A17 PCR-RFLP Analysis of cpDNA in the Genus *Oryza*

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Objectives

This study was aimed to determine the potential utility of PCR-RFLP approach for detecting genetic variation of chloroplast DNA (cpDNA) in the genus *Oryza*, and to clarify the phylogenetic relationships among nine analyzed species in the genus *Oryza*.

Materials and Methods

Plant materials and DNA isolation: The present study involved 94 strains in the genus *Oryza*, which consisted of nine species in two major complexes, *Oryza sativa* complex and *Oryza officinalis* complex. In addition, two strains from genera *Zizania* and *Leerisia*, which are closely related to the genus *Oryza*, were included for comparative analysis. Total DNA was extracted from approximately 10-15g of fresh leaves of young seedling with CTAB protocol (Ausubel et al. 1993; Sun et al. 1996).

PCR-RFLP: Five regions of the chloroplast genome were amplified with the plant cpDNA universal primer pairs including *rpo*C2-*rpo*C1, *psb*C-*trn*S, *trn*M, *ORF*106, and *ORF*100 (Taber et a. 1991; Demesure et al. 1995; Dumolin-Lapegue et al. 1997), and then digested with several restriction enzyme combinations among *Hind III, EcoR I, Pst I, Sca I, Xba I, Dra I, Alu I, Sau 3A I, Cfo I, Hpa II,* and *Msp I.* The digested PCR products were finally separated on 1.8-2.8% agarose gels (or on 6% denaturing polyacrymide gels) in 1X TBE buffer.

Results and Discussions

7. The five primer pairs used in present study successfully amplified the corresponding regions of cpDNA in all the materials investigated, supporting the utility of PCR-RFLP method for detecting variations of cpDNA and mtDNA in the genus *Oryza*.
8. Among four primer pairs, only two primer pairs, *trn*M, *ORP*100, directly generated polymorphic markers as showed in Figure 1. In regions of *rpo*C2-*rpo*C1 and *ORF*106, all the species in the genus *Oryza* including the genera *Zizania* and *Leerisia* shared the same genetic markers in size.

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9. In general, there were no substantial differences in size between the original undigested fragments and the sums of the sizes of the digested fragments. The differences among the variants detected with the same fragment/enzyme combination ranged around 50bp to 100bp. One exception was found in Rhizome with the *psb*C-*trn*S/*Cfo I* combination, the digested fragment was about 500bp difference from others (Figure 2).

10. Genetic variations in region of *psb*C-*trn*S were observed, suggesting that *Oryza officinalis* complex was clearly diverged from other species investigated in the study. Noticeably, at this same region in chloroplast genome, Rhisome was highly diverged from other species in the genus *Oryza*.

11. Genetic differentiations of cpDNA were found among and within the species of *Oryza* in the present study, especially in the region of *ORF*100, the patterns of differentiation is consistence with the previous studies (Ishii et al 1993; Huang et al. 1996; Sun et al. 1996). It implied that differentiations of cpDNA probably resulted in species and/or subspecies diverged from the genus *Oryza*.

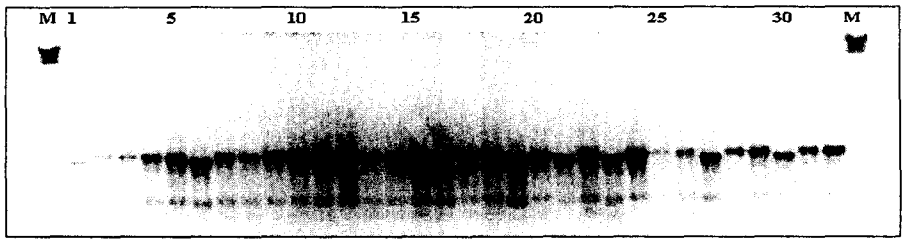

Fig 1. Amplified fragment patterns of cpDNA in region of ORF100. M, size marker (λ/*Hind III*) Lane 1, deletion type; Lane 2, non-deletion type.

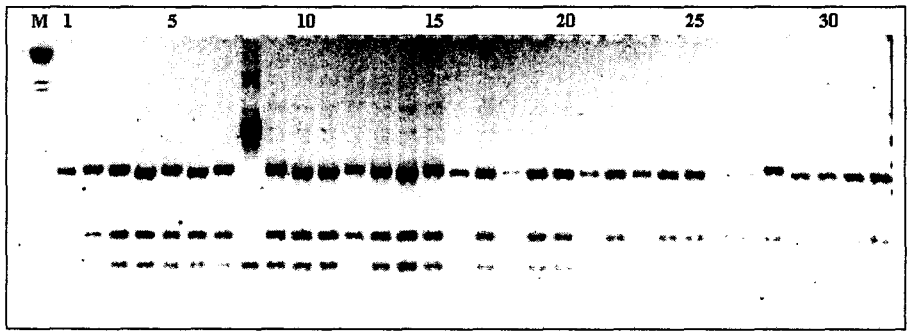

Fig 2. The restriction fragment patterns of 3 variants detected in *psb*C-*trn*S/*CfoI* from nine species in the genus *Oryza*. M, size marker (λ/*Hind III*). Lane 8, rhizome.