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Introgression and molecular identification of quantitative trait loci for yield components in rice (*Oryza sativa* L.) variety Basmati 370

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Abstract

To improve yield potential of export quality traditional Basmati rice (*Oryza sativa* L.) variety Basmati 370, QTL for yield components were introgressed from a high yielding tropical *japonica* cultivar Palawan through marker assisted selection in BC_2F_2 population. Transgressive segregants were observed for all traits. Enhancement over recurrent parent for panicle weight (155.5%), panicle number (141.5%), grains per panicle (35.7%), 100-grain weight (35%), panicle length (20%) and for overall grain yield was 168.7%. A set of 201 SSR markers was used to identify and tag a total of 19 putative QTL by bulked segregant analysis. Six putative QTL for panicle number were tagged on chromosome 1, 2, 7, 8, and 12, five for panicle weight on chromosome 1, 5, 7, 9 and 12, three QTL for 100 grain weight 1, 6 and 7 and five QTL for grain yield per plant on chromosome 1, 2, 7, 8 and 12 were detected. The QTL identified in this study will be useful in molecular breeding of Basmati for improvement of yield.

Key words: Rice, Basmati 370, AB-QTL analysis, SSR markers, yield components

Introduction

Basmati rice (*Oryza sativa* L.) traditionally grown in northwestern states of Indian sub-continent holds a special place in international as well as domestic markets commanding the highest premium [1]. It is due to its exquisite aroma, superfine grain characteristics, and excellent cooking qualities like soft and flaky texture of cooked rice, high volume expansion during cooking by linear kernel elongation with minimum breadth wise swelling fluffiness, palatability, easy digestibility and longer shelf life [2]. Traditional Basmati varieties like Basmati 370, Taraori Basmati and Dehradun Basmati (Type 3) occupy a premium position in the export market.

India accounts for about 23.60% share from the Basmati of the total rice export [3]. Traditional Basmati varieties have several undesirable agronomic traits like tall plant stature, weak culm, long crop duration, photosensitivity, poor response to fertilizers and are more susceptible to various pests resulting in to low yield. Breeding efforts have been made to genetically improve Basmati rice by crossing with novel high yielding indica rice varieties but only few varieties like Pusa Basmati 1, Haryana Basmati 1, Kasturi etc, have been successful. The progress has been limited due to higher degree of divergence between Basmati and *indica* rice varieties, hybrid sterility and polygenic nature of aroma and grain /cooking quality traits. Molecular marker assisted selection, bulk segregant analysis [4] and advanced backcross QTL analysis [5] can be used to monitor introgression in the genetic background of an elite recurrent Basmati parent. A high yielding, non glutinous, early maturing, aromatic and having wide compatibility, upland tropical *japonica* traditional variety from Philippines, Palawan was chosen as donor to introgress and identify the novel QTL alleles for yield and yield components in the background of fine grain variety Basmati 370, as Basmati is closer to *japonica* than it is to *indica* rice [6] by using advanced backcross QTL analysis.

Materials and methods

Development of experimental population: A single plant of Basmati 370 (a fine grain, strongly aromatic traditional Basmati released from erstwhile Punjab) was used as the recurrent parent for cross with upland, aromatic, non-glutinous, wide compatible, early maturing tropical *japonica* variety Palawan from Philippines. Seven most vigorous F_1 plants, whose hybrid status was confirmed with SSR markers, were backcrossed to Basmati 370 (now used as male parent) to generate 300 BC_1F_1 plants. The best 32 BC_1F_1 plants selected for desirable plant type, maturity and fertility and yield component traits were backcrossed a second time to Basmati 370 to produce more than 3500 BC_2 seeds. 40 seeds from each selected 32 BC_1F_1 plants were grown in off-season

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nursery at Central Rice Research Institute (CRRI), Cuttack. One hundred and twenty agronomical superior BC_2F_1 plant progenies were selected based on their phenotypic performance and selfed to produce BC_2F_2 progenies. BC_2F_2 population development and evaluation was carried out at Punjab Agricultural University, Ludhiana.

Phenotypic evaluation: The recurrent parent and the 120 BC_2F_2 plant progenies were evaluated in an augmented block design with two replications. Each progeny was planted in three rows (1 m each) with 11 plants per row with a row-to-row spacing of 30 cm. High yielding progenies phenotypically similar to Basmati 370 were selected for phenotypic traits. Five BC_2F_2 plants from each of the selected progenies were evaluated for days to heading (DTH), days to maturity (DTM), plant height (PH), panicle length (PL), panicle weight (PW), panicle number (PN), grains per panicles (GPP), 100 grain weight (100 GW) and grain yield per plant (Yld) [7]. Grains were categorized into four categories extra long (>7.50), long (6.61-7.5), medium (5.51-6.6), short (<5.5) for grain size [8] on the basis of average length of ten kernels of each of the selected plants.

Marker genotype determination: DNA from the parents Basmati 370 and Palawan was surveyed for polymorphism using SSR markers. Twenty seeds from each of the selected BC_2F_3 plants were bulked for DNA extraction from young leaves by CTAB method [9] 201 anchored SSR markers [10] representing both the arms of all the 12 chromosomes of rice were chosen for parental polymorphism. Bulked segregant analysis [11] was followed for detecting markers linked to QTL for various yield components *i.e.*, 100 GW, PW, PN, GPP, grain length and grain yield per plant (Yld). Five to seven BC_2F_2 plants each from the selected plant progenies with high and low mean performance for the target trait were chosen for making bulks with high and low phenotypic value for the trait, respectively. Polymorphism was determined by resolution of PCR amplified products on 2.5% MetaPhore gel following ethidium bromide staining.

Data analysis: Skewness and kurtosis tests [12] were applied to the distribution of the phenotypic traits. The analysis of variance (ANOVA) was performed using the software programme CPCS1 [13]. Trait correlations were evaluated by regressing the phenotypic value of one trait on those of another trait by using the CS11 software programme [14]. Identified QTL were designated according to the rice nomenclature [7].

Results and discussion

Polymorphism of markers: 93 markers of the 201 (approximately 50%) SSR markers screened were found to be polymorphic between Palawan and Basmati 370 and were used to genotype the BC_2F_2 population.

Phenotypic evaluation of BC_2F_2 plant progenies: Trait means over replications were considered for analysis based on phenotypic data of selected 120 BC_2F_2 plant progenies. Frequency distribution of selected 120 BC_2F_2 plant progenies for eleven traits is presented in Fig. 1. Except for panicle length and grains per panicle, Palawan was superior in all traits examined and transgressive segregants were observed for all traits under study. For grain yield per plant, 26 (81%) of the selected BC_2F_2 progenies out performed recurrent parent Basmati 370 with maximum gain of 168.7%. For 100-grain weight, 29 (91%) of the BC_2F_2 plant progenies out performed Basmati 370 with maximum gain of 35%. Improvement of panicle weight was as high as 155.5% over the recurrent parent (31 progenies). Improvement in grains per panicle was in 14 families with maximum gain of 35.7%. Fifteen progenies outperformed recurrent parent improving panicle number by 141.5%. A maximum gain of 20% was observed for panicle length in 25 out-performing progenies. Phenotypic evaluation of backcross derivatives demonstrates that the sub-specific hybridization has been successful for introgressing superior QTL alleles for yield and its components.

Trait correlations: The correlation between traits was estimated by regressing phenotypic values of one trait on those of another trait. Pairwise trait correlation is presented in Table 1. The significant positive

Table 1. Correlation coefficients (r) among traits in rice var. Basmati 370*3/Palawan derived BC_2F_2 population

	GY	100 GW	PW	GPP	PN	PL	PH	SPP	DTH	DTM
100-grain weight (100 GW)	.000									
Panicle weight (PW)	.319	.015								
Grains per panicle (GP)	.367	−.026	.505							
Panicle number (PN)	.534	−.012	−.061	.007						
Panicle length (PL)	.134	.287	.324	.298	.232					
Plant height (PH)	.102	.284	−.158	−.128	−.097	.093				
Spikelets per plant (SPP)	.325	.029	.589	.612	.024	.226	−.285			
Days to heading (DTH)	−.193	.408	−.145	.083	.126	.359	.048	−.044		
Days to maturity (DTM)	−.188	.463	−.064	.095	−.012	.323	.137	.023	.913	
Tiller number (TN)	.374	−.021	−.199	−.054	.927	.132	−.196	−.047	.108	−.027

r = .349 at P = 0.05

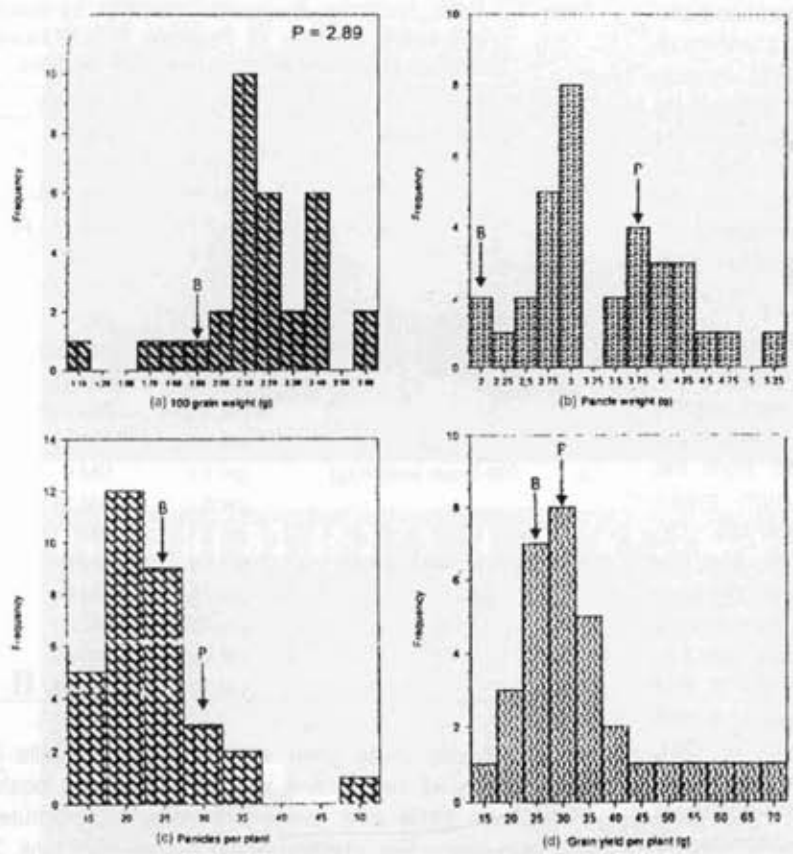

Fig 1. Frequency distribution of phenotypic value of each trait in the selected 32 elite BC_2F_2 families. Arrow shows phenotypic value of recipient rice var. Basmati 370(B) and donor Palawan (P). Phenotypic value have been indicated at the top when it falls out of the range of BC_2F_2 families.

correlated ($P < 0.05$) traits included GPP × GY (0.367), GPP × PW (0.505), PN × GY (0.534), SPP × PW (0.589), SPP × GPP (0.612), DTH × 100 GW (0.408), DTM × 100 GW (0.463), DTM × DTH (0.913), TN × GY (0.374), TN × PN (0.927). As expected grain yield was most influenced by panicle numbers.

Identification of QTL: Out of 98 polymorphic markers between Basmati 370 and Palawan, 39 were found linked to QTL for 100 GW, PW, PN, GPP, Yld and grain length in the respective bulks, which were debulked for selective genotyping.

QTL controlling panicle number: Five plants each, constituted high panicle number bulk (218.18%-63.7% over the upper range of recurrent parent) and low panicle number bulk (38.46%-50%, below the lower range of recurrent parent) selected from the BC_2F_2 plant progenies representing high mean performance for panicle number (Table 2). On selective genotyping of the individual plants of the high and low bulks, 6 out of 20 linked markers (as detected by BSA) were found to be cosegregating with panicle number. Six Palawan derived putative QTLs were *pn* 1.1, *pn* 2.1, *pn* 2.2, *pn* 7.1, *pn* 8.1, and *pn*.1 were found linked to RM 9, RM 11, RM 11, RM 42, RM 110, RM 247 on chromosomes 1, 2, 7, 8 and 12 respectively, contributing 6.62%-27.88%

Table 2. Phenotypic details of the components of the bulks used for bulked segregant analysis for yield, yield components and grain length of rice var. Basmati 370*3/Palawan BC_2F_2 plants

Bulk/plant No.	Plant No.	GY (g)	Plant No.	PN	Plant No.	PW (g)	Plant No.	GPP	Plant No.	100GW (g)	Plant No.	GS
HIGH												
1	D-11/1-10	99.19	6-2/2-3	40	7-4/3-4	5.26	7-4/1-2	185.67	D-5/2-1	2.86	2-1/1-5	11.0
2	6-2/3-8	78.54	D-11/1-4	38	6-8/1-6	4.54	7-4/3-4	182.67	D-12/2-10	2.68	6-5/1-7	9.1
3	D-10/3-8	60.19	D-11/1-10	37	7-4/1-2	4.51	D-4/3-3	170.67	6-9/1-3	2.61	7-2/1-8	8.9
4	6-8/1-6	57.42	D-10/3-8	36	6-7/3-2	4.32	7-5/2-9	165.00	7-1/2-9	2.61	7-2/2-8	8.8
5	6-9/3-2	55.29	6-2/3-8	36	6-9/3-2	4.32	7-10/3-2	163.67	6-6/1-7	2.55	9-5/3-9	8.8
6	6-7/3-2	54.76	-	-	6-10/2-2	4.23	-	-	6-7/3-2	2.51	-	-
7	7-4/1-2	53-72	-	-	7-10/3-2	4.19	-	-	D-10/3-8	2.51	-	-
LOW												-
1	9-5/3-5	8.41	7-2/3-7	9	D-12/3-1	1.30	2-1/3-7	35.00	6-1/3-6	1.06	6-3/3-9	5.5
2	7-2/1-1	8.57	7-9/3-3	12	6-3/2-10	1.46	2-1/3-7	46.67	D-5/2-9	1.30	D-4/1-5	5.5
3	9-5/3-9	10.25	7-2/1-1	13	7-9/2-8	1.61	2-1/1-5	51.67	7-7/1-5	1.56	6-3/3-5	5.5
4	D-12/3-1	12.81	D-4/1-5	13	6-3/3-9	1.74	9-5/3-5	58.33	D-4/3-3	1.71	7-2/1-3	5.5
5	7-2/3-7	12.95	7-9/3-6	13	9-2/3-3	1.82	9-2/2-9	60.00	7-7/3-3	1.74	6-3/1-4	5.7
6	7-9/2-8	12.46	-	-	9-2/2-9	1.87	-	-	7-2/2-8	1.81	-	-
7	7-2/1-8	13.53	-	-	7-2/1-9	1.88	-	-	D-5/3-10	1.84	-	-
Basmati 370		23.16		20.50		2.02		127.50		1.98		2

of phenotypic variation. Palawan alleles increased the average number of panicles per plant by a minimum of 16.9 panicles (Table 3 and Fig. 2a (RM 11). Panicle number per plant is a quantitative trait with a relatively low heritability, which is highly influenced by environment and the cultural practices/spacing of plants. Different studies on mapping of QTL for panicle number reported many regions influencing this trait on chromosome 12 [15-16, 20] chromosome 2 [7, 17] chromosomes 7, 8 [15, 20] supporting this study.

QTL controlling 100-grain weight: Seven plants each constituted the high 100-grain weight bulk (41.5%-26.24% over the upper range of recurrent parent) and for low 100-grain weight bulk (37.6% below the lower range of recurrent parent) selected from the BC_2F_2 plant progenies representing high mean performance for 100-grain weight (Table 2). On genotyping the individual plants of the high and low bulk for 100-grain weight, 3 out of 23 linked markers (as detailed by BSA) cosegregated with 100-grain weight. Three Palawan derived putative plus alleles *gw* 1.1, *gw* 7.1 and *gw* 6.1 were found linked to RM 9, RM 11 and RM 162 (Fig. 2b) on chromosomes 1, 6 and 7 respectively (Table 3). When positions of QTL associated with the same trait were compared from different studies, [15-18, 20] a major QTL for 100-grain weight was found to be on the same region chromosome 1. These QTL contributed 21.58% to 28.86% of phenotypic variation. The sum of their absolute effect amounted to 2.62 g per 100 grains.

QTL controlling panicle weight: Seven plants each from both the extreme phenotypic classes (107.11%-65% over the recurrent plant and 5.7-34.34% below the lower range of recurrent parent) was pooled from the selected progenies for panicle weight (Table 2). On selective genotyping the individual plants of the high and low bulk for panicle weight only 5 out of 21 linked markers were found to be segregating with panicle weight. Thus for panicle weight 5 putative QTL on chromosome1, 5, 7, 9 and on chromosome 12 (Fig. 2c) associated with RM 9, RM 163, RM 11, RM 242 and RM 247 respectively were identified by bulk segregant analysis (Table 3). The increasing alleles pw 1.1 *pw* 5.1, *pw* 7.1 *pw* 9.1 and *pw* 12.1 contributed 10.73% to 26.4% of phenotypic variation with absolute effects of 2.14 g in panicle weight.

QTL controlling grains per panicle: The phenotypic details of the bulk for high grains per panicle (9.1%-23.33% over the recurrent parent) and bulk for low grains per panicle (40%-65% below the recurrent parent) are presented in Table 2. On selective genotyping, out of 13 markers linked with grains per panicle, none was found to be segregating with phenotypic values.

Table 3. QTLs for yield components identified by bulked segregant analysis of Basmati 370*3/Palawan derived BC_2F_2 plants using rice SSR markers

S.No.	Trait	QTL	Markers
1.	Panicle number	*pn 1.1*	RM 9
		pn 2.1	RM 71
		pn 2.2	RM 110
		pn 7.1	RM 11
		pn 8.1	RM 42
		pn 12.1	RM 247
2.	Panicle weight (g)	*pw 1.1*	RM 9
		pw 5.1	RM 163
		pw 7.1	RM 11
		pw 9.1	RM 242
		pw 12.1	RM 247
3.	100-Grain weight (g)	*gw 1.1*	RM 9
		gw 6.1	RM 162
		gw 7.1	RM 11
4.	Grain yield/plant (g)	*yld 1.1*	RM 9
		yld 2.1	RM 71
		yld 7.1	RM 11
		yld 8.1	RM 42
		yld 12.1	RM 247

QTL controlling grain size: Basmati 370 falls in the category of long grains with rank 2 on 1-4 scale. Plants with extra long grains with rank 1, constituted the high bulk. The medium sized grains with rank 3, constituted the low bulk (Table 2). Six BC_2F_2 progenies were found to have extra long grains longer than 7.0 mm out performing the recipient parent Basmati 370 which have medium grain length equal or less than 6.7 mm. On genotyping the individual plants of the high and low bulk for grain size, out of 14 linked markers none was found to be segregating with grain length.

Even though transgressive segregants were observed for grains per panicle and grain length introgression could not be identified probably due to a low degree of polymorphism in certain regions or it could be located in a terminal position on the chromosomes and could not be mapped. Additional molecular markers could be employed to enrich the polymorphic markers.

QTL controlling grain yield: Plants for high grain yield bulk (219.35%-73.29% over the upper range of recurrent parent) and low grain yield bulk (52%-32% below the lower range of recipient) was taken from the selected progenies for high and low grain yield (Table 2). On selective genotyping the individual plants of the bulks for grain yield per plant, 11 out of 19 linked markers were found to be segregating with grain yield per plant. Five putative QTL were found to be linked with RM 9, RM 71, RM 11, RM 42 and RM 247 on

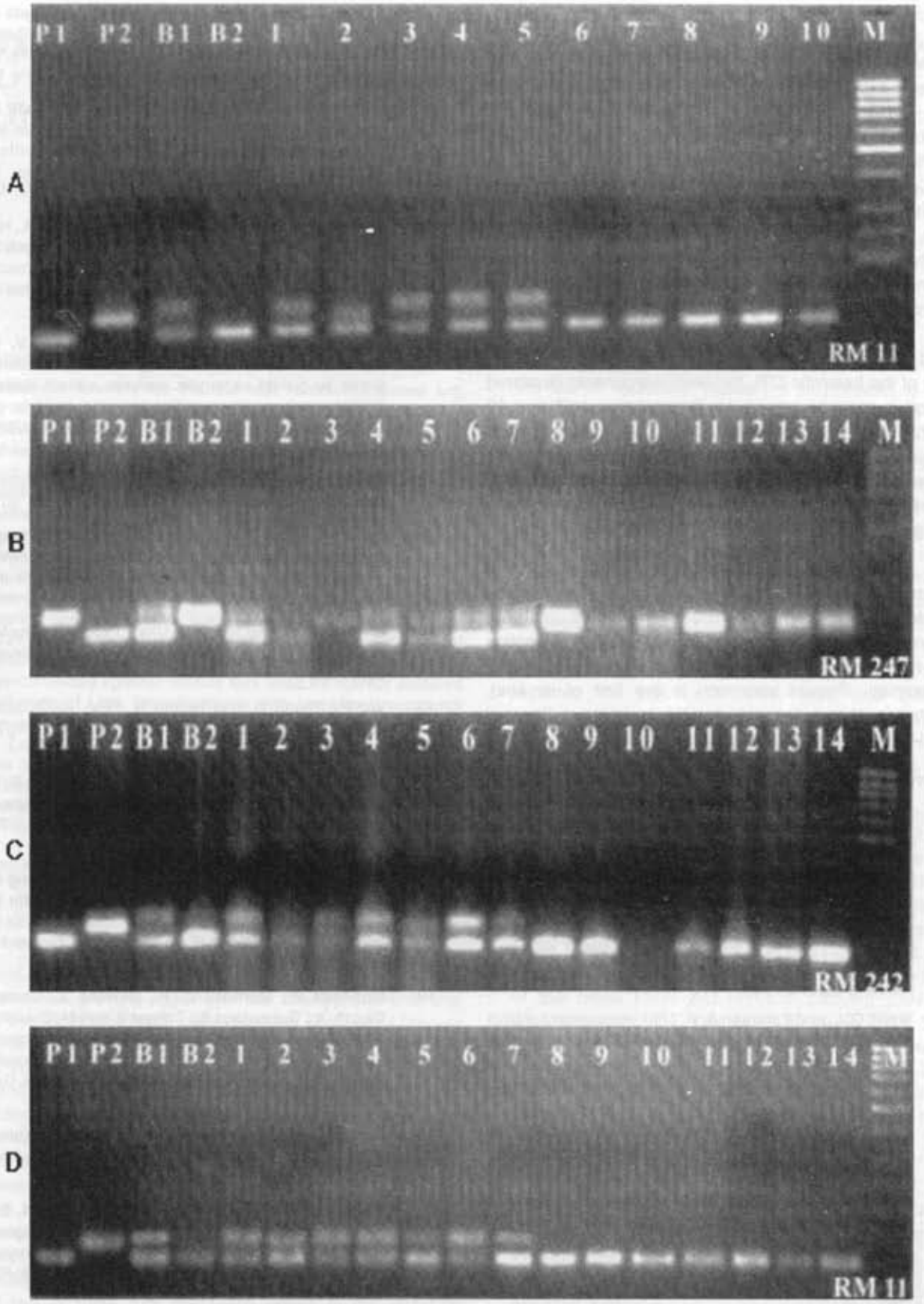

Fig 2. Bulk segregant analysis and selective genotyping of rice Basmati 370*3(P1)/Palawan(P2) derived elite BC_2F_2 plants representing extreme bulks (B1 High bulk and B2, Low bulk). A). Panicle number (RM11); B) 100 Grain weight (RM 247); C) Panicle weight (RM242); D) Grain Yield per plant (RM11). M is the 50bp marker in the extreme right of each photograph.

chromosome1, 2, 7, 8 and 12 respectively (Table 3, Fig. 2d) A similar genomic region on chromosome 12 influencing grain yield per plant is reported [7, 18, 20] Three putative QTL detected for grain yield per plant were *yld* 1.1, *yld* 2.1, *yld* 7.1, *yld* 8.1 and *yld* 12.1. Alleles of yield enhancing QTL contributed 4.25%-18.24% of phenotypic variation with cumulative absolute effect of was 65.59g. Grain yield has been reported to be positively associated with panicles per plant, panicle length, grains per panicle and panicle weight [19]. Plants with these QTL alleles from Palawan, which enhanced the yield or yield component were heterozygous suggesting that the increase in yield or yield component was due to heterotic effect, which could be effectively exploited through hybrid breeding. Most of the heterotic QTL for yield components clustered around a common region on chromosome 1, 7 and 12 which could be either due to pleiotropy or tight linkage of multiple QTL [20]. Fine mapping and further genetic dissection of the target regions containing these QTL would be needed to distinguish between pleiotropy and linkage of multiple genes.

It is essential to combine peculiar quality characteristic of Basmati with high level of expression of yield components to break the yield plateau. Conventional breeding based on phenotypic selection has not been very successful to improve productivity of Basmati. Present approach is the first of its kind, which has shown a great promise to improve Basmati yield through marker-assisted introgression.

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