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Marker assisted selection: a paradigm shift in Basmati breeding

A. K. Singh, S. Gopalakrishnan, V. P. Singh, K. V. Prabhu, T. Mohapatra[1], N. K. Singh[1], T. R. Sharma[1], M. Nagarajan[2], K. K. Vinod[2], Devinder Singh, U. D. Singh[3], Subhash Chander[4], S. S. Atwal[5], Rakesh Seth[5], Vikas K. Singh, Ranjith K. Ellur, Atul Singh, Deepti Anand, Apurva Khanna, Sheel Yadav, Nitika Goel, Ashutosh Singh, Asif B. Shikari, Anita Singh and Balram Marathi

Division of Genetics, [1]NRCPB, [3]Division of Plant Pathology, [4]Division of Entomology, Indian Agricultural Research Institute, New Delhi 110 012
[2]IARI-RBGRC, Aduthurai, Tamil Nadu 612 101; [5]IARI-Regional Station, Karnal, Haryana 132 001

Abstract

Marker assisted backcross breeding (MABB) provides a great opportunity for precise transfer of desirable donor segment by minimizing the linkage drag into a recurrent parent. In our lab, MABB was used for incorporating bacterial blight (BB) resistance genes (*xa13* and *Xa21*) into the genetic background of Pusa Basmati1, which led to development of Improved Pusa Basmati 1 (Pusa 1460) as one of the first products of molecular breeding. Further, the parental lines of superfine grain aromatic rice hybrid Pusa RH 10 namely, Pusa 6A, Pusa 6B and PRR78 were improved for resistance to BB and blast by transferring genes *xa13* + *Xa21* and *Pi54* + *Piz5*, respectively. Presently, the pyramiding of genes for resistance to BB (*xa13* and *Xa21*), blast (*Piz5* and *Pi54*) and brown plant hopper (BPH; *Bph3*, *Bph17*, *Bph18*, *Bph20* and *Bph21*) into Basmati rice varieties viz., Pusa Basmati 1121 and Pusa Basmati 6 is under way. In addition, a major QTL for salt tolerance (*Saltol*) is being transferred to Pusa Basmati 1121, which is widely grown in Haryana, the state having problem of salinity owing to underground brackish water. In order to develop genetically enhanced donor sources for resistance to biotic (BB, blast and BPH) and abiotic (salt tolerance, and phosphorus uptake) stresses in Basmati background, isogenic lines are being developed for major resistance genes/QTLs for respective stresses in the background of Pusa Basmati 1. Molecular mapping of fertility restorer gene(s) in Basmati restorer line PRR78 led to identification of two *Rf* gene linked molecular markers, RM258 and RM6100. Of these, RM6100 on validation in a set of rice germplasm showed 97.4% efficacy in identifying restorer lines from germplasm.QTL mapping using RIL population has unveiled several novel QTLs for grain and cooking quality traits. Molecular markers are also being routinely used for establishing variety/hybrids identity and authentication of genetic purity of hybrid seed lots.

Key words : Basmati Rice Breeding, MAS, QTL, Molecular markers.

Introduction

Basmati rice from the Indian subcontinent is highly priced in the international market for its unique quality. The traditional Basmati cultivars are tall, prone to lodging, photoperiod and temperature sensitive and low yielding. Genetic improvement of Basmati rice at Indian Agricultural Research Institute (IARI) has led to the development of number of high yielding Basmati/aromatic rice varieties and hybrids (Pusa Basmati 1, Pusa Basmati 1121, Improved Pusa Basmati 1, Pusa Basmati 6, Pusa Sugandh 2, Pusa Sugandh 3, Pusa Sugandh 5 and hybrid Pusa RH10), wherein the duration of traditional Basmati rice varieties has been reduced from 160 days to 115-140 days with enhancement of productivity from 2.5 tons/ha to 6-8 tons/ha (Fig. 1.) [1]. As a result, India's forex earning from export of Basmati rice has gone up from Rs. 294 crores in 1990-91 to Rs. 12,000 crores in 2009-10 (www.apeda.gov.in) (Table 1) to which the contribution of IARI varieties is more than 60 per cent. Albeit superiority in the quality and consumer acceptance, these varieties, parental lines of the hybrid and hybrid in general are susceptible to several biotic stresses such as bacterial blight (BB) caused by *Xanthomonas oryzae pv. oryzae*, blast caused by *Magnoporthae oryzae* and Brown Plant Hopper (BPH) infection by *Nilaparvata lugens* reducing yield and quality of rice.

The most effective and environmental friendly management strategy of combating these stresses is exploitation of host plant resistance. Till date 34 bacterial BB genes [2], 85 blast resistance genes [3] and 21 BPH resistance genes [4] have been identified, mapped to specific chromosomal location and tightly linked

*Corresponding author's e-mail: aks_gene@yahoo.com
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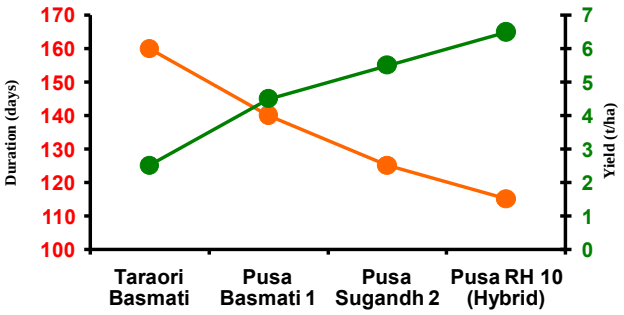

Fig. 1. Breaking the jinx of unholy relationship between yields and duration

molecular markers have been developed. However, all these genes are available in non-Basmati sources and their transfer to Basmati background impairs the grain and cooking quality traits of Basmati rice varieties. Under such situations, marker assisted backcross breeding (MABB) offers a great opportunity for transferring desirable genes from unadapted donors to otherwise agronomically superior cultivars having specific weakness. With the availability of molecular markers and saturated molecular genetic map of rice, MAS has now become feasible both for traits controlled by major genes as well as QTLs. Given the information available, molecular markers can be successfully deployed for foreground as well as background selection in order to confirm the presence of resistance gene(s) and speedy recovery of recurrent parent genome (RPG) and phenome. With a view to develop BB, Blast, BPH and salt resistance/tolerance cultivars in our Basmati breeding program, number of resistance genes/QTLs are being incorporated into the Basmati varieties using the MABB strategy. A list of genes, markers their chromosomal locations, distance between marker and genes, donors used, recurrent parents being improved, are given in Table 2. The progress in MABB for improvement of Basmati rice varieties is briefly presented trait wise.

Table 1. Trends in Basmati export since past two decades

Year	Quantity (m.t.)	Value (Rs. Crores)
1990-91	0.23	261
1995-96	0.37	765
2000-01	0.84	1,930
2005-06	1.16	2,727
2006-07	1.04	2,511
2007-08	1.18	3,900
2008-09	1.50	7,200
2009-10	2.00	12,000

Source: APEDA, Ministry of Commerce, Govt, of India

Improvement of Pusa Basmati1 for BB resistance

Pusa Basmati 1 (PB1) was the first semi-dwarf, high yielding Basmati quality rice variety released for commercial cultivation in 1989. Over period of time, this variety became highly susceptible to BB disease caused by *Xanthomonas oryzae pv. oryzae*. MABB approach was used to incorporate BB resistance in PB1 using IRBB55 (an isogenic line of IR24) as a donor for BB resistance genes *xa13* and *Xa21*. The CAPS marker RG136 linked to *xa13*, and STS marker *pTA248* linked to *Xa21* were used for the foreground selection. MAS for foreground genes *xa13* and *Xa21* was coupled with phenotypic selection for agronomic, grain and cooking quality traits in BC_1F_1, BC_1F_2 and BC_1F_3 generations. Background analysis with 252 polymorphic AFLP markers quantified the RPG recovery ranging from 80.4% to 86.7% in different BC_1F_3 progenies [17]. Marker assisted background analysis was effectively integrated with foreground selection to identify superior BB resistant recombinants with minimal linkage drag [18]. One of these selections was released as Improved Pusa Basmati 1 (Pusa 1460), for commercial cultivation in 2007 as one of the first products of molecular breeding in the country.

Improvement of parental lines of Pusa RH10 for BB resistance

PusaRH10 (PRH10), the first super fine grain aromatic rice hybrid developed by IARI, has the inherent advantage of earliness, high yield and better quality, but it is susceptible to BB. In order to incorporate BB resistance in PRH10, its maintainer line Pusa6B and restorer line PRR78 were first improved using Pusa 1460, as donor for *xa13* and *Xa21* through MABB approach. Later, the improved Pusa 6B was used as donor for *xa13* and *Xa21* for improving Pusa 6A. The markers RG136 and *pTA248* linked to BB resistance genes *xa13* and *Xa21*, respectively, were used for foreground selection. Seventy-four STMS markers polymorphic between Pusa6B and Pusa1460, and 54 STMS markers polymorphic between PRR78 and Pusa1460, were utilized for background selection to recover the RPG ranging from 85.14 to 97.30% and 87.04 to 92.81% in the 10 best BC_2F_5 families of Pusa6B and PRR78, respectively [19, 20]. Stringent phenotypic selection coupled with the cooking quality analysis in the back cross generations aided in hastening the

Table 2. Genes/QTLs and their linked markers used for improvement of Basmati rice varieties

Trait	Gene/ QTL	Donor Parent(s)	LG	Marker	Forward primer Sequence	Reverse Primer Sequence	Linkage distance	Ref
BB	*xa13*	Pusa 1460	8	*xa13 prom*	GGCCATGGCTCAGTGTTTAT	GAGCTCCAGCTCTCCAAATG	Gene Based	A
	Xa21		11	*pTA248*	AGACGCGGAAGGGTGGTTCCCGGA	AGACGCGGTAATCGAAGATGAAA	Gene Based	[5]
	Piz-5	C101A51, Pusa 1602 and IRBLZ5-a	6	AP4007	CGACGAACAACAACTCAAC	GTTCTCTCGGTTTGGACTTC	< 0.1 cM	[6]
				AP5930	CATGAAAGAAAGGAGTGCAG	ACAGAATTGACCAGCCAAG	0.10cM	
	Pi54	Tetep, Pusa 1603 and DHMAS-70Q164-2a	11	RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	0.6 cM	[7]
Blast	*Pita*	DHMAS-70Q164-2a	12	RM247	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	<5 cM	[8]
	Pi-1		11	RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	0 cM	[9]
	Pi-5	IRBLB5-M	9	S04G03	CTTAACAATCAATGTTTAATGAAA	GTTATATTATACTAATTGTTTATC	0.8 cM	[10]
	Pi-9	IRBL9-W	6	AP5930	CATGAAAGAAAGGAGTGCAG	ACAGAATTGACCAGCCAAG	0.05cM	B
	Pi-b	IRBLb-B	2	RM208	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	1.2 cM	[11]
	Bph-3	Rathu Heenathi	6	RM589	ATCATGGTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	0.9 cM	[12]
	Bph-17		4	RM5953	AAACTTTCTGTGATGGTATC	ATCCTTGTCTAGAATTGACA	3.2 cM	[13]
BPH	*Bph-18*	IR65482	12	RM6217	GCAGCAAGAGCAAGAAATCC	GTTCCTGCCGTACCAGCAG	Gene based	[14]
	Bph-20	IR71033	4	BP-20-2	AACCAAAGTTGGTAACGAGAGC	CGCAATCTATTAGACACCGTTC	Gene Based	B
	Bph-21		12	B121	AACCAAAGTTGGTAACGAGAGC	CGCAATCTATTAGACACCGTTC	Gene based	[15]
Salt tolerance.	*Saltol*	FL478	1	RM3412	AAAGCAGGTTTTCCTCCTCC	CCCATGTGCAATGTGTCTTC	QTL based	[16]
Restoration	*Rf1*	PRR78	10	RM6100	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC	7 cM	[25]
Aroma	*badh2*	-	8	*nksbadh2*	GGTTGCATTTACTGGGAGTTATG	TCCACAGAAATTTGGAAACAAAC	Gene based	[31]

A: Personal Communication with R M Sundaram, Senior Scientist, DRR, Hyderabad, B: Personal communication with R. Rathore, Associate Professor, HPKVV, Palampur

recovery of the recurrent parent genotype and phenotype.

The extent of donor segments in the improved version of Pusa6B was estimated to be <0.97 and <2.15 Mb in the genomic regions flanking *xa13* and *Xa21*, respectively, whereas in improved PRR78, it was estimated to be<2.07 and <3.45 Mb in the corresponding genomic regions. For a Basmati quality hybrid, grain and cooking quality traits are extremely important and thus emphasis was laid on analyzing such traits before advancing generations. Since, the background analysis using a sufficiently large number of markers and plants is expensive, phenotypic selection was combined with marker-assisted background selection for making the marker assisted breeding protocol economic and effective [18]. Improved lines of Pusa6B and PRR78 showed yield advantages of up to 8.24 and 5.23%, respectively. The performance of the BB resistant version of PRH10 produced by intercrossing the improved parental lines was on par with or superior to PRH10. Improvement of PRH10 for BB resistance was facilitated by the availability of BB resistance genes *xa13* and *Xa21* in the Basmati background i.e., Improved Pusa Basmati 1.

Improvement of Pusa Basmati 1121 and Pusa Basmati 6 for BB resistance

At present Pusa Basmati 1121 is the most widely grown Basmati rice variety. During Kharif 2010, this variety was planted on 1.2 m.ha. area out of the total Basmati cultivating area of 2 m ha. Pusa Basmati 6 (Pusa

1401), a recently developed variety surpasses Pusa Basmati 1121in several attributes such as non-lodging and non-shattering habit, response to input use, dwarf stature, higher yield, non-chalky grains, strong aroma and better cooking quality. However, both these varieties are also susceptible to BB disease. In order to incorporate BB resistance in both these varieties, Pusa 1460 was again used as the donor parent for marker assisted transfer of BB resistance genes *xa13* and *Xa21 in Pusa Basmati 1121 and Pusa Basmati6* . Superior plants with desirable grain and cooking quality have been recovered in BC_2F_1 and the rapid recovery of Basmati quality traits is attributed to the utilization of Basmati quality donor parent, Improved Pusa Basmati 1.

Development of blast resistant isogenic lines of PRR78

PRR78 is a Basmati quality restorer line and male parent of PRH10, is susceptible to blast disease. Simultaneous but step wise gene transfer strategy of MABB was deployed to transfer two blast resistance genes, *Pi54* and *Piz*-5, from the rice cultivars Tetep and C101A51, respectively into PRR78. The BC_2F_1 populations were generated from individual backcross breeding program using marker assisted foreground selection with gene linked markers RM206 (linked to *Pi54*) and AP5930 (linked to *Piz-5*)coupled with evaluation for morphological traits and blast resistance. BC_2F_1plants were advanced to BC_2F_6 by pedigree selection along with selection for grain and cooking quality traits in each of the generations. Based on background analysis, four lines *viz.,* Pusa1603-06-10-2-12 and Pusa1603-06-58-3-91 with *Pi54* gene; and Pusa1602-06-30-1-51 and Pusa1602-06-24-5-45 with *Piz-5* gene showed RPG recovery of 89.01%, 88.25%, 87.88% and 86.66%, respectively. To pyramid both the blast resistant genes (*Pi54* and *Piz-5*), the best BC_2F_1s from individual backcross were intercrossed and subjected to foreground selection in the segregating generation to identify the plants homozygous for both the genes. The superior lines were carried forward through pedigree selection to develop Advanced Backcross Inbred Lines. Background analysis with 72 polymorphic STMS markers providing genome wide coverage revealed that Pusa1609-09-3-60, Pusa1609-09-3-4 and Pusa1609-09-11-30 carrying *Piz-5* and *Pi54* had RPG of 91.62%, 90.21%, and 88.80%, respectively. The hybrids produced with Pusa6A x improved ABILs were found to be on par with original PRH10 in yield, grain and cooking qualities with the advantage of blast resistance.

Development of near isogenic lines carrying major blast resistance genes in the background of Pusa Basmati 1

Until now, 83 major blast resistance genes have been documented in rice. However, all these genes are present in non-Basmati background and their transfer to Basmati varieties poses the problem of impairment of grain and cooking quality traits. Therefore, for the first time an attempt has been made to develop near isogenic lines for seven major blast resistance genes in the genetic background of PB1. The development of near isogenic lines was carried out using the series of IRBL lines (IRBL5-M: *Pi5*; IRBLb-B: *Pib*;IRBL9-W: *Pi9* and IRBLz5-CA: *Piz-5*) and a doubled haploid line carrying three blast resistant genes (*Pi1, Pi54*, *Pita*) as the donors, and PB1 as the recurrent parent in independent backcross breeding programs. The BC_4F_1 generation for the genes *Pi1, Pi54* and *Pita*, BC_2F_1s for the genes *Piz-5* and *Pib* and BC_3F_1s for the genes *Pi5 and Pi9* have been developed. Marker assisted foreground selection using gene linked molecular markers was performed in each generation to identify plants positive for the respective genes. Background selection with polymorphic markers between parents coupled with stringent phenotypic selection for morphological, grain and cooling quality traits was carried out for rapid RPG recovery. The RPG recovery in the selections for each cross combination namely, Pusa1633-6 (PB-1+*Pita*), Pusa1636-1 (PB-1+*Pi5*) and Pusa1637-1 (PB-1+*Pi9*) was found to be in the range of 86.2-92.7%, 71.5-88.7% and 89.06-93.7 respectively. The isogenic lines carrying different blast resistance genes developed are likely to be released as direct varieties after extensive testing and evaluation. Further, these lines will also serve as excellent donors for blast resistance genes in Basmati breeding program, and as genetic material for functional genomics to understand molecular mechanism of blast resistance.

Improvement of Pusa Basmati 1121 and Pusa Basmati 6 for blast resistance

The Blast resistant donors developed by marker assisted breeding namely Pusa 1602 (PRR78 + *Piz-5*) and Pusa 1603 (PRR78 + *Pi54*) were used to transfer the respective genes into Pusa Basmati1121 and Pusa Basmati6. The strategy involving foreground selection with gene linked molecular markers followed by stringent selection for morphological, grain and cooking quality traits coupled with background selection for accelerated recovery of RPG was adopted. In the BC_3F_1 generation, the lines of Pusa Basmati 1121 with *Pi54* and *Piz-5* are

being intercrossed to pyramid both blast resistance genes. Similarly the lines of Pusa Basmati6 with *Pi54* and *Piz-5* separately have also been intercrossed to pyramid both blast resistance genes.

Improvement of Pusa Basmati 1121 and Pusa Basmati 6 for BPH resistance

The donors for BPH resistance Rathu Heenathi (*Bph3*, *Bph17*), IR65482 (*Bph18*) and IR71033 (*Bph20*, *Bph21*) were screened for their resistance level in the green house using the standard protocol [21]. The genotype, Rathu Heenathi was found to be highly resistant followed by IR65482 and IR71033.These donors have been crossed with Pusa Basmati 1121 and Pusa Basmati 6, and the F_1s were backcrossed with respective recurrent parents. Our ultimate goal is to pyramid genes for all these biotic stresses (BB, Blast and BPH) together in Pusa Basmati 1121 and Pusa Basmati 6.

Improvement of Pusa Basmati 1121 and Pusa Basmati 6 for salt tolerance

The salt tolerant genotype FL478 [22], a recombinant inbred line from the cross IR29 x Pokkali was used as a donor to transfer the QTL '*Saltol'* governing salt tolerance at seedling stage into the recurrent parents Pusa Basmati 1121 and Pusa Basmati 6 through MABB in two independent backcross programs. A total of three markers linked to *Saltol* locus namely RM8094, RM3412 and RM493 were found to be polymorphic between the recurrent and donor parents. Foreground selection with the QTL linked molecular marker in each back cross generation to identify the gene positive plants, followed by stringent phenotypic selection for rapid recovery of RPG and phenome with salt tolerance is being adopted.

Genetic survey of Pup1 gene among aromatic and non-aromatic rice varieties using gene based markers

Development of rice varieties that can extract P from P-fixing soils with higher P fertilizer use efficiency is considered an important breeding goal. *Pup1* (*Phosphorous uptake* 1) is a major QTL conferring tolerance to the P deficiency under field conditions and is a complex locus~130kb long, probably harbouring many uncharacterized genes [23]. It has been found to occur more in *indica* rice than in *japonica,* especially among upland varieties of both the sub-types. Gene based molecular markers K29, K46, K59 and K41spanning the *Pup1* locus were used to screen, 105 rice varieties, including major Basmati varieties, with Vandana as positive, N22 as partial, IR64 and Anjali as negative checks. All Basmati/aromatic rice varieties were *Pup1* positive while most of the non-aromatic varieties were devoid of *Pup1* locus. A sub-set of genotypes from each of the groups were evaluated under hydroponic conditions, under restricted P nutrition. A marked difference in root length and plant height was observed between the groups in hydroponic screening. The presence of *Pup1* locus in all the Basmati varieties is reported for the first time. Further, validation of 'P' use efficiency of Basmati varieties vis-à-vis presence of *Pup1* locus is being undertaken in P sick plot and the findings has great relevance in management and use of phosphorous in Basmati rice.

Molecular mapping and MAS for fertility restorer gene(s) in Basmati Rice

The inheritance and molecular mapping of a fertility restorer gene in Basmati quality restorer line PRR78 was carried out using an F_2 mapping population from the cross IR58025A X PRR78 employing microsatellite markers. Dominant monogenic control of fertility restoration was observed in the F_2, and further confirmed by test cross data. Bulked segregant analysis (BSA) revealed the marker RM258 located on chromosome 10, linked to the restorer gene at a distance of 9.5 cM [24].

In order to discover a more tightly linked marker for *Rf* gene, mapping was carried out in the F_2 population derived from the crossIR62829A/MTU9992 through BSA. The microsatellite molecular marker RM6100located on chromosome 10 was identified to be closely linked to *Rf* gene at a distance of 7cM. Further to assess the potentiality of the marker for utilization in the restorer breeding program for identification of restorers, RM6100 was validated with a set of 175 germplasm lines and found to highly effective in identifying most of the restorer lines with an efficacy of 97.4% [25]. Thus, RM6100 has become the integral part of hybrid breeding program for identification and breeding new restorer lines.

QTL mapping for grain dimension traits

A mapping population developed from the cross Sonasal x Pusa Basmati 1121 was used to identify QTLs for grain dimension traits. Sonasal is a short grain aromatic rice landrace and Pusa Basmati 1121 is the most popular Basmati rice variety with long slender grains. Phenotyping of 300 F_2 plants for grain dimensions before and after cooking was done using F_3 seeds harvested from individual plants. Data for grain dimension traits

(milled rice length and breadth, L/B ratio, cooked kernel length and elongation ratio) in 10 seeds/sample were recorded (Table 3). For polymorphism survey between parents >1000 markers (HvSSR, STMS and RGNMS) were used and physical map was generated with genotypic data of 141 markers having genome-wide coverage. A major QTL was mapped for milled rice length explaining phenotypic variance of 74% at 149cM (Fig. 2.) position. At the same position, QTLs for other grain dimension traits namely, milled rice breadth, milled rice length/breadth ratio, cooked kernel length were also mapped (Table 4) explaining phenotypic variance of 19.7 %, 76.5 % and 69.5 % respectively. A QTL for elongation ratio (ER) was also discovered in the marker interval RM130- RM514 spanning a region of 2.04 cM.

Validation and use of fragrance gene linked markers

The aromatic character of Basmati rice has been largely attributed to 2-acetyl-1-pyrroline (2-AP) even though more than 100 volatile aroma compounds have been identified in cooked rice [26]. Sensory evaluation is often carried out by plant breeders to select aromatic plants in segregating generations. However, it is labour intensive and varies from individual to individual and the ability to distinguish between fragrant and non-fragrant samples diminishes with each successive analysis due to saturation of sensory organ and/or physical abrasions to the tongue.

A recessive gene *badh2* for aroma has been cloned on chromosome 8 [27, 28, 29, 30]. Analysis with the gene based perfect marker has revealed that grain aroma is due to an eight base pair deletion in aromatic varieties compared to non-aromatic varieties [29]. However, these markers, when validated in a set of Basmati and non-Basmati rice genotypes, their efficacy was found to be low. In order to mitigate the problem, another marker *nksbadh2* based on *badh2* gene sequence information was designed [31]. This marker was validated on 25 aromatic and 28 non-aromatic rice genotypes with 100% efficacy. In aromatic rice varieties, 82bp fragment was amplified while in case of nonaromatic genotypes a 90bp fragment was amplified (Fig.

Table 3. Variation for grain dimension traits in F_2 population of cross Sonasal X Pusa Basmati 1121.

Trait	Sonasal	Pusa Basmati 1121	300 F_2 population		
			Mean	Range	CV
Milled rice length (mm)	2.93	7.66	5.30	3.63- 8.01	18.20
Milled rice breadth(mm)	1.56	1.93	1.94	1.60-2.40	5.89
Length: breadth ratio	1.87	3.95	2.76	1.79-4.70	21.98
Cooked kernel length(mm)	5.13	17.36	8.86	5.76-15.23	21.61
Elongation ratio(mm)	1.75	2.26	1.67	1.00-2.30	11.54

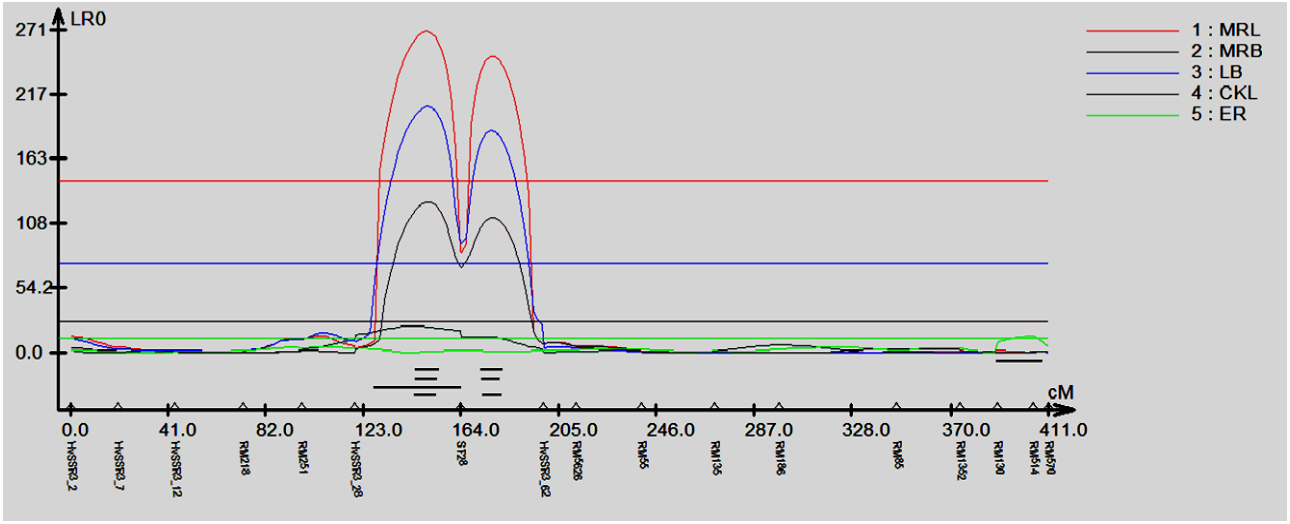

Fig. 2. QTL cartographer LOD plot for grain dimension traits on chromosome 3 (each trait is represented as different colours)

Table 4. QTLs identified for grain dimension traits on chromosome 3 using composite interval mapping.

S.No	Trait	QTL name	Marker interval	LOD	R^2
1	MRL	mrl3_1	HvSSR3_28-SF28	58.71	0.74
2	MRL	mrl3_2	SF28- HvSSR3_62	54.13	0.74
3	MRB	mrb3_1	HvSSR3_28- SF28	5.00	0.19
4	L/B	mrl/b3_1	HvSSR3_28- SF28	45.03	0.76
5	L/B	mrl/b3_2	SF28- HvSSR3_62	40.63	0.77
6	CKL	ckl3_1	HvSSR3_28-SF28	27.55	0.69
7	CKL	ckl3_2	SF28- HvSSR3_62	24.65	0.70
8	ER	er3_1	RM130- RM514	3.04	0.06

MRL: milled rice length, MRB:milled rice breadth, L/B: milled rice length/ breadth ratio, CKL: cooked kernel length, ER: elongation ratio, NLM: nearest left marker, NRM: nearest right marker

3). Further, the marker was also validated in a RIL population consisting of 184 RIL developed from cross (Pusa 1121 x Pusa1342), segregating for aroma, the expected fragment size of 82bp and 90bp were amplified in aromatic and non-aromatic RILs, respectively (Fig. 4).

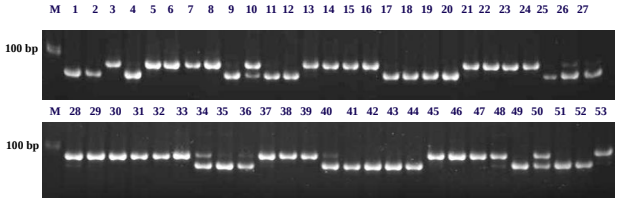

Fig. 3. Validation of *badh2* gene based marker (*nksbadh2*) in aromatic/non-aromatic rice genotypes. Aromatic varieties (25 varieties): Bindli (1), Muskan (2), Super Basmati (4), Shah Pasand (9), Ketaki Joha (11), Badshah Bhog (12), Sonasal (17), Type-3 (18), Barasitwa (19), Chini Kamini (20), Lalmati (25), Pusa 1121 (26), Basmati-370 (27), Seond Basmati (34), Dhushura (35), Hasan Sarai (36), Madhumati (40), Pusa Basmati-1 (41), Katak Tara (42), Tilak Chandan (43), Kala Jeera (44), Lal Basmati (49), Kalnamak (50), Taraori Basmati (51), Ramdilal (52). Non- Aromatic Varieties (28 varieties): Malviya Dhan 36 (3), PNR 136 (5), Jyoti (6), Rasi (7), Red Triveni (8), ADT-37 (10), IRBB-60 (13), IR-36 (14), Ananda (15), Nagina 22 (16), Neela (21), IR 4630 (22), Pusa 44 (23), Sona Mahsuri (24), VL Dhan 221 (28), Pant Dhan 4 (29), TKM-6 (30), Swarna (31), IR 24 (32), IR 20 (33), Krisna Hamsa (37), Sarju 52 (38), Madhu Vijaya (39), Ratna (45), Tetep (46), Vikash (47), Narendra -97 (48), IR-108(53). Note: The figures within parenthesis indicate the lane number

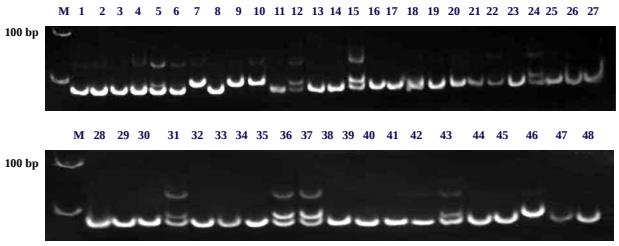

Fig. 4. Validation of *badh2* gene based marker (*nksbadh2*) in F9 RILs from cross Pusa 1342 X Pusa Basmati 1121. M: 50 base pair DNA ladder, Lanes 1-48: RILs

Conclusion

Basmati rice breeding, like all other plant breeding programmes was solely dependent on conventional tools of plant breeding based on phenotypic selection which inspite of achieving significant impacts in Basmati rice improvement was still needing refinements for making progress in breeding for stress resistance. The evolution of reliable molecular marker technologies has provided us a reliable tool by enabling us to map important Basmati quality traits in rice and pyramid genes for stress resistance with impeccable precision through marker assisted selection. Marker assisted breeding has been successfully employed for the development of Improved Pusa Basmati 1 and the improved versions of PRR78 and has become an integral component in the Basmati rice breeding program at IARI, New Delhi. It has been made possible by adopting cost effective MAS strategy complemented by phenotypic selection for precise gene transfer and improvement of Basmati rice varieties. MAS based on molecular markers linked to genes for resistance to biotic

stresses (BB, Blast and BPH), abiotic stress (Salt tolerance and phosphorous use efficiency), grain and cooking quality traits and aroma has enhanced the efficiency and precision of breeding program.

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