Accelerated molecular breeding of novel cytoplasmic male sterility lines in rice using *orfH79* or *orf290* haplotypes

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Abstract

The identification and development of new cytoplasmic male sterility (CMS) lines in higher plants is important for the preservation of grain security and the prevention of homogenization of hybrid rice. Molecular markers assisted selection (MAS) based on CMS-associated genes or mitochondrial-specific chimeric sequences are important for rapid and effective breeding of new CMS lines and hybrids. In our study, the distribution and allele variation of *orfH79* and *orf290* genes were characterized from 273 wild and cultivated rice in the AA genome species. Based on the alignment of nucleotide and amino acid sequences, four accessions with *orfH79* and three accessions with *orf290* were screened. Four novel CMS lines carrying *orfH79* haplotypes and three novel CMS lines carrying *orf290* haplotypes were then developed using multiple backcross generations with a maintainer line under MAS. The breeding process used in our study provides an efficient and feasible approach for selecting new CMS lines. CMS lines selected in our study are important for enriching rice germplasm resources and guaranteeing rice breeding programs.

Background

Chimeric genes encoded by the mitochondrial genome in androgynous angiosperms results in cytoplasmic male sterility (CMS), a male reproductive defect; CMS is inherited as maternal inheritance which does not follow Mendelian inheritance. To date, CMS has been identified in more than 150 plants, with 28 CMS types associated to 13 crop species (Chen and Liu, 2014). More than 50 mitochondrial genes associated with CMS have been identified in plants, and the majority of plant CMS-related genes are related to the rearrangement of mitochondrial functional genes or chimeric genes of unknown mitochondrial DNA sequences. At least 10 mitochondrial functional genes have been found to be involved in the formation of CMS genes (Chen and Liu, 2014).

According to cytological and genetic characteristics, CMS types in rice include WA-CMS (wild abortion), BT-CMS (Boro-II), HL-CMS (Honglian), LD-CMS (Lead rice), CW-CMS (Chinese wild rice), RT102-CMS (RT102C) and RT98-CMS (RT98C) (Kim and Liu, 2018). To date, analysis of CMS and the mechanism of fertility restoration for WA-CMS, BT-CMS and HL-CMS types has successfully advanced. At the molecular level, WA-CMS is associated with the mitochondrial gene WA352. Pollen associated to this gene is abortive in the mononuclear stage and fertility was restored using Rf3 and Rf4 restorer genes (Luo et al., 2013). BT-CMS is related to the mitochondrial chimeric atp6-orf79 gene. The ORF79 protein has cytotoxicity and accumulates in microspores, inducing the gametophyte abortion of BT-CMS rice. Rf1A and Rf1B related to BT-CMS can inhibit pollen abortion at the tricellular stage (Wang et al., 2006). HL-CMS, similar to BT-CMS, belongs to a gametophytic CMS that exhibits pollen abortion at the bicellular stage. In HL-CMS, atp6-orfH79 is the CMS-associated transcript. Although identity between orfH79 and orf79 sequences (from HL-CMS and BT-CMS, respectively) has a commonality of 98%, they show significant divergence in the intergenic region (Yi et al., 2002). Fertility in HL-CMS can be independently restored using Rf5 or Rf6 restorer genes (Hu et al., 2012; Huang et al., 2012), and a novel CMS-related

chimeric DNA fragment L-sp1 was recently identified in the HL-CMS line, Yuetai A (YTA) and other wild rice strains (Tan et al., 2015).

Furthermore, four alloplasmic CMS lines carrying different orf(H)79 haplotypes were developed by crossing with the Honglian maintainer line Yuetai B (YTB); test-crossing analysis indicated these to display various fertility-restoring models (Li et al., 2008). This finding suggests that, although the CMS lines all contain orf(H)79, the gametophytic CMS lines have different CMS-related genes. A new CMS-related gene orf290 (GenBank No. MF669484) was cloned and confirmed to induce CMS in the binucleate pollen stage of rice (Yang et al., 2018). In our previous study (Tan et al., 2015; Yang et al., 2018), it was found that orfH79 and orf290 predominantly existed in the same cytoplasm, with a few independent genes in different cytoplasms. In this study, therefore, we screened several CMS-associated cytoplasms with orfH79 and orf290 haplotypes from 273 wild and cultivated rice accessions in AA genome species using the molecular markers of these two CMS-associated genes. A series of new haplotype CMS lines with either orfH79 or orf290, having shared similar maintainer lines, were then bred.

Materials And Methods

Plant materials

A total of 273 rice accessions were used in this study, including 125 accessions of AA-genome wild rice (Table S1) from Wuhan university (Wuhan, China), 143 accessions of cultivated rice (Table S2) from Hubei Academy of Agricultural Sciences (Wuhan, China) and 5 CMS lines or maintainer lines related to HL-CMS. All plant materials were planted in the experimental field of the South-Central University for Nationalities in Wuhan, China, from 2015 to 2017.

Isolation of nuclear and mitochondrial DNA

Total nuclear genomic DNA was isolated from green leaves using the modified CTAB method (Zhang et al., 1992). DNA quality and quantity were estimated spectrophotometrically by visualizing under ultraviolet light, using a specific amount of lambda DNA (MBI, USA) on an agarose gel.

Mitochondrial DNA (mtDNA) was isolated by initially harvesting pure mitochondria using etiolated seedlings by differential centrifugation and DNase I processing (Promega, USA), following the modified method of Yi et al. (2002). Mitochondria was then resuspended using lysis buffer, following phenol-chloroform extraction, and mtDNA was isolated using precipitated ethanol.

Polymerase chain reaction amplification

DNA amplification was undertaken using a programmable thermal controller (PTC-100, MJ Research, USA) with the following process: 4 min at 94°C denaturation; 35 cycles of 30 sec at 94°C denaturation, 30 sec(*orfH79*) or 60sec (*orf290*) at 58°C annealing, 1 min at 72°C; 8-min final extension at 72°C. Polymerase chain reactions (PCRs) were performed using a 20-μL reaction volume (10 mM Tris-HCl (pH 8.8), 25 mM KCl, 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.2 mM primers, 100 ng genomic DNA, and 1 unit of Taq polymerase (Takara, Japan)). The primer sequences were: *orfH79* primers (H1: 5'-ATGACAAATCTGCTCCGAT-3'; H2: 5'-TTACTTAGGAAAGACTACAC-3') and *orf290* primers (O1: 5'-ATGCTGCGCTTCGAACGTATC-3'; O2: 5'-CTAGGAGGCTGAGTTTTGTCC-3'). PCR products were electrophoretically separated using 1.0% agarose gels, and they were stained using ethidium bromide. A Gel Doc2000 (Bio-Rad, USA) was used to photograph samples under ultraviolet light.

DNA sequencing and sequence alignment

DNA polymorphism bands were collected from agarose gels using a AxyPrepTM DNA Gel Extraction Kit 50-prep according to the manufacturer's specifications (Axygen, USA). Purified PCR products were cloned using a TA-cloning® kit pCR®2.1 (TakaRa, Japan) and sequenced by Qingke Corporation (Wuhan, China). DNA and amino acid sequences of ORFH79 and ORF290 from the different candidate accessions were aligned using the ClustalX program.

Southern hybridization

orfH79 and *orf290* probes were amplified using *orfH79* sequence-specific primers (H1 and H2) and *orf290* sequence-specific primers (O3: 5'-ATCCAAACGGAGTGAGTGGTTC-3'; O4: 5'-ACCCTTCTCTCAGCCATGTCTAG-3'), respectively. MtDNA (20 μg) was separated on 0.8% agarose gels following digestion with either EcoRV or XhoI (New England Biolabs, USA) before being transferred to Hybond N^+-nylon membranes. Probes were radioactively labeled using the North2South® Biotin Random Prime Labeling Kit (Thermo scientific, USA). Southern hybridization was performed using North2South® Chemiluminescent Hybridization (Thermo scientific, USA) at 55°C for 16 h. The membrane was washed twice at room temperature using a Detection Kit (Thermo scientific, USA) before being exposed using a gel imaging system.

Fertility scoring of hybrid plants

Hybrid plants were obtained by crossing wild or cultivated rice with the Honglian maintainer YTB as the male or female parent. Fertility evaluation was performed according to pollen stainability in a 1% I_2–KI solution and the seed-setting rate of spikelets. All fertility results were recorded as mean ± SD.

Results

Distribution of *orfH79* and *orf290* in AA genome rice

orfH79 and *orf290* distribution in the AA genome of wild and cultivate rice accessions was determined using PCR amplification with *orfH79* sequence-specific primers H1 and H2 and *orf290* sequence-specific primers O1 and O2, respectively. Specific bands of both *orfH79* and *orf290* were recorded in 11 rice accessions, similar to those in YTA and CGA belonging to Honglian CMS lines; one accession IRRI81 was from cultivated rice and 10 accessions were from wild rice. The 10 wild rice accessions belonged to three species: two were from *Oryza glumaepatula* (W1 and W2), five were from *Oryza nivara* (W3, W4, W7, W9 and W11), and three were from *Oryza rufipogon* (W14, W15 and W18). The 11 rice accessions came from Cambodia, China, India, Sri Lanka, Suriname, Brazil, Bangladesh, the Philippines and Laos. Eight accessions deriving from *Oryza nivara* and *Oryza rufipogon* (cultivated rice IRRI81 and wild rice W5, W6, W8, W10, W12, W16 and W17) contained only specific bands of *orfH79* (or *orf79*). Only one accession in wild rice (W18) only contained a specific band of *orf290* (Fig. 1). 13 accessions of cultivated rice (Z593, Z595, Z597, Z604, Z612, Z613, Z615, Z616, Z620, Z624, Z740, Z743 and Z747) contained a specific band of *orf290* without a specific band of *orfH79* (Fig. 2). All plant materials containing either *orfH79* or *orf290* in our study are shown in Table 1.

Alignment of DNA and amino acid sequences

Due to the very high similarity between *orfH79* and *orf79*, it is impossible to distinguish between them using band size of the PCR products. PCR amplification products with primers H1 and H2 from mtDNA of YTA, BYA, IBBI81, W5, W6, W8, W10, W12 and W17 were retrieved, cloned and sequenced. All sequence alignments indicated that the four accessions of W6, W8, W12 and W17 were identical to *orfH79* sequences in YTA or CGA in the HL-CMS lines. IRRI81, W5, W10 and W16 accessions also had a high level of similarity, with IRRI81, W5 and W10 being identical to *orf79* sequences in SJA or BYA in the Boro-II-CMS lines. W16 differed in only one base in the nucleotide site 147 from A to T compared with the *orf79* sequence in SJA or BYA. Amino acid sequences differed at sites 48aa (Leu to Met), 49aa (Asp to Glu) and 60aa (Tyr to His) between ORFH79 in YTA and ORF79 in SJA. Amino acid sequences in W6, W8, W12 and W17 were identical to the ORFH79 sequence in YTA or CGA, but they were not identical among IRRI81, W5, W10, W16 and SJA or BYA (Fig. 3a). These results suggest that YTA, CGA, W6, W8, W12 and W17 shared the same mitotype holding *orfH79* and SJA, BYA, IRRI81, W5, W10 and W16 shared the similar mitotype holding *orf79.*

Of the 14 cultivated rice accessions containing *orf290*, 11 nucleotide sites were different. Three of the 14 accessions (Z597, Z615 and W18) are identical to nucleotide sequences in YTA. Compared with the YTA nucleotide sequence, up to five nucleotide differences were identified in the other 11 accessions. Among these accessions, Z747 had a differential nucleotide site (533nt); Z593, Z595, Z604, Z612, Z613, Z616, Z740 (199nt, 265nt and 533nt) and Z743 had three different nucleotide sites (349nt, 580nt and 863nt);

Z620 (199nt, 216nt, 265nt, 533nt and 797nt) and Z624 (199nt, 265nt, 328nt, 533nt and 551nt) had five different nucleotide sites. For the amino acid sequences, there were eight different loci (Fig, 3b): Z747 had a differential amino acid site (178aa) and Z593, Z595, Z604, Z612, Z613, Z616 and Z740 had two different amino acid sites (66aa and 178aa). Z620 (66aa, 178aa and 265aa) and Z743 (117aa, 194aa and 287aa) had three different amino acid sites whilst Z624 had four different amino acid sites (66aa, 110aa, 178aa and 184aa) (Table 2).

Southern blotting

To further assay the specificity mitotype and copy number of *orfH79*, mitochondrial genomic DNA of four accessions with only specific bands of *orfH79* and the control accessions YTA, YTB and BYA were digested with *EcoR* I and hybridized using a *orfH79* probe. Only one band was detected around 300 bp for W6, W8, W12 and W17 as YTA, suggesting that *orfH79* is a single copy gene in these mitochondrial genomes. In order to verify the absence of the mitotype with *orf290*, all of the over accessions were digested with *EcoR* V and *Xho* I and hybridized with a *orf290* probe. Results for southern blotting indicated that neither the sequence nor homologous sequence of *orf290* was detected in the mitochondrial genomes of W6, W8, W12 and W17, and a special band was recorded for *orf290* in YTA around 1700 bp (Fig. 4).

The same method was used to evaluate the mitotype and copy number of *orf290* and exclude the existence of gene *orfH79* in accessions of W18, Z593, Z595, Z597, Z604, Z612, Z613, Z615, Z616, Z620, Z624, Z740, Z743 and Z747. Although results from this analysis indicated that homologous sequences of *orfH79* were not present, the special band at 1700 bp of *orf290* was detected in these mitochondrial genomes (Fig. 5).

Development of new CMS lines via backcross from accessions containing either *orfH79* or *orf290*

CMS is a maternally inherited trait that, when induced by one or more mitochondrial chimeric genes, contains partial fragments of mitochondrial function genes. Nuclear-cytoplasmic incompatibility, showing a CMS phenotype is the result of the combination of a nuclear genome lacking *Rf* genes and a mitochondrial genome containing CMS-inducing mitotypes. In order to develop new CMS lines only holding *orfH79* or *orf290*, interspecies crosses were performed using four accessions (W6, W8, W12 and W17) only carrying *orfH79* and three accessions (W18, Z597 and Z615) only carrying *orf290* in the mitochondrial genome as maternal parents with YTB as the male parent. Fertility analysis of the backcross offspring indicated that the percentage of stainable pollen grains of F1 hybrids were significantly low, and over 50% of abortive pollen grains were spherical. The seed-setting rates of bagged spikelets were also noticeably reduced. Backcrosses were then undertaken repeatedly between the offspring of low-fertility as maternal parents and YTB as recurrent male parents (Fig. 6a). The fertility of

backcross progeny decreased with an increase of backcross generations (Table 3), and fertility of backcross offspring BC7F1 was almost close to complete sterility (Table 3). On the other hand, another interspecies cross was performed using YTB of artificial emasculation as maternal parents with four wild rice accessions (W6, W8, W12 and W17) and three accessions (W18, Z597 and Z615) as male parents. The offspring plants as female parents crossed with four wild rice as recurrent male parents (Fig. 6b). Compared with YTB, the fertility of these combinations was still very high (Table 3). For these results, the following conclusions can be drawn: (i) W6, W8, W12 and W17 shared the same *orfH79* mitotype and W18, Z597 and Z615 shared the same *orf290* mitotype; (ii) the fertility of offspring between YTB as male parent and YTB as maternal parent was completely different; (iii) inconsistent fertility between reciprocal and positive crosses indicates that low fertility belongs to cytoplasmic inheritance, and low fertility may be caused by the cytoplasmic male sterility gene.

Discussion

CMS genes are usually chimeric genes derived from the rearrangement between the fragment of mitochondrial functional genes and other unknown sequences (Hanson and Bentolila 2004). To date, several types of CMS-associated genes or sequences have been reported in rice, including orfH79 and orf290 for CMS-HL (Yi et al. 2002; Yang et al., 2018), orf79 for CMS-BT and CMS-LD (Wang et al. 2006; Kazama et al. 2016), orf307 for CMS-CW (Fujii et al. 2010), orf113 for CMS-RT98 (Igarashi et al. 2013), WA352c and WA352a for CMS-WA (Tang et al. 2017), and orf352 for CMS-RT102 (Okazaki et al. 2013). The same CMS related gene has been recorded to exist in different CMS types, such as orf79 for CMS-BT and CMS-LD, and WA352a (orf352) for CMS-WA and CMS-RT102. Two CMS related genes or sequences have also been isolated from the same CMS line, for example orfH79 and orf290 for CMS-HL and WA352c and WA352a for CMS-WA. All of these genes or specific sequences can be used as molecular markers in the breeding of CMS lines.

The identification of CMS cytoplasm and the development of new CMS lines in higher plants mainly depends on traditional test-cross breeding and molecular markers based on mitochondrial genome sequences. Due to over dependence on breeders' experience and uncertainty of field judgment, it is difficult to create a new sterile line in a short time period using traditional breeding methods. Due to its fast, efficient, cost-effective and accurate characteristics, molecular marker assisted selection (MAS) has been widely used in crop breeding. Based on the report of distinguish different cytoplasmic using molecular markers to backgrounds in radish, rapeseed and rice (Kim et al., 2007; Zhao et al., 2010; Fang et al., 2015), specific sequences or ORFs in mitochondrial genomes can assist in the selection of new strains or varieties of crop. Of the mitotype-specific sequences (MSS) tested, 14 MSS were related to CMS, including nine MSS specific to sporophytic CMS, three specific to gametophytic CMS, and two shared by all types of CMS. Many mitotypes in wild rice can be differentiated and new CMSs can be developed using MSS molecular markers (Xie et al. 2014). Previous studies documented four completely sterile alloplasmic CMS lines developed from wild rice by successive recurrent backcrossing of sterile

plants from a BC1F1 population with the HL maintainer YTB, respectively. These alloplasmic CMS lines, carrying different orf(H)79 haplotypes, displayed various fertility-restoring models through test-crossing (Li et al., 2008). Using the same method, sterile CMS lines were developed from wild rice accessions carrying L-sp1, a CMS-associated mitochondrial sequence (Tan et al., 2015). Recently, based on the sequence of L-sp1, a novel CMS-related mitochondrial chimeric gene (orf290) which can induce CMS in the binucleate pollen stage of rice was cloned and confirmed from HL-CMS line YTA (Yang et al., 2018). In our study, we developed several new CMS lines via backcross (using the same male parent YTB) from accessions containing either orfH79 or orf290 using molecular marker assistance.

It has been suggested that CMS-associated mitotypes have a parallel evolutionary relationship with Rf-candidate-related nucleotypes within plant species, and that different Rf alleles interact with CMS in a gene-for-gene manner (Taylor et al., 2001; Van Damme et al., 2004; Tan et al., 2011). In our study, two new haplotype CMS lines were bred with either orfH79 or orf290, having shared similar maintainer lines. Fertility of these lines could be restored to different degrees by independently restoring either Rf5 or Rf6 (Unpublished data). Our results indicate that the corresponding relationship between Rf genes and CMS-associated genes is not a simple one-to-one relationship.

Abbreviations

BT: Boro II; CMS: Cytoplasmic Male Sterlity; CW: Chinese Wild rice; HL: HongLian; LD: Lead rice; MAS: marker Assisted Selection; MSS: Mitotype-Specifific Sequences; *Rf*: Fertility Restore; RT102: RT102C; RT98: RT98C; WA: Wild Abortive; YTA: Yuetai A; YTB: Yuetai B

Declarations

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Authors' Contributions

YP Tan and CT Wang designed the research experiments. YP Tan, T Chen, Z Tian, JY Li, XY Tang and XQ Liu performed all the experiment and breeding. YP Tan, X Xu, and G Cheng analyzed the data. YP Tan and XQ Liu wrote and prepared the manuscript. The authors declare that there are no conflicts of interest.

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Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Tables

Table 1 All the plant materials containing either *orfH79* or *orf290* in this study

NO	Name	Character or IRGC Accessions number	Species	Country
1	YTA (Yuetai A)	CMS line, Honglian type	*O. Sativa*	China
2	YTB (Yuetai B)	Maintainer line, Honglian type	*O. Sativa*	China
3	SJA (Sijin A)	CMS line, Boro type	*O. Sativa*	China
4	BYA (Baoyuan A)	CMS line, Boro type	*O. Sativa*	China
5	CGA (Congguang41 A)	CMS line, Honglian type	*O. Sativa*	China
6	IRRI81	IRRI81	*O. Sativa*	Philippines
7	W1	100968	*O. glumaepatula*	Suriname
8	W2	105661	*O. glumaepatula*	Brazil
9	W3	101978	*O.nivara*	India
10	W4	103415	*O.nivara*	Sri Lanka
11	W5	103419	*O.nivara*	Sri Lanka
12	W6	103824	*O.nivara*	China
13	W7	103835	*O.nivara*	Bangladesh
14	W8	103841	*O.nivara*	Bangladesh
15	W9	105712	*O.nivara*	Cambodia
16	W10	105728	*O.nivara*	Cambodia
17	W11	106153	*O.nivara*	Laos
18	W12	101974	*O.rufipogon*	India
19	W13	103423	*O.rufipogon*	Sri Lanka
20	W14	104057	*O.rufipogon*	China
21	W15	105349	*O.rufipogon*	India
22	W16	105491	*O.rufipogon*	Malaysia
23	W17	105696	*O.rufipogon*	Nepal
24	W18	105698		Nepal
25	Z593	O. *Rufipogon*		China
26	Z595		*O. Sativa*	China
27	Z597		*O. Sativa*	China
28	Z604		*O. Sativa*	China
29	Z612		*O. Sativa*	China
30	Z613		*O. Sativa*	China
31	Z615		*O. Sativa*	China
32	Z616		*O. Sativa*	China
33	Z620		*O. Sativa*	China
34	Z624		*O. Sativa*	China
35	Z740		*O. Sativa*	China
36	Z743		*O. Sativa*	China
37	Z747		*O. Sativa*	China
			O. Sativa	

Table 2 Differences in nucleotides and amino acids compared to YTA

Accessions	Differential nucleotide loci	Differential amino acid loci
Z593	199 (G- A), 265 (T-C), 533 (G- A)	66(E-K), 178 (G-E),
Z595	199 (G- A), 265 (T-C), 533 (G- A)	66(E-K), 178 (G-E),
Z597	None	None
Z604	199 (G- A), 265 (T-C), 533 (G- A)	66(E-K), 178 (G-E),
Z612	199 (G- A), 265 (T-C), 533 (G- A)	66(E-K), 178 (G-E),
Z613	199 (G- A), 265 (T-C), 533 (G- A)	66(E-K), 178 (G-E),
Z615	None	None
Z616	199 (G- A), 265 (T-C), 533 (G- A)	66(E-K), 178 (G-E),
Z620	199 (G- A), 216 (T-C), 265 (T-C), 533 (G- A), 797 (A-T)	66(E-K), 178 (G-E), 265 (E-V)
Z624	199 (G- A), 265 (T-C), 328 (T-C), 533 (G- A), 551 (C-T)	66(E-K), 110 (F-L), 178 (G-E), 184 (S-F)
Z740	199 (G- A), 265 (T-C), 533 (G- A)	66(E-K), 178 (G-E),
Z743	349 (A-G), 580 (T-C), 863 (C-T)	117 (M-V), 194 (W-R), 287(S-L)
Z747	533 (G- A)	178 (G-E),
W18	None	None

Table 3 Fertility analysis for the different backcross generations

Accessions	Fertility for different backcross generations								
	YTB as maternal parent		YTB as male parent						
	BC1F1	BC2F1	BC1F1	BC2F1	BC3F1	BC4F1	BC5F1	BC6F1	BC7F1
W6	91.3±2.3	92.3±1.5	49.6±2.0	30.2±2.2	19.3±1.1	9.8±1.1	5.1±1.1	1.2±0.3	0
W8	90.3±2.2	91.2±1.9	47.9±3.0	23.1±1.7	11.2±0.9	10.2±1.3	4.2±1.2	0	0
W12	90.5±3.1	92.3±2.6	46.3±1.8	19.6±1.9	12.6±2.1	8.7±0.8	4.2±0.9	1.5±0.2	0
W17	89.6±2.7	92.1±2.9	48.7±1.6	24.3±2.3	12.3±2.4	8.2±0.7	3.6±1.2	0	0
W18	93.2±2.6	91.8±3.2	51.3±2.3	17.5±1.4	10.9±1.6	8.2±0.3	3.6±0.9	0	0
Z597	94.1±1.8	93.6±2.4	50.7±2.1	30.2±1.7	15.3±1.9	7.6±0.6	2.8±1.0	0	0
Z615	92.6±1.3	93.4±1.1	42.3±1.7	25.7±1.6	14.2±1.3	5.6±0.7	2.1±0.7	0	0

Figures

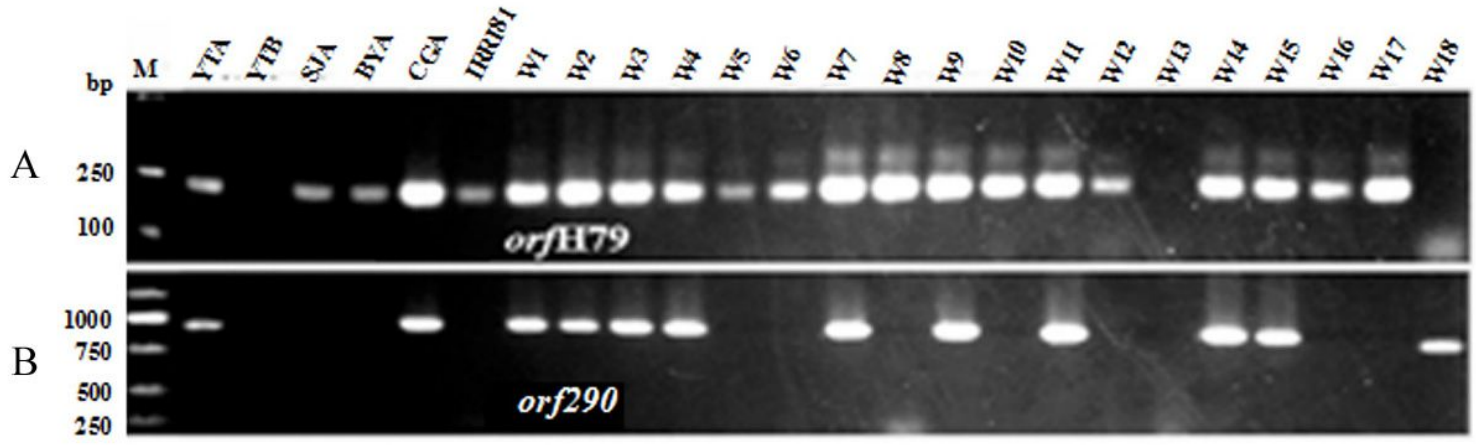

Figure 1

The distribution of orfH79 and orf290 in wild rice in this study. A: orfH79; B: orf290

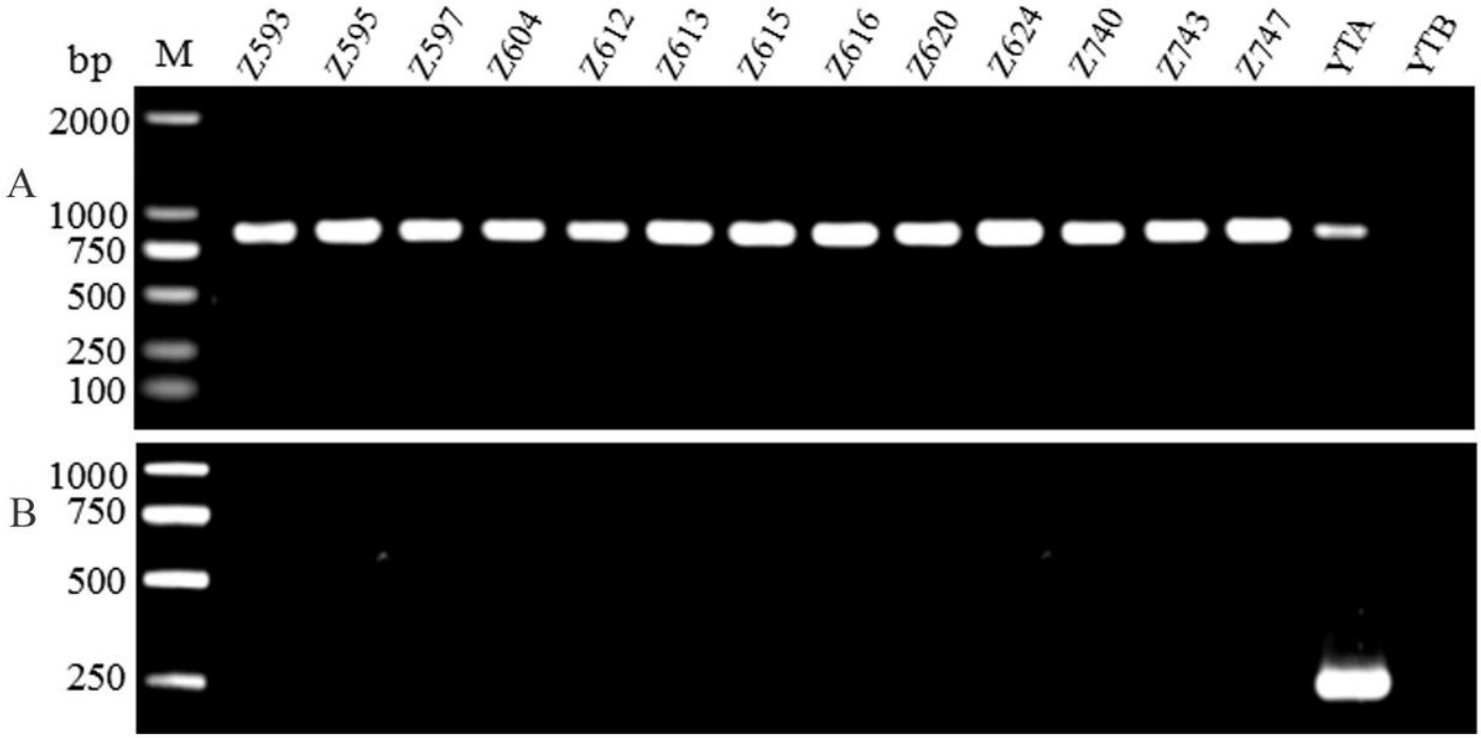

Figure 2

The distribution of orfH79 and orf290 in cultivated rice in this study. A: orf290; B: orfH79

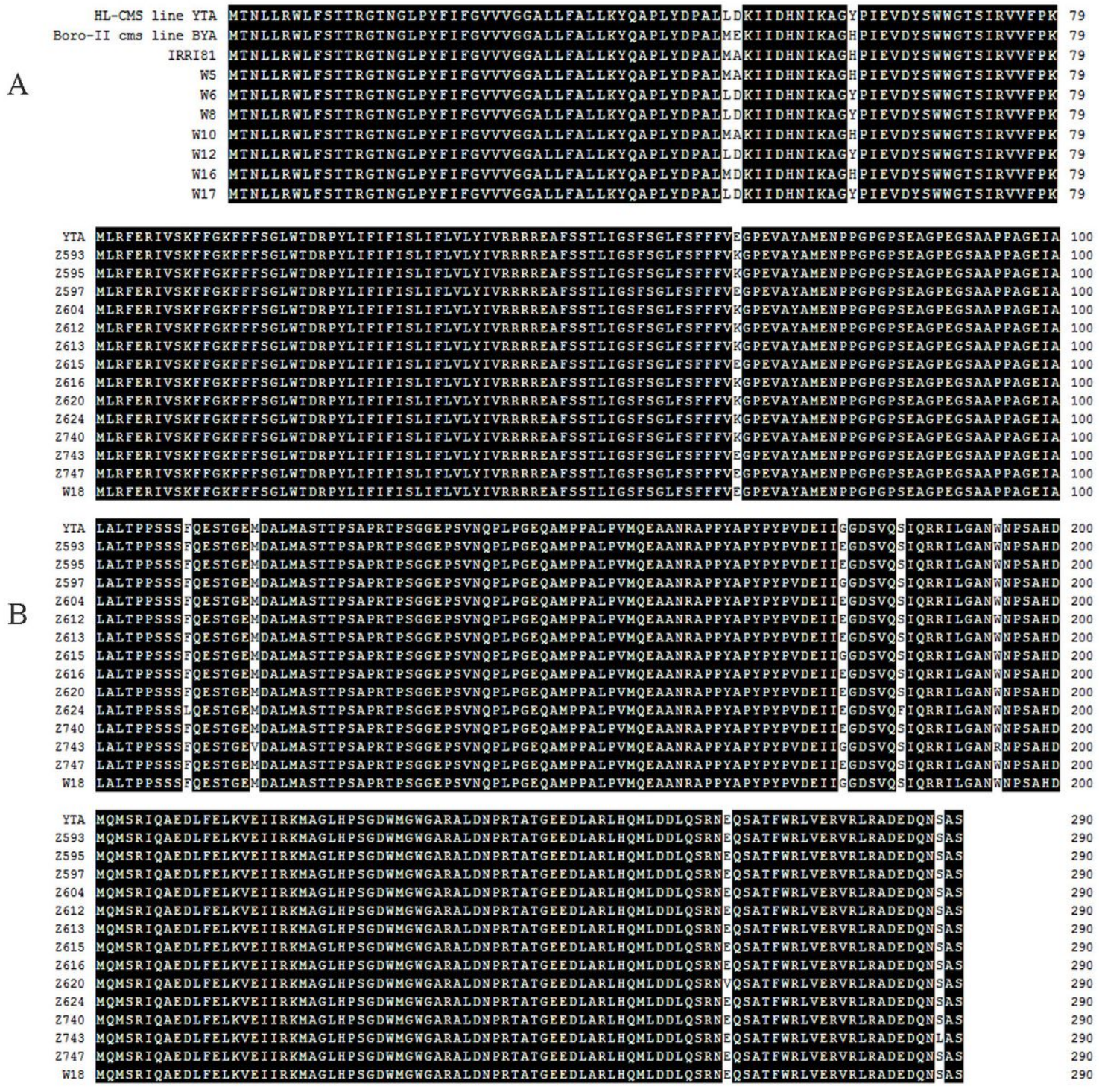

Figure 3

Alignment for amino acid sequence of ORFH79 and ORF290. A: ORFH79; B: ORF290

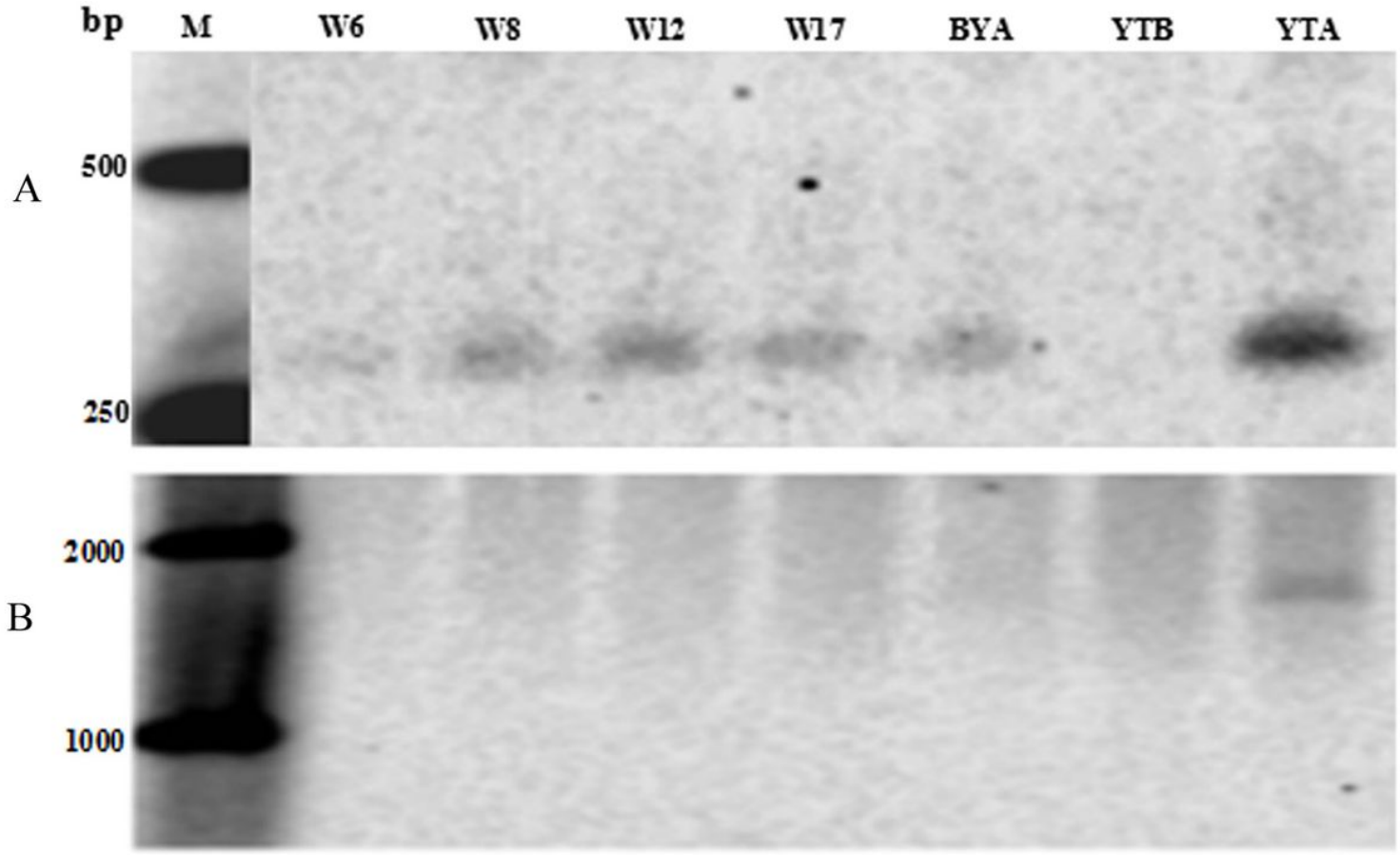

Figure 4

Southern blotting of wild rice screened with probes orfH79 and orf290. A: orfH79; B: orf290

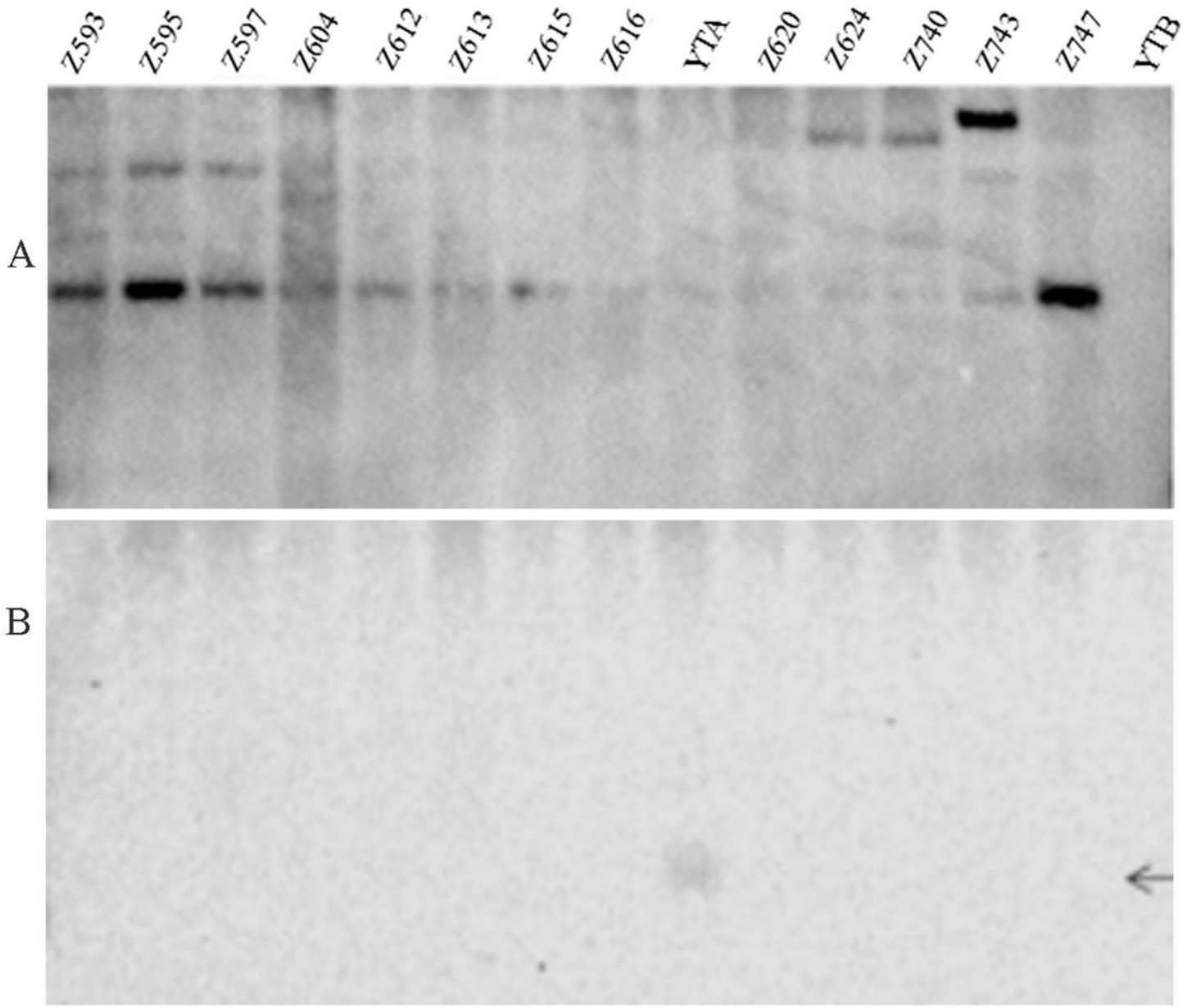

Figure 5

Southern blotting of cultivated rice screened with probes orfH79 and orf290. A: orfH79; B: orf290

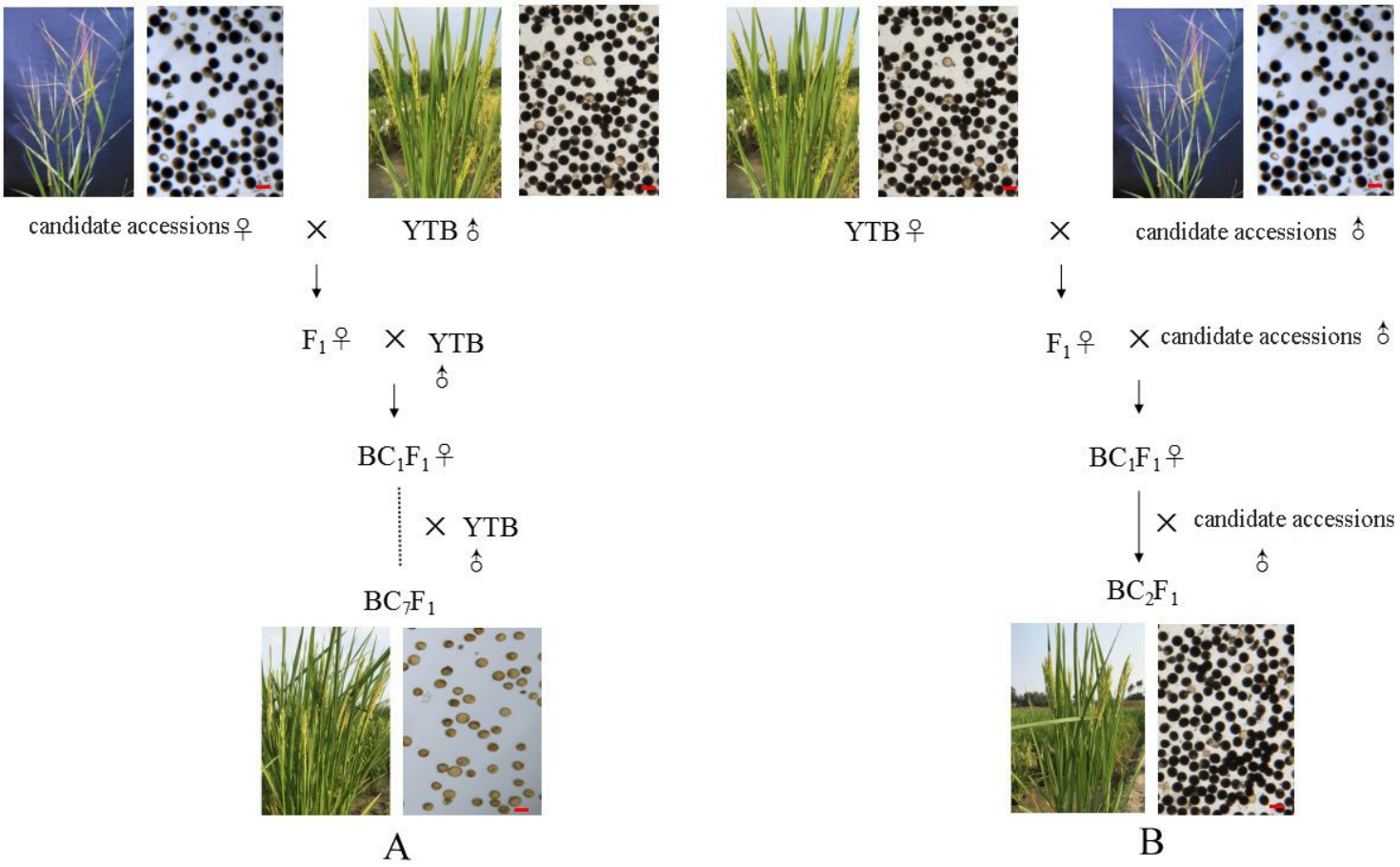

Figure 6

Breeding process of CMS lines and identification of pollen fertility. A: Forward hybridy, CMS lines; B: Reverse hybridy, fertile lines

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementTable2cultivaterice.doc
- supplementTable1wildrice.doc