

CONFIRMATION OF *Xa* GENES INTROGRESSED INTO ELITE BASMATI DERIVED LINES

M.Y.V. Reveche, A. Das, E.F.L. Mercado, M.G.C. Carrillo, I. P. Oña, S.N. Begum, V. Lopena, G.S. Capilit, P. Virk and C.M. Vera Cruz
Plant Breeding, Genetics and Biotechnology Division, IRRI, Los Baños, Laguna, Philippines

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

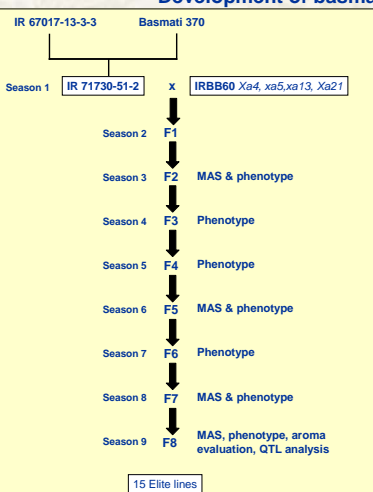


Fig. 1 Pedigree and selection scheme of the cross IR 71730-51-2 x IRBB60 from F1 to F8 generation.



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')	Type	Marker type	Gene type	Reference
MP	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP1	TGCTGAAAGAGGCTGCTG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP2	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP3	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP4	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP5	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP6	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP7	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP8	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP9	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP10	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP11	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP12	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP13	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP14	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP15	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP16	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP17	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP18	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP19	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP20	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP21	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP22	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP23	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP24	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP25	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP26	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP27	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP28	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP29	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP30	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP31	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP32	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP33	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP34	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP35	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP36	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP37	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP38	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP39	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP40	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP41	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP42	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP43	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP44	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP45	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP46	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP47	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP48	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP49	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP50	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP51	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP52	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP53	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP54	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP55	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP56	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP57	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP58	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP59	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP60	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP61	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP62	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP63	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP64	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP65	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP66	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP67	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP68	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP69	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP70	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP71	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP72	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP73	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP74	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP75	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP76	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP77	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP78	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP79	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP80	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP81	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP82	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP83	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP84	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP85	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP86	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP87	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP88	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP89	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP90	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP91	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP92	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP93	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP94	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP95	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP96	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP97	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP98	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP99	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia et al. 1994
MP100	ATGTCAGGATGCTGACGAG	SNP	Linked marker	Cell based	Ma Bo Jia 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 con't.

- 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.

Line	Yield (t/ha)	Grain yield (t/ha)	Grain quality	BB resistance	Insect resistance
IR 71730-51-2	10.5	8.5	High	Low	Low
IRBB60	12.0	10.0	Low	High	High
Basmati 370	11.0	9.0	High	Low	Low
IR 77542-220-2-3-2-3	11.5	9.5	High	High	High
IR 77542-147-1-1-1-1-2	12.0	10.0	High	High	High
IR 77542-345-1-1-1-1-2	11.0	9.0	High	High	High
IR 77542-345-1-1-1-1-3	11.5	9.5	High	High	High
IR 77542-345-1-1-1-1-4	12.0	10.0	High	High	High
IR 77542-345-1-1-1-1-5	11.0	9.0	High	High	High
IR 77542-345-1-1-1-1-6	11.5	9.5	High	High	High
IR 77542-345-1-1-1-1-7	12.0	10.0	High	High	High
IR 77542-345-1-1-1-1-8	11.0	9.0	High	High	High
IR 77542-345-1-1-1-1-9	11.5	9.5	High	High	High
IR 77542-345-1-1-1-1-10	12.0	10.0	High	High	High
IR 77542-345-1-1-1-1-11	11.0	9.0	High	High	High
IR 77542-345-1-1-1-1-12	11.5	9.5	High	High	High
IR 77542-345-1-1-1-1-13	12.0	10.0	High	High	High
IR 77542-345-1-1-1-1-14	11.0	9.0	High	High	High
IR 77542-345-1-1-1-1-15	11.5	9.5	High	High	High
IR 77542-345-1-1-1-1-16	12.0	10.0	High	High	High
IR 77542-345-1-1-1-1-17	11.0	9.0	High	High	High
IR 77542-345-1-1-1-1-18	11.5	9.5	High	High	High
IR 77542-345-1-1-1-1-19	12.0	10.0	High	High	High
IR 77542-345-1-1-1-1-20	11.0	9.0	High	High	High



Fig. 3A & 3B Examples of Basmati-derived progenies from the cross of IR71730-51-2/IRBB60 used to evaluate grain quality and bacterial blight resistance genes. (B) A - IR 77542-234-1-1-1-1-3, B - IR 77542-220-2-3-2-3-3, C - Basmati 370, D - IRBB60-1

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