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Morpho-Phenological and Molecular Traits for Yield-Associated Genes/QTLs in Advanced Rice Breeding Lines (*Oryza sativa* L.)

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ABSTRACT

An investigation was conducted at the Indian Institute of Rice Research (IIRR) farm located at Rajendranagar, Hyderabad. The experimental field was laid out in an RBD design using 30 advanced breeding lines. BPT-5204 is used as a quality check. The experimental field was laid in RBD replicated thrice with 30 high yielding rice genotypes which includes 26 advanced breeding lines (ABL) (SP-351, SP-352, SP-353, SP-354, SP-355, SP-356, SP-357, SP-358, SP-359, SP- 360, SP-70, SP-72, SP-63, SP-61, SP-69, SP-55, SP-80, SP-25, SP-13, SP-03, SP-02, SP-34, SP-37, SP-08, SP-75 and SP-57) and four checks(NDR-359, BPT-5204, IR-64, Jaya).

Morphological parameters of plant height among genotypes ranged from 92 to 131cm. Genotype, SP-70 (126 cm), showed maximum plant height and was followed by SP-355 (124 cm), compared to BPT-5204 (98 cm). Mean plant height increased by 44.3% from active tillering to panicle initiation stage and then by 28.4% from PI to physiological maturity. A higher number of tillers per plant was observed in SP-08 and SP-70 (23) compared to check BPT-5204 (18). Among the duration groups, the short duration (116 to 122 days) lines were SP-08, SP-72, SP-70, and SP-69. The medium duration (125 to 133 days) lines, SP-351, SP-353, SP-355, SP-357, SP- 80, and SP-25, and long duration (140 to 148 days) lines, SP-02, SP-03, and SP-37 found superior in terms of grain yield as compared to their respective check varieties. Two advanced breeding lines, namely SP-08, SP-70, can be further probed thoroughly for further increasing yield and yield attributes. The morphological and physiological parameters of advanced breeding lines were correlated with the molecular analysis. SP-08 showed better morpho-phenological parameters like the number of tillers and also showed the presence of seven (Gn1, Gn2, Yld12.1, and Yld2.1) yield contributing genes/QTLs. Genotype, SP-70 showed better in several physiological parameters photosynthetic rate, SPAD meter readings, dry matter accumulation, and also contains six (Gn1, Yld2.1, and Yld12.1) genes/QTLs. Genotype, SP-69 higher panicle length and also contains six corresponding genes governing (Gn1, Gn2, and Yld2.1) genes/QTLs.

Keywords: *Rice (Oriza sativa), Advanced Breeding Lines(ABL), Plant Height, Number of tillers, yield contributing genes/ QTLs (Gn1, Gn2, Yld12.1, Yld4.1and Yld2.1).*

Introduction

Rice (*Oryza sativa* L.) belongs to the family Gramineae and subfamily *Oryzoideae*. As a cereal grain, it is the most important staple food crop in the world. In Asia, more than two billion people are getting 60-70 per cent of their energy requirement from rice and its derived products. In the twenty-first century, the world faces a serious challenge in that agricultural land area has sharply decreased in contrast to a population explosion. To solve the crisis of food shortage, there is a necessity to increase the crop productivity of rice, as rice is the primary staple food for one- third of the world population after wheat and maize.

So far, several high-yielding and management-responsive varieties have been developed and released for improved crop production. Among which Samba Mahsuri, a hybrid derived from the cross (GEB 24 x TN1) rice, is otherwise called Sona Mahsuri/ Samba Mahsuri/ BPT- 5204, which is a premium quality aromatic and light-weight rice. Due to its excellent grain character, the variety is regularly used in hybridization programmes to meet current breedingobjectives. Therefore, use of advanced breeding lines generated from BPT-5204 would only be appropriate, and evaluation of available germplasm or mutants for various physiological and yield attributes is essential [1].

Keeping the above-mentioned points in view, it was proposed to characterize the advanced EMS-mutated lines for their important morphological, physiological, yield yield- attaining traits, grain characteristics as compared to the wild type (untreated Samba Mahsuri). Further, molecular characterization of these selected lines would confirm the specific characteristics of a variety.

Material and Methods

Location of the experimental site

This research was conducted during the *Kharif* season 2014 and 2015 at the research farm of the Indian Institute of Rice

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Research (IIRR), Rajendranagar, Hyderabad, located at a Latitude of $17^{0}19'$ N, a Longitude of $78^{0}28'$ E, and at an altitude of 542 m above the Mean Sea Level.

Plant height (cm)

The height of the five plants was measured in centimeters from the ground level to the tip of the youngest leaf at active tillering, panicle initiation, and to the tip of the panicle at physiological maturity.

Total number of tillers per hill

The number of tillers in five hills was counted at harvest.

Number of productive tillers per hill

At harvest the tillers bearing panicles per hill were counted and expressed as number of productive tillers per hill.

Molecular Analysis

Advanced breeding lines used for molecular characterization are the pre released lines which are homozygous mutant lines. These constitute an important source of genetic variation for utilization in breeding of high yielding rice varieties and hybrids. These lines were characterized for genes and *QTLs*, seven yield associated genes *viz., Gn1, Gn2, SCM2, SCM3, Gs3, Gw5, Spl14* and three *QTLs viz., Yld12.1, Yld2.1 and Yld4.*1.For characterization of advanced breeding lines for presence of genes and *QTLs*, seven yield genes *viz.,Gn1, Gn2, SCM2, SCM3, Gs3, Gw5, Spl14* and three *QTLsviz., Yld12.1, Yld2.1 and Yld4.*1 were selected. Each genes/*QTLs* used functional and linked markers showed in table 1.

Table 1: High yield contributing genes/QTLs and related markers with references

S.No	Gene/*QTLs*	*Chromosome*	Location	Markers	References
1	*Gn1*	*Chromosome-1*	5.5Mb-7.9Mb	Gn1A*	[2]
				Gn1A17*	[3]
				Gn1INDE*	[4]
				RM10499	
				RM151	
				RM10382	
2	*Gn2*	*Chromosome-2*	32.7Mb-35.1Mb	RM250 RM208	[5] [6]
3	*Yld-12.1*	*Chromosome-12*	45.2Mb-67.3Mb	RM511	[7]
				RM28166	[8]
				RM28163	[9]
				RM280130	
				RM28099	
4	*Yld-2.1*	*Chromosome-2*	20.7Mb-25.8Mb	RM262	[10]
				RM263	
					[11]
					[12]
					[13]
5	*Yld-4.2*	*Chromosome-4*	25.8Mb-31.5Mb	RM261	[14]
				RM16338	
				RM16373	

**Functional markers*

Results and Discussion

Morpo-Physiologcal studies

Plant height at active tillering (AT), panicle initiation (PI), and physiological maturity (PM)stages

The plant height is a major trait that influences the seed yield in paddy. Plant height increased with the age of the crop and reached a maximum at physiological maturity. Similarly, [15] showed that plant height increases progressively with the advancement of time from 15to 75 DAT. In the present study, at active tillering (AT), panicle initiation (PI), and physiological maturity (PM), plant height differed significantly among the genotypes (Table 2). The mean plant height across the seasons at the active tillering stage, PI and PM was 77, 102, and 126 cm, respectively highest in SP-70 against the check variety, BPT-5204 (48, 75, and 98 cm, respectively). The least plant height was observed in the genotype, SP-03 (49, 69, and 87 cm, respectively).

Plant height increases mainly during the vegetative phase (active tillering to panicle initiation), and it was by 44.3% across the varieties and 56.3% in BPT-5204. The plant height has shown a positive relationship with both biomass and grain yield, but significantly with yield only at the physiological maturity stage (Table 3). However, maximum yield was attained in a variety having plant heights of 84cm and 111 cm.

Table 2: Plant height (cm) at active tillering, panicle initiation and physiological maturity in advanced breeding lines of rice

Plant height (cm)										
		Active Tillering			Panicle Initiation			Physiological Maturity		
S.No	Genotypes	2014	2015	Pooled	2014	2015	Pooled	2014	2015	Pooled
1	SP-351	59	61	60	95	100	98	119	121	120
2	SP-352	56	53	55	89	92	91	111	114	113
3	SP-353	56	59	58	89	91	90	109	111	110
4	SP354	54	50	52	85	81	83	114	112	113
5	SP-355	75	73	74	97	104	101	123	125	124
6	SP-356	68	72	70	88	93	91	120	115	118
7	SP-357	62	65	64	92	103	98	110	107	109
8	SP-358	66	61	64	91	98	95	116	120	118
9	SP-359	63	65	64	94	99	97	111	114	113
10	SP-360	56	58	57	91	93	92	120	123	122
11	SP-70	78	75	77	98	106	102	124	127	126
12	SP-72	61	65	63	86	91	89	113	115	114
13	SP-63	65	62	64	92	90	91	110	114	112
14	SP-61	64	66	65	94	95	95	113	119	116
15	SP-69	70	68	69	90	89	90	120	123	122
16	SP-55	55	54	53	92	90	91	110	115	113
17	SP-80	69	73	71	88	94	91	103	105	104
18	SP-25	63	64	64	96	98	97	110	114	112
19	SP-13	61	68	65	86	89	88	114	119	117
20	SP-08	60	66	63	82	86	84	109	112	111
21	SP-75	63	65	64	78	79	79	108	109	109
22	SP-57	64	68	66	76	81	79	114	121	118
23	SP-03	43	52	49	67	71	69	85	89	87
24	SP-02	59	61	60	80	84	82	115	120	118
25	SP-34	69	70	70	81	87	84	103	107	105
26	SP-37	53	55	54	80	88	84	116	119	118
27	NDR-359	54	59	57	75	80	78	101	104	103
28	BPT-5204	52	50	48	72	77	75	97	100	98
29	IR-64	51	53	51	73	83	78	100	103	101
30	Jaya	52	56	54	85	88	87	113	114	114
	Mean	60	62	61	86	90	88	111	114	113
	SE (m)	9.73	8.63	2.36	11.98	13.08	12.78	6.9	11.8	2.95
	CD at 5%	19.9	17.65	14.67	18.52	16.76	14.62	20.13	21.15	20.85
	CV (%)	2.23	2.6	4.87	3.25	3.53	5.54	4.31	6.88	3.08

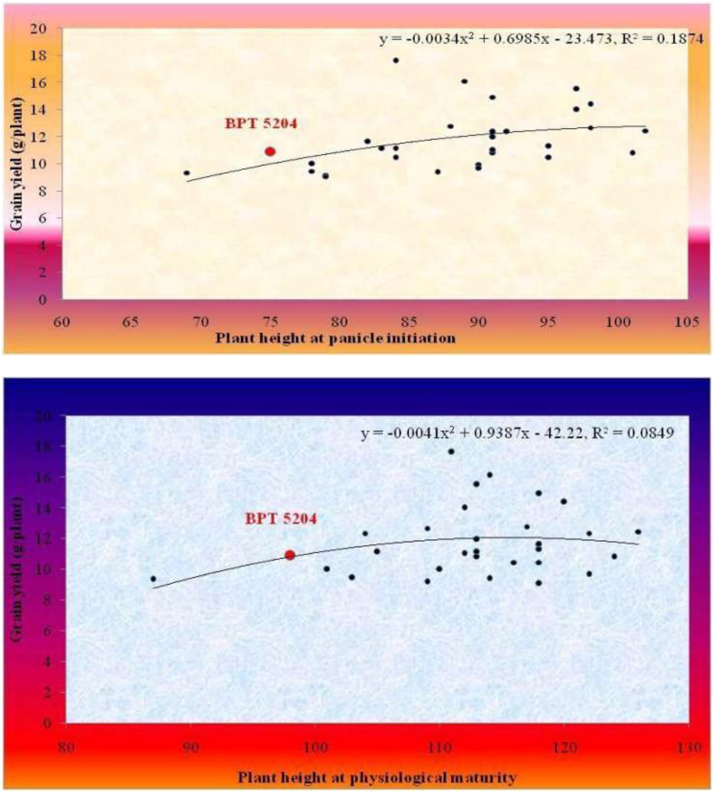


Figure 1. Relationship between plant height at different crop growth stages and grain yield

(Note: Red spot denotes BPT-5204; blue spots indicates advanced breeding lines)

respectively at PI and PM stage, beyond which there was no improvement in grain yield (Figure 1). The dwarf types have less biomass and thus lower seed yield, but a medium plant height would produce more biomass, panicle length and higher seed yields [16]. The tall plant height may lead to lodging problem; photosynthates may locked up in stem with longer distance for translocation of photosynthates from leaves to panicle.Therefore, it appears that plant height in paddy for better yields would be nearly 110 cm, a medium plant height types as compared to BPT-5204.

Table 3. Correlation coefficient values between plant height at different stages with biomass production and grain yield at harvest in elite rice genotypes

Crop stage	Plant height with biomass	Plant height with grain yield
Active Tillering (AT)	0.351*	0.278
Panicle Initiation (PI)	0.196	0.416*
Physiological Maturity (PM)	0.231	0.231

**: denotes significance at 5 % level of confidence.*

Total number of tillers and productive tillers

The total number of tillers generally determines the biomass production, the more tillers per hill, more will be the biomass production. In the present study, except the released varieties including BPT-5204, all the breeding lines possess higher biomass compared to BPT-5204. The tiller number in BPT-5204 is 21 per hill as against the genotypic mean of 24 tillers per hill. The relationship between the tiller number and biomass is not significant ($r = 0.16^{NS}$) because all the breeding lines, except four lines (SP-355, SP-360, SP-70, SP-69 significantly superior over BPT- 5204), were similar medium plant height that of BPT-5204.

Therefore, it is not only the number of tillers but also tiller height that is important in biomass production.

With respect to yield, it is not the total tillers per hill, but it is the effective tillers (productive tillers) that are important. A direct relationship between productive tillers and grain yield has been reported with significant genotypic variations in paddy [17],[18]. The ratio of effective tillers to total tillers per hill is 87.4%. Similar observation of >85% effective tillers in paddy has been documented. The mean productive tillers over the seasons were 21 per hill. The BPT-5204 had 18 tillers per hill, and the breeding lines with significantly

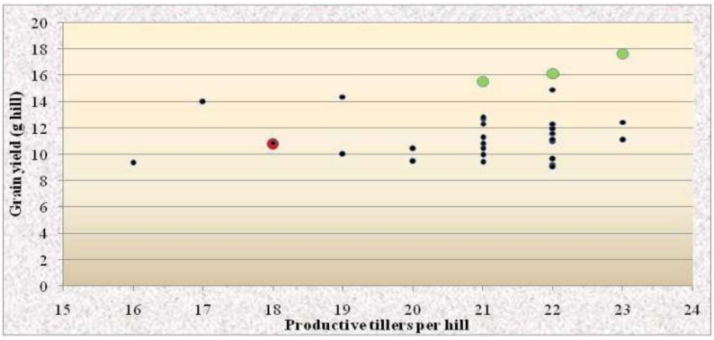


Figure 2. Relationship between productive tillers and grain yield in advanced rice breeding lines

(Note: Red spot denotes BPT-5204; green spots indicate SP-359, SP-72, and SP-08 from lower to higher grain yield)

High-yielding varieties possess higher productive tillers. No significant relationship was observed between productive and grain yield ($r = 0.24^{NS}$) might be due to all the breeding lines being derived from the same parentage, although the range statistic is 23.8 per cent. However, the highest-yielding varieties had higher productive tiller numbers per hill (Figure 2). It appears that the productive tillers could be 23 per hill for higher productivity in derivatives of BPT-5204.

Phenological parameters

Rice crop phenology or duration forms the major determinant of the agro-ecological and agronomic fit of rice cultivars into any cropping situation. The physiological age of the crop has been characterized by the formation of the various organs and their appearance [19]. Based on the duration of growth, rice genotypes have been classified into early duration (110-120), medium duration (125-135), and long duration (more than 150). Days to flowering and maturity of different rice varieties grown under field conditions are presented in Table 4.

Days to 50% flowering

Flowering time plays an important role in regulating the biomass of crops by affecting their duration of basic vegetative growth, and thereby grain yield. In the present study significant difference was observed for days to 50% flowering among genotypes. During the first season, the genotype SP-03 and BPT-5204 (95 days and 93 days) recorded maximum values, SP-08and SP-72(80 days and 81 days) recorded minimum days for 50% flowering. During second season, the genotype SP-03 and BPT-5204 (97days), recorded maximum value respectively while, SP-08 and SP-72 recorded (82days and 83 days) minimum values. Pooled data revealed that SP-03 and BPT-5204 showed maximum value under control (96 days and 95 days). The minimum value was recorded under control and elevated temperature in the genotypes SP-08 and SP-72 (81days and 82days).

Table 4: Days to 50% flowering and days to physiological maturity in advanced breeding lines of rice

		Days to 50% flowering			Days to Physiological Maturity		
S.No	Genotypes	2014	2015	Pooled	2014	2015	Pooled
1	SP-351	88	86	87	131	132	132
2	SP-352	83	86	85	145	143	144
3	SP-353	89	86	88	126	128	127
4	SP354	82	87	85	135	131	133
5	SP-355	84	87	86	127	129	128
6	SP-356	80	91	86	122	124	123
7	SP-357	86	86	86	124	126	125
8	SP-358	85	86	85	141	140	140
9	SP-359	87	90	89	139	136	138
10	SP-360	83	90	87	120	121	121
11	SP-70	83	88	86	117	119	118
12	SP-72	80	82	81	114	118	116
13	SP-63	82	84	83	119	121	120
14	SP-61	86	94	90	142	142	142
15	SP-69	92	87	90	125	126	126
16	SP-55	82	89	86	128	130	129
17	SP-80	82	88	85	124	125	125
18	SP-25	82	88	85	124	126	125
19	SP-13	86	87	87	120	124	122
20	SP-08	81	83	82	114	113	114
21	SP-75	84	85	85	120	122	121
22	SP-57	84	88	86	128	130	129
23	SP-03	95	97	96	145	147	146
24	SP-02	86	88	87	143	142	142
25	SP-34	84	95	90	140	141	140
26	SP-37	81	84	83	144	142	143
27	NDR-359	82	90	86	125	122	124
28	BPT-5204	93	97	95	146	149	148
29	IR-64	84	87	86	130	132	131
30	Jaya	91	85	88	134	132	133
	Mean	84	88	86	126	127	126
	SE (m)	6.65	6.18	5.64	10.28	11.28	9.41
	CD at 5%	8.91	4.13	6.26	21.04	20.44	18.76
	CV (%)	18.23	12.64	14.29	5.7	5.62	4.25

Days to physiological maturity

The crop duration was reduced under elevated temperature [20]. In the present study significant difference was observed among the genotypes for days to maturity. During the first season, BPT- 5204 (146 days), SP-03 (145 days), SP-352 (145 days) and SP-37 (144 days) recorded maximum values, SP-08 (114 days), SP-72 (114 days), and SP-70 (117 days) recorded minimum values respectively. During the second season, BPT-5204 (149 days), SP-03 (147 days), SP-352 (143 days) and SP-37 (142 days) recorded maximum values, while, SP-08(113 days), SP-72 (118 days) and SP-70(119 days) recorded minimum values, respectively. Pooled data revealed thatBPT-5204 (148 days), SP-03 (146 days), SP-352 (144 days), and SP-37 (143 days) showed maximum value, while, minimum in SP-08 (114 days), SP-72 (116 days) and SP-70 (118 days) respectively. This reduction in days to maturity might be due to earliness in the flowering in the elevated temperature stress environment. Similar observations were also reported by [21].

Among the duration groups, the short duration (116 to 122 days) lines were SP-08, SP-72, SP-70, and SP-69. The medium duration (125 to 133 days) lines, SP-351, SP-353, SP-355, SP-357, SP- 80 and SP-25 and long duration (140 to 148 days) lines, SP-02, SP-03, and SP-37 found superior in terms of grain yield as compared to their respective check varieties. Two advanced breeding lines, namely SP-08, SP-70, can be further probed thoroughly for further increasing yield and yield attributes.

Molecular studies

In view of characterization of advanced breeding lines for genes and *QTLs*, five yield-associated genes *viz.,Gn1, Gn2*(*grain number*), and three *QTLs viz., Yld12.1, Yld2.1, and Yld4.1* (*grain yield*) were selected.

The *Gn1* gene was characterized using three functional markers (Gn1A, Gn1A17 and Gn1INDEL) and three linked markers (RM10499, RM151 and RM10382). For *Gn2,* two linked markers (RM250 and RM208) were used and presented Table 5. *Yld12.1* with five markers (RM511, RM28166, RM28163, RM28130 and RM28099), *Yld2.1* with two (RM262 and RM263) markers and*Yld4.1*with three (RM261, RM16338, RM16373) markers were assessed. The functional ladder size was used 50bp and linked markers ladder size was used 100bp.

Three major traits (grain size, grain number per panicle, and panicle number per plant) are directly associated with yield, and these traits strongly depend on the genetic potential of rice. However, these traits are complex and quantitative in nature. Through *quantitative trait loci (QTL)* analysis with fine mapping or positional cloning using rice mutants, about 20 genes that are involved in yield-related traits have been isolated in rice [22].

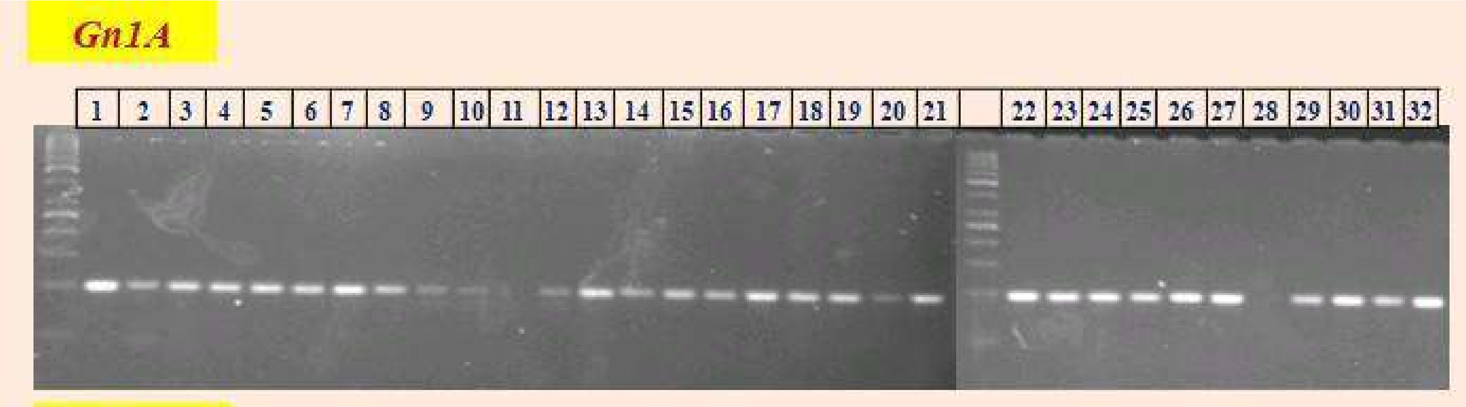
Table 5: Selected genes and QTLs related to yield showed in present study

S.No	Gene / *QTLs*	Trait	*Chromosome*	Location	Markers
1	*Gn1*	Grain Number	*Chromosome -1*	5.5Mb-7.9Mb	Gn1A*Gn1A17*Gn1INDE L*RM10499 RM151 RM10382
2	*Gn2*	Grain Number	*Chromosome-2*	32.7Mb-35.1Mb	RM250 RM208
3	*Yld-12.1*	Yield (Drought tolerance)	*Chromosome-12*	45.2Mb-67.3Mb	RM511 RM28166 RM28163 RM280130 RM28099
4	*Yld-2.1*	Yield	*Chromosome -2*	20.7Mb-25.8Mb	RM262 RM263
5	*Yld-4.2*	Yield	*Chromosome-4*	25.8Mb-31.5Mb	RM261 RM16338 RM16373

**Functional markers*

4.3.1*Grain Number* (*Gn1* and *Gn2*)

The grain number will be determined by the panicle branching and length of the panicle [23]. The genes, *LAX1, Gn1a,* and associated with grain number are regulated number of branches [24]. In the present study, for *Gn1,* "Habataki," a japonica cultivar was selected as a positive control, whereas BPT-5204 was used as a negative control. Yield-positive *Gn1a* allele was originally isolated from the high-yielding rice variety Habataki through *QTL* analysis with positional cloning [25], [26]. To detect the allelic status of *Gn1* in the advanced breeding lines, three functional markers (Gn1A, Gn1A17, and Gn1INDEL) and three linked markers (RM10499, RM151, and RM10382) were used (Table 7). Upon genotyping with these six markers, seven advanced breeding lines (SP-351, SP-70, SP-61, SP-69, SP-25, SP-08, and SP- 75) showed positive alleles with more than two markers (Figure 3).



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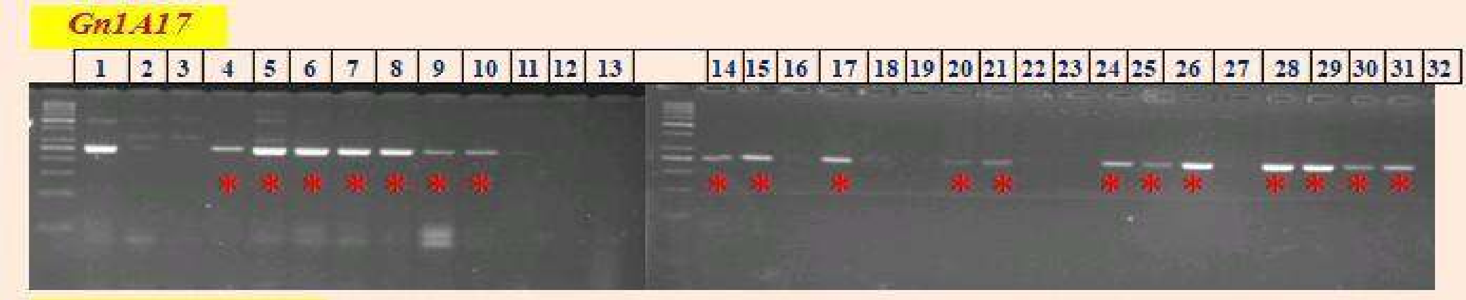

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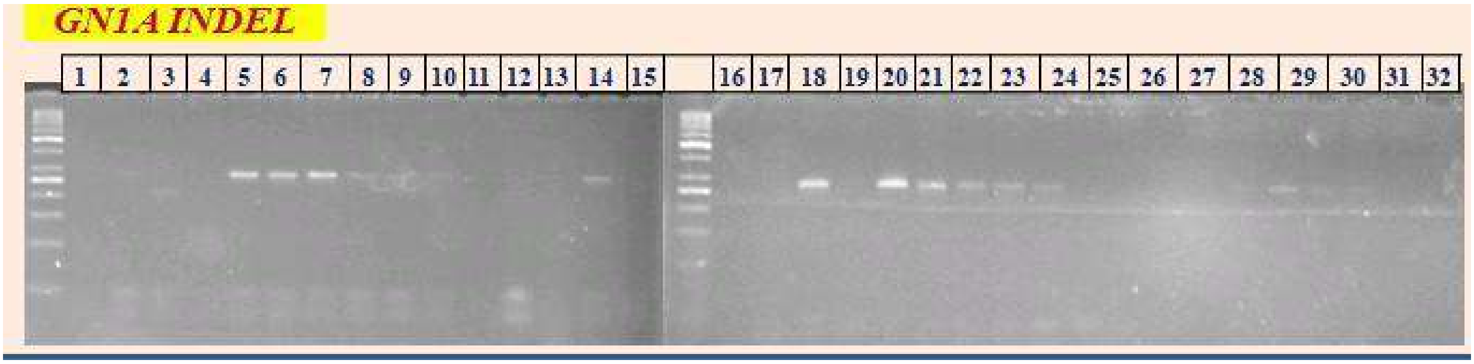

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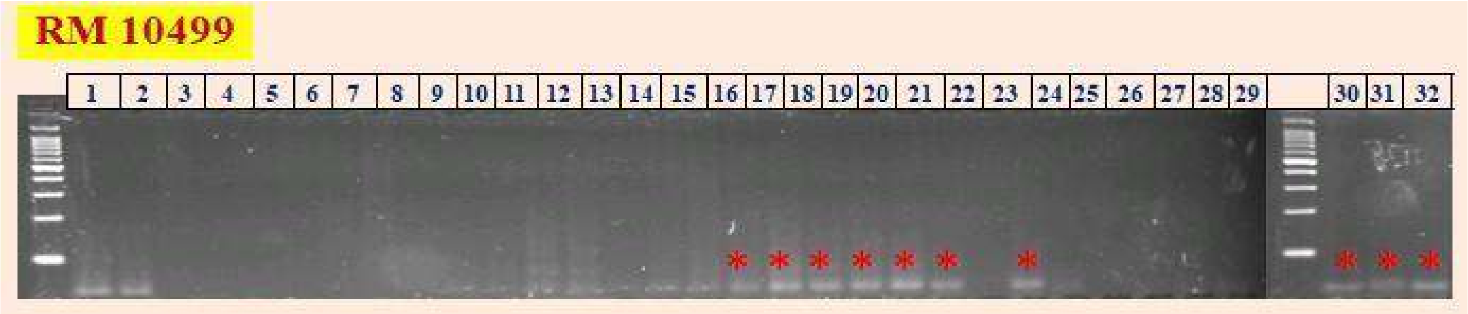

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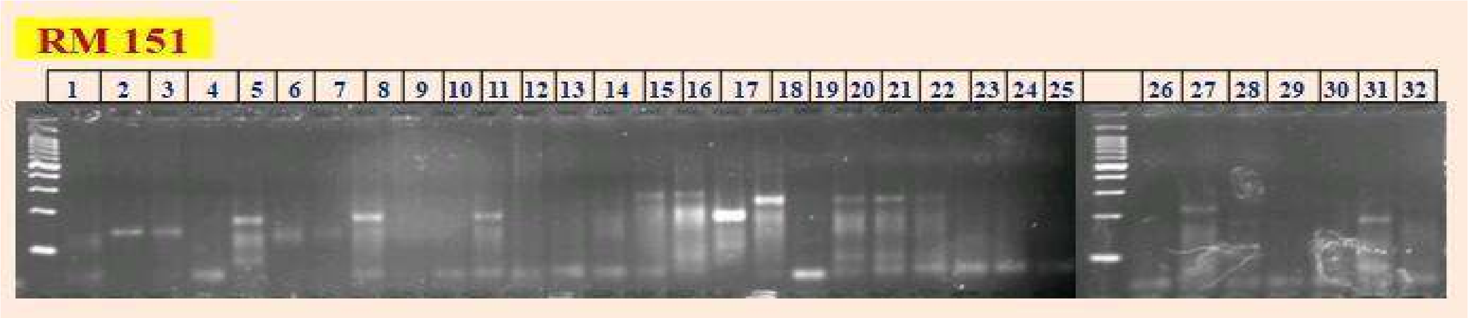

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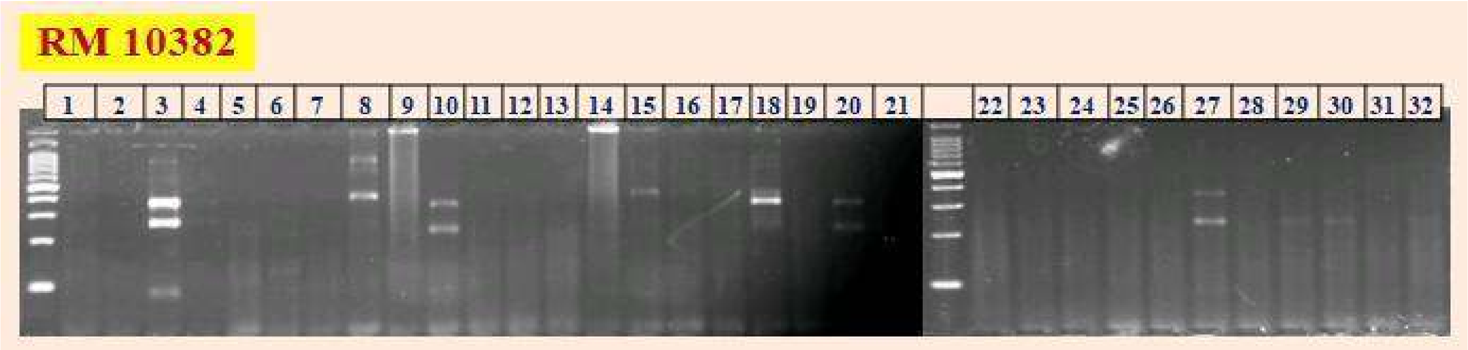

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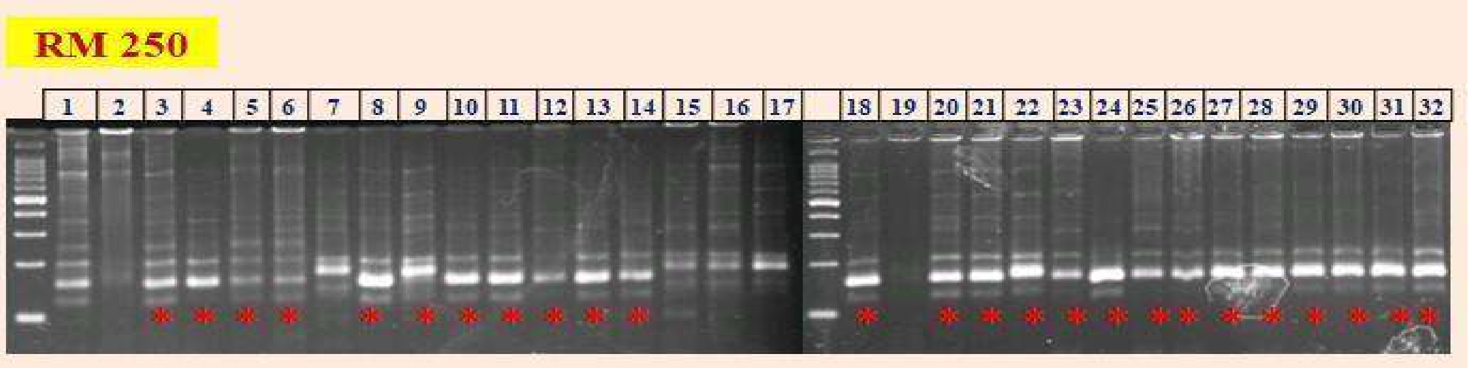
1.MTU 10101 (H-2-4), 2.BPT-5204, 3.IR-64, 4.NDR-359, 5.Jaya, 6.BPT-5204, 7.SP-351, 8.SP-352, 9.SP-353, 10.SP-354, 11.SP-355, 12.SP-356, 13.SP-357, 14.SP-358, 15.SP-359, 16.SP-360, 17.SP-70, 18.SP-72, 19.SP-63, 20.SP-61, 21.SP-69, 22.SP-55, 23.SP-80, 24.SP- 25, 25.SP-13, 26.SP-03, 27.SP-01, 28.SP-34, 29.SP-37, 30.SP-08, 31.SP-75, 32.SP-57.
** Genotypes have positive alleles for grain number (Gn1).*

Figure 3. Genotyping of Gn1 among advanced breeding lines with markers

Grain number is an important factor determining the grain production of rice (*Oryza sativa* L.). The molecular and genetic basis for grain number is complex. [27] identified *GRAIN NUMBER 2 (GN2),* a novel gene that is responsible for rice grain number, from "Yuanjiang" common wild rice (*O. rufipogon Griff.).* Transgenic plants over expressing *GN2* showed more grain number, reduced plant height, and longer heading date than wild-type plants. Recently, a few *quantitative trait loci (QTL*) and genes regulating rice grain number have been identified. Among which *DEP1, DEP2, LP, SP1, PAP2*, and *sped1-D*alter panicle architecture [28], whereas *Gn1a, LAX1, SPA1, TAW1, DST,*and *FZP* control the initiation and outgrowth of branches and spikelets [29]. In addition, some genes, such as *Ghd7, Ghd8, PROG1, IPA1, FUWA, PAY1,*and *An1*, underlying rice grain number show pleiotropic effects in many significant agronomic-or domestication- related traits, including plant height, heading date, plant architecture, awn habit, and grain size [30].

In the present study, for assessing the presence *Gn2* allele among advanced breeding lines, the "(H-2-4)" an *indica* cultivar was selected as a positive control, whereas BPT-5204 negative control. The lines, SP-61, SP-69, SP-55, SP-80, SP-25, SP-13, SP-03, SP-02, SP-34, SP-37, SP-08, SP-75 and SP-57 were found to have the *Gn2* positive alleles for both the linked markers,RM250 and RM208 (Table 4 and depicted figure 4).



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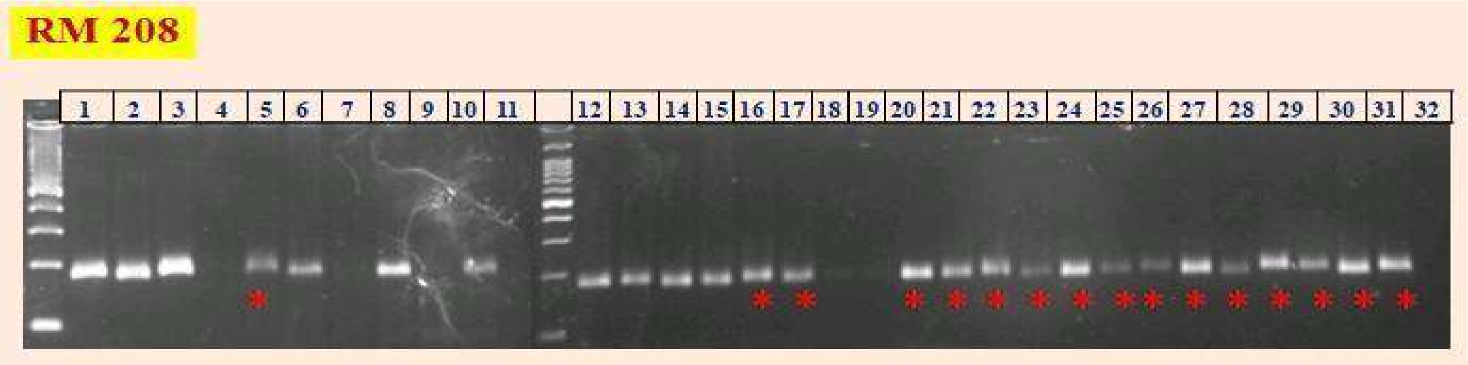

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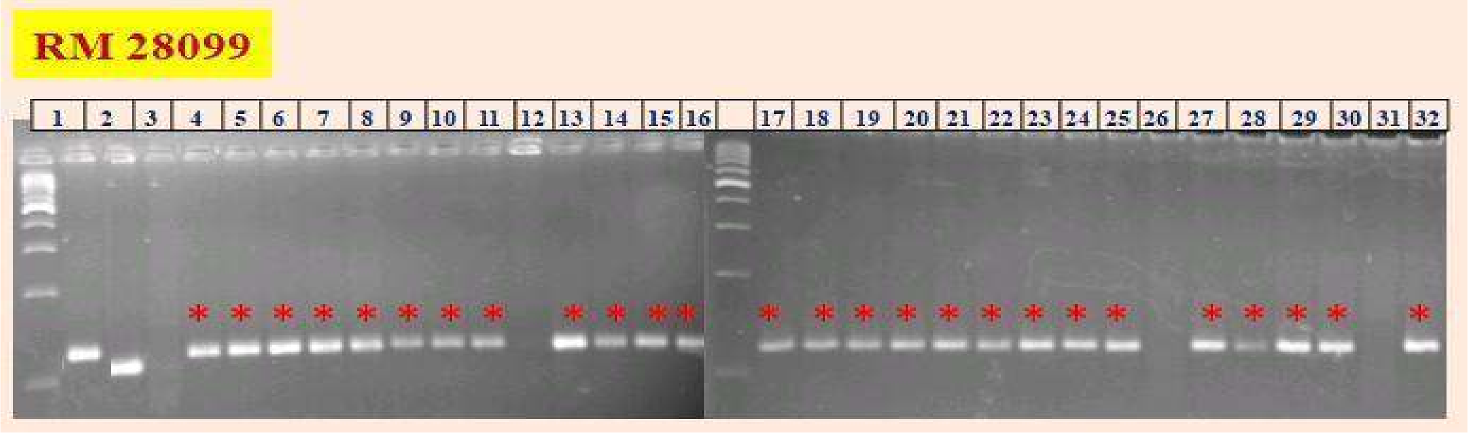
1.MTU 10101 (H-2-4), 2.BPT-5204, 3.IR-64, 4.NDR-359, 5. Jaya, 6.BPT-5204, 7.SP-351, 8.SP-352, 9.SP-353, 10.SP-354, 11.SP-355, 12.SP-356, 13.SP-357, 14.SP-358, 15.SP-359, 16.SP-360, 17.SP-70, 18.SP-72, 19.SP-63, 20.SP-61, 21.SP-69, 22. SP-55, 23.SP-80, 24.SP- 25, 25.SP-13, 26.SP-03, 27.SP-01, 28.SP-34,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

Figure 4. Genotyping of Gn2 among advanced breeding lines with markers

Yield contributing major *QTLs* (*Yld 12.1, Yld 2.1,* and *Yld 4.1*)

For *Yld 12.1,* "Near isogenic line of Vandana" was selected as the positive control, whereas "Varalu" was the negative control. To detect the allelic status of *Yld 12.1* in the test lines, five linked markers were used (RM511, RM28166, RM28163, RM28130, and RM28099). Upon genotyping with these five markers, twelve advanced breeding lines (SP-351, SP-353, SP-357, SP-359, SP-360, SP-70, SP-63, SP-25, SP-37, SP-08, SP-75, and SP-57) were identified with positive alleles for *Yld12.1*with more than two markers (Table 4 and depicted figure 5).

[31] showed that *qDTY12.1* and *qDTY2.2,* in single or in combination with other *qDTYs*, contributed to amajor effect when introgressed into *MRQ74*. The results clearly imply the necessity to identify *qDTY* combinations with positive interaction against different genetic backgrounds for appropriate yield increase under drought. *MRQ74 PLs* with two*qDTY* combinations stood as top performers for grain-yield in severe RS trials as well as in NS trials. This was further supported by the findings of [32].



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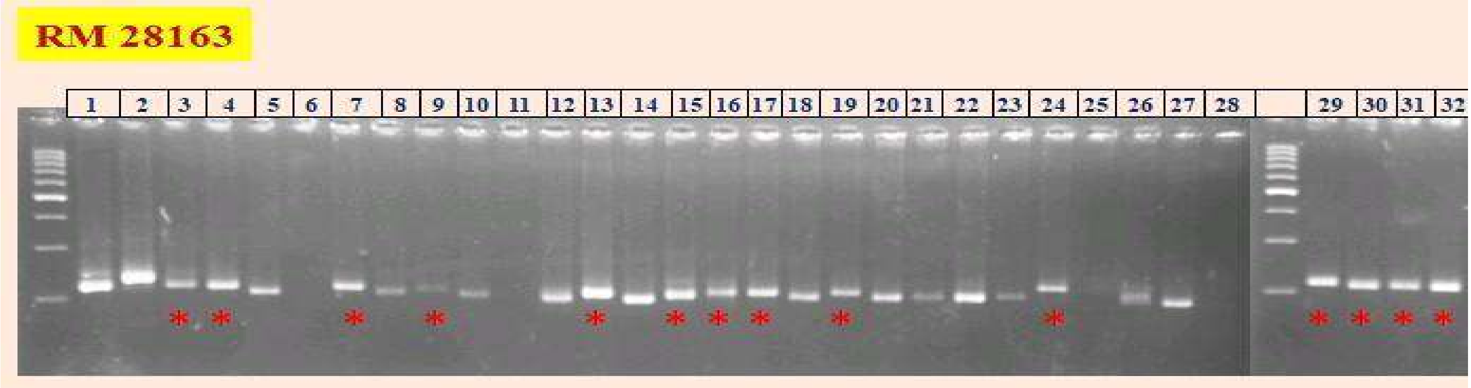

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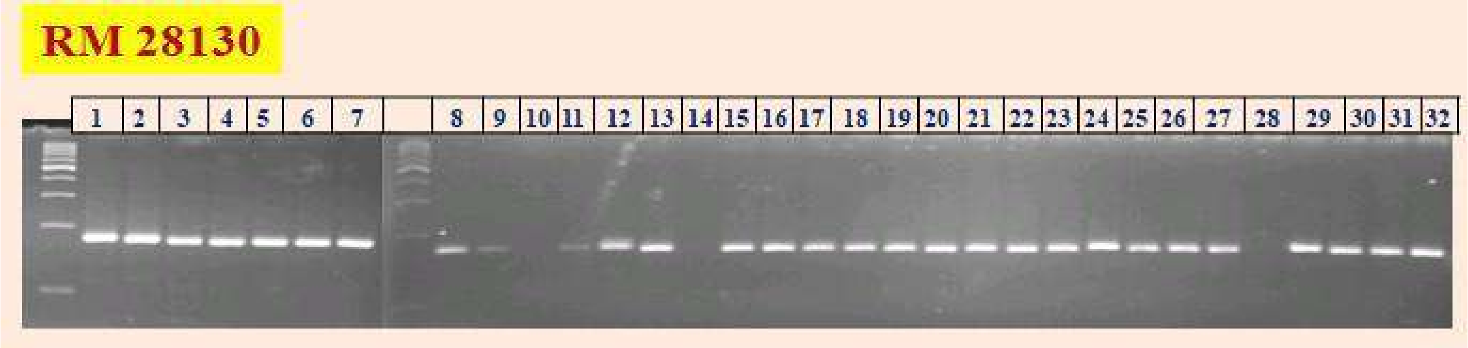

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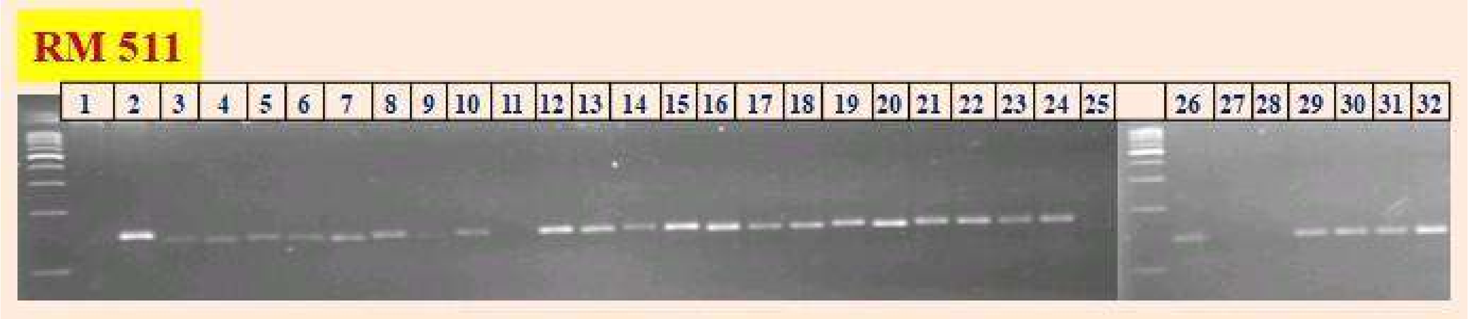

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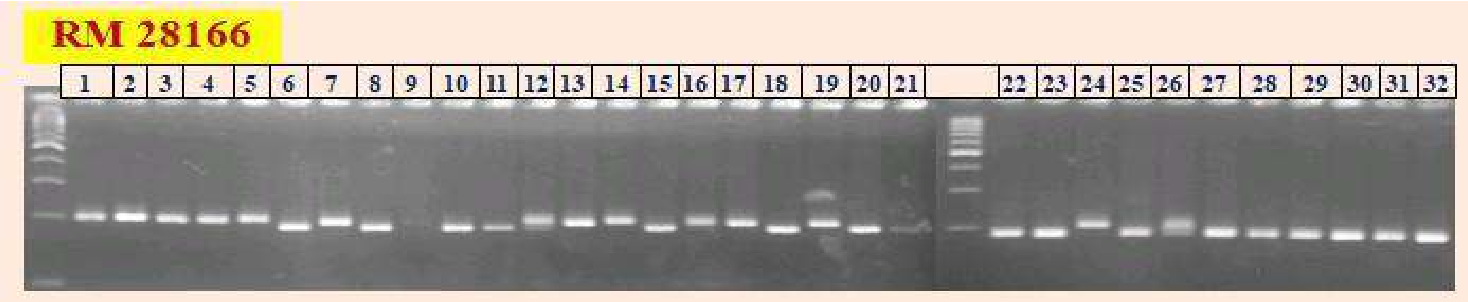

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1.Vandana, 2.BPT-5204, 3.IR-64, 4.NDR-359, 5.Jaya, 6.BPT-5204, 7.SP-351, 8.SP-352, 9.SP-353, 10.SP-354, 11.SP-355, 12.SP-356, 13.SP-357, 14.SP-358, 15.SP-359, 16.SP-360, 17.SP-70, 18.SP-72, 19.SP-63, 20.SP-61, 21.SP-69, 22.SP-55, 22. SP-80, 24.SP-25,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

Figure 5. Genotyping of Yld12.1 among advanced breeding lines with markers

For assessment of *Yld2.1,* "DRR-50-7" was selected as the positive control, whereas "BPT-5204" negative control. To detect the allelic status of *Yld2.1* in the test lines, two linked markers were used (RM262 and RM263).Advanced breeding lines, SP-357, SP-70, SP-72, SP-61, SP-69, SP- 55, SP-03, SP-02, SP-34, SP-37 and SP-08showed positive alleles for *Yld2.1* with more than two markers. Similarly, [33] reported that lines with two and three *QTL* combinations performed better. This result indicates (i) non-linear interaction between multiple *qDTYs* and(ii) the presence of differential synergistic relationships between *qDTY* combinations.

For assessing *Yld, 4.1* "DRR-50-7" was selected as the positive control, whereas "BPT-5204" negative control. None of the advanced breeding lines showed the presence of positive alleles for linked markers (RM261, RM16338, RM16373). [34] reported that *qDTY2.2*, *qDTY4.1* combinations showed higher yield advantage under drought over single *QTLs* in Samba Mahsuri background, indicating the effectiveness as well as positive interactions between these two *QTL*s in multiple genetic backgrounds.

Conclusion

Among the duration groups, the short duration (116 to 122 days) lines were SP-08, SP-72, SP-70, and SP-69. The medium duration (125 to 133 days) lines, SP-351, SP-353, SP-355, SP-357, SP- 80 and SP-25, and long duration (140 to 148 days) lines, SP-02, SP-03, and SP-37 found superiorin terms of grain yield as compared to their respective check varieties. Two advanced breeding lines, namely SP-08, SP-70, can be further probed thoroughly for further increasing yield and yield attributes.

- The morphological and physiological parameters of advanced breeding lines were correlated with the molecular analysis. The advanced breeding lines SP-08, followed by SP-70, showed better morphological and physiological parameters, like the number of tillers, and also showed the presence of seven (*Gn1+Gn2 +Yld12.1+Yld2.1*) yield contributing genes/*QTL*s. So this line can serve as a potential donor for the rice improvement program.
- SP-69 advanced breeding lines showed higher panicle length and also contain six corresponding genes governing (*Gn1, Gn2, and Yld2.1*) genes/*QTL*s.

Future line of work

- Two advanced breeding lines, namely SP-08, SP-70, can be further probed thoroughly for yield and yield attributes.
- The early duration check is NDR-359, which is a better yielder than BPT-5204 may be utilized as a donor in breeding programmes aimed at short duration varieties for higher yield and quality.

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