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Genomic analysis for the selection of superior donor genotypes in rice improvement

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Abstract
The field experiment was conducted following a Randomized Block Design (RBD) with three replications, involving 30 advanced breeding lines, with BPT-5204 included as a standard quality check. For molecular characterization, a set of seven genes (*Gs3, Gw5, Gn2, Gn1, SCM3, SCM2, and Spl14*) and three yield-related *QTLs* (*Yld12.1 and Yld2.*1) associated with grain yield were employed. Among these, *Gn1* and *Gn2* were linked to grain number, *SCM2* and *SCM3* to culm strength, *Gw5* to grain weight, *Gs3* to grain size, and *Spl14* to the number of filled grains per panicle. Genotyping results revealed that only one advanced breeding line, SP-08**,** possessed all seven yield-associated genes and *QTLs*, suggesting a strong potential for enhanced yield performance. Two breeding lines exhibited the presence of six yield-contributing loci**:** SP-69**,** which carried *Gn2, Gn1, Gw5, SCM3, Yld2.1, and Spl14,* and SP-70, which harbored *Gn1, SCM3, Gw5, Gs3, Yld2.1, and Yld12.1*. In addition, four advanced breeding lines were identifies with 5 yield-related genes/*QTLs***,** line SP-37 (*Gn2, SCM3, Gw5, Yld2.1, Yld12.1*), SP-55 (*Gn2, SCM2, SCM3, Gw5, Yld2.1*), SP-75 (*Gn2, Gn1, SCM3, Gw5, Yld12.1*), and SP-61 (*Gn2, Gn1, SCM2, Gw5, Yld2.1*).

Keywords: Advanced breeding lines, Gn2, Gn1, SCM3, SCM2, Gs3, Gw5, Spl14, QTLs, Yld2.1, Yld4.1 and Yld12.1

Introduction
Rice (*Oryza sativa* L.) is the family *Poaceae* and the subfamily *Oryzoideae*. As one of the principal cereal crops, rice serves as the primary food source for a large proportion of the global population. Along in Asia, over two billion people derive nearly 60-70 percentage of their daily food habits intake from rice and rice-based products. Among rice-producing nations, India ranks first in cultivated area (42.27 million hectares) and second in total production (105.24 million tonnes), following China, which produces approximately 144 million tonnes. However, India's average rice productivity (2.49 t ha^{-1}) remains considerably lower than the global mean yield of 4.36 t ha^{-1} (FAOSTAT, 2019). With an annual population growth rate of about 1.9 percentage, India's projected rice demand is expected to reach nearly 125 million tonnes by 2030 [1]. Ensuring national food security under such conditions necessitates substantial improvements in rice yield despite constraints on land and water availability. Approximately, increasing grain output per unit number of natural resource has become a critical challenge.

The closely low yield levels of rice in India pose a serious threat to the food and nutritional security of more than 45 Percentage of the population, who depend heavily on this crops. Moreover, the growing lower of irrigation water is emerging as a major limitation to sustainable rice production, particularly in leading rice-growing countries such as India, USA and China, where escalating comparative for fresh water resources from without agricultural sectors is anticipated in the becoming years [2].

Plant height and flowering duration are key detections of rice plant architecture and productivity. In this study, attempts were made to conduct a morphological and molecular characterization of Ethyl Methane sulfonate-induced dwarf and early flowering mutants developed from the rice variety Nagina 22, along with an assessment of their inheritance patterns. A total of 9 stable, true-breeding mutants, previously generated through Ethyl Methane sulfonate mutagenesis, were evaluated for variation in phenotypic traits following the national guidelines for (DUS) [3].

Materials and Methods

1. DNA Extraction

Genomic DNA was extracted from fresh leaf samples using Murray's method [4]. Quality and concentration of the isolate DNA was assessed using a Nano Drop spectrophotometer and further verified through agarose gel electrophoresis [5].

2. Gene Profiling

Polymerase Chain Reaction (PCR) assays were performed to screen for high-yield-associated genes and quantitative trait loci (*QTLs*)**.** The analysis included positive controls and susceptible check genotypes to confirm amplification accuracy. A total of ten major yield-related loci**,** comprising seven genes (*Gn2, Gn1, SCM3, SCM2, Spl14 Gs3, and Gw5*,) and three *QTLs* (*Yld2.1, Yld12.1 and Yld4.1*), were in thisstudy using gene-specific molecular markers following the protocol. Details of the markers used for each gene and *QTL* are provided in Table 1**.**

Table 1: Identified genes and *QTL*s realative to yield showed in present study

S. No.	Gene /*QTL*	Chromosome	Markers
1	Gn2	Chr-2	RM250 RM208
2	Gn1	Chr -1	Gn1A* Gn1A17* Gn1INDEL* RM10499 RM151 RM10382
3	SPL-14	Chr -8	Spl14-12* Spl14-4* RM23237 RM23386
4	SCM-2	Chr -6	SCM2-1* SCM2-2* SCM2-3* SCM2-4* RM20615 RM20458
5	SCM-3	Chr -3	SCM3-1* SCM3-2* SCM3-3* SCM3-4* RM1350
6	Gs-3	Chr -3	DRR-GL
7	Gw-5	Chr -5	RM437 RM18161 RM18089
8	Yld-4.2	Chr -4	RM261 RM16338 RM16373
9	Yld-2.1	Chr -2	RM262 RM263
10	Yld-12.1	Chr -12	RM511 RM28166 RM28163 RM280130 RM28099
	* Functional markers.		

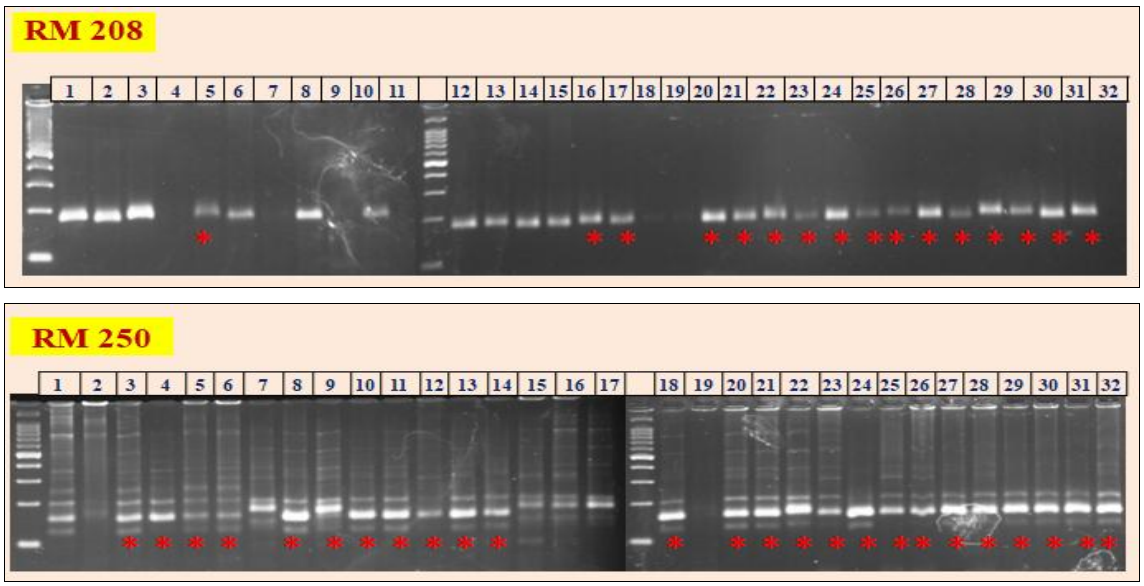
Results and Discussion

1. Grain Number (Gn1 and Gn2)

Two major genes, *Gn1* and *Gn2***,** were targeted. For *Gn1*, the japonica cultivar Habataki was used as a positive control, while BPT-5204 served as the negative control [6]. The allelic variation of *Gn1* among the ABL was examined using **6** molecular markers (Table 1), existing of 3 functional markers (*Gn1A17, Gn1INDEL and Gn1A*,) and 3 closely linked markers (*RM10382, RM10499 and RM151*). Genotypes with these markers revealed that 7 ABL-SP-351, SP-70, SP-61, SP-69, SP-25, SP-08, and SP-75-carried the positive *Gn1* allele, as confirmed by amplification with more than 2 markers.

The analysis of *Gn2*, the indica genotype H-2-4 was employed as a positive control, whereas BPT-5204 was included as a negative check. 2 linked markers, *RM208 and RM250*, were utilized to determine the allelic status of *Gn2* in the ABLs (Table 1; Figure 1). Genotyping results indicated that 13 ABLs- SP-13, SP-03, SP-01, SP-61, SP-69, SP-55, SP-80, SP-25, SP-34, SP-37, SP-08, SP-75, and SP-57-exhibited positive alleles for *Gn2* with both markers (Figure 1).

Grain number is a critical yield-determining trait in rice (*Oryza sativa* L.), governed by a complex genetic network. *Gn2***,** originally identified from the wild rice accession *Oryza rufipogon* Griff. ("*Yuanjiang*"), has been reported to play a significant role in regulating this trait. ABLs over expressing *Grain Number2* observed an increase in GN, accompanied by reduced plant height and delay in flowering compares to control in paddy plants [7].



1a.MTU 10101 (H-2-4), 2a.BPT-5204, 3a.IR-64, 4a.NDR-359, 5a.Jaya, 6a.BPT-5204, 7a.SP-351, 8a.SP-352, 9a.SP-353, 10a.SP-354, 11a.SP-355, 12a.SP-356, 13a.SP-357, 14a.SP-358, 15a.SP-359, 16a.SP-360, 17a.SP-70, 18a.SP-72, 19a.SP-63, 20a.SP-61, 21a.SP-69, 22a.SP-55, 23a.SP-80, 24a.SP- 25, 25a.SP-13, 26a.SP-03, 27a.SP-01, 28a.SP-34, 29a.SP-37, 30a.SP-08, 31a.SP-75, 32a.SP-57. * Genotypes have positive alleles for (Gn2).

Fig 1: Genotyping of *GrainNumber2* among lines with markers

2. Spike length (Spl 14)

Spike Length gene, *Spl 14*"Aikava (273)" were selected positive control, where as BPT-5204 negative control. To detect the allelic status of *Spl 14* 4 markers were used (Table 1). 2 were functional markers (*Spl14-4 and Spl14-12*) and 2 linked markers (*RM23386 and RM23237*). Upon genotyping only one ABLs (SP-69) showed positive alleles for *Spl 14* with more than 2markers.

3. Strong Culm (SCM2 and SCM3)

To assess culm strength, 2 key genes, *SCM2* and *SCM3*, identified. Both loci, the japonica cultivar Habataki was included as a positive control, while BPT-5204 served as the negative check. The allelic variation of SCM2 among the advanced breeding lines was analyzed using six molecular markers (Table 1), comprising four functional markers (*SCM2-1, SCM2-2, SCM2-3, and SCM2-4*) and two tightly linked markers (*RM20615 and RM20458*).

Genotyping results indicated that five advanced breeding lines- SP-351, SP-61, SP-55, SP-08, and SP-57-carried the favorable *SCM2* allele, as confirmed by amplification with more than two markers. Previous studies have shown that *near-isogenic lines (NILs)* of Koshihikari carrying the *SCM2* allele introgressed from Habataki exhibited a marked improvement in culm strength, along with increased spikelet number and enhanced grain yield. These findings suggest that *SCM2* is a valuable genetic resource for improving lodging resistance and yield potential**,** particularly in japonica rice backgrounds.

4. Grain Weight (Gw5)

Grain WeighT_5 gene was targeted to evaluate variation in grain weight among the ABLs. Line of Swarna (272)**,** an indica genotype, was used as the positive control, while BPT-5204 served as the negative check. To determine the allelic status of *Gw5*, 4 closely linked molecular markers- *RM18065, RM18089, RM437 and RM18161*-were employed (Table 1). Genotyping results showed that twenty breeding lines (SP-70, SP-72, SP-63, SP-61, SP-69, SP-55, SP-25, SP-01, SP-37, SP-08, SP-75, SP-351, SP-352, SP-353, SP-354, SP-355, SP-356, SP-357, SP-359 and SP-360,) carried the favorable *Grain Weigh*T_5 allele, as confirmed by amplification with more than 2 markers.

The grain weight-associated *QTL qGw5* was initially identified using *Asominori/IR24 recombinant inbred lines* and *chromosome segment substitution line* populations. Subsequent fine mapping localized this *QTL* to a 59.7 kb genomic interval**,** enabling its effective utilization in rice improvement programs globally [8].

5. Grain Size (Gs3)

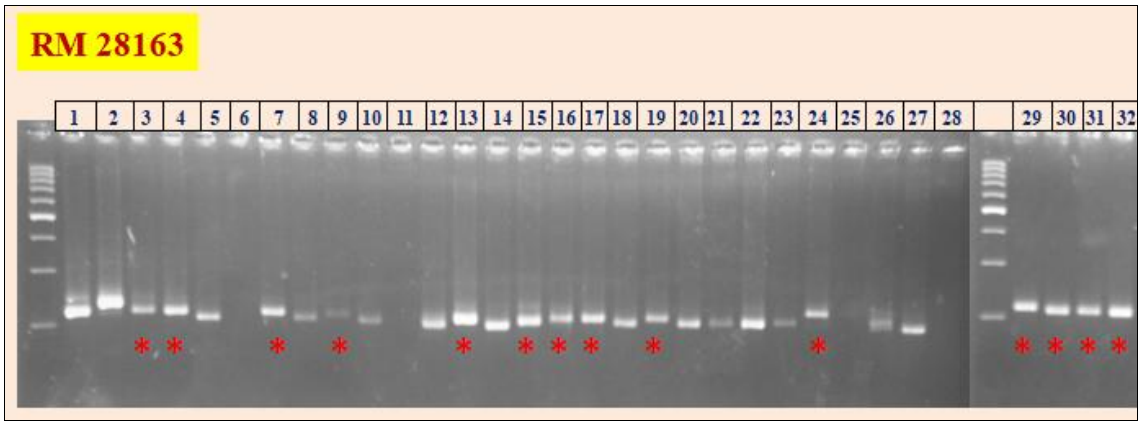
To assess grain size, the *Gs3* gene was selected for analysis. The long-grain aromatic cultivar Basmati 370 was used as a positive control, whereas BPT-5204, characterized by medium-slender grains, was included as the negative control. The allelic variation of *Gs3* was detected using a single linked functional marker (*DRR-GL*) (Table 1). Genotyping revealed that four advanced breeding lines SP-70, SP-63, SP-80 and SP-360, possessed the favorable *Gs3* allele**.**

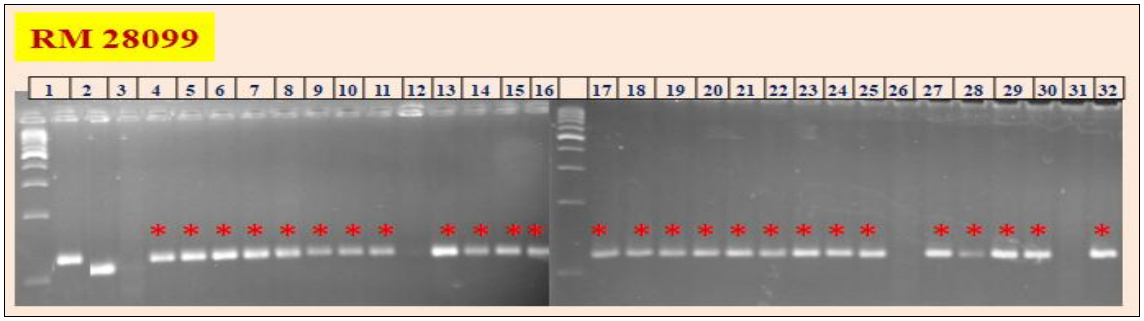
Conventional cleaved amplified polymorphic sequence (CAPS) markers used earlier for this gene were found to be labor-intensive. As an alternative, the functional marker *DRR-GL*, designed to target a C/A *single nucleotide polymorphism* within the *Gs3* gene, was developed and extensively validated across diverse rice germplasm, proving its effectiveness for grain size selection [9].

6. Grain Yield (Yld12.1, Yld2.1, and Yld4.1)

Three key quantitative trait loci associated with grain yield- *Yld12.1, Yld2.1, and Yld4.1*-were selected for molecular analysis. Among these, *Yld12.1* was examined in detail using of Vandana as the positive control, while Varalu was included as the negative control. The allelic status of *Yld12.1* in the experimental genotypes was assessed using five closely linked *SSR* markers (*RM28163, RM511, RM28166, RM28099 and RM28130*) (Table 1; Figure 2). Marker analysis indicated that twelve advanced breeding lines- SP-70, SP-63, SP-25, SP-37, SP-08, SP-75, SP-57 SP-351, SP-353, SP-357, SP-359 and SP-360 -carried the favorable *Yld12.1* allele, as confirmed by amplification with more than 2 markers.

Previous studies have shown that a large proportion of grain yield-related *QTLs* under stress conditions are clustered near the centromeric region of *chromosome 12*, particularly around the *qtl12.1* locus. *Composite interval mapping* conducted over 2 consecutive years using a population of 566 lines localized this *QTL* within the interval flanked by *RM28048* (47.2 cM) and *RM511* (55.5 cM)**.** The rare allele at *qtl12.1* exhibited an additive effect of 172 kg ha^{-1} and accounted for nearly 33% of the phenotypic variation in grain yield under stress conditions. In addition to grain yield, significantly associations were also observed in this genomic region for biomass production, final plant height, flowering delay, harvest index, flowering time, panicle number and drought-response index highlighting the pleiotropic influence of *qtl12.1* [10].



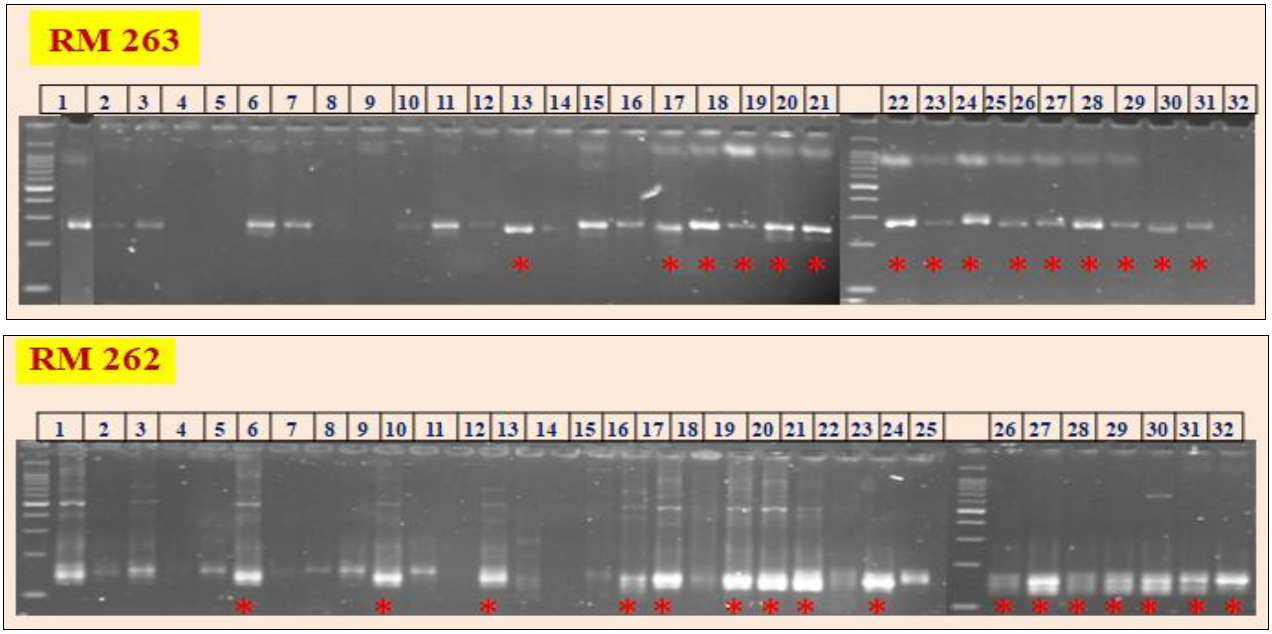


1b.Vandana, 2b.BPT-5204, 3b.IR-64, 4b.NDR-359, 5b.Jaya, 6b.BPT-5204, 7b.SP-351, 8b.SP-352, 9b.SP-353, 10b.SP-354, 11b.SP-355, 12b.SP-356, 13b.SP-357, 14b.SP-358, 15b.SP-359, 16b.SP-360, 17b.SP-70, 18b.SP-72, 19b.SP-63, 20b.SP-61, 21b.SP-69, 22b.SP-55, 23b.SP-80, 24b.SP- 25, 25b.SP-13, 26b.SP-03, 27b.SP-01, 28b.SP-34, 29b.SP-37, 30b.SP-08, 31b.SP-75, 32b.SP-57.
* Genotypes have positive alleles for *Grain Yield* (*Yld12.1*).

Fig 2: Genotyping of *Yld12.1* among ABLs with markers

For the analysis of *Yld2.1*, the genotype *DRR-50-7* was used as a positive reference, while BPT-5204 served as the susceptible control. The allelic composition of *Yld2.1* in the evaluated breeding lines was determined using two closely linked SSR markers**,** *RM262* and *RM263* (Table 1; Figure 3). Marker-based screening revealed that eleven advanced lines-, SP-70, SP-72, SP-357, SP-03, SP-01, SP-34, SP-37, SP-61, SP-69, SP-55 and SP-08-carried the favorable *Yld2.1* allele, as indicated by consistent amplification with morethan one markers.

Earlier efforts to identify this *QTL* employed a marker-assisted selection strategy using three peak markers, with one diagnostic marker assigned to each *qDTY* locus. During foreground selection, previously reported peak markers, *RM520***,** *RM511*, were utilized to validate the presence of *qDTY3.1 and qDTY12.1*, respectively [11].



1c.DRR-50-7, 2c.BPT-5204, 3c.IR-64, 4c.NDR-359, 5c.Jaya, 6c.BPT-5204, 7c.SP-351, 8c.SP-352, 9c.SP-353, 10c.SP-354, 11c.SP-355, 12c.SP-356, 13c.SP-357, 14c.SP-358, 15c.SP-359, 16c.SP-360, 17c.SP-70, 18c.SP-72, 19c.SP-63, 20c.SP-61, 21c.SP-69, 22c.SP-55, 23c.SP-80, 24c.SP- 25, 25c.SP-13, 26c.SP-03, 27c.SP-01, 28c.SP-34, 29c.SP-37, 30c.SP-08, 31c.SP-75, 32c.SP-57.
* Genotypes have positive alleles for Grain Yield (*Yld2.1*).

Fig 3: Genotyping of *Yld2.1* among ABLs with markers

Conclusion

- Among the evaluated materials, SP-08 emerged as the most promising advanced breeding line, as it harbored seven key genes/*QTLs* associated with grain yield. these genotypes therefore represents a valuable donor source for use in future rice improvement and molecular breeding programs.
- The breeding lines SP-69 and SP-70 were also notable, as they possessed the panicle length-related gene (*Spl14*) along with six yield-enhancing genes/*QTLs*, indicating their potential contribution to yield improvement.
- In addition, the advanced breeding lines SP-37, SP-55, SP-75, and SP-61 were found to carry five yield-related genes/*QTLs*, suggesting moderate but consistent potential for inclusion in breeding strategies aimed at yield enhancement.

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