

CONFIRMATION OF Xa GENES INTROGRESSED INTO ELITE BASMATI DERIVED LINES

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It is essential in any rice breeding program to develop high yielding varieties and breeding lines with resistance to biotic and abiotic stresses in combination with desirable agronomic traits such as grain quality and aroma. Bacterial blight (BB) is a major disease in rice and introgression of effective BB resistance genes (Xa genes) singly or in combination into susceptible varieties is the most economical approach to manage the disease. Basmati rice is an aromatic rice favored for its good eating and cooking qualities especially in South and Southeast Asia, but is susceptible to BB. To improve its resistance, Xa genes were pyramided into basmati rice by crossing a basmati-derived line IR 71730-51-2 with IRBB60 (a pyramid line containing Xa4, xa5, xa13 and Xa21 genes). Elite breeding lines were selected for aroma, BB resistance, and grain yield and other desired agronomic traits, including grain quality related physico-chemical traits. To select for BB resistance, phenotyping against selected diagnostic strains of Xoo was done until F8. Marker-assisted selection or confirmation was practiced at F2, F5, F7 until F8 generations. Fifteen promising lines with genes for aroma, BB resistance, along with good agronomic performance and other grain quality traits were identified. These elite materials were evaluated in large replicated yield trials at IRRI. The resistance of these elite lines to BB was assessed based on their reaction to 12 strains which represent 10 Philippine races. The fragrance gene was also detected using a PCR based marker. To confirm the BB resistance genes in each line, we used several PCR based applications including a gel-free dot blot assay using gene specific primers and SNP-based probes for xa5, xa13 and Xa21. The objective of this study was to confirm the presence of the introgressed genes so as to ensure the stability of the genes in the elite lines which can serve as potential donors in the breeding program.

MATERIALS AND METHODS

Development of basmati derived lines

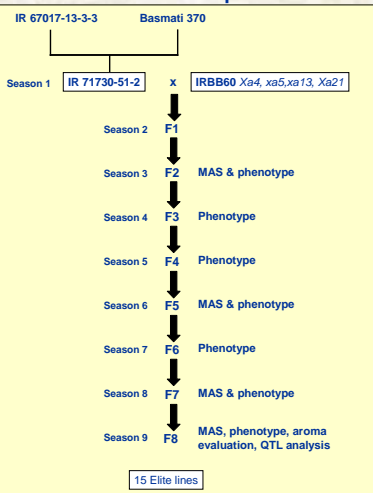


Fig. 1 Pedigree and selection scheme of the cross IR 71730-51-2 x IRBB60 from F1 to F8 generation.



Figure 2. (A) One of the elite basmati-derived lines planted in the replicated yield trial plot at IRRI, Philippines during wet season 2007 (B) Resistance reaction of a Basmati-derived line to Xoo race 2 at 14 d post inoculation in IRRI field.

Confirmation of the presence of Xa genes and fragrance gene

- Genotyping of the 15 elite lines was performed in two batches. (1) the first batch consisted of a bulk of 10 plants per line, (2) the second batch consisted of 9 individual plants per line.
- PCR-based marker applications were used to confirm the presence of Xa4, xa5, xa13 and Xa21. The presence of the fragrance gene was also confirmed with a PCR-based marker designed from the sequences of the cloned gene following the report of Bradbury et al., 2005
- Xa4 was confirmed using a linked marker, MP. The genes xa5 and xa13 were confirmed using gene specific primers based on published cloned sequences of these genes (Iyer and McCouch 2004, Chu et al., 2006, respectively). Xa21 was confirmed using primers designed from the protein kinase domain of the gene (Song et al., 1995), and a SNP-based probe in a gel-free dot blot assay. Table 1 is a list of the markers used in the genotyping of the lines.

RESULTS AND DISCUSSION

- The 15 elite lines were chosen based on over all performance based on all target traits for basmati-derived lines. Table 2 is a summary of the lines and the respective genes they contain, and their characteristics based on yield, BB resistance, grain quality traits (i.e. amylose content), and other agronomic traits. Figure 3 shows the grains (hulled and

Marker	Sequence (5' - 3')	Gene	Marker type	Gene type	Reference
MP	ATCCTCATAGGACAGCCAG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP1	TGCTTCAAGGAGGCTGGT	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP2	ATCTGGCGGCTGCAAGGCTG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP3	ACTTCCTGATGATGATTTGGAGG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP4	ATCCTGGAGGAGGCTGAGG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP5	ATCCTGGAGGAGGCTGAGG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP6	ATCCTGGAGGAGGCTGAGG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP7	ATCCTGGAGGAGGCTGAGG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP8	ATCCTGGAGGAGGCTGAGG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP9	ATCCTGGAGGAGGCTGAGG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994
MP10	ATCCTGGAGGAGGCTGAGG	Xa4	Linked marker	Cell based	Ma Bo Sun et al., 1994

dehulled) of parental lines IR71730-51-2 and IRBB60, Basmati 370 and two out of the 15 elite lines, IR 77542-220-2-3-2-3 and IR 77542-147-1-1-1-1-2.

- The genotyping data done on a bulk of 10 plants per line are shown in Table 3. This procedure is typically done for materials at the most advanced generation (e.g. F8 or later generation). Segregation in any of the individual plants will result in heterozygous condition. Since, no heterozygotes were detected, it is assumed that the lines are fixed for the traits of interest and are no longer segregating.

RESULTS AND DISCUSSION cont'

- However, a single plant with a different genotype than the rest in the bulk may not be detectable. Alternatively, genotyping on individual plants would detect single plant segregation. We have applied this procedure on lines which are potential donors for the target traits of interest, and where seed purity is important. Figures 4, 5, 6 and 7 show the genotypes of the lines containing the aroma gene, xa5, xa13 and Xa21 genes, respectively. Segregating lines containing xa13 and Xa21 were detected. A further selfing and selection may be needed for these lines.



Table 2. Morpho-agronomic, disease and insect resistance, and grain quality data from 15 elite basmati-derived lines and 3 parents.

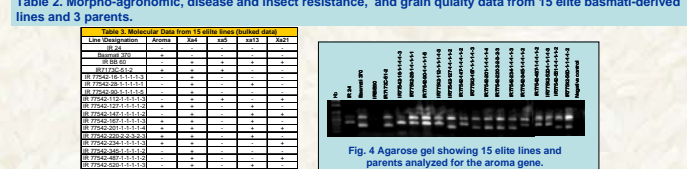


Fig. 4 Agarose gel showing 15 elite lines and parents analyzed for the aroma gene.

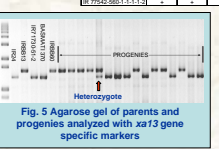


Fig. 5 Agarose gel of parents and progenies analyzed with xa13 gene specific markers

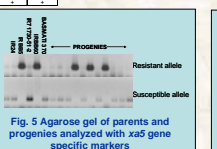


Fig. 5 Agarose gel of parents and progenies analyzed with xa5 gene specific markers

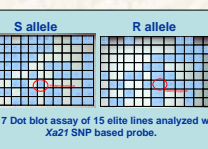


Fig. 7 Dot blot assay of 15 elite lines analyzed with Xa21 SNP based probe.

- Consistency of phenotype data is more evident when the elite lines are grouped based on their Xa genes. Three elite lines contain the single gene Xa4. One line IR 77542-345-1-1-1-1-2, is susceptible to only 2 races while the others to 3. Among the five lines containing Xa4 and xa13, one line, IR 77542-220-2-3-2-3 is distinct because it is resistant to all 10 Xoo races. Four lines contain Xa4, xa13 and Xa21. Two of these lines are resistant to all races while the other two are moderately susceptible and susceptible to 3 and 2 races, respectively. The two lines containing Xa4 and Xa21 consistently show moderately susceptibility to two races while they are resistant or moderately resistant to the other eight races. The single line containing Xa4, xa5 and Xa21 is resistant to six of the 12 Xoo races (Fig. 4).

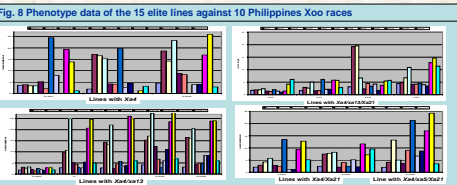


Fig. 8 Phenotype data of the 15 elite lines against 10 Philippines Xoo races

Code	Gene	Genes
A1632	IR 77542-16-1-1-1-1-3	Xa4
A1661	IR 77542-90-1-1-1-1-5	Xa4
A1642	IR 77542-345-1-1-1-2	Xa4
A1633	IR 77542-28-1-1-1-1-1	Xa4, xa13
A1645	IR 77542-535-1-1-1-1-3	Xa4, xa13
A1636	IR 77542-127-1-1-1-1-2	aroma, Xa4, xa13
A1638	IR 77542-623-1-1-1-1-3	aroma, Xa4, xa13
A1640	IR 77542-220-2-3-2-3-3	aroma, Xa4, xa13
A1639	IR 77542-147-1-1-1-1-2	Xa4, xa13, Xa21
A1644	IR 77542-201-1-1-1-1-4	aroma, Xa4, xa13, Xa21
A1646	IR 77542-201-1-1-1-1-2	aroma, Xa4, xa13, Xa21
A1647	IR 77542-560-1-1-1-1-2	aroma, Xa4, xa13, Xa21
A1643	IR 77542-487-1-1-1-1-2	Xa4, Xa21
A1641	IR 77542-234-1-1-1-1-3	Xa4, xa5, Xa21
A1635	IR 77542-112-1-1-1-1-3	Xa4, xa5, Xa21
BASMATI 370		aroma
IR 71730-51-2		aroma
IR BB 60-1		Xa4, xa5, xa13, Xa21

CONCLUSION

It is important for basmati rice, a popular aromatic rice but highly susceptible to bacterial blight, to have resistance to the disease. Fifteen elite lines derived from the cross between basmati derived line IR71730-51-2 and IRBB60 have been selected which contain combinations of the genes Xa4, xa5, xa13 and Xa21 for bacterial blight resistance and the aroma gene. Bulk analysis of these lines is sufficient in confirming the presence of the genes when the lines are to be released as varieties. Breeders retain some amount of diversity which is more desirable when grown under farmers' field condition. On the other hand, breeding for resistant varieties require a stable source of genes for resistance. Analysis of individual plants will ensure that the lines are pure and the genes are in the homozygous condition.

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