ANALYSIS OF INTROGRESSION FROM A TETRAPLOID WILD SPECIES (*Oryza minuta*) INTO CULTIVATED RICE (*O. sativa*)

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ABSTRACT. *Oryza minuta, a tetraploid wild relative of cultivated rice, O. sativa, is a potential source of gene(s) for resistance to an important bacterial blight (BB) disease caused by Xanthomonas oryzae pv. oryzae. Interspecific crosses of O. sativa and O. minuta had produced advanced backcross progenies (MAALs and introgression lines; using O. sativa as recurrent parent). A total of 59 putative MAALs and 158 introgression lines have been examined for the presence of BB resistance genes(s) from O. minuta using chromosome analysis, screening for BB resistance in the field, isozyme analysis and simple sequence repeat (SSR) analysis. Of the 59 putative MAALs, 17 plants had a 2n chromosome complement of O. sativa and one chromosome of O. minuta. These plants have been identified as MAALs. For BB resistance screening, the result showed that 17 out of 158 introgression lines were found to be resistant to race 1 (PXO61) of BB of Philippines. However, none of the putatives MAALs were resistant. Isozyme analysis of 158 introgression lines did not reveal any introgression from O. minuta. By using 76 polymorhpic SSR markers, however, 28 markers detected introgression in some of the derived lines. The results showed SSR markers are efficient for detecting the introgression of chromosome segment(s) from O. minuta into rice.*

KEYWORDS. *Oryza minuta*, *O. sativa*, introgression, chromosome analysis, BB resistance screening, isozyme, SSR markers

INTRODUCTION

Wild species are an important source of useful traits for cultivated plants improvement. The genus *Oryza* has two cultivated (2n=24, AA) and 22 wild (2n=24 or 2n=48) species. These wild species are represented by AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and HHKK genomes (Vaughan, 1994; Aggarwal, *et al*., 1997; Ge, *et al*., 1999). Among these species, *O. minuta* (2n=48, BBCC), is an important source of useful gene(s) for resistance to bacterial blight (BB), brown planthopper, whitebacked planthopper, green leafhopper, blast and sheath blight (Heinrichs *et al*., 1985; Sitch, 1990).

Introgression lines have been produced from crosses of new plant type rice (NPT, IR6500-81-5-3-2) with *O. minuta* (Acc. 101089) using rice as recurrent parents (Buang, 2000). These progenies have been produced to transfer genes for resistance to bacterial blight from *O. minuta* into *O. sativa*.

Several markers have been developed to detect introgression, such as restriction fragment length polymorphism (RFLP), randomly-amplified polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite or

commonly known as simple sequence repeat (SSR). The advantages and the applications of those markers have been summaried by Mohamad *et al*. (2005). Among these markers, SSR have been widely used in breeding, bulk segregant analysis, diversity studies, F_1 identification, fingerprinting, framework/region specific maps, genetic maps, marker-assisted selection (MAS), seed testing and varietal/line identification. Preference to SSR is due to it advantages such as, it is PCR-based markers, found abundance in cell, is a co-dominant markers, need only small amount of DNA, and did not require radioactive detection.

MATERIALS AND METHODS

Advanced backcross progenies (monosomic alien additional lines, MAALs and introgression lines) have been derived from interspecific crosses of *O. sativa* (NPT, IR65600-81-5-3-2) and *O. minuta* (Acc. 101089) using *O. sativa* as recurrent parent (Buang, 2000). A total of 59 putative MAALs and 158 introgression lines were examined for the presence of resistance gene(s) from *O. minuta*. Chromosome analysis, screening for BB resistance, isozyme analysis and SSR analysis were carried out to detect alien introgression.

Chromosome analysis

Young panicles were collected between 0730-0900H and fixed in Carnoy's solution. The young panicles were transferred to 70% ethanol after 24 hours of fixation. Anthers of suitable stage were selected and squashes were made on glass slides in 2% acetocarmine. Data on chromosome counts were recorded at diakinesis or metaphase I. A few representative cells were used for photomicrography using plain 10x eyepieces and 100x objective lenses.

BB resistance screening

A clipping technique was used for inoculation of BB (Kauffman *et al*., 1973). All materials, including the parents were inoculated with race 1 (*PX061*) of BB of Philippines after 30 days of transplanting. Observations on disease symptoms were recorded after 14 days of inoculation.

Isozyme analysis

The method described by Glaszman *et al*. (1988) was used in isozyme analysis. The analyzed isozymes included glutamate oxaloacetate transaminase (GOT), shikimic dehydrogenase (SDH), alcohol dehydeogenase (ADH), isocitrate dehydrogenase (ICD), aminopeptidase (AMP), phosphogluconate dehydrogenase (PGD) and malic enzyme (MAL).

SSR analysis

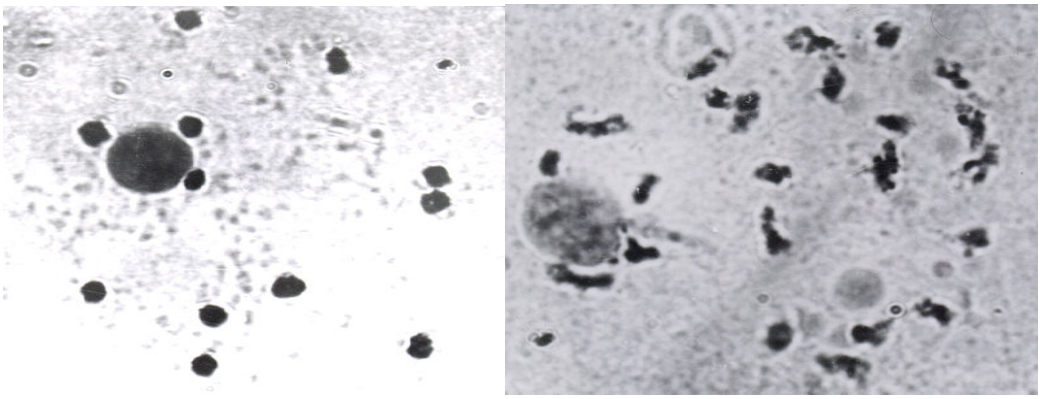
Total genomic DNA was extracted from leaves of three week-old seedlings following the modified cetyl-trimethyl ammonium bromide (CTAB method; Doyle and Doyle, 1987). A subset of 301 SSR markers was used to detect polymorphism between the parents (*O. sativa* and *O. minuta*) and to determine introgression segment in the advanced backcross progenies. PCR reactions were performed by using Thermal Cycler (MJ PTC-100) with 35 PCR cycles profile started from one minute at 94^0C for denaturation, followed by one minute at 94^0C annealing at $55/58/61^0$C and one minute

of polymerization at 72^0C. Each PCR was performed in a volume of 20 µl PCR cocktail containing 10mM Tris-HCl (pH8.3), 50mM KCL, 1.5mM $MgCl_2$, 0.01% gelatine, 2µl dNTP, 0.25 units of Taq, 2µl primers and 50ng genomic DNA. The PCR products were analyzed using agarose gel stained with ethidium bromide and polyacrylamide gel with silver staining.

RESULTS AND DISCUSSIONS

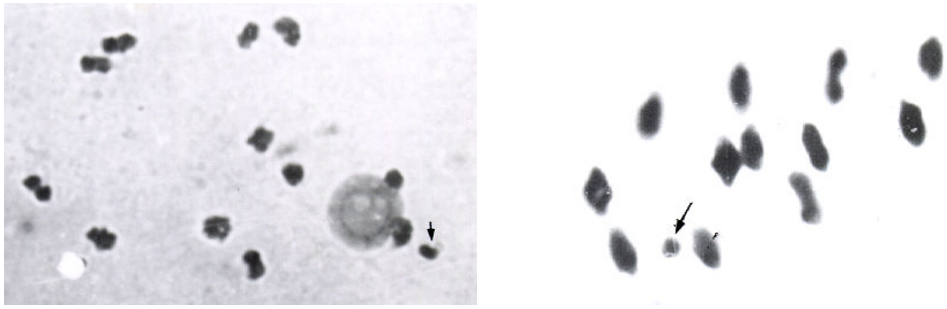
Chromosome analysis

The parents (*O. sativa* and *O. minuta*) showed a normal meiosis with regular formation of 12 and 24 bivalents at diakinesis and metaphase, respectively (Figure 1). Most of the progenies resembled the recurrent parent, *O. sativa*. Only seventeen plants were detected with an extra chromosome through cytogenetic analysis and have been identified as MAALs (Figure 2). Meiotic analysis of the MAALs showed the extra chromosome as unpaired. Multani *et al*. (1994) and Jena and Khush (1989) reported that chromosome association of MAALs was 12 bivalent plus one univalent (12 II + 1 I), indicating the extra chromosome is univalent. The occurrence of unpaired (univalent) chromosome, in this study, indicates that the extra chromosome is from *O. minuta*, which has limited homology with the *O. sativa* chromosome.



(a) (b)

Figure 1. Diakinesis showing (a) 12 bivalents in *O. sativa* NPT IR65600-81-5-3-2 and (b) 24 bivalents in *O. minuta* Acc. 101089.



(a) (b)

Figure 2. Diakinesis (a) and metaphase I (b) showing 2n=25 (12 II + 1 I) in MAAL of O. minuta (univalent from O. minuta is marked by arrow).

BB resistance screening

A subset of 158 introgression lines and 59 putative MAALs derived from *O. sativa* × *O. minuta* were screened for BB resistance in the field. Of 158 introgression lines, 17 were found to be resistant to race 1 (*PXO61*) of BB of Philippines, but none of the putative MAALs were resistant (Figure 3). *Oryza minuta* was known to carry resistance genes of BB (Khush and Kinoshita, 1991; Kinoshita, 1995; Lin *et al*., 1996). Among these resistance genes, six have been identified to be resistance to race 1 of BB of Philippines (Buang, 2000). The introgression segment in this study is likely to have a wide spectrum of resistance. All putative MAALs were found to be susceptible to BB. This suggested that the extra chromosome does not carry genes for BB resistance.

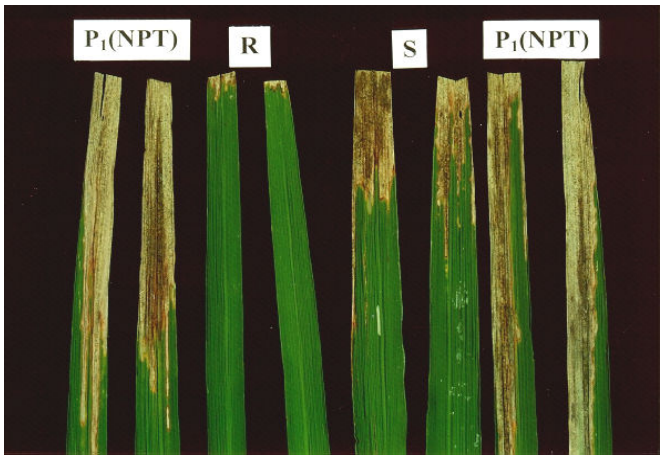


Figure 4 Reaction of *O. sativa* × *O. minuta* introgression lines to race 1 (*POX*61) of bacterial blight (BB) of Philippines. From left Lane 1, 2, 7 and 8 = *O. sativa* NPT IR65600-81-5-3-2 (susceptible); Lane 3 and 4 = *O. sativa* × *O. minuta* derivatives, BC_4F_4 (introgression lines – resistant); Lane 5 and 6 = *O. sativa* × *O. minuta* derivatives, BC_4F_4 (introgression lines – susceptible).

Isozyme analysis

Starch gel electrophoresis was used in isozyme analysis of *O. sativa*, *O. minuta*, F_1 and their derivatives (BC_4F_4). Six isozyme loci were identified to showed polymorphism in *O. sativa* and *O. minuta*. These six loci are *Got*1, *Got*2, *Sdh*1, *Pgd*2, *Amp*3 and *Amp*4 (Figure 4). Isozyme analysis of 110 backcross derivatives did not reveal introgression from *O. minuta* in all isozymes studied. However, 11 progenies showed allele 2 of *Adh*1 (Figure 5) and four progenies showed allele 2 of *Pgd*1 (Figure 6), while another 17 progenies showed allele 2 in both *Adh*1 and *Pgd*1. Both parents are monomorphic (allele 1) for *Adh*1 and *Pgd*1. Loci *Adh*1 and *Pgd*1 are located on chromosome 11 of *O. sativa* (Ranjhan *et al*., 1988 and Wu *et al*., 1988). The result suggested interaction between the alien and recipient segments involving loci on chromosome 11. The same phenomenon was found in the study of Multani *et al*. (1994), which a different allele from the parents, *Oryza sativa* and *O. australiensis* was found. Both parents were polymorphic for *Amp*3, however, investigation of introgression in BC_2F_1 revealed a band which moves slower than *O. australiensis* band and they named it as modified allele. Unfortunately, none of these progenies was resistant to race 1 of BB of Philippines. Isozyme analysis has been used to identify MAALs, for examples, Romero *et al*. (1993) have identified MAALs of *O.*

minuta by using endopeptidase (ENP), PGD and SDH. Two *O. minuta* derived plants, with chromosome number of 2n=25, exhibited two *O. minuta* specific isozymes. However, in this study, none of the MAALs of *O. minuta* could be identified. This could be due to the lack of complete series of MAALs, especially MAALs of *O. minuta*.

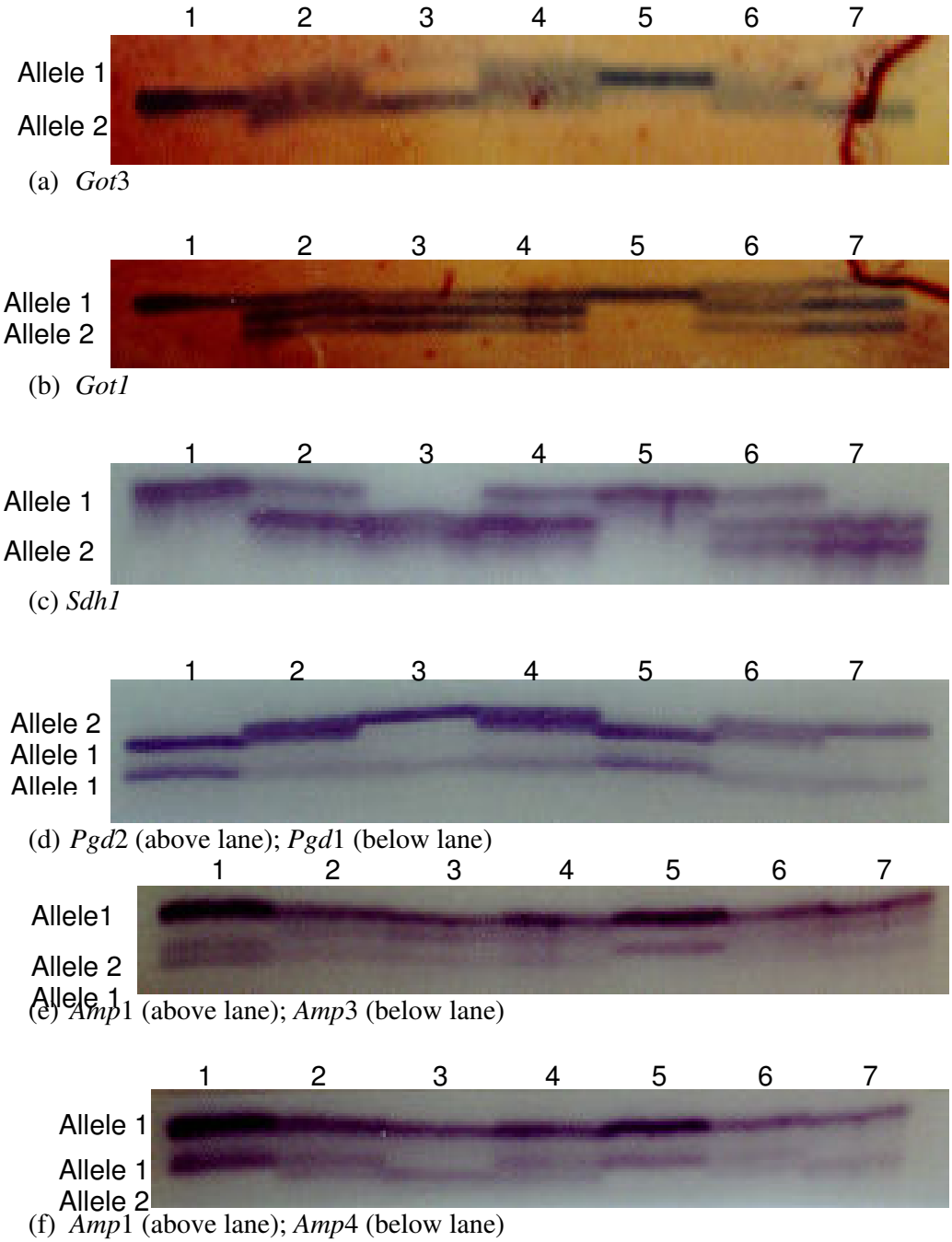
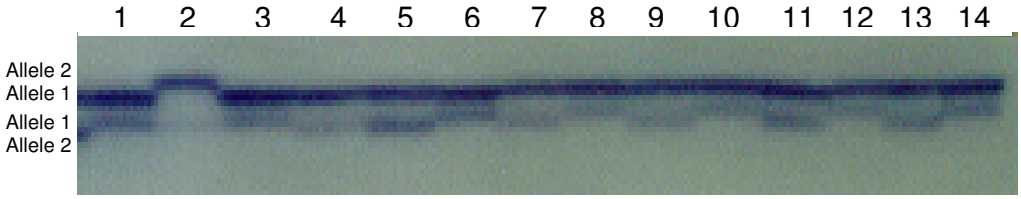


Figure 4 Banding patterns for the enzymes loci (Got3, Got1, Sdh1, Pgd2, Pgd1, Amp1, Amp2, Amp3, Amp4, Mal1, Icd1 and Adh1) in O. sativa, O. minuta and their F_1 hybrids. 1 = O. sativa (IR77981-81-5-3-2); 2 = F_1 (IR77981-81-5-3-2 x O. minuta, Acc. 101089); 3 = O. minuta (Acc. 101089); 4 = F_1 (IR68552-55-3-2 x O. minuta, Acc. 101089);

5 = O. sativa (IR68552-55-3-2); 6 = F_1 (IR68552-55-3-2 x O. minuta, Acc. 101141); 7 = O. minuta (Acc. 1011141).

Figure 5. Banding pattern for *Adh*1 in some of the putative MAALs of *O. sativa* (NPT IR65600-81-5-3-2) × *O. minuta* (Acc. 101089). 1 = *O. sativa*, NPT, IR65600-81-5-3-2 (genotype = 11); 2 = *O. minuta* Acc. 101089 (genotype = 11); 3 – 18 = BC_4F_4 (putative MAALs). Both parents are monomorphic (genotype=11). However, some progenies showed different genotype from the parents (genotype=22; lane 3, 4 and 16).



*Pgd*2 (above lane); *Pdg*1 (below lane)

Figure 6 Banding pattern for *Pgd*1 and *Pdg*2 in some of the putative MAALs of *O. sativa* NPT IR65600-81-5-3-2 × *O. minuta* (Acc. 101089). 1 = *O. sativa*, NPT IR65600-81-5-3-2 (genotype = 11); 2 = *O. minuta* Acc. 101089 (genotype = 11); 3 – 14 = BC_4F_4 (putative MAALs). Both parents are monomorphic (genotype = 11). However, some progenies showed different genotype from the parents (genotype = 22; lane 4, 5, 7, 9, 11 and 13).

SSR analysis

Introgression was detected using SSR marker. SSR markers showed about 28% polymorphism between *O. sativa* and *O. minuta* (Figure 8). Low polymorphism between *O. sativa* and *O. minuta* may due to their different genomes, where *O. sativa* belongs AA genome and *O. minuta* belongs to BBCC genome. Polymorphism can range from 38% to 92%, between *O. sativa* and others AA genome of wild realative species, such as *O. glaberrima* (Jason Talag per. comm.) and *O. longistamanata* (Chen *et al*., 1997). Of the 76 polymorphic SSR markers used, only 28 markers were detected with introgression from *O. minuta* (Figure 9). Based on the map developed by Temnykh *et al*. (2000, 2001) the introgression occurred on chromosomes 1, 2, 4 and 7. This study showed only small segment(s) of introgression was detected. Jena *et al*. (1992), Ishii *et al*. (1994) and Brar and khush (1997, 2002) also reported only small segment(s) of introgression found in the intraspecies of rice hybrid using single RFLP markers. The introgressed segments of *O. minuta* were found in homozygous as well as in heterozygous forms in BC_4F_4 progenies. This suggested that the mechanism of introgression is probably through exchange of segments involving crossing over between chromosomes of *O. sativa* and *O. minuta*. The result showed SSRs as efficient markers for detecting segments from *O. minuta* into rice.

1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 3 5 7 1 3 5 7

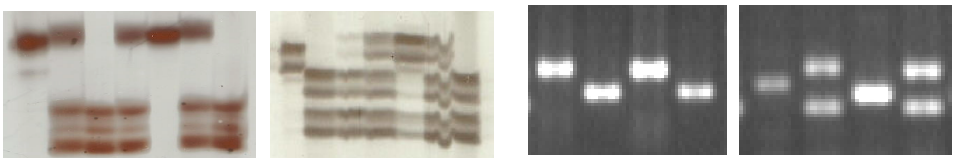


Figure 7. Polymorphism between *O. sativa* and *O. minuta* using SSR markers (a) RM202; (b) OSR12; (c) RM151; and (d) RM306. Lane 1, *O. sativa* (MR1); 2, MR1 × *O. minuta* 101141, F1; 3, *O. minuta* 101141; 4, NPT × *O. minuta* 101141, F1; 5, *O. sativa* (NPT - IR65600-81-5-3-2); 6, NPT × *O. minuta* 101089, F1; 7, *O. minuta* 101089.

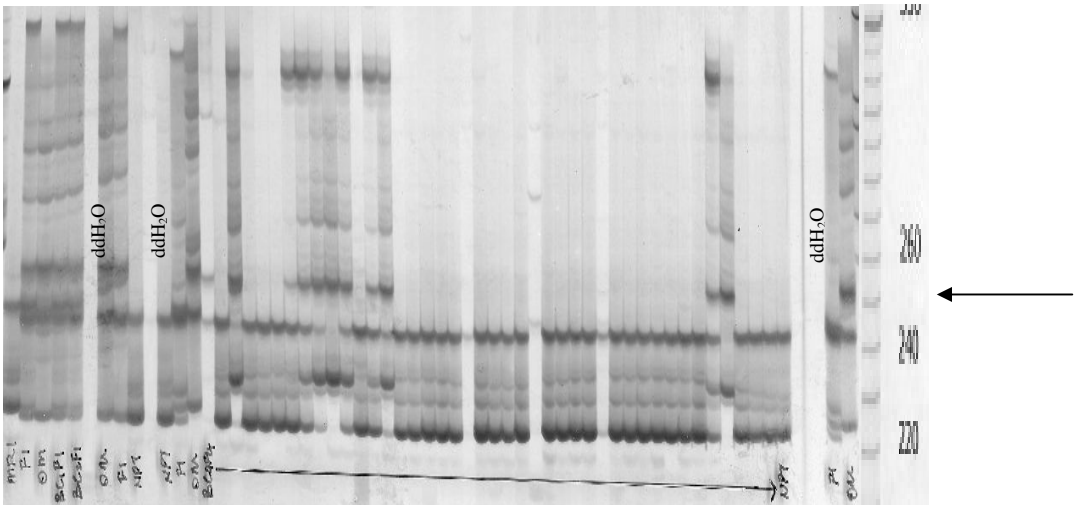


Figure 8. SSR analysis of *O. sativa* × *O. minuta* derivatives (BC_4F_4) using polyacrylamide gel OSR15 (chromosome 4). Arrow indicates introgression segments from *O. minuta*.

CONCLUSION

SSR analysis of the putative MAALs and introgression lines indicated that small segments from *O. minuta* had been transferred into *O. sativa*. The results from this study showed SSR markers are efficient for detecting introgression.

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