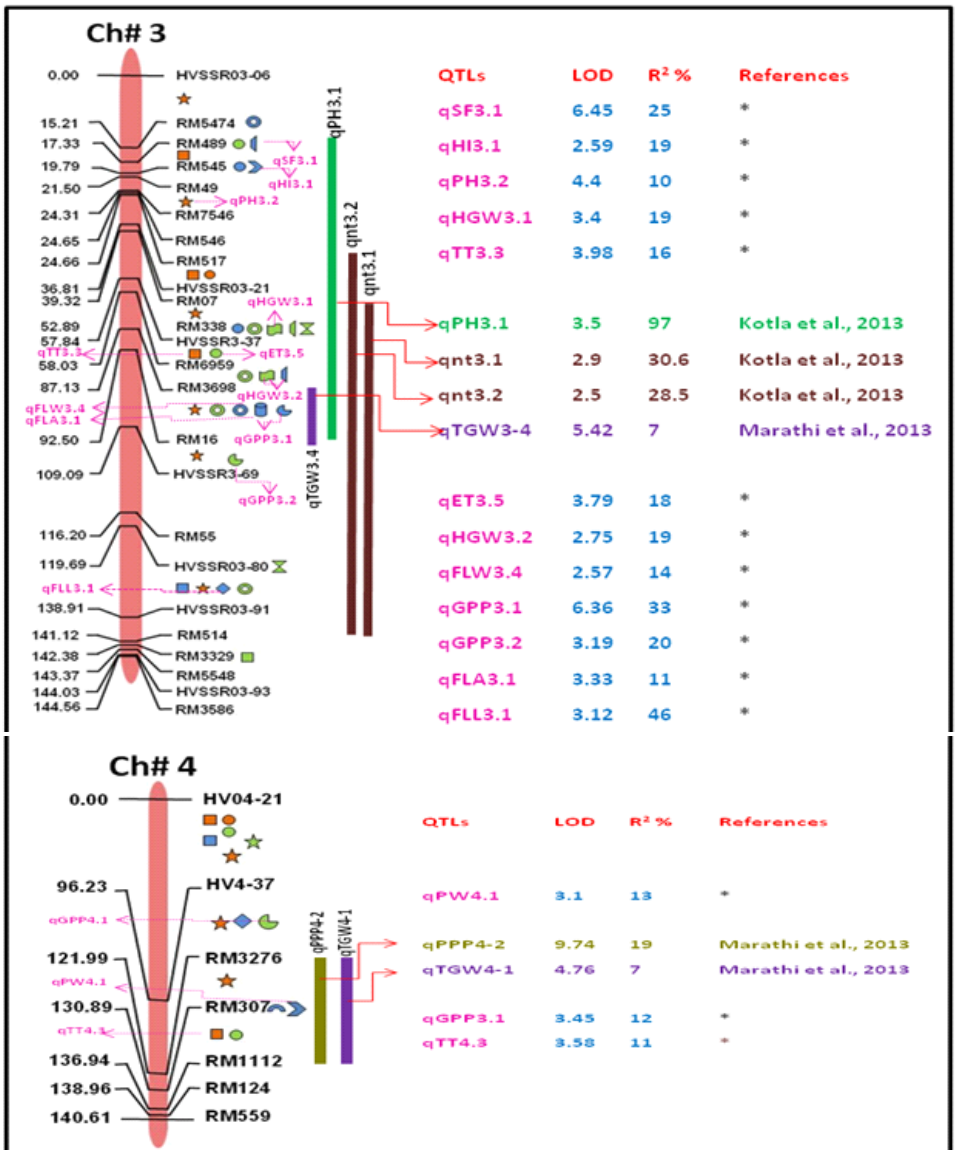


Fig. 2. Molecular linkage map showing the position of major effects QTLs for yield and yield contributing 13 traits in rice chromosomes



Pink color: Indicate identified Major effects QTLs. Validated QTLs were indicated by purple, green and brown color bar's: LOD score with R^2% of validated QTLs and identified major effects QTLs were presented by their respective color beside linkage map. (*: identified QTLs in present study)

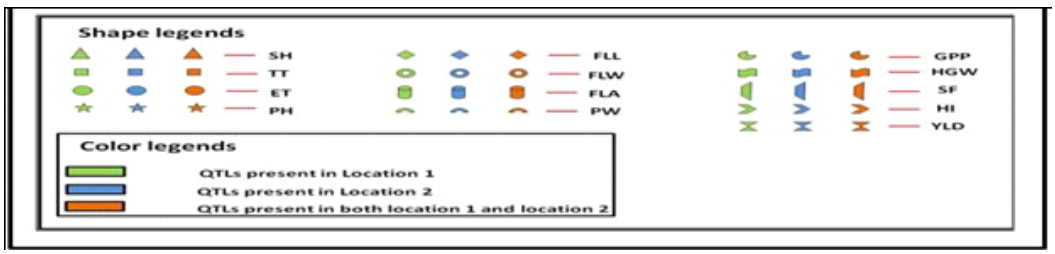


Fig. 3. Position of validated QTLs (qTGW3.4, qPH3.1, qnt3.1 and qnt3.2; qPPP4-2, qTGW4-1) in molecular linkage map of chromosome 3 in and 4 respectively along with the identified QTLs hotspots and major effects QTLs

Table 1. Trait means, range, standard deviation (SD), Coeficient of variance (CV %) and number of favourable transgressive segregants over better parent for yield and yield contributing traits

SN	Traits	Location 1								Location 2					
		Mean	Parent 1 Swarna	Parent 2 IR8693 1B-6	SD	Range Min	CV (%) Max	No of Transgressive Segregants	Mean	Parent 1	Parent 2 Swarna	SD IR8693 1B-6	Range	Min	Max	CV (%)	No of Transgressive Segregants
1	Seedling Height (cm)	28.68	20	26.5	2.20	19.50	29.90	10.23	15.00	18.82	19.1	25	2.05	14.01	28.20	7.57	2.00
2	Total Tiller per m2	109.65	134.33	102.6	24.26	68.33	197.83	7.00	10.00	127.92	140.8	106.33	24.85	61.67	183.33	5.13	23.00
3	Effective Tiller per m2	96.91	126.66	86.5	20.43	62.33	160.33	7.72	6.00	116.38	128.4	103.33	21.45	51.67	175.00	5.29	21.00
4	Plant Height (cm)	97.01	86.58	106.5	9.82	81.83	131.67	4.67	10.00	99.10	90	103	10.55	82.50	133.50	5.33	14.00
5	Flag Leaf Length (cm)	25.38	22.5	30	3.26	19.33	34.84	7.05	12.00	29.35	23	32	4.62	20.83	44.33	6.56	20.00
6	Flag Leaf Width (cm)	1.24	1.43	1.37	0.18	0.86	1.66	3.90	13.00	1.36	1.5	1.4	0.18	1.05	1.85	4.66	13.00
7	Flag Leaf Area (cm)	31.60	32.18	41.1	7.18	19.27	48.58	8.66	11.00	39.95	34.5	44.8	8.64	28.78	71.77	8.62	20.00
8	Days to 50% Flowering	104.45	110	92	5.94	91.50	117.50	4.19	03.00	106.09	110	94	6.71	92.16	124.67	3.02	04.00
9	Panicle Length (cm)	21.15	20	23	1.56	16.67	24.27	4.33	14.00	22.84	21.5	24.5	1.60	19.17	27.00	5.62	10.00
10	Panicle Weight (gm)	4.77	5.23	4.12	0.45	2.98	5.97	5.59	11.00	4.85	5.83	4.48	0.52	3.61	6.30	5.44	3.00
11	Grain per Panicle	166.49	185	95	42.63	76.50	290.33	12.58	28.00	142.21	195	116	34.04	70.75	247.39	4.65	8.00
12	100 Grain Weight (gm)	2.17	2.74	2.3	0.25	1.49	2.75	8.27	1.00	2.06	2.41	2	0.23	1.31	2.60	5.36	3.00
13	Spikelet Fertility (%)	70.08	88	77	10.99	32.96	93.66	11.17	2.00	74.60	90	80	7.55	55.20	90.80	6.43	1.00
14	Harvest Index (%)	21.99	27.5	14.2	4.29	10.18	30.57	6.56	8.00	22.39	37.45	18.87	8.44	8.96	42.35	9.66	5.00
15	Yield per m^2 (gm)	166.49	210	125	42.63	76.50	290.33	12.58	12.00	118.10	215	138	28.23	30.79	194.51	6.49	0.00

Axygen Scientific Inc. Union city CA, USA. The 20 µl master mix consisted of 50 çg of genomic DNA, 1 U of *Taq* DNA polymerase, 1 X PCR assay buffer with 1.5 mM $MgCl_{2,}$ 10µM each of forward and reverse primer and 1 mM of dNTP mix was prepared on ice and the PCR plate was immediately loaded in the thermal cycler (Verity, Applied Biosystem USA) for PCR using conditions of (1) Initial denaturation at 94 °C for 5 min; (2) 35 cycles of 94°C for 1 min; 55-60°C (depending on marker) for 1min; 72°C for 2 min; (3) final extension at 72°C for 5min.

A set of 343 marker loci comprising of 156 HvSSR (Highly variable simple sequence repeats), 176 SSR (Simple sequence repeats) and 11 belong to RGNMS (Rice genic non coding micro satellites) were used for polymorphism survey between Swarna and IR86931B-6. Out of 176 SSR used, 38 were previously reported (Marathi *et al*. 2012, Kotla *et al*. 2013) QTL specific markers for yield and yield contributing traits. The primer sequences for RM series markers were obtained from (McCouch *et al*. 2002, Temnykh *et al*. 2000, Chen *et al*. 1997) and gramene SSR markers web resources (www.gramene.org), RGNMS markers (Parida *et al*. 2009) and HvSSR (Harvinder *et al*. 2010). Genotypic data was generated with a set of 83 polymorphic primers providing genome wide coverage *i.e*. RGNMS, SSR and HvSSR markers. Chi square test (÷2) was done for these polymorphic markers to check the marker segregation pattern.

Development of linkage map and QTL mapping

Linkage map based on 83 polymorphic markers and position of QTLs on Chromosomes was identified using Single marker analysis (SMA) and composite interval mapping (CIM) performed using programme QTL cartographer 2.5 (Wang *et al*. 2005) with a 2.5 threshold value. Identified major effects QTLs along with their markers were mapped in 12 rice chromosomes with the help of MapChart version 2.3. Nomenclature for QTLs was first two or three letter abbreviation followed by the identity of the chromosome on which the QTL is found and a terminal suffix with unique identifier to distinguish multiple QTL on a single chromosome was used (McCouch 1997). QTLs identified in the present study were compared with earlier reported QTLs and the QTLs available in gramene database to detect common QTLs across populations for 13 traits. QTLs on the same chromosomal region as

found in the present study were selected for detailed comparisons. The rice genetic linkage map (the Cornell SSR map) were used to compare QTL locations found in the present study and co localized with validated QTLs. QTLs were identified as potentially novel if the marker intervals harboring QTLs were not significantly overlapping the previously reported marker intervals. QTL hotspots were identified manually as reported from Marathi *et al*. (2012) by searching in a sliding window of 20 cM in the original QTL data and the regions with more than three co-locating QTLs in each window region were recorded. The window was advanced in 5 cM steps across the entire genetic map and the maximum number of QTL in a window region was recorded.

RESULTS AND DISCUSSION

Trait Performance

The mean performance of parents and minimum and maximum trait values of F_5 RILs at two locations are presented in table 1. The results of ANOVA revealed highly significant mean sum of squares for all the traits in both location, suggesting presence of sufficient variation among the genotypes for these traits. Hence, there is scope to select desirable F_5 lines with higher productivity combining favorable yield contributing traits. In the mapping population developed for the present study, wider variability was observed for all the traits as indicated by the range and co-efficient of variation, this provides more opportunities to select plants with different combination of desirable traits. Maximum variability was observed for grain per panicle and yield per m^2 in location 1($L\text{-}_1$) and for harvest index at location 2 (L_2) whereas, Minimum variability was observed for flag leaf width and days to fifty percent flowering at location 1 and two respectively.

The transgressive variation was noticed for all the traits and it was noticed in both direction which indicated that neither of the parents carried the entire positive or all the negative alleles. The frequency distribution showed continuous variation and approximately followed normal distribution for most of the traits, indicated the polygenic (quantitative) nature of these yield contributing traits (figure 1).

Heritability, Genetic Advance, Variability and Correlation

In the present study, considerably high genetic variability, heritability and genetic advance (GA) were observed in the F_5 mapping population for most of yield contributing traits (table 2). This indicated that, the advanced breeding combined with direct selection for yield along with important productivity traits in irrigated condition could be

Table 2. Broad sense heritability, Genetic advance (GA) as per cent of mean, phenotypic coefficient of variance (PCV) and genotypic Coefficient of variance (GCV) for yield and yield contributing traits

Character	Location 1				Location 2			
	Heritability	Genetic advance	GCV	PCV	Heritability	Genetic advance	GCV	PCV
Seedling Height (cm)	45	02.34	02.15	08.9	70	3.22	09.91	11.84
Total Tiller per m2	74	21.44	10.18	11.84	93	48.46	19.06	19.75
Effective Tiller per m2	08	05.82	10.30	33.20	92	41.5	18.05	18.81
Plant Height (cm)	79	17.21	10.61	10.85	78	17.91	09.95	11.29
Flag Leaf Length (cm)	66	08.98	05.09	06.26	80	11.61	05.95	06.67
Flag Leaf Width (cm)	50	02.73	06.83	09.63	84	8.34	15.05	16.42
Flag Leaf Area (cm)	86	00.27	10.76	11.61	88	0.34	12.77	13.59
Days to 50% Flowering	72	08.89	14.15	16.61	85	15.76	20.74	22.46
Panicle Length (cm)	60	01.79	05.07	06.53	51	1.93	05.75	08.04
Panicle Weight (gm)	57	00.48	06.36	08.42	77	0.87	09.96	11.35
Grain per Panicle	52	00.23	10.12	10.16	79	0.4	10.53	11.81
100 Grain Weight (gm)	78	52.72	18.8	21.33	96	68.16	23.71	24.17
Spikelet Fertility (%)	44	07.91	10.23	12.01	66	11.32	09.04	11.09
Harvest Index (%)	65	04.22	11.4	14.09	94	16.54	37.06	38.30
Yield per m^2 (gm)	76	45.01	10.02	11.08	92	55.01	23.46	24.34

Table 3. Genotypic and Phenotypic correlation coefficient between yield and yield contributing traits (Location 1 and Location 2)

Coefficient of Location 1: right half of table in bold letters ; coefficient of Location 2: left half of table in normal font

SN	Character	Correlation type	SH	TT	ET	PH	FLL	FLW	FLA	DFF	PL	PW	GPP	HGW	SF	HI	YLD
1	Seedling Height (SH)	G	1	0.024	0.045	0.027	0.062	-0.203*	-0.096	0.025	0.034	-0.06	-0.256**	-0.054	0.098	-0.025	-0.227*
		P	1	-0.002	0.033	-0.014	0.057	-0.220*	-0.11	-0.03	0.081	-0.014	-0.206*	0.002	0.087	0.001	-0.078
2	Total Tiller per m2 (TT)	G	0.062	1	0.998**	-0.201*	-0.263**	0.009	-0.133	0.143	-0.12	0.086	-0.088	0.133	-0.181	-0.177	0.702**
		P	0.034	1	0.936**	-0.121	-0.259**	0.006	-0.135	0.103	-0.136	0.044	-0.073	0.055	-0.043	-0.11	0.171
3	Effective Tiller per m2 (ET)	G	0.002	0.935**	1	-0.144	-0.225*	-0.026	-0.133	0.171	-0.126	0.072	-0.085	0.144	-0.107	-0.173	0.945**
		P	-0.005	0.919**	1	-0.083	-0.212*	-0.03	-0.132	0.134	-0.138	0.052	-0.099	0.073	0.001	-0.137	0.200*
4	Plant Height (PH)	G	0.339**	0.009	-0.035	1	0.123	0.154	0.17	-0.225*	0.420**	0.219*	0.134	-0.028	0.022	-0.181	-0.697**
		P	0.326**	0.009	-0.016	1	0.08	0.053	0.081	-0.111	0.347**	0.156	0.095	-0.038	0.050	-0.138	-0.059
5	Flag Leaf Length (FLL)	G	-0.181	-0.033	0.045	-0.170	1	0.414**	0.800**	-0.27	0.336**	0.230*	0.436**	0.388*	-0.598**	-0.187*	-0.158
		P	-0.120	-0.005	0.041	-0.142	1	0.317**	0.772**	-0.159	0.241*	0.232*	0.300**	0.279	-0.221*	-0.066	-0.046
6	Flag Leaf Width (FLW)	G	0.149	0.055	0.108	0.081	-0.090	1	0.875**	-0.224*	0.233*	0.414**	0.486**	0.302**	-0.680**	-0.056	-0.307**
		P	0.090	0.047	0.083	0.041	-0.048	1	0.843**	-0.203*	0.213*	0.218*	0.387**	0.113	-0.302**	-0.023	-0.003
7	Flag Leaf Area (FLA)	G	0.189*	0.086	0.063	0.141	-0.211*	0.058	1	-0.290**	0.329**	0.405**	0.551**	0.396**	-0.742**	-0.142	-0.252*
		P	0.172	0.082	0.068	0.141	-0.176	0.079	1	-0.226*	0.207*	0.232*	0.426*	0.170	-0.326**	-0.056	-0.021
8	Days to 50% Flowering (DFF)	G	0.241*	0.079	0.103	0.151	-0.199*	0.777**	0.668**	1	-0.051	-0.053	-0.162	0.231	0.163	0.05	0.614**
		P	0.188*	0.071	0.088	0.117	-0.145	0.788**	0.668**	1	-0.039	-0.028	-0.103	0.06	0.069	0.081	0.096
9	Panicle Length (PL)	G	-0.130	-0.126	-0.027	0.368**	0.268*	0.442**	0.225*	0.265**	1	0.348**	0.195*	0.009	-0.014	-0.101	-0.472**
		P	-0.016	-0.102	-0.038	0.363**	0.228*	0.371**	0.221*	0.265**	1	0.232*	0.100	-0.013	0.136	-0.007	-0.026
10	Panicle Weight (PW)	G	0.162	-0.396**	-0.392**	0.075	0.248*	0.265**	0.077	0.255*	0.426**	1	0.483**	0.041	-0.437**	-0.02	0.920**
		P	0.139	-0.310**	-0.312**	0.113	0.292**	0.211*	0.051	0.195*	0.313**	1	0.306*	0.040	-0.211*	-0.074	0.069
11	Grain Per Panicle (GPP)	G	-0.007	-0.083	-0.079	-0.058	0.327**	0.276**	0.280**	-0.296**	0.194*	-0.007	1	-0.258**	-0.858**	-0.189*	-0.396**
		P	-0.058	-0.047	-0.051	-0.062	0.288**	0.277*	0.259**	-0.281**	0.077	-0.031	1	-0.140	-0.371**	-0.102	-0.079
12	100 Grain Weight (HGW)	G	0.075	-0.071	-0.117	0.242*	0.265**	0.261**	0.444**	0.330**	0.183*	0.363**	-0.180	1	0.677**	0.207*	0.275**
		P	0.080	-0.062	-0.101	0.222*	0.219*	0.240*	0.401**	0.283**	0.115	0.340**	-0.181	1	0.161	0.141	0.199*
13	Spikelet Fertility (SF)	G	-0.184*	0.022	0.053	-0.114	-0.038	-0.238*	-0.459**	-0.462**	-0.232*	-0.166	0.198*	-0.239*	1	0.596**	0.989**
		P	-0.140	0.010	0.020	-0.042	-0.017	-0.135	-0.327**	-0.304**	0.123	-0.101	0.121	-0.179	1	0.273**	0.158
14	Harvest Index (HI)	G	-0.150	-0.151	-0.120	-0.063	-0.020	-0.178	-0.346**	-0.333**	0.242*	0.094	0.304**	-0.072	0.008	1	-0.495**
		P	-0.102	-0.138	-0.114	-0.070	-0.012	-0.170	-0.332**	-0.316**	0.124	0.076	0.291**	-0.066	0.006	1	-0.004
15	Yield per m2 (YLD)	G	-0.049	0.029	0.072	0.039	0.109	-0.003	-0.015	-0.008	0.154	0.162	0.156	-0.051	0.175	0.282**	1
		P	-0.034	0.029	0.066	0.059	0.090	-0.009	-0.005	-0.007	0.137	0.142	0.138	-0.050	0.145	0.270**	1

**Significant at 0.01; *Significant at 0.05; G: Genotypic correlation coefficient; P: Phenotypic correlation coefficient

Table 4. Major effects QTLs detected by composite interval mapping across two locations

Trait/SN	QTLs	CHR	Flanking marker	QTL Size (cM)		Peak Position (cM)		QTL interval		QTL interval		LOD Score		Additive effect		Dominance effect		Phenotypic variance (R^2) %	
				L1	L2	L1	L2	L1		L2		L1	L2	L1	L2	L1	L2	L1	L2
Seedling Height																			
1	qSH1.1	1	HVSSR01-07 - RM283	-	2.5	-	18.6	-	-	17.35	19.85	-	4.57	-	1.26	-	0.79	-	17
Total Tiller																			
2	qTT3.3	3	RM338 - RM3698	14	3	55.9	55.9	48.9	62.9	54.4	57.4	3.7	3.98	3.7	5.21	54.33	63.45	8	16
3	qTT4.3	4	RM307 - RM1112	4	3.1	133.9	134.9	131.9	135.9	133.35	136.45	3.58	3.45	1.48	5.51	54.35	60.97	3	11
Effective Tiller																			
4	qET3.5	3	RM338 - RM3698	21.9	-	55.9	-	44.95	66.85	-	-	3.79	-	5.64	-	56.65	-	18	-
5	qET10.1	10	HVSSR10-30 - HVSSR10-34	46.9	44	38	38	14.55	61.45	16	60	3.71	5.8	5.77	4.82	52.05	53.6	1	11
Plant Height																			
6	qPH1.2	1	RM3746 - RM1003	45.5	71	68.6	118.6	45.85	91.35	83.1	154.1	4.37	4.08	4.14	1.43	22.88	24.83	10	8
7	qPH3.2	3	RM49 - RM7576	2.1	1.8	22.5	22.5	21.45	23.55	21.6	23.4	3.9	4.4	0.11	0.47	30.31	33.61	7	10
Flag Leaf Length																			
8	qFLL3.1	3	HVSSR03-80 - HVSSR03-91	-	7.4	-	128.7	-	-	125	132.4	-	3.12	-	2.51	-	4.97	-	46
Flag Leaf Width																			
9	qFLW2.3	2	HVSSR02-50 - HVSSR02-60	9	13.5	86.7	88.7	82.2	91.2	81.95	95.45	4.63	2.56	0.06	0.06	0.33	0.36	21	10
10	qFLW2.4	2	HVSSR02-60 - RM6318	2.2	2.5	95.6	95.6	94.5	96.7	94.35	96.85	4.28	3.72	0.05-	0.05	0.28	0.38	10	6
11	qFLW3.4	3	RM6959 - RM16	-	10.8	-	89.1	-	-	83.7	94.5	-	2.57	-	0.08	-	0.2	-	14
12	qFLW6.1	6	HVSSR06-43 - HVSSR06-44	12.9	28.4	38	14.2	31.55	44.45	0	28.4	4.44	3.43	0.07	0.08	0.38	0.38	8	18
13	qFLW6.2	6	HVSSR06-43 - HVSSR06-44	-	12.8	-	31	-	-	24.6	37.4	-	2.52	-	0.09	-	0.38	-	23
14	qFLW9.1	9	RM316 - RM278	27.3	-	34	-	20.35	47.65	-	-	3	-	0.04	-	0.34	-	10	-
15	qFLW10.1	10	HVSSR10-30 - HVSSR10-34	46.7	-	40	-	16.65	63.35	-	-	3.41	-	0.01	-	0.34	-	14	-
Flag Leaf Area																			
16	qFLA3.1	3	RM3698 - RM16	-	4	-	90.1	-	-	88.1	92.1	-	3.33	-	2.85	-	10.04	-	11
Panicle Weight																			
17	qPW2.1	2	HVSSR02-77 - HVSSR02-86	-	15	-	133.2	-	-	125.7	140.7	-	2.53	-	0.17	-	0.2	-	11
18	qPW4.1	4	RM3276 - RM1112	-	5.5	-	130.9	-	-	128.15	133.65	-	3.1	-	0.18	-	0.41	-	13
Grain Per Panicle																			
19	qGPP1.1	1	HVSSR01-07 - RM283	-	2	-	18.6	-	-	17.6	19.6	-	4.03	-	21.75	-	2.91	-	13
20	qGPP3.1	3	RM3698 - RM16	-	3	-	91.1	-	-	89.6	92.6	-	6.36	-	27.07	-	0.68	-	33
21	qGPP3.2	3	RM3698 - HVSSR03-69	7	-	91.1		87.6	94.6	-	-	3.19		17.78		2.95		20	-
22	qGPP4.1	4	HVSSR04-37 - RM3276	28.5	-	96.2	-	81.95	110.45	-	-	3.45	-	12.44	-	29.09	-	12	-
23	qGPP9.1	9	RM316 - RM278	22.5	41	34	62	22.75	45.25	41.5	82.5	2.99	3.34	20.32	27.32	74.78	24.97	4	30
Hundred Grain Weight																			
24	qHGW2.1	2	RM452 - HVSSR02-60	30	-	79.7	-	64.7	94.7	-	-	3.24	-	0.09	-	0.42	-	10	-
25	qHGW3.1	3	RM07 - HVSSR03-37	9	-	53.9	-	49.4	58.4	-	-	3.4	-	0.11	-	0.27	-	19	-
26	qHGW3.2	3	RM6959 - RM3698	-	23	-	71	-	-	59.5	82.5	-	2.75	-	0.08	-	0.3	-	19
27	qHGW6.1	6	HVSSR06-43 - HVSSR06-44	-	13	-	52	-	-	45.5	58.5	-	2.56	-	0.1	-	0.38	-	28
Spikelet Fertility																			
28	qSF1.1	1	RM1003 - RM5389	6	-	137.9	-	134.9	140.9	-	-	2.56	-	4.29	-	5.65	-	11	-
29	qSF3.1	3	RM5474 - RM49	-	1.5	-	19.8	-	-	19.05	20.55	-	6.45	-	6.19	-	4.28	-	25
Harvest Index																			
30	qHI2.1	2	RM12368 - HVSSR02-50	24.3	-	34	-	21.85	46.15	-	-	4.48	-	3.25	-	12.18	-	12	-
31	qHI3.1	3	RM489 - RM49	-	3	-	19.3	-	-	17.8	20.8	-	2.59	-	4.02	-	2.03	-	19
32	qHI7.1	7	RM11 - RM70	-	9	-	82	-	-	77.5	86.5	-	2.94	-	5.86	-	1.3	-	25
33	qHI8.1	8	RM25 - HVSSR08-46	43.2	-	91.5	-	69.9	113.1	-	-	2.62	-	3.28	-	1.54	-	22	-
Yield per meter^2																			
34	qYLD1.1	1	HVSSR01-07 - RM3746	-	4.3	-	21.6	-	-	19.45	23.75	-	2.69	-	10.13	-	42.91	-	13
35	qYLD2.1	2	RM12368 - HVSSR02-50	9.5	-	38.3		33.55	43.05	-	-	6.97		5.25		45.66		10	-
36	qYLD5.1	5	RM3683 - HVSSR05-57	7.1	-	89.7	-	86.15	93.25	-	-	2.68	-	6.75	-	48.48	-	13	-

Where; SH: Seedling Height; TT: Total Tiller/m^2 ; ET: Effective Tiller/m^2; PH: Plant Height; FLL: Flag Leaf Length (cm); FLW: Flag Leaf Width (cm); FLA: Flag Leaf Area (cm^2); PW: panicle Weight (gm); GPP: Grain Per panicle; HGW: Hundred Grain Weight (gm); SF: Spikelet Fertility (%); HI: Harvest Index (%); YLD: Yield/m^2; L_1: location 1; L_2: Location 2; LOD: Log of Odds; R^2: Phenotypic Variance; TR^2; Total Phenotypic Variance; cM: centi Morgan

highly effective in improvement of yield in rice. According to Johnson *et al*. (1955), high heritability coupled with high GA, are normally more helpful than heritability alone and it indicates that the heritability is due to additive gene action and selection may be effective.

Coefficient of variation truly provides a relative measure of variance among the different traits. In our study close relationship between GCV and PCV were found in all the traits in both location except for number of effective tillers per m^2 in location 1, here PCV is more than twice of GCV (table 2). This finding was in agreement with Zahid *et al*. (2006), who also observed similar results also in yield and yield related traits. Association of yield with yield related traits revealed the Significant correlations between yield and yield related traits in F_5 mapping population. Correlation coefficients with > 0.707 are considered as highly significant, because at this level each trait influences the other trait to an extent of more than 50 per cent (Snedecor and Cochreham, 1989). We found such significant relationship between TT and ET in both locations (table 3) will be useful for selection programmes to increase the chances of simultaneously improving two or more traits. Similar results for TT and ET was also reported by Kumar *et al*. 2014.

Identification of QTLs for yield and its contributing traits

Parental Polymorphism Survey and construction of linkage map

Parental polymorphism survey at 343 loci revealed that 83 loci (24.20%) were found polymorphic between parents. Out of total polymorphism percentage, HvSSR, SSR and RGNMS marker showed 19.23% (30), 28.98% (51) and 18.18% (2) polymorphic ratio respectively. The highest percentage of polymorphism was obtained on chromosome 2 (41.67%) and lowest on chromosome 12 (6.67%). In chromosome three, 24 polymorphic markers were obtained, which was maximum among all chromosomes. Based on these polymorphic markers linkage map was constructed with a total map length of 1275.24 cM. The average interval size was 21.68 cM, the smallest size in chr 3 (6.02 cM), and the largest in chr.8 (32.93 cM). Marker segregation analysis of 83 polymorphic markers showed that only 14 (16.87 %) markers followed mendelian segregation, rest of the 69 markers, 50 (60.24%) were skewed towards Swarna and 19 (22.89%) were skewed towards IR86931B-6. The single marker analysis showed that, A total of 158 loci (consisting commonly associated markers for some traits) were significantly associated with yield and yield contributing traits in both locations exactly.

Identification of major and minor effects QTLs by Composite Interval Mapping (CIM)

The genotypic data thus generated along with phenotypic date recorded in the field was used for identification and mapping of QTLs by calculating threshold logarithm of odds (LOD) for each trait by performing test with 1000 permutations. The experimental threshold LOD mean were 2.5 at 5% level of significance in both locations. A total of 130 QTLs (major and minor) spread over 11 linkage groups were detected for all phenotyped yield and related traits except DFF and PL, across two locations. Out of 130 identified QTLs for yield and related traits, 36 QTLs were found as major effects QTLs across two locations with minimum LOD threshold value 2.5 and having e”10% phenotypic variance (table 4) and rest of the other QTLs were minor effects QTLs with less than 10% phenotypic variance.

All major effects QTLs were mapped in 12 rice chromosomes based on their positions (figure 2). Out of these 9 QTLs were common in both locations whereas 12 QTLs were present in location one and 15 QTLs were present in location two. Maximum 7 major effects QTL was identified for FLW (qFLW2.3, qFLW2.4, qFLW6.1, qFLW6.3, qFLW9.1 and qFLW10.1) in chromosome 2, 6, 9 and 10 followed by 5 major effects QTLs were observed for GPP (qGPP1.1, qGPP3.1, qGPP3.2, qGPP4.1 and qGPP9.1) in chromosome 1, 3, 4 and 9 whereas minimum major effects QTLs were identified for SH (qSH1.1), FLL (qFLL3.1), FLA (qFLA3.1) in chromosome one and three. Maximum number (11 QTLs) of major effects QTLs were found in chromosome three. Among these QTL qFLL3.1 (LOD-3.12) has highest phenotypic variance with additive effects of 2.1, dominance effects of 4.97and variance (R^2%) of 46 percent.

Seedling height is also important trait for initial level screening of plant condition. Only one major effect QTL (qSH1.1) was detected for this trait on location 2 on chromosome1 with LOD value 4.57 and phenotypic variance 17 percent. This might be novel QTL region for this trait because

previously identified QTL for this trait by Abe *et al*. 2012 was presented on chromosome 3.

Appropriate total tiller, number of effective tillers and plant height are prerequisites for attaining the increased yield level in rice breeding programme. For these traits, two major effects, for TT (qTT3.3 and qTT4.3) and ET (qET3.5 and qET10.1) were present in Location two. Kotla *et al*. 2013 also reported QTLs for tiller number in chromosome number 2 and 3 respectively. Similarly in RIL population, Zhou *et al*. 2013 reported Five QTLs for the tiller number per plant on chromosomes 3, 4, 6, 9, and 12, respectively. The major effect QTLs contributed the most, up to 10%, indicating that the number of tiller per plant was controlled by multiple quantitative trait genes.

Plant height is an important agronomic trait that determines the yielding potential of rice variety. Plant height is known to be controlled in both major and minor genes but most of the relevant reports confirmed its polygenic nature (Rahman *et al*. 2007). Only two major effect QTL, qPH1.2 with LOD value 4.37 in $L_1$and 4.08 in L_2) and qPH3.1 (with LOD value3.9 in $L_1$and 4.4 in L_2) has been observed for this trait in both locations. Detecting the major effect QTLs controlling plant height at vegetative and maturity is useful in practical breeding and agriculture. Similarly Wang *et al*. 2012, Marathi *et al*. 2012 and Kotla *et al*. 2013 also reported QTLs for plant height in different mapping population.

It was reported that the source leaves, particularly the flag leaves, were associated with improved grain filling, 1000-grain weight and panicle weight as well as other yield-related traits in cereal crops (Quarrie *et al*. 2006). In our present study similar results observed where FLL, FLW and FLA positively correlated (both genotypically and phenotypically) with PL, PW, GPP and HGW which confirms the previous findings. Compared to other leaves, the flag leaf contributes the most photosynthetic assimilates in rice therefore; it assumes the Flag leaf length, width and area has greatest importance in terms of grain yield (Lupton, 1973). The above sentences proven by the 9 major effects QTLs identified related to these traits. Out of 9 QTLs, one QTL for FLL (qFLL3.1), 7 QTLs for FLW (qFLW2.3, qFLW2.4, qFLW3.4, qFLW6.1, qFLW6.2, qFLW9.1, qFLW10.1 and one QTL for FLA (qFLA3.1). The QTL, qFLL3.1 was found for flag leaf length has highest phenotypic variance (46%) among all the traits with 3.12 LOD value. Similar findings also observed by Wang *et al*. (2012) on BRILs (backcross recombinant inbreed lines, Marathi *et al*. (2012) also reported 14 QTLs for FLL in RIL population across three locations.

Simple selection criterion for yield can be increased by selecting plants with many heavy panicles, increasing the number of grain per panicle, heavy grain weight and high percentage of spikelet fertility. Total of 13 major effects QTLs found for these traits *viz* PW (qPW2.1 and qPW4.1), GPP (qGPP1.1, qGPP3.1, qGPP3.2, qGPP4.1, and qGPP9.1), HGW (qHGW2.1, qHGW3.1, qHGW3.2 and qHGW6.1) and SF (qSF1.1 and qSF3.1). Similar results also observed by Wang *et al*. (2012), and Zhou *et al*. (2013) in different mapping populations for the same traits, which confirms our findings.

Harvest index is also has a major importance in rice yield. Higher the HI value may increase the yield also. A total of 4 major effects QTLs identified (two QTLs in each locations) for HI *viz* qHI2.1, qHI3.1, qHI7.1, qHI8.1. Previously Sabouri *et al*. (2009) reported eight significant QTLs across ten environments which showed the importance of this trait.

In the present study, for yield three major effects QTLs *viz* qYLD1.1, qYLD2.1, qYLD5.1 were identified in chromosome 1, 2 and 5. The additive effects of this QTL were also high with 6.25 and 5.25. For plot yield Swamy *et al*. (2011) also reported three QTLs *qPYLD3.1*, *qPYLD4.1* and *qPYLD4.2* were detected on two chromosomes. Marathi *et al*. (2012) also reported 5 QTL for Grain yield per plant. A major QTL, qYLD4-1, was identified with LOD 3.28 explaining 15% of phenotypic variation.

Co localization of Known QTLs

In the present study, total 6 known QTLs from study of Kotla *et al*. (2013) and Marathi *et al*. (2012) were validated by single marker analysis and co localized, in the background of Swarna and IR86931B-6 derived mapping population flanked by 8 significant marker loci governing the QTLs for plant height (qph3.1, flanked with RM16 and RM489), number of tillers (qnt3.1, flanked with RM7 and RM514; qnt3.2, flanked with RM514 and RM517) and thousand grain weight (qTGW3-4, flanked with RM3698 and RM16; qTGW4-1, flanked with RM3276 and RM1112) and panicles per plant (qPPP4-2, flanked with RM3276 and

RM1112) in 3 and 4, chromosome. These co localized regions of yield related traits will be further useful for identification of putative candidate gene by fine mapping and used for the cloning purpose and further breeding programme. Liu *et al*. (2010) also validated QTLs, SPP3b and TGW3b in the BC3F2 population governing the trait spikelet per panicle and thousand grain weights in chromosomal 3. This gives strength of our validated QTLs, for hunderd grain weight in same chromosomal region (Figure 3).

QTL Hot Spots (Clusters)

While considering phenotypic and genetic correlation it is very interesting to examine co localized QTLs for breeding perspective. One of the central concepts in genetical genomics is the existence of QTL hotspots, where a single polymorphism leads to widespread downstream changes in the expression of distant genes, which are all mapping to the same genomic locus (Schadt *et al*. 2003). In this study 5 QTLs hotspots containing 37 QTLs affecting many traits were identified in chromosome 3 and chromosome 4 between colocalized QTLs flanking marker regions of which, some are either genetically correlated or allometrically related. Out of 37 QTLs, 14 was major effects QTLs with more than 10% phenotypic variance having minimum 3 LOD value and 23 minor effects QTLs less than 10 % phenotypic variance.

Out of 14 major effects QTLs 12 found in chromosome 3 and only 2 major effect QTLs (qTT4.3 and qPW4.1) were found to be present on chromosome 4 for total tiller and panicle weight (Table 5; Figure 3).

According to Marathi *et al*, 2012, it is very difficult to know the contributing mechanism between all these traits in a hotspot as correlations do not suggest link between them. It is possible that these clusters represent more than one gene but the present mapping population resolution is not sufficient to differentiate whether it is due to either linkage or pleiotropy. It is observed that some hotspots contain QTLs that are not allometrically linked. It may possible that these loci represent trans acting QTL (most likely transcription factors) where the effect of alterations in regulation or structural characteristics would be expected to have smaller effects on many traits (Rae *et al*, 2009). It can be concluded that each QTLs present within a QTL hotspot region give strengthen to results that these all traits are relative to each other and might donate a small positive effect, but co- locality of some traits point out that choice for valuable allele at these loci will result in a cumulative increase in yield due to the integrative positive effect of various QTLs.

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