

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:** **BB5555** (*Xa4* + *Xa5* + *Xa7* + *Xa13* + *Xa21*) — *Xa4* and *Xa7* are the main priorities because of temperature stability of resistance
 - **Blast:** **BB222-28** (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
BB555	<i>Pisum2</i>	BB55-15-75-2-127
BB5-2	<i>Pisum2</i>	BB55-15-75-2-127
<i>Xa4</i>	<i>Xa4</i>	BB55-5
<i>Xa5</i> -B	<i>Xa5</i> -resistant	BB55-5
<i>Xa7</i> -B	<i>Xa7</i> -resistant	BB55-5
<i>Xa13</i>	<i>Xa13</i>	BB55-5
<i>Xa21</i>	<i>Xa21</i>	BB55-5

Screening using artificial inoculation

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

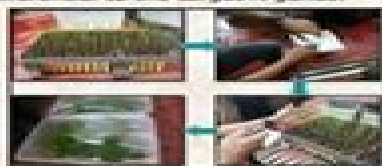


Fig. 1. Blast artificial inoculation setup. Fourteen days after sowing (DAS), seedlings were inoculated with blast fungal spore suspension differential to Pi9. Monogenic line screening with different isolates revealed that *Ca43* discriminates the Pi9 gene from other blast-resistance genes (data not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



Fig. 2. Artificial inoculation of Pi9061 (*Xa4*) and Pi9065 (*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. Probes were taken 14 days post-inoculation.

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

Line	<i>Ca43</i> score ^a	BB-1	BB-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 = complete 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				
		<i>Xa4</i>	<i>Xa5</i>	<i>Xa7</i>	<i>Xa13</i>	<i>Xa21</i>
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.

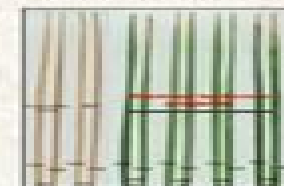


Fig. 4. Reactions of leaves inoculated with isolates differential to *Xa4* and *Xa7*. Pi9061 and Pi9065, respectively.



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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