

## Molecular and phenotypic analyses of yield components QTLs in IR64 backcross progenies and popular high yielding rice varieties of India

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Received : 31 December 2017

Accepted : 20 March 2018

Published : 27 June 2018

### ABSTRACT

The projected rice demand for the increasing population across the globe is going to be very tough beyond 2040. The option of increasing yield potential of rice varieties need to be accelerated. Yield potential improvement of popular variety IR64 was taken up by introducing yield component QTLs *Gn1a*, *Gw2*, *gw5*, *OsSPL14* and *SCM2*. The backcross derived lines at BC<sub>2</sub>F<sub>3</sub> generation and popular varieties showed presence of QTLs *Gn1a*, *Gw2*, *gw5*, *OsSPL14* and *SCM2*. Expected phenotypic effects were also observed in the derived lines and popular varieties. Seven derived lines were observed to possess all five yield component QTLs. These genotypes may serve as useful donors for improvement of yield potential in rice.

**Key words:** Yield QTL, *Gn1a*, *Gw2*, *gw-5*, *OsSPL14*, *SCM2*, backcross breeding

### INTRODUCTION

Rice is the world's most important cereal food crop. It is 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. It is considered as the model cereal crop due to its relatively small genome size, vast germplasm collection, enormous repertoire of molecular genetic resources, and efficient transformation system (Paterson et al., 2005). Rice is globally grown in about 163 million hectares annually with a total production of around 750 million tons (500 mt milled rice). To meet the future staple food requirement of the increasing population expecting 9 billion by 2050, rice varieties with higher yield potential and greater yield stability under climatic order need to be developed. Quantitative trait locus (QTL) analysis can provide information relevant to agronomic traits using molecular markers to identify specific regions of the genome affecting any measurable trait (Tanksley, 1993).

In rice, >8000 QTLs have been identified and mapped relating to various traits. The important yield

component traits such as grain number (*Gn1a*, *Ghd7*, *DEP1* and *WFP*), grain weight (*GS3* and *GW2*), grain size (*GS3* and *GW5*) grain filling (*GIF1*) and panicle number (*DEP1* and *WFP*) have already been sequenced and cloned. These genes/QTLs are useful for increasing yield potential directly or indirectly in rice. Ashikari et al. (2005) were able to increase grain number by 45% along with reducing plant height by 20% by combining the grain number QTL (*Gn1a*) and the semi dwarfing gene (*sd1*) gene using Marker-assisted approach. In another study, Ando et al. (2008) have pyramided two QTLs viz., *qSBN1* (for secondary branch number on chromosome 1) and *qPBN6* (for primary branch number on chromosome 6) and showed more spikelets than the independent NIL lines containing QTLs *qSBN1* and *qPBN6*. Recently, Wang et al. (2011) demonstrated that the pyramid line NIL (*qHD8* + *GS3*) had higher yield potential, longer grains, and a more suitable heading date than recipient variety.

Morphological characterization and molecular analysis using gene specific markers for four yield component traits were taken up in backcross progenies of IR 64 variety along with some popular varieties. The

present investigation aims to validate the yield component QTLs viz., *Gn1a*, *Gw2*, *gw5*, *OsSPL14*, *SCM2* through molecular and phenotypic analysis in backcross progenies and popular varieties. The genotypes carrying these QTLs may be used cultivar or as donor for yield potential breeding program.

## MATERIALS AND METHODS

The seeds of fifty three genotypes comprising of IR64 backcross progenies and high yielding popular varieties were used for genotyping and phenotyping work (Table 2). The IR 64 derived backcross lines were in BC<sub>2</sub>F<sub>4</sub> generation and were derived from the cross of IR64 with variety 'Maudamani'. Rice variety Maudamani possesses the yield component QTLs *Gn1a*, *Gw2*, *OsSPL14* and *SCM2*. Field experiment was conducted during dry and wet season, 2016 for characterization of yield and yield component traits. The 53 genotypes were grown in randomized block design with two replications during wet season, 2016. Number of effective tillers, panicle length, panicle weight, total grains/panicle, grain length, grain width and section modulus (mm<sup>3</sup>) were recorded in both the seasons. Cross section modulus (mm<sup>3</sup>) =  $\pi/4 \times (a_1 b_1 - a_2 b_2)/a_1$ , where  $a_1, b_1$  indicate short radius and long radius of the outside, while  $a_2, b_2$  were the short radius and long radius of the measured IV internode from top, respectively. Mean phenotypic data were analyzed using software crop stat.

Leaves were collected from 15 days old seedlings to extract genomic DNA for molecular screening. Total genomic DNA was extracted after crushing in liquid nitrogen in microfuge tubes using CTAB extraction buffer (100mM Tris-HCl pH 8, 20 mM EDTA pH 8, 1.3M NaCl, 2% CTAB) and phenol-

chloroform-Isoamyl alcohol extraction followed by RNAase treatment and isopropanol precipitation. Agarose gel electrophoresis was used to estimate DNA concentration using Lambda DNA as standard, and each sample was then diluted to approximately 25ng/ $\mu$ L. DNA amplification was performed in a Thermal Cycler (Veriti, Applied BioSciences) with a reaction volume of 10ml containing 1.5mM Tris HCL (pH 8.75), 50mM KCL, 2mM MgCl<sub>2</sub>, 0.1% TrotonX-100, 200mM each of dATP, dCTP, dTTP, dGTP, 4 pMole of each forward and reverse primers, 1 unit of Taq Polymerase and 30ng of genomic DNA. The reaction mixture was first denatured for 4 mins at 94 °C and then subjected to 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55-58°C, and 1 min extension at 72°C; and then a final extension for 10 mins at 72°C.

Five gene specific markers namely *Gn1a*, *Gw2*, *Gw5*, *Osspl14*, *Scm2* were used to screen for yield qtls. PCR amplification products were loaded in 2.5-3% gel containing 0.8mg/ml Ethidium Bromide for electrophoresis in 1X TBE (pH 8.0). Lanes were loaded with 50bp and 100bp DNA ladder. The gel was run at 120v (2.5 V/cm) for 4 hrs and photographed using a Gel Documentation System (SynGene). Data scored were analyzed on the basis of the presence or absence of amplified products for each genotype-primer combination.

## RESULTS AND DISCUSSION

Genomic DNA of three genotypes was amplified by using gene based direct markers for grain number, strong culm, grain width and weight, heavy panicle and ideal plant architecture (Table 1). Gene specific marker *Gn1a* screens for the presence of higher grain number in a rice genotype. Presence of QTL *SCM2* indicates the

**Table 1.** Molecular markers used for tracking of yield component QTLs in 53 IR64 derived lines.

Sl No	Gene name	Primer name	Oligo-nucleotide primer sequence
1.	High grain number	Gn1aM2(F)	5' TGAGGATGCCGTGGAAGACGA3'
		Gn1aM2 (R)	5' TTCGTGTTCCGCGCAGGACGT 3'
2.	Grain width	GW2(F)	5' CCAATAAAGATGTCCATTCTGTTA 3'
		GW2 (R)	5' GCTCTTCCTGTAACACATATTATG 3'
3.	Grain weight	GW5 (F)	5' GCGTCGTCAGAGGTAGA 3'
		GW5 (R)	5' GACCTAACCCATCTCATTCCA 3'
4.	Strong Culm	(SCM 2) (F)	5' ATCAGATCAATAGGTTGAGTGT 3'
		(SCM 2) (R)	5' TGCTATGTATATCCTATCGGTTTC 3'
5	Heavy Panicle & Plant architecture	OsSPL 14(F)	5'CAAGGGTTCCAAGCAGCGTAA3'
		OsSPL 14(R)	5'TGCACCTCATCAAGTGAGAC3'

**Table 2.** Genotyping of IR64 derived lines and popular varieties for yield component QTLs *Gn1a*, *Gw2*, *gw5*, *OsSPL14* and *SCM2*.

Sl.No.	Genotype name	Gn1a (180bp)	Gw2(180bp)	gw5(1050bp)	OsSPL14(500bp)	SCM2(180bp)
1	YP64-27-12-8	Absent	Absent	absent	absent	absent
2	YP64-27-12-23	absent	Absent	absent	absent	present
3	YP64-27-12-27	absent	Absent	absent	absent	absent
4	YP64-27-12-32	present	Absent	absent	absent	Present
5	YP64-27-12-29	absent	Absent	absent	present	Present
6	YP64-27-12-25	absent	Absent	absent	absent	Absent
7	YP64-27-12-41	absent	Absent	absent	present	Absent
8	YP64-27-12-23	absent	Absent	absent	absent	Absent
9	YP64-27-12-37	present	present	absent	present	present
10	YP64-27-12-41	absent	Absent	absent	absent	Absent
11	YP64-27-12-43	present	Absent	absent	present	Present
12	YP64-27-12-45	present	Present	absent	present	Present
13	YP64-27-12-47	present	Present	absent	present	Present
14	YP64-27-12-52	absent	Present	absent	present	Present
15	YP64-27-12-5	absent	Present	absent	present	Present
16	YP64-27-12-6	absent	Absent	absent	present	Present
17	YP64-27-12-63	present	Present	absent	present	Present
18	Durga	present	Present	absent	present	Present
29	MTU1010	present	Present	absent	present	Present
20	Pooja	present	Present	absent	present	Present
21	Madhya Vijaya	present	Present	absent	present	Present
22	YP64-27-12-52	present	Present	absent	present	Absent
23	YP64-27-12-48	present	Present	absent	absent	Present
24	YP64-27-12-53	present	Present	absent	absent	Absent
25	Varshadhan	present	Present	absent	present	Present
26	Mashuri	present	Present	absent	absent	Present
27	Ranjeet	present	Present	absent	absent	Present
28	Annada	absent	Absent	absent	absent	Present
29	Sahabhagi dhan	absent	Absent	absent	absent	Absent
30	Tapaswini	absent	Absent	absent	absent	Absent
31	Rajalaxmi	absent	Absent	absent	absent	Absent
32	Lalat	absent	Absent	absent	absent	Present
33	MTU 1001	absent	Absent	absent	absent	Absent
34	Jaya	absent	Absent	absent	absent	Absent
35	Pratiksyia	absent	Absent	absent	absent	Absent
36	YP64-27-12-1	present	Present	absent	present	Present
37	YP64- 27-12-2	absent	Present	absent	present	Present
38	YP64-27-12-9	present	Present	present	present	Present
39	YP64- 27-12-10	present	Present	absent	present	Present
40	YP64- 27-12-22	present	Present	present	present	Present
41	YP64-27-12-12	present	Present	present	present	Present
42	YP64- 27-12-3	present	Present	present	present	Present
43	YP64- 27-12-15	present	Present	absent	present	Present
44	YP64- 27-12-7	present	Present	present	present	Present
45	YP64-27-12- 19	present	Present	present	present	Present
46	YP64- 27-12-4	present	Present	absent	present	Present
47	YP64- 27-12-9	present	Present	present	present	Present
48	YP64- 27-12-11	present	Present	absent	present	Present
99	YP64- 27-12-18	present	Present	absent	present	Present
50	YP64- 27-12-11	present	Present	absent	present	Present
51	YP64-12-12-10	absent	Absent	absent	absent	Absent
32	YP64-27-12-23	absent	Absent	absent	absent	Absent
53	YP64-27-12-14	absent	Absent	absent	absent	Absent

presence of strong culm in the studied genotypes. *Gw5* and *Gw2* for grain weight and grain width and *OsSPL14*

**Table 3.** Yield component traits of 53 studied genotypes during wet season, 2016.

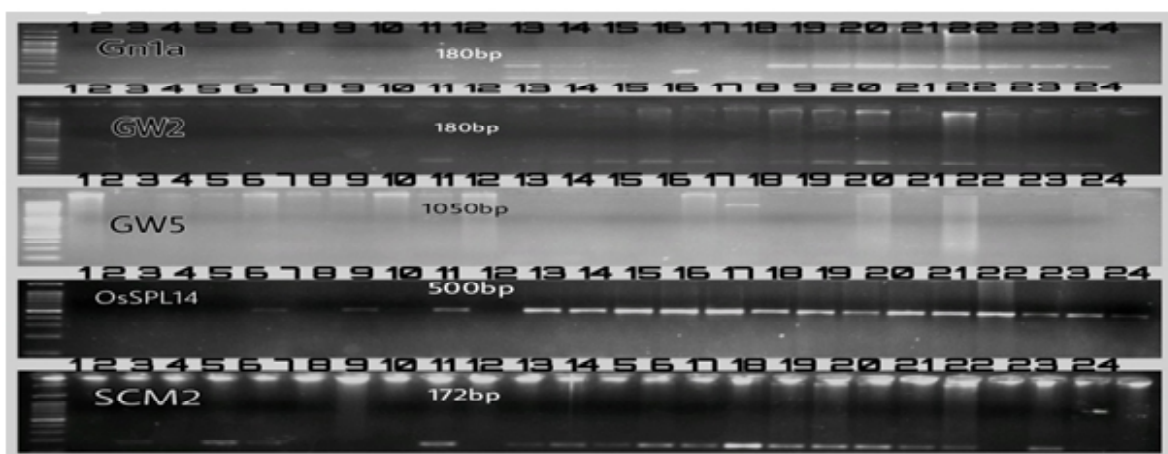
Sl no	Verity name	Plant height	Tiller no	Panicle length	Panicle weight	Grains/panicle	Grain length	Grain width	Section modulus(mm <sup>3</sup> )
1	YP64-27-12-8	81	8	22	2.5	130	7.5	2.7	5.5
2	YP64-27-12-23	82	7	2.6	2.2	142	7.7	2.7	8.3
3	YP64-27-12-27	80	8.6	20.5	2.0	127	7.9	2.8	5.4
4	YP64-27-12-32	85	8.2	26	5.2	195	8.0	2.9	8.4
5	YP64-27-12-29	84	9.0	23	2.3	128	8.2	2.8	8.5
6	YP64-27-12-25	85	7	23.6	2.1	147	8.5	2.7	8.1
7	YP64-27-12-41	85	8.2	23.5	2.7	149	7.6	2.9	5.2
8	YP64-27-12-23	82	8.4	24	3.1	160	7.8	2.8	5.6
9	YP64-27-12-37	83	9.2	236	5.3	210	8.1	2.4	8.5
10	YP64-27-12-41	80	9.8	22.8	2.1	152	8.4	2.7	5.5
11	YP64-27-12-43	81	10	24.8	2.9	200	7.6	2.9	8.0
12	YP64-27-12-45	83	10.1	25	2.9	195	7.5	2.3	8.1
13	YP64-27-12-47	84	8.2	25.2	3.8	210	7.9	2.4	8.1
14	YP64-27-12-52	85	8.6	23.6	2.9	150	7.7	2.5	8.1
15	YP64-27-12-5	85	8.9	23.4	2.7	147	8.0	2.6	8.2
16	YP64-27-12-6	82	9.0	23.4	5.4	148	8.1	2.9	8.3
17	YP64-27-12-63	83	9.6	24.0	5.2	180	8.6	2.8	8.6
18	Durga	121	12.0	23.6	3.4	229.0	7.9	2.3	7.2
29	MTU1010	98	7.	23.4	2.8	174.4	10.0	2.8	7.4
20	Pooja	116	8.2	27.2	3.1	175.0	8.6	2.8	7.4
21	Madhya Vijaya	98	11.0	26.1	3.8	205	8.4	2.7	7.2
22	YP64-27-12-52	170	8.6	28	5.1	200	8.0	2.8	8.3
23	YP64-27-12-48	129	6.8	25.4	3.1	218	7.5	2.4	7.4
24	YP64-27-12-53	96	9.1	26.2	3.2	170	7.9	2.8	6.7
25	Varshadhan	94	6.0	26.0	5.1	100	7.8	3.2	6.8
26	Mashuri	108	9.0	25.0	2.7	154.0	8.7	3.3	6.5
27	Ranjeet	95	10.8	25.2	3.2	149.0	8.3	2.4	6.7
28	Annada	118	8.6	30.6	4.1	184.0	9.6	2.7	7.8
29	Sahabhagi dhan	110	9.0	27.4	3.6	160	9.6	2.9	7.1
30	Tapaswini	113	7.6	24.0	3.7	172	8.3	2.7	7.4
31	Rajalaxmi	105	7.8	26.0	3.2	178.0	9.1	2.9	7.1
32	Lalat	107	7.0	26.2	4.3	120	8.7	2.9	7.3
33	MTU 1001	105	7.0	26	4.9	190	8.2	2.7	8.4
34	Jaya	101	8.1	25	2.2	132	8.7	2.6	8.7
35	Pratiksyia	103	8.3	27	2.4	179	9.2	2.8	8.5
36	YP64-27-12-1	105	8.7	24	5.1	203	8.7	2.5	8.6
37	YP64- 27-12-2	100	9.0	28	3.0	204	10.0	2.4	8.2
38	YP64-27-12-9	106	10.0	29	3.0	206	7.8	2.3	8.7
39	YP64- 27-12-10	96	9.6	26.5	5.2	172	9.1	2.5	8.8
40	YP64- 27-12-22	98	9.8	26.4	5.1	184	8.8	2.4	8.6
41	YP64-27-12-12	103	9.8	26.2	5.1	156	8.5	2.5	8.5
42	YP64- 27-12-3	107	11.0	30	2.9	208	8.6	2.2	8.5
43	YP64- 27-12-15	96	9.2	24	5.2	201	8.3	2.6	8.2
44	YP64- 27-12-7	99	8.7	29	2.6	209	9.3	2.5	8.1
45	YP64-27-12- 19	100	8.6	28	5.3	216	8.2	2.2	8.7
46	YP64- 27-12-4	105	7.7	25	2.4	211	9.0	2.4	8.3
47	YP64- 27-12-9	106	10.2	29	3.2	201	8.6	2.6	8.1
48	YP64- 27-12-11	102	11.1	24	5.1	206	9.6	3.0	8.8
99	YP64- 27-12-18	101	9.7	23	3.0	205	9.0	2.6	8.5
50	YP64- 27-12-11	98	9.6	22	2.6	208	9.2	2.7	8.4
51	YP64-12-12-10	107	706	24.3	2.4	146	7.4	2.7	6.2
32	YP64-27-12-23	100	6.9	25	5.3	133	7.5	2.6	6.4
53	YP64-27-12-14	93	7	22	2.6	142	7.6	2.7	6.6
	CD5%	12.8	1.32	2.54	2.87	18.54	0.921	0.324	0.878
	CV%	4.24	9.43	5.16	10.32	9.78	8.64	10.35	9.36

for screening of heavy panicle and ideal plant height. In our study, we surveyed the IR64 derived lines and popular varieties to find out the yield component QTLs for validation of the markers in indica background and tracking the QTLs in the progenies. Five markers (*Gn1a*, *Gw2*, *Gw5*, *Osspl14*, and *Scm2*) were used to identify the yield QTL. Banding analysis showed the presence of yield component QTL *Gn1a* in 29 genotypes of which 22 are tracked in BC<sub>2</sub>F<sub>4</sub> progenies; 31 genotypes for QTL *Gw2*; 7 genotypes for QTL *gw5*; 31 genotypes for QTL *OsSPL14* and 36 genotypes for QTL *SCM2*. However, presence of bands for all five QTLs was seen in 7 genotypes viz., Durga, Pooja, Madhya Vijaya, YP64-27-12-52, YP64-27-12-48, YP64-27-12-53 and Varshadhan.

Out of the 21 plants (Fig. 1) showing positive for *Gn1a* QTL, phenotyping results of these genotypes for grain number also showed high grain number (>150 grains/panicle) in these genotypes (Table 3). Hence, genotypes namely YP64-27-12-32, YP64-27-12-23, YP64-27-12-37, YP64-27-12-41, YP64-27-12-43, YP64-27-12-45, YP64-27-12-52, YP64-27-12-63, YP64-27-12-47, Durga, MTU1010, Pooja, Madhya Vijaya, Varshadhan, Mashuri, Ranjeet, YP64-27-12-1, YP64-27-12-9, YP64-27-12-10, YP64-27-12-22, YP64-27-12-12, YP64-27-12-3, YP64-27-12-15, YP64-27-12-7, YP64-27-12-19, YP64-27-12-4, YP64-27-12-9, YP64-27-12-11, YP64-27-12-18 and YP64-27-12-11 may be used in the yield potential breeding programme. The QTL *Gn1a* enhances grain yield significantly whenever the allele present in the rice genotype.

Mohanty et al. 2016 has also reported the QTL in indica-tropical japonica derived lines. *Gw5* is a major QTL, which is associated with reduced grain width and influences grain yield in rice. Detection of *gw5* was observed in 7 genotypes through banding analysis. All these 7 genotypes showed longer grain than other studied genotypes (Table 3). Therefore, the genotypes namely YP64-27-12-9, YP64-27-12-22, YP64-27-12-12, YP64-27-12-3, YP64-27-12-15, YP64-27-12-7 and YP64-27-12-19 are useful for increasing yield through *gw5*. Grain width also influences grain yield in rice (Zhang et al., 2013; Gao et al., 2015; Mohanty et al., 2016). The QTL for the trait, *Gw2* determines the grain dimension of rice. In our study, we could detect 32 genotypes with *Gw2* genes.

The number of grains per panicle under the control of the *Gn1a* locus along with WFA/IFA1 (*OsSPL14*) may be useful QTLs for panicle traits for increasing yield potential. The direct marker *OsSPL14* was employed to genotype the indica-tropical japonica derivative lines that showed the presence of *OsSPL14* in 196 genotypes. Heavy panicle QTL, *OsSPL14* band is observed in 31 genotypes. Phenotyping for heavy panicle (>5g.) was observed in 11 genotypes (Table 3). These eleven genotypes namely YP64-27-12-29, YP64-27-12-37, YP64-27-12-6, YP64-27-12-63, Varshadhan, Pratiksyia, YP64-27-12-1, YP64-27-12-10, YP64-27-12-15, YP64-27-12-19 and YP64-27-12-23 may serve as donor for heavy panicle. Earlier reports also indicate the presence of *OsSPL14* in rice (Mohanty et al., 2016). Strong culm is a desirable trait of rice plant that provides



**Fig. 1.** Representative electrophoregram of IR64 derived lines and high yielding varieties using yield component QTLs markers *Gn1a*, *Gw2*, *gw5*, *OsSPL14* and *SCM2*. The numbers on the top of the lane indicate the genotype used as in the Table 2. L: 100 bp DNA ladder.

non-lodging habit to the crop plant. Rice plant without strong culm may not be able to bear a big panicle load which may lodge leading to a loss to the growers. Presence of *SCM2* increases the cross section modulus of culm and thereby increases lodging resistance. Majority of the derived lines possess the QTL, *SCM2*. Also, popular varieties like YP64-27-12-8, YP64-27-12-23, YP64-27-12-27, YP64-27-12-32, YP64-27-12-29, YP64-27-12-25, YP64-27-12-41, YP64-27-12-23, YP64-27-12-45, YP64-27-12-6, YP64-27-12-63, Durga, MTU1010, Pooja, Madhya Vijaya, YP64-27-12-52, YP64-27-12-48, YP64-27-12-53, Varshadhan, Mashuri, Ranjeet, Annada, Sahabghidhan and Tapaswini showed bands of *SCM2*.

## CONCLUSION

Yield potential improvement of popular variety IR64 was possible using component trait QTLs *Gn1a*, *Gw2*, *gw5*, *OsSPL14* and *SCM2*. Presence of the yield QTLs were detected in the backcross progenies in BC<sub>2</sub>F<sub>3</sub> generation. Phenotypic effects were also observed in the derived lines and popular varieties. Presence of bands for all five QTLs was seen in genotypes Durga, Pooja, Madhya Vijaya, YP64-27-12-52, YP64-27-12-48, YP64-27-12-53 and Varshadhan. These genotypes are useful donors for improvement of yield potential in rice.

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