

Temperate rice in the tropics: upgrading MS11 disease resistance through early-generation morphological field screening and marker-assisted backcrossing

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We conducted an early-generation morphological selection in IRRI fields followed by marker-assisted backcrossing, increasing the possibility of detecting successful breeding lines with useful disease resistance (R) genes for further evaluation. Screening with isolates specific to the target R genes revealed a high correspondence between genotype and phenotype, suggesting the usefulness of molecular foreground markers in the MS11 background.

Materials and methods

Plant materials

- ◊ **Recurrent parent (RP):**
 - MS11 (a high-quality japonica, Jinmi2yeo/Cheolwon 46, 20% yield gain in the Philippines)
- ◊ **Resistance donors (DR):**
 - **Bacterial blight:** **IS5856** ($Xa4 + xa5 + Xa7 + xa13 + Xa21$) — $Xa4$ and $Xa7$ are the main priorities because of temperature stability of resistance
 - **Blast:** **ISBL2-28** (Pi9 — broad-spectrum)

Early-generation field selection

BC₁F₂ segregating generation was grown in the field without genotyping. Plants were selectively harvested with the following criteria:

- Good fertility
- MS11-like plant shape

Foreground genotyping

Bulk segregant analysis (BSA) was done for the selected lines of BC₁F₂. Panicles from plants selected from the BSA were propagated in the field.

Table 1. List of markers used for foreground genotyping.

Marker	Target R gene	Donor
PR11	PR11	IR64 (15-75-2-127)
PR2	PR2	IR64 (15-75-2-127)
Xa4	Xa4	IR64
xa5-6	xa5-resistant	Aus Basmati
xa5-9	xa5-susceptible	IR64
Xa7	Xa7	IR64
Xa13-58-547	Xa13	IR64 (Aus Basmati)
Xa21	Xa21	IR64 (Aus Basmati)

Screening using artificial inoculation

BC₁F₂ plants were grown in screenhouses and screened for isolates differential to the target R genes.

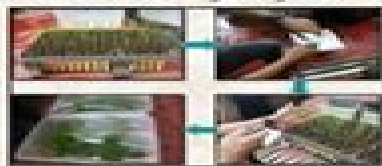


Fig. 1. Blast artificial inoculation setup. Fourteen days after sowing (DAS), seedlings were inoculated with blast fungal spore suspension differential to Pi9. Monogenic line screening with different isolates revealed that Ca43 discriminates the Pi9 gene from other blast resistance genes (data not shown).



Fig. 2. Artificial inoculation of PR11 (Xa4) and PR28 (Xa7) by clipping method 45 DAS.

Results and discussion

There was a high correspondence between marker scores and screening results.

Blast (Pi9)



Fig. 3. Ca43 isolate reactions of RP, DR, and improved backcross progenies. Protoplasts were taken 14 days post-inoculation.

Table 2. Blast scores of RP, DR, and backcross progenies together with marker scores.

Line	Ca43 score ^a	RP-1	RP-2
RP	5	absent	absent
DR	0	present	present
MSB-24	1	present	present
MSB-29	0	present	present
MSB-34	0	present	present
MSB-44	2	present	present
MSB-47	0	present	present
MSB-50	0	present	present
MSB-55	1	present	present
MSB-57	0	present	present
MSB-61	2	present	present
MSB-63	0	present	present

RP = completely resistant, 5 = completely susceptible.

Bacterial blight ($Xa4 + Xa7 +$ more R genes)

Table 3. Lesion length and marker scores of the RP, DR, and 4 lines selected for additional backcrossing.

Line	Lesion length (cm)	R genes ^a				
		Xa4	xa5	Xa7	xa13	Xa21
RP	81.32 ± 2.26	0	0	0	0	0
DR	15.58 ± 2.36	0	0	0	0	0
MSB-112	2.56 ± 0.33	0	0	0	0	0
MSB-148	2.43 ± 0.38	0	0	0	0	0
MSB-542	2.72 ± 0.40	0	0	0	0	0
MSB-548	2.78 ± 0.73	0	0	0	0	0

0, 0, and 0 are resistant, susceptible, and heterozygous, respectively.

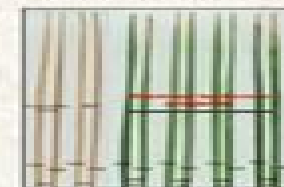


Fig. 4. Reactions of leaves inoculated with isolates differential to Xa4 and Xa7, PR11 and PR28, respectively.



Fig. 5. Segregation of backcross progenies at the R-gene loci.

Conclusions

- ◊ Disease resistance conferred by the R genes from different donors is highly expressed in the MS11 background, a temperate japonica.
- ◊ With high correspondence to the screening results, the molecular markers used in this study can be used for additional backcrossing of selected progenies.
- ◊ In the advanced backcross generation, high-throughput background genotyping will be applied together with field inoculation of bacterial blight and blast isolates.

Acknowledgments

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