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Note

Rice tungro spherical virus resistance into photoperiod-insensitive japonica rice by marker-assisted selection

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Rice tungro disease (RTD) is one of the destructive and prevalent diseases in the tropical region. RTD is caused by *Rice tungro spherical virus* (RTSV) and *Rice tungro bacilliform virus*. Cultivation of japonica rice (*Oryza sativa* L. ssp *japonica*) in tropical Asia has often been restricted because most japonica cultivars are sensitive to short photoperiod, which is characteristic of tropical conditions. Japonica1, a rice variety bred for tropical conditions, is photoperiod-insensitive, has a high yield potential, but is susceptible to RTD and has poor grain quality. To transfer RTD resistance into Japonica1, we made two backcrosses (BC) and 8 three-way crosses (3-WC) among Japonica1 and RTSV-resistant cultivars. Among 8,876 BC_1F_2 and 3-WCF_2 plants, 342 were selected for photoperiod-insensitivity and good grain quality. Photoperiod-insensitive progenies were evaluated for RTSV resistance by a bioassay and marker-assisted selection (MAS), and 22 BC_1F_7 and 3-WCF_7 lines were selected based on the results of an observational yield trial. The results demonstrated that conventional selection for photoperiod-insensitivity and MAS for RTSV resistance can greatly facilitate the development of japonica rice that is suitable for cultivation in tropical Asia.

Key Words: *Rice tungro spherical virus*, rice tungro disease, marker-assisted selection, photoperiod-insensitivity, japonica rice (*Oryza sativa* L. ssp *japonica*).

Introduction

Rice tungro disease (RTD) is one of the most destructive diseases of rice in tropical Asia (Hibino *et al.* 1991). Rice (*Oryza sativa* L.) plants affected by RTD show stunted growth, yellow to orange leaf discoloration, and few reproductive tillers (Thomas *et al.* 1980). RTD is caused by *Rice tungro bacilliform virus* (RTBV) and *Rice tungro spherical virus* (RTSV). Both RTSV and RTBV are transmitted by green leafhoppers (GLH) in a semi-persistent manner (Hibino *et al.* 1991). RTSV is independently transmitted by GLH, whereas RTBV can be transmitted by GLH only in the presence of RTSV (Hibino *et al.* 1990).

An evaluation of more than 40,000 rice germplasm accessions for RTSV and RTBV showed that dozens of traditional cultivars are resistant to RTSV, whereas only two cultivars are resistant to RTBV (Hibino *et al.* 1990, Shahjahan *et al.* 1990, Zenna *et al.* 2006). A genetic analysis of RTD-resistant rice cultivar Utri Merah showed that RTSV and RTBV resistance are independently inherited, and the interaction between both resistance traits is necessary to suppress RTD effectively (Encabo *et al.* 2009). RTSV resistance is a recessive trait controlled by the translation initiation factor 4 gamma (eIF4G) gene located between 22.05 and 22.25 Mb in chromosome 7 (Lee *et al.* 2010). The molecular marker, RM336 was successfully used for mapping the RTSV resistance gene (Lee *et al.* 2010). Sequence analysis of the eIF4G gene in cultivars resistant to RTSV identified single nucleotide polymorphisms (SNPs) in five combinatorial patterns that are associated with RTSV resistance (Lee *et al.* 2010).

The need for japonica rice (*O. sativa* L. ssp *japonica*) in Asia is increasing due to the increasing japonica rice consumers and trading (Magno and Yanagida 2000). Typical temperate japonica rice cultivars require a long-day photoperiod and are not adaptable to the short-day length conditions of the tropical regions. Photoperiod-sensitive japonica rice cultivars usually yield less than 1.2 ton/ha whereas photoperiod-insensitive japonica cultivars can yield up to 5.5 ton/ha in tropical regions (Philippine Rice Research

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Table 1. Selection for photoperiod-insensitivity and good grain quality at BC_1F_2 and 3-WCF_2

Cross combinations	No. of plants examined	No. of selected photoperiod-insensitive plants	No. of selected plants with good grain quality	Selection intensity (%)
IR97705 (Hwaseong/MS11)/Japonica1	225	17	9	4.0
IR97707 (Hwaseong/Japonica1)/MS11	101	12	7	6.9
IR97708 (Hwaseong/Japonica1)/Japonica1	1,025	124	64	6.2
IR97709 (Hwaseong/Japonica1)/Jinmi	1,000	119	71	7.1
IR97711 (Dongjin/MS11)/Japonica1	750	37	25	3.3
IR97715 (Dongjin/Japonica1)/Jinmi	1,775	97	53	3.0
IR97717 (Sangju/MS11)/Japonica1	1,000	19	18	1.8
IR97719 (Sangju/Japonica1)/MS11	1,300	56	26	2.0
IR97720 (Sangju/Japonica1)/Japonica1	700	21	14	2.0
IR97721 (Sangju/Japonica1)/Jinmi	1,000	91	55	5.5
Total	8,876	593	342	3.9

Institute 2014). The rice variety Japonica1, which has been bred for tropical regions, has a high yield but is highly susceptible to RTD and has poor grain quality. Breeding of rice varieties for resistance to tungro viruses had relied exclusively on phenotypic selection. Here we report the application of marker-assisted selection (MAS) to transfer RTSV-resistance into photoperiod-insensitive japonica rice breeding lines to assure their stable yield in tropical environment. Numerous rice molecular markers linked to specific traits have been developed (Jena and Mackill 2008), but only a limited number of these molecular markers are actually being used for conventional rice breeding. This is the first case of molecular MAS for tungro virus disease resistance in the course of developing a japonica variety that is adaptable to tropical conditions.

Materials and Methods

Plant materials

Photoperiod-insensitive japonica varieties (Jinmi, Maligaya Special 11 (MS11) and Japonica1), photoperiod-sensitive varieties (Dongjin and Hwaseong) and an intermediately photoperiod-sensitive japonica variety (Sangju) were used in 10 cross combinations of backcrosses and three-way crosses (3-WC) (**Table 1**). Japonica1 has a high yield but has a poor grain quality and is susceptible to RTSV. Dongjin (Lee *et al.* 2010), Hwaseong, Sangju, Jinmi, and MS11 are resistant to RTSV. MS11 and Jinmi were included in the 3-WC as a source of good grain quality and photoperiod insensitivity. Jinmi, Dongjin, Hwaseong, Sangju, MS11, and Japonica1 have short and bold grains with an average of 1.8 length to width ratio and an average of 17–19% amylose content. Grains of these 6 varieties are clear and translucent with no significant chalkiness.

MAS for RTSV resistance

MAS for RTSV resistance was carried out for F_1, F_3, F_4, and F_5 generations (**Fig. 1**). The F_2 generations were excluded from MAS for RTSV resistance because of the selection of the F_2 generations for photoperiod insensitivity. Genomic DNA samples were prepared from the young leaves of plants using the modified TPS method (Miura *et al.* 2009). The tips of rice leaves (5 cm) were excised and ground in TPS buffer (100 mM Tris-HCl [pH 8.0], 1 M KCl, 10 mM EDTA) using a GenoGrinder (OPS Diagnostics). After centrifugation, the supernatant was recovered and an equal volume of isopropyl alcohol was added. The isopropyl alcohol-insoluble material was recovered by centrifugation, and the pellet was rinsed with 75% ethanol. The pellet was then dried and dissolved in TE (10 mM Tris-HCl [pH 8.0], 1 mM EDTA, RNase A [10 mg/ml]). The DNA samples were then used for genotyping using the simple sequence repeat marker, rice microsatellite 336 (RM336, McCouch *et al.* 2002, forward primer: CTTACAGAGAA ACGGCATCG, reverse: GCTGGTTTGTTTCAGGTTCG, 21.87 Mb of chromosome 7, according to IRGSP 1.0 of the rice annotation project database at http://rapdb.dna.affrc.go.jp/) that is tightly linked to the RTSV resistance gene

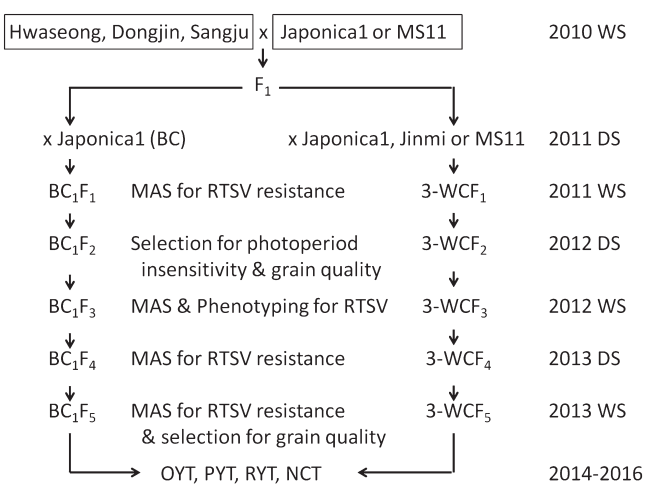

Fig. 1. Breeding scheme for the development of *Rice tungro spherical virus* (RTSV)-resistant photoperiod-insensitive rice via selection for RTSV resistance and grain quality. RTSV-resistant varieties, Dongjin, Hwaseong, and Sangju were crossed with Japonica1 to produce F_1. MS11 and Jinmi were used as the donor for photoperiod insensitivity. DS) dry season, WS) wet season, OYT) observatory yield trial, PYT) preliminary yield trial, RYT) replication yield trial.

(Lee *et al.* 2010). The PCR profile was as follows: predenaturation for 5 min at 95°C, 35 cycles of denaturation for 1 min at 95°C—annealing for 30 sec at 55°C—extension for 30 sec at 72°C, and final extension for 7 min at 72°C. The PCR products were resolved on TAE (Tris-Acetate-EDTA) agarose gel (3%) for 1 h at 250 volts.

Background selection for photoperiod insensitivity and grain quality

The photoperiod-sensitive japonica rice varieties typically exhibit early flowering and poor vegetative growth when grown under tropical conditions (12–14 hour day length; 25–33°C average day temperature). In the tropical condition of Philippines, the average height of highly photoperiod-sensitive japonica varieties is about 55 cm; they flower earlier than 45 days after seeding (**Fig. 2**A); and their panicles are shorter than 15 cm (**Fig. 2**B). Stricter criteria were applied for background selection of the BC_1F_2 and 3-WCF_2 generations for photoperiod insensitivity. Plants that were taller than 75 cm; had panicles longer than 22 cm; and that flowered at or after 60 days after seeding were considered photoperiod-insensitive. The selection criteria for good grain quality include grain chalkiness, opacity, color, boldness and appearance (Webb *et al.* 1985). The grains were dehulled and examined by visual test. Plants with grains that are not chalky, clear translucent, bold (length/width ratio less than 1.9), and short (shorter than 5.5 mm in length) were selected (**Fig. 2**C).

Evaluation for reaction to RTSV

RTSV strain A (Cabauatan *et al.* 1995) was used as the source of inoculum. The GLH-mediated inoculation of plants with RTSV was carried out using the modified water tray method as described by Azzam *et al.* (1999). BC_1F_2 and 3-WCF_2 plants from the respective cross combinations that had been selected for good grain quality and photoperiod insensitivity were advanced to BC_1F_3 and 3-WCF_3. Twenty BC_1F_3 and 3-WCF_3 plants per line were grown in seed boxes. At 10 days after germination, the seedlings were placed inside a water tray and covered with a screen cage. RTSV-viruliferous GLH that had been allowed to feed on RTSV-infected plants for 4 days were released into the cage at an average of seven GLH per seedling for 3 hours to effect RTSV transmission. After inoculation, the trays were filled with water until the test seedlings were submerged to remove the GLH. One month after inoculation, leaves were collected from each plant and RTSV infection in the seedlings was examined by a double-antibody sandwich-enzyme-linked immunosorbent assay (Bajet *et al.* 1985). The presence of RTSV in the leaf extracts was determined by measuring the absorbance of the leaf extracts at 405 nm (Cabunagan *et al.* 1993). Plants whose 10-fold-diluted leaf extracts exhibited an absorbance value greater than 0.1 were considered to be infected with RTSV. F_3 lines with an infection rate of <20% were classified as resistant, those with 21 to 79% infection as segregating, and those with >80% infection as susceptible (Lee *et al.* 2010).

Results

Hybridization between photoperiod-insensitive and RTSV-resistant varieties

F_1 plants were obtained by crossing the photoperiod-insensitive varieties Japonica1 or MS11 with three RTSV-resistant varieties Hwaseong, Sangju, or Dongjin (**Fig. 1**). The F_1 plants from two crosses (Hwaseong/Japonica1 and Sangju/Japonica1) were backcrossed to Japonica1, and the F_1 plants from the other six crosses (Hwaseong/Japonica1, Hwaseong/MS11, Dongjin/Japonica1, Dongjin/MS11, Sangju/Japonica1, and Sangju/MS11) were used for 3-WC with Japonica1, Jinmi, or MS11 to produce 10 different cross combinations (**Fig. 1**, **Table 1**). Among the 236 BC_1F_1 and 3-WCF_1 plants, 110 were identified to have a homozygous RTSV resistance allele, 22 have a homozygous susceptible

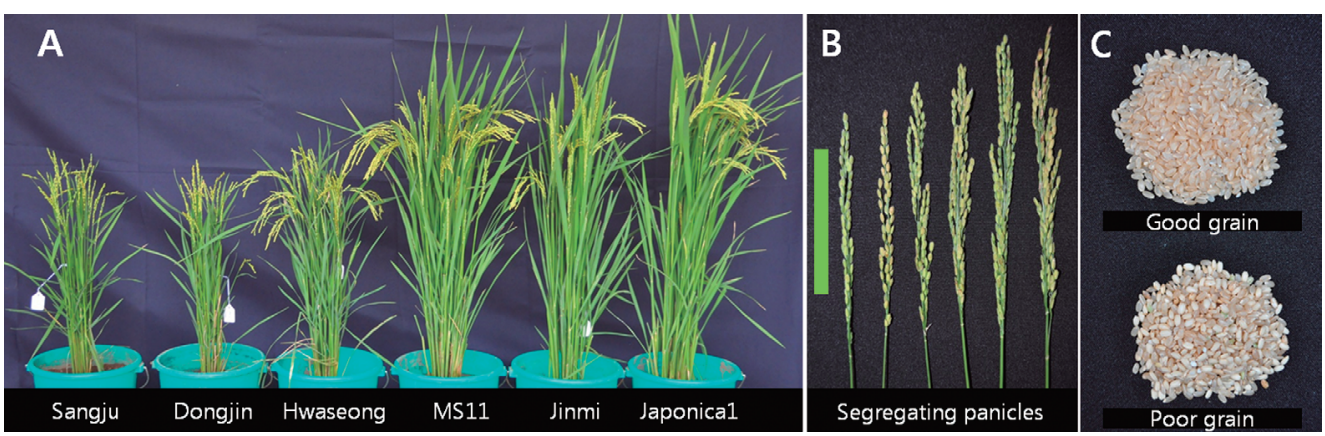

Fig. 2. Phenotypic selection criteria for photoperiod insensitivity and grain quality. (a) Difference in height among photoperiod-sensitive varieties (Sangju, Dongjin and Hwaseong) and photoperiod-insensitive varieties (MS11, Jinmi and Japonica1). (b) Typical segregation of panicle length and grain number in BC_1F_2 plants. Panicles from BC_1F_2 plants of IR97705. Scale bar equals 10 cm. (c) Typical segregation in grain chalkiness and opacity in BC_1F_2 plants. Grains from BC_1F_2 plants of IR97705.

Table 2. Phenotypic selection for RTSV resistance at BC_1F_3 and 3-WCF_3

Cross combinations	No. of lines examined	No. of resistant lines	No. of segregating lines	No. of susceptible lines	No. of lines selected[a]
IR97705 (Hwaseong/MS11)/Japonica1	9	2	5	2	5
IR97707 (Hwaseong/Japonica1)/MS11	7	3	2	2	5
IR97708 Hwaseong/Japonica1)/Japonica1	64	22	23	19	39
IR97709 (Hwaseong/Japonica1)/Jinmi	71	71	0	0	27
IR97711 (Dongjin/MS11)/Japonica1	25	16	9	0	13
IR97715 (Dongjin/Japonica1)/Jinmi	53	33	1	19	26
IR97717 (Sangju/MS11)/Japonica1[b]	18	–	–	–	7
IR97719 (Sangju/Japonica1)/MS11	26	7	2	17	9
IR97720 (Sangju/Japonica1)/Japonica1	14	5	0	9	5
IR97721 (Sangju/Japonica1)/Jinmi	55	50	2	3	18
Total	342	209	44	71	154

[a] Three panicles were harvested from each line selected.
[b] Penotyping for RTSV infection was not conducted for IR97717, and the selection was made on the basis of field performance.

allele, and 104 have heterozygous RTSV resistance/susceptible alleles by MAS using RM336. All the 110 BC_1F_1 and 3-WCF_1 plants with homozygous resistance alleles were advanced to the next generation. Also, 25 of the 104 heterozygous BC_1F_1 and 3-WCF_1 plants that are over 70 cm tall, with panicles that are 20 cm long and that had wide, deep green and erect leaves were advanced to the next generation.

Selection of BC_1F_2 and 3-WCF_2 for photoperiod insensitivity and grain quality

The F_2 progenies derived from the 10 cross combinations segregated for plant height, panicle length, vegetative growth period, and flowering date. Among the 8,876 BC_1F_2 and 3-WCF_2 plants generated from the 10 cross combinations, 593 plants were selected as photoperiod-insensitive (**Table 1**). F_2 plants that were shorter than 75 cm in height were considered as photoperiod-sensitive and were discarded (**Fig. 2**A). The average panicle length of plants selected as photoperiod-insensitive was 22 cm. F_2 plant panicles that were shorter than 22 cm, and flowered earlier than 60 days after seeding were considered photoperiod-sensitive and were discarded in the field (**Fig. 2**B). A total of 593 photoperiod-insensitive F_2 plants were harvested and dehulled for grain quality test. Grains that were chalky, opaque, or irregularly-shaped were discarded (**Fig. 2**C). A total of 342 BC_1F_2 and 3-WCF_2 plants were selected for photoperiod insensitivity and good grain quality and were advanced to BC_1F_3 and 3-WCF_3 (**Table 1**). Only 3.9% of the F_2 plants were selected and advanced to the next generation indicating that photoperiod sensitivity resulting in short plant height, early flowering and short panicle length, as well as poor grain quality, is highly heritable in tropical regions.

Evaluation of BC_1F_3 and 3-WCF_3 by RTSV bioassay

Among the 342 BC_1F_3 and 3-WCF_3 lines, 324 were examined for their reaction to RTSV (18 lines of IR97717 were excluded from phenotyping for RTSV infection). Among the 324 lines examined, 209 were classified as resistant to RTSV, 44 as segregating for RTSV resistance, and 71 as susceptible to RTSV. From the 209 resistant lines and 44 segregating lines, a total of 154 plants were selected. Three panicles were harvested from each of the 154 plants, and a total of 462 plants were advanced to the next generations (**Table 2**).

MAS of BC_1F_4 and 3-WCF_4 for RTSV resistance

A total of 462 BC_1F_4 and 3-WCF_4 lines were planted in the field. Among the 462 lines, 78 lines were selected for MAS based on field performance. Three plants from each of the 78 lines were subjected to genotyping for RTSV resistance using RM336 (**Fig. 3**, **Table 3**). Among the 78 lines, all plants of 50 lines were found to have homozygous RTSV resistance alleles, whereas 11 lines segregated into resistant,

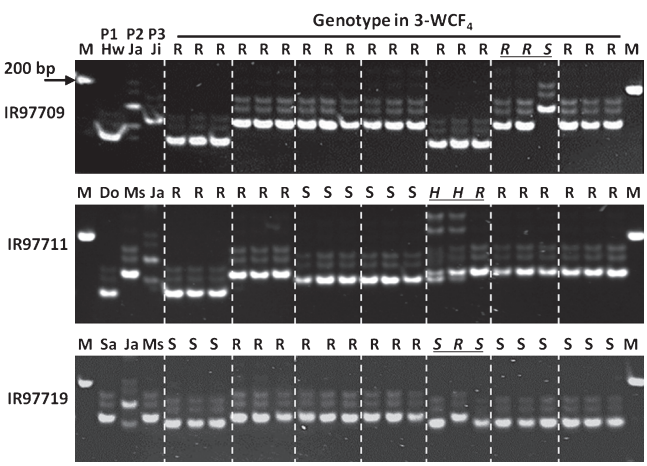

Fig. 3. Representative genotypes of 3-WCF_4 plants using the SSR marker RM336 which is tightly linked to RTSV resistance. Japonica1 is susceptible whereas Hwaseong, Dongjin, Sangju, MS11, and Jinmi are resistant to RTSV. Three plants per line were examined for genotypes with RM336. Genotypes from different lines were separated by dashed lines. Underlined italic genotypes indicate segregation of genotypes in a line. M) marker, Hw) Hwaseong, Ja) Japonica1, Ji) Jinmi, Do) Dongjin, Ms) MS11, Sa) Sangju, R) resistant, S) susceptible, H) heterozygous.

Table 3. Marker-assisted selection for RTSV resistance at BC_1F_4 and 3-WCF_4 using RM336

Cross combination	No. of lines planted[a]	No. of lines selected for genotyping	Genotypes			No. of lines selected
			Resistant	Heterozygous	Susceptible	
IR97705 (Hwaseong/MS11)/Japonica 1	15	2	2	0	0	2
IR97707 (Hwaseong/Japonica1)/MS11	15	5	3	0	2	3
IR97708 (Hwaseong/Japonica1)/Japonica1	117	17	4	4	9	8
IR97709 (Hwaseong/Japonica1)/Jinmi	81	14	13	1	0	14
IR97711 (Dongjin/MS11)/Japonica1	39	7	4	1	2	5
IR97715 (Dongjin/Japonica1)/Jinmi	78	6	5	1	0	6
IR97717 (Sangju/MS11)/Japonica1	21	3	2	1	0	3
IR97719 (Sangju/Japonica1)/MS11	27	7	3	1	3	3
IR97720 (Sangju/Japonica1)/Japonica1	15	5	4	0	1	4
IR97721 (Sangju/Japonica1)/Jinmi	54	12	10	2	0	12
Total	462	78	50	11	17	60

[a] Three panicles were harvested from each of the previous 154 BC_1F_3 and 3-WCF_3 lines; therefore 462 BC_1F_4 and 3-WCF_4 lines were planted. Of the 462 lines, 78 were selected for MAS for RTSV.

Table 4. Marker-assisted selection for RTSV resistance at BC_1F_5 and 3-WCF_5 using RM336

Cross combination	No. of lines planted[a]	Genotypes			No. of lines selected
		Resistant	Heterozygous	Susceptible	
IR97705 (Hwaseong/MS11)/Japonica1	6	6	0	0	3
IR97707 (Hwaseong/Japonica1)/MS11	9	9	0	0	6
IR97708 (Hwaseong/Japonica1)/Japonica1	24	3	0	21	1
IR97709 (Hwaseong/Japonica1)/Jinmi	42	42	0	0	11
IR97711 (Dongjin/MS11)/Japonica1	15	12	2	1	10
IR97715 (Dongjin/Japonica1)/Jinmi	18	16	2	0	7
IR97717 (Sangju/MS11)/Japonica1	9	9	0	0	0[b]
IR97719 (Sangju/Japonica1)/MS11	9	9	0	0	2
IR97720 (Sangju/Japonica1)/Japonica1	12	12	0	0	7
IR97721 (Sangju/Japonica1)/Jinmi	36	28	3	5	15
Total	180	146	7	27	42

[a] Three panicles were harvested from each of the previous 60 BC_1F_4 and 3-WCF_4 lines; therefore 180 BC_1F_5 and 3-WCF_5 lines were planted.
[b] No IR97717 lines were selected due to bacterial blight infection.

susceptible, or heterozygous genotypes (underlined italic genotypes in **Fig. 3**, **Table 3**). It appeared that the locus linked to RM336 is heterozygous in Japonica1, and that only either of the two alleles in Japnoica1 was passed on to some progenies (genotypes indicated as 'S' in **Fig. 3**). In case of IR97709, no segregating or susceptible lines were found among the previous 71 lines of 3-WCF_3 examined for phenotypes for RTSV infection (**Table 2**); however, the genotype data showed segregation in the 3-WCF_4 generation of IR97709 (underlined italic genotypes in **Fig. 3**), suggesting that the contradictory results may be due to missed inoculation of RTSV via GLH on some 3-WCF_3 plants of IR97709 that might have occurred during the phenotyping of the 3-WCF_3 lines. Three panicles were harvested from each of the 60 BC_1F_4 and 3-WCF_4 lines that have homozygous or heterozygous RTSV resistance alleles (**Table 3**), and a total of 180 lines were advanced to the next generation (**Table 4**). Another MAS for RTSV resistance for 180 BC_1F_5 and 3-WCF_5 lines identified 62 lines to be homozygous for RTSV resistance alleles. The grains of 62 lines were dehulled and evaluated by visual examination. Forty-two lines were selected for good grain quality (**Table 4**). Three panicles were taken from each of the 42 lines and consequently a total of 126 lines were advanced to the next generation for observatory yield trial (OYT). Based on yield performance and agronomic traits in the OYT, we finally selected 22 lines (**Table 5**). The 22 lines selected showed a yield higher compared to MS11 and Japonica1 (**Table 5**).

Discussion

RTSV and RTBV resistance traits in rice are independently inherited (Encabo *et al.* 2009). Both RTBV resistance and RTSV resistance may be necessary for the effective management of RTD in fields. However, MAS for RTSV resistance alone might have a significant impact on the management of RTD because RTBV cannot be transmitted by GLH without the helper virus RTSV. Moreover, RTSV enhances the damages caused by RTBV (Hibino *et al.* 1990). The DNA marker RM336 is tightly linked to the RTSV resistance gene (Lee *et al.* 2010). Therefore, transfer of RTSV resistance by MAS into varieties to be cultivated in RTD-prone areas is a practical approach toward RTD resistance

Table 5. Yield and agronomic characteristics of the 22 advanced breeding lines selected by observatory yield trial

Designation	Heading date (Days after seeding)[a]	Culm length (cm)[b]	Panicle length (cm)[b]	Tiller number[b]	Reproductive panicle number[b]	Total plant mass (kg)[a]	Total grain weight (kg)[a]	Yield (kg)[a]	Yield (ton/ha)
IR 97705-8-1-1-1-2	83	75	19	12	10	3.61	0.93	0.77	4.73
IR 97705-8-1-2-2-1	75	77	20	15	13	3.84	0.93	0.75	4.55
IR 97708-15-1-3-3-1	77	71	18	12	11	3.53	1.01	0.79	4.89
IR 97708-15-1-3-3-2	80	72	24	13	12	3.40	0.97	0.79	4.80
IR 97708-23-1-1-1-3	75	64	20	11	10	3.53	0.96	0.78	4.79
IR 97708-23-1-1-2-2	74	66	23	15	14	3.55	0.98	0.75	4.66
IR 97709-30-1-3-3-1	77	69	19	16	16	4.11	0.77	0.83	5.07
IR 97709-30-1-3-3-2	75	74	24	13	13	3.56	1.10	0.83	5.25
IR 97709-30-1-3-3-3	77	71	22	11	11	3.26	0.95	0.76	4.73
IR 97711-25-1-1-3-3	75	62	19	12	11	3.26	0.96	0.73	4.52
IR 97711-25-1-3-1-2	70	60	21	11	9	3.25	0.94	0.73	4.50
IR 97719-22-1-3-3-3	74	77	24	16	16	3.62	0.94	0.73	4.51
IR 97721-29-1-3-3-2	72	72	21	16	16	4.19	1.19	0.74	4.59
IR 97721-29-1-3-3-3	75	76	22	15	14	4.36	1.01	0.77	4.77
IR 97721-38-3-2-1-1	74	77	24	17	17	4.40	1.01	0.75	4.63
IR 97721-38-3-2-1-2	74	72	18	16	14	3.98	1.04	0.76	4.73
IR 97721-38-3-2-1-3	65	66	17	16	15	3.98	1.01	0.75	4.67
IR 97721-43-2-1-3-1	70	67	15	14	15	4.03	1.27	0.78	4.82
IR 97721-43-2-1-3-2	70	65	20	16	14	4.16	0.99	0.77	4.74
IR 97721-43-2-2-3-1	72	61	14	16	15	4.29	1.13	0.75	4.67
IR 97721-43-2-2-3-3	77	70	21	22	21	4.73	1.36	0.93	5.78
IR 97721-45-2-1-3-3	83	73	21	19	18	4.07	1.29	0.84	5.17
Japonica1	79	71	20	12	11	3.28	0.77	0.57	3.50
MS11	76	68	19	15	14	3.18	0.83	0.58	3.55

[a] Average value among 30 plants.
[b] Average value among 3 plants.

breeding. Several virus species have been recognized to cause serious damages to rice production (Hibino 1996). Locations of resistance genes for rice viruses such as RTSV (Lee *et al.* 2010), *Rice yellow mottle virus* (Albar *et al.* 2003, Thiémélé *et al.* 2010), and *Rice strip virus* (RSV) (Hayano-Saito *et al.* 2000) have already been determined. MAS for resistance to RSV has been successfully implemented to breed RSV-resistant rice varieties (Chen *et al.* 2010).

Photoperiod sensitivity is a trait closely associated with flowering time. Rice is a facultative, short-day plant that requires certain periods of dark to flower (Ichitani *et al.* 1998, Yano *et al.* 2000). Photoperiod-sensitive japonica rice varieties in temperate regions are usually planted during periods of long day-length to ensure that plants achieve their full vegetative growth. Once subjected to short day-length (more than 14 hours of darkness), the plants start flowering (Vergara and Chang 1985). Cultivation of photoperiod-sensitive japonica rice varieties under the consistently short-day condition in tropical regions usually results in short vegetative growth, early flowering, short plant height, and short panicles that eventually lead to very low yield. Japonica rice cultivars such as Dongjin, Sangju and Hwaseong are photoperiod-sensitive and yield an average of 1.2 ton/ha at 40% grain filling ratio in tropical regions. The average number of filled grains/reproductive tiller of these varieties is about 25 grains (data not shown). On the other hand, photoperiod-insensitive cultivar Japonica1 yields an average of 5.5 ton/ha at 80% grain filling ratio, and at 150 grains/tiller. Therefore, selection of japonica cultivars for photoperiod insensitivity is important to improve the vegetative growth of the plants and to increase crop yield.

Photoperiod insensitivity is a complex and quantitative trait (Yano *et al.* 2000), thus multiple molecular markers might be required for MAS for the trait. *Heading date 1* (*Hd1*) confers long vegetative growth (Yano *et al.* 2000) whereas *Hd3a* is closely associated with photoperiodic flowering time in rice (Zhang *et al.* 2012). The expression of *Hd3a* promotes flowering under short-day length conditions, and suppresses it under long-day length conditions (Tamaki *et al.* 2007). At least seven other genes (*Hd1*, *Hd2*, *Hd4*, *Hd5*, *Hd6*, *Hd7*, and *Hd9*) are also reported to be associated with vegetative growth and flowering time (Lin *et al.* 1998, 2002, Yamamoto *et al.* 1998, 2000, Yano and Sasaki 1997), suggesting that photoperiod sensitivity is a trait too complicated for MAS application. Despite of the genetic complexity associated with photoperiod insensitivity, the phenotypes resulting from photoperiod insensitivity distinctively segregated among the japonica rice populations examined in this study (**Fig. 2**A, **2**B). Therefore, the evaluation for measurable phenotypes such as flowering date, plant height, and panicle length appears to be a more practical option than the use of genetic markers for selection for photoperiod insensitivity. The results of this study demonstrated that MAS for RTSV resistance can be adopted to facilitate breeding of RTSV-resistant, photoperiod-insensitive rice

varieties for the stable production of rice in RTD-prone areas.

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