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Marker aided improvement of Pusa 6B, the maintainer parent of rice hybrid Pusa RH10, for resistance to bacterial blight

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Abstract

The present study was under taken to transfer two bacterial blight resistance genes, *xa13* and *Xa21* into Pusa6B, the maintainer parent of Pusa RH10 (PRH 10), the first superfine grain aromatic rice hybrid. Marker aided foreground selection for genes *xa13* and *Xa21* was carried out using a CAPS marker RG136 and STS marker pTA248, respectively. Forty Six STMS markers polymorphic between Pusa 6B and Pusa1460 providing genome wide coverage were used for background selection for recovering recurrent parent genome. Marker aided selection was combined with phenotypic selection for agronomic, grain and cooking quality traits to hasten the breeding process. In each backcross generation, based on background selection, a single plant positive for genes *xa13* and *Xa21* was selected for further backcrossing/ selfing. The extent of recurrent parent genome recovery in the 9 BC_2F_2 plants, carrying both the genes in homozygous condition, ranged from 92.3% to 95.6%. All the plants of BC_2F_3 families were found highly resistant to BB on artificial inoculation with the most virulent Kaul isolate of *Xoo*. The improved lines of Pusa 6B showed highly resistant reaction with susceptibility index (SI) ranging from 0.5 to 1.5 as compared to 1.2 of donor parent Pusa 1460 and 9.2 of recurrent parent Pusa 6B. The agronomic performance, grain and cooking quality attributes of majority of the BC_2F_3 plants were similar to Pusa 6B. However, some plants showed improvement in spikelet fertility, aroma, grain length and L/B ratio. Some of the BC_2F_3 families matured a few days earlier than the recurrent parents, which will be useful in synchronization of parental lines in hybrid seed production.

Key words: Foreground selection, Background selection, Bacterial Blight, STS & CAPS

Introduction

India is endowed with enormous diversity in rice including aromatic rices. Among these, Basmati, because of its unique aroma, flavor and cooking quality is the pride of India. Basmati rice is characterized by long grain, pleasant aroma, delicate flavor, fluffy texture of cooked rice, high volume expansion on cooking made up by linear kernel elongation with minimum breadth wise swelling, palatability, easy digestibility and longer self life [1]. PRH10, the world's first superfine grain aromatic rice hybrid is a CGMS based hybrid with Pusa 6A and Pusa Rice Restorer78 (PRR78) as its female and male parents, respectively. This hybrid is very popular among the farmers. However, both the parents and the hybrid PRH 10 are susceptible to Bacterial Blight (BB) disease caused by *Xanthomonas oryzae pv oryzae*. In India, it is a serious problem during South West monsoon period causing yield losses ranging from 20% to 74% [2]. It also severely affects the quality of the grains. Considering the severity of the disease and susceptibility of the hybrid, the occurrence of BB is likely to become a bottleneck in popularity of this hybrid in future.

Although, various chemicals have been recommended for the control of BB, they are less effective and environmentally unsafe. In absence of effective chemical or other control agents, exploitation of host resistance by means of developing resistant varieties has gained enormous importance in management of the disease [3]. Till now 33 BB resistance (R) genes have been identified [4] many of which are deployed in breeding lines to control the disease. However, most of these major resistance genes have been overcome by newly evolved, indigenous and uncharacterized pathogen races [5]. One of the effective ways to delay this breakdown is to incorporate multiple resistance genes each giving resistance to different races of the pathogen. This

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becomes extremely difficult through conventional breeding, as it is difficult to identify plants having more than one gene because of similar phenotypic expression of the segregating genes and also some of the genes like *xa13*, which confer resistance in recessive condition, cannot be identified in heterozygous condition. With the advent of molecular markers, it has become possible to tag the individual genes giving resistance to different races of the pathogen and identify the presence of multiple genes in plants by indirectly selecting for the markers linked to the genes. Marker assays can be of advantage in backcross breeding for foreground selection and background selection. In foreground selection, the presence of target allele is diagnosed using the gene-linked marker(s) and the background selection accelerates the recovery of recurrent parent genome [6]. In rice the feasibility of Marker Aided Backcross Breeding (MABB) to pyramid BB resistance genes has been well demonstrated [7-9].

Considering the economic impact of PRH10 and the fact that it is susceptible to BB, incorporation of multiple BB resistance genes into its parental lines, Pusa6A and PRR78 was rightly perceived as an essential requirement. Since the female parent, Pusa6A is male sterile, the BB resistance genes have to be first transferred into its maintainer, Pusa6B, and then to Pusa6A. Also, since PRH10 is a basmati quality hybrid, retention of basmati quality traits in the hybrid through its parental line is of utmost importance during parental line improvement through stringent selection for quality traits.

The present study was taken up to pyramid two BB resistant genes *xa13* and *Xa21* into the maintainer parent (Pusa 6B) of the Basmati hybrid PRH10, using marker assisted foreground and background selection combined with phenotypic selection for agronomic performance, disease resistance and cooking quality traits.

Materials and methods

Plant materials

The experimental material for the present study consisted of Pusa6B, the maintainer parent of PRH10, which was used as the recurrent parent for incorporation of BB resistance genes *xa13* and *Xa21*. Pusa1460, an improved version of Pusa Basmati 1, which carries genes *xa13* and *Xa21,* was used as resistant donor. The F_1 obtained from this was designated as Pusa 1605 and backcrossed with the recurrent parent Pusa6B to obtain BC_1F_1 and BC_2F_1 generations. After foreground selection for *xa13* and *Xa21* and background selection for recurrent parent genome recovery in BC_1F_1 and BC_2F_1, selfing was done to obtain BC_2F_2 and BC_2F_3 generations. The nine BC_2F_3 families carrying *xa13* and *Xa21* in homozygous condition were screened in the field for disease resistance, agronomic performance and Basmati quality traits.

Screening for BB disease resistance

Single colony of the most virulent *Xoo* isolate, i.e. the Kaul isolate maintained in the Division of Plant Pathology, IARI, New Delhi laboratory was used for field inoculation. Inoculum was prepared by suspending each pure culture in sterile distilled water adjusting the inoculum concentration of about 10^9 cells per milliliter. The inoculations were done at the maximum tillering stage of the crop in the second fortnight of August, as the weather conditions were conducive for disease development during this period. Five emerging young leaves in each plant were inoculated through clip inoculation method [10]. The plant reaction to the pathogen was scored 21 days after inoculation taking average of the inoculated lines following the standard scale [11]. Plants with an average lesion length up to 6cm were considered as resistant and those with more than 6 cm lesion length as susceptible. The BC_2F_3 families were screened for disease resistance under the field conditions.

Evaluation for agronomic and quality traits

Thirty-day-old seedlings of the parents and the improved lines in BC_2F_3 generation were transplanted in the field with a spacing of 20 x 15 cm. Data were recorded for agronomic traits, namely, plant height, tillers/plant, number of effective tillers/plant, panicle length, number of filled grains/panicle and 1000-grain weight. To facilitate the evaluation of the performance of the newly developed lines, the parents Pusa6B and Pusa1460 were used as checks.

Seeds harvested from the BC_2F_3 plants were analyzed for physico-chemical characters like kernel dimensions of milled and cooked rice, aroma and alkali spreading value (ASV). For testing aroma, one gram milled rice kernels were soaked in 10 ml of 1.7% KOH at room temperature in covered Petri plates for 10 minutes [12]. Coded samples were evaluated by a panel of five experts who have rich experience in Basmati quality evaluation. Pusa6B and Pusa1460 were used as standard checks. The samples were scored on 0-3 scale with 0, 1, 2 and 3 corresponding to absence of aroma, mild aroma, strong aroma and very strong

aroma, respectively. For calculating length/breadth ratio (L/B ratio), five fully developed wholesome milled rice kernels were measured for their length and breadth using a photo enlarger. The kernel elongation ratio (KER) was expressed as the ratio of the average length of the cooked kernels to that of the uncooked kernels. For the estimation of ASV, five whole milled kernels were incubated in 10 ml of 1.7% potassium hydroxide solution taken in a Petridish for 24 hours at 30°C.

Molecular marker analysis

DNA isolation was carried out using micro-extraction method (13). CAPS marker, RG136 (3.7cM away from *xa13)* and STS marker pTA248 (a gene sequence based marker for *Xa21*), were used to confirm the presence of genes *xa13* and *Xa21,* respectively as described earlier [14]. Forty six STMS markers polymorphic between Pusa6B and Pusa1460 were used for background selection to identify lines with greater genetic similarity with the recurrent parent. The recurrent parent genome recovery as revealed by all the polymorphic STMS markers was expressed in percentage.

Results and discussion

Foreground selection for BB resistance

Fifteen F_1 plants were obtained from the cross of Pusa6B with Pusa1460 and their hybridity was confirmed using markers linked to *xa13 and Xa21* and two STMS markers that were polymorphic between parents. One confirmed hybrid plant was used as female parent and backcrossed with the recurrent parent Pusa6B. Ninety two BC_1F_1 plants were grown in glass house in National Phytotron Facility. On analysis with the gene specific markers, 22 plants were found to be carrying *xa13* and *Xa21* genes in heterozygous condition. The segregation among the BC_1F_1 plants is presented in Figs. 3 and 4. Based on the background selection information with 24 STMS markers (two on each chromosome), one plant carrying maximum recurrent parent genome was backcrossed with the recurrent parent Pusa6B to generate BC_2F_1 seeds. Of the 85 BC_2F_1 plants, 20 were found to be heterozygous for the markers linked to both the genes *xa13* and *Xa21*. Based on the background selection with all the 46 polymorphic STMS markers, one plant carrying maximum proportion of recurrent genome was selected to generate BC_2F_2 generation, by selfing. Based on the molecular analysis with RG136 and pTA248, nine plants were identified to be homozygous for *xa13* and *Xa21* (Figs. 5 and 6). The BC_2F_3 families derived from these nine plants were grown in field during *Kharif* 2007 and their disease resistance, agronomic performance and Basmati quality traits were evaluated.

Background selection using microsatellite markers for recovery of recurrent parent genome

A polymorphism survey was done between Pusa 6B and Pusa 1460, with 309 STMS markers spanning all the 12 rice chromosomes. Among these, 46 markers (14.9%) were found to be polymorphic. These markers were used for background selection in backcross generations to hasten the recovery of Pusa6B genome by selecting the plant with highest number of Pusa 6B specific markers in homozygous condition. In the BC_1F_1, background selection was carried out among 22 plants carrying *xa13* and *Xa21* in heterozygous condition, using 24 STMS markers. The proportion of recurrent genome recovery ranged from 71% to 81%. The plant carrying 81% of recurrent parent genome was used to generate BC_2F_1 seeds. In the BC_2F_1 generation, background selection was done in seven plants which were heterozygous for markers RG136 and pTA248, and also having superior quality traits, using the remaining 22 markers and the ones for which the selected BC_1F_1 plants was heterozygous. Among these seven plants, the proportion of recurrent parent genome based on total 46 markers, ranged from 87.75% in plant Pusa1605-05-25 to 92.2% in plant Pusa1605-05-30. The plant Pusa1605-05-30 having the highest percentage of the recurrent parent genome was carried forward to generate BC_2F_2 population. In the BC_2F_2 generation, nine plants carrying *xa13* and *Xa21* in homozygous condition were further subjected to background selection using markers, which remained heterozygous in BC_2F_1. Based on all the 46 markers, the proportion of recurrent parent genome in BC_2F_2 plants ranged from 92.3% in plant Pusa1605-05-114 to 95.6% in plant Pusa1605-05-14. Thus, in the BC_2F_2 generation it became possible to identify a plant carrying recurrent parent genome of more than 95%, which could have been very difficult by morphological selection alone.

Analysis of disease resistance, agronomic performance and Basmati quality in BC_2F_3 generation

The BC_2F_3 families derived from nine BC_2F_2 plants which were found to be homozygous for *xa13* and *Xa21* were grown in the field during *Kharif* 2007. All the plants of the nine families showed disease lesion of less than 2 cm, 21 days after artificial inoculation with Kaul isolates of the bacterium, which was comparable to the resistance shown by the donor parent Pusa1460. All the plants were confirmed to be homozygous for *xa13*

Table 1. Performance of selected BC_2F_3 plants with respect to yield components, disease resistance and quality traits and their parents

BC_2F_3 plant designation	Plant ht (cms)	No. of tillers	Panicle length (cm)	No.of filled grains/ panicle	1000 grain wt. (gm)	Awn (yes/ no)	Yield/ plant (gm)	BB lesion length (cm)	Grain length (mm)	Grain breadth (mm)	L/B ratio	KER	Grain shape	ASV	Aroma
Pusa1605-05-14-1	79	13	28	144	21.9	No	39.5	1.2	8.0	1.6	4.8	1.7	EL,S	7	Strong
Pusa1605-05-14-2	80	17	23	149	22.3	No	38.3	1.5	7.6	1.6	4.6	1.7	EL,S	1/5, 5/6	Mild
Pusa1605-05-14-3	90	14	26	155	19.5	No	36.5	0.8	7.6	1.3	5.8	1.8	EL,S	3/6, 3/7	Mild
Pusa1605-05-14-4	80	16	24	143	21.7	No	41.3	1.3	7.6	1.6	4.6	1.8	EL,S	1/7, 5/6	Mild
Pusa1605-05-26-1	85	14	25	141	21.7	No	55.4	1.1	8.0	1.6	4.8	1.7	EL,S	3/5, 3/6	Mild
Pusa1605-05-26-2	87	15	24	163	23.8	No	53.3	1.4	7.6	1.6	4.6	1.7	EL,S	4/6, 2/7	Strong
Pusa1605-05-31-1	89	13	26	143	20.7	No	39.5	1.1	7.6	1.6	4.6	1.8	EL,S	6	Mild
Pusa1605-05-31-2	82	12	28	134	22.4	No	38.8	1.4	8.0	1.6	4.8	1.7	EL,S	7	Mild
Pusa1605-05-31-3	88	13	28	151	21.3	No	38.6	0.9	8.0	1.3	6.0	1.7	EL,S	3/6,2/5,1/7	Mild
Pusa1605-05-31-4	88	12	24	135	22.0	No	53.0	1.1	7.6	1.6	4.6	1.7	EL,S	2/6, 4/7	Strong
Pusa1605-05-70-1	97	16	31	154	22.8	No	47.1	0.9	7.6	1.6	4.6	1.7	EL,S	7	Mild
Pusa1605-05-70-2	84	11	29	95	22.0	No	52.0	1.1	8.0	1.6	4.8	1.7	EL,S	1/6, 5/7	Mild
Pusa1605-05-70-3	90	15	29	172	23.1	No	43.6	1.1	8.0	1.3	6.0	1.6	EL,S	4/6, 2/7	Mild
Pusa1605-05-73-1	92	21	28	158	24.1	No	46.7	0.5	7.3	1.6	4.4	1.7	L,S	2/6, 4/7	Mild
Pusa1605-05-73-2	90	16	26	153	23.6	No	51.0	0.9	7.0	1.6	4.2	1.7	L,S	4/6, 2/7	Mild
Pusa1605-05-73-3	89	15	26	139	21.5	No	41.4	1.1	7.0	1.6	4.4	1.7	L,S	6	Mild
Pusa1605-05-73-4	91	20	27	141	23.1	No	39.8	0.6	7.3	1.3	4.2	1.6	EL,S	3/6, 3/7	Mild
Pusa1605-05-73-5	94	19	27	150	23.3	No	43.1	0.9	7.0	1.6	4.4	1.7	L,S	2/5, 4/6	Mild
Pusa1605-05-97-1	87	14	26	132	22.8	No	48.2	0.7	8.0	1.6	4.8	1.7	EL,S	5/6, 1/7	Strong
Pusa1605-05-114-4	85	14	25	141	23.1	No	41.6	0.9	7.3	1.3	5.5	1.8	L,S	2/6, 4/7	Mild
Pusa1605-05-117-1	89	17	24	139	20.6	No	51.6	1.2	8.0	1.6	4.8	1.7	EL,S	4/6, 2/7	Mild
Pusa1605-05-117-2	91	16	27	140	22.2	No	46.5	0.9	7.6	1.3	5.8	1.7	EL,S	3/6, 3/7	Strong
Pusa1605-05-117-3	88	19	25	128	23.3	No	37.8	1.3	7.3	1.3	5.5	1.7	**L,S**	3/6, 3/7	Mild
Pusa6B	**83**	**14.8**	**25**	**145**	**22.1**	**No**	**43.3**	**9.2**	**7.0**	**1.6**	**4.0**	**1.7**	**L,S**	**5**	**Mild**
Pusa1460	**88**	**16.4**	**34**	**161**	**23.3**	**Yes**	**45.6**	**1.2**	**8.0**	**1.3**	**6.0**	**1.8**	**EL,S**	**6**	**Strong**
SE±	**0.88**	**0.51**	**0.49**	**2.92**	**0.22**		**1.13**	**0.33**	**0.07**	**0.03**	**0.12**	**0.01**			

Note: 1. (*for awns, presence is represented as Yes and absence as No, Grain Shape: L, S implies Long Slender grains and EL, S implies Extra Long Slender Grains, ASV-Alkali Spreading Value).
2. SE± is given where applicable.

and *Xa21* genes as was revealed by the gene specific primers and the phenotypic reaction in BC_2F_3 families. The plant height in the BC_2F_3 progenies ranged from 79-94 cm and it was higher than the recurrent parent (Table 1). For other traits like number of tillers, panicle length, 1000grain weight and yield most of the plants were comparable to Pusa6B. All the nine families matured 4 to 5 days earlier than the recurrent parent Pusa6B. All the families had awnless grains similar to Pusa6B, whereas the donor parent had awned grains. The performance of selected BC_2F_3 plants for disease resistance, agronomic and quality traits is presented in Table 1.

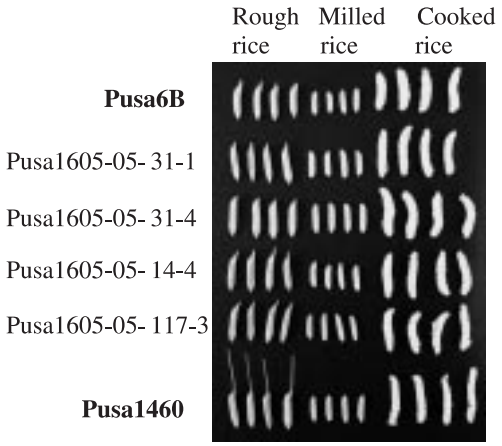

Fig. 1. Comparison of rough rice, milled rice and cooked rice of selected BC_2F_3 recombinants and their parental lines, Pusa 6B and Pusa 1460.

All the plants of the nine BC_2F_3 families were evaluated for basmati quality, based on which 20 plants were selected across the families. There was improvement for various quality parameters (Figs. 1 and 2). All the selected lines except Pusa1605-05-73-1, Pusa1605-05-73-2, Pusa1605-05-73-3, Pusa1605-05-73-4, Pusa1605-05-114-4 and Pusa1605-05-117-3 had Extra Long grains (>7.5mm milled rice grains). This is an improvement over Pusa6B which has long grains (7mm). The KER ranged from 1.6 to 1.8. Majority of the plants had KER equal to that of Pusa6B (1.7). Four plants Pusa1605-05-14-3, Pusa1605-05-14-4,

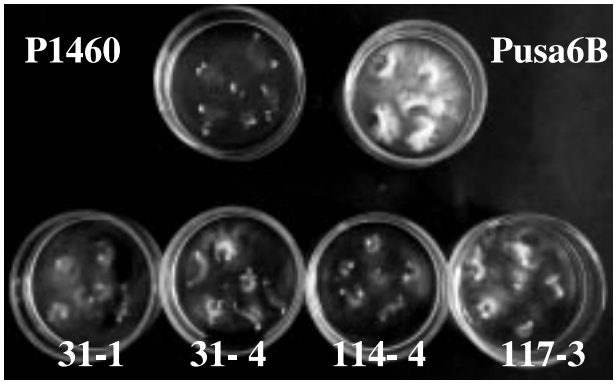

Fig. 2. Alkali spreading value in selected BC_2F_3 recombinants and their parental lines, Pusa 6B and Pusa 1460. The recombinants are Pusa 1605-05-31-1, Pusa 1605-05-31-4, Pusa 1605-05-114-4, Pusa 1605-05-117-3, respectively from left to right.

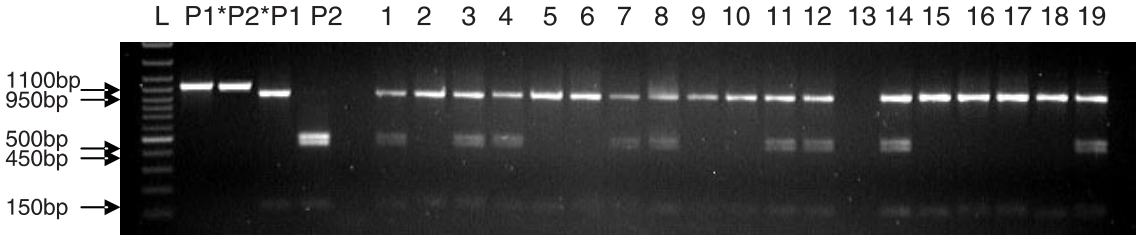

Fig. 3. Molecular profile BC_1F_1 plants of PRR78/P1460/PRR78 for presence of *xa13* gene using CAPS marker RG136. L-100bp ladder, P1*-Pusa 6B uncut, P2* - Pusa 1460 uncut, P1 - Pusa 6B cut with *Hinf1*, P2-cut with *Hinf1*, lane 1-9 plants segregating for *xa13* linked marker RG 136.

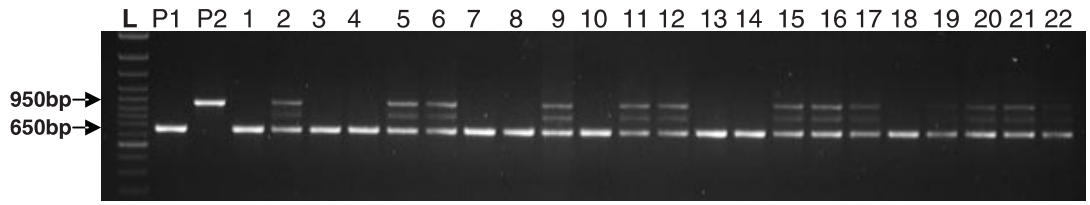

Fig. 4. Molecular profile BC_1F_1 plants of P6B/P1460/P6B for presence of *Xa21* using pTA248. L-100bp ladder, P1-P6B, P2-P1460, lane 1-22 plants showing presence of 950 bp and 650 bp are heterozygous for *Xa21* and those showing single 650 bp are homozygous for *xa21*.

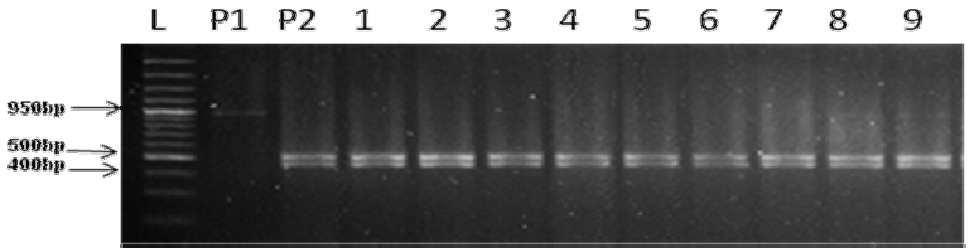

Fig. 5. Molecular profile of BC_2F_2 plants of P6B × P1460 cross confirming their homozygocity for *xa13* gene linked marker RG136.

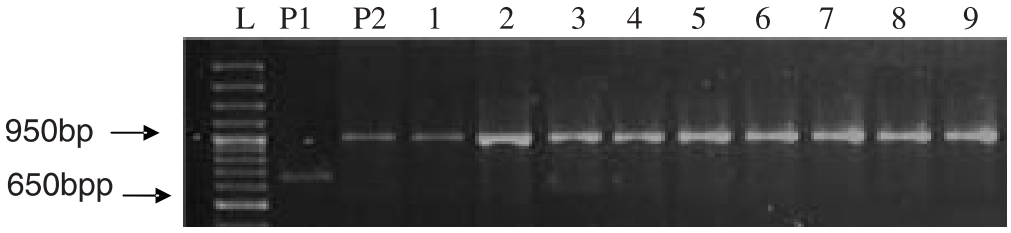

Fig. 6. Molecular profile of BC_2F_2 plants of P6B × P1460 cross which were homozygous for *Xa21* linked marker pTA248. 100bp ladder, P1-Pusa6B, P2-Pusa 1460, lane 1-9 BC_2F_2 plants homozygous for *Xa21* gene

Pusa1605-05-31-1 and Pusa1605-05-114-4 had KER of 1.8. With respect to Alkali Spreading Value (ASV), there was variation between the lines and also within the lines. All the BC_2F_3 lines had ASV more than that of Pusa6B. Some of the grains within the lines had ASV of 7, which is like that of Pusa1460. So, there is a need for further selection among the progenies of these lines to stabilize this trait. Selected lines showed mild to strong aroma. Six of the lines had strong aroma, which is an improvement over Pusa 6B, which has mild aroma. Also, there was improvement with respect to grain filling in some of the families, Pusa1605-05-30-14, Pusa1605-05-30-26, Pusa1605-05-30-70 and Pusa1605-05-30-114 had completely filled grains, whereas in Pusa 6B grains lack complete filling towards tip. Thus, along with improvement for disease resistance, there was significant improvement for basmati quality traits of the Pusa RH10 maintainer line Pusa6B, which would contribute towards improvement of the hybrid.

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