A18 PCR-RFLP Analysis of mtDNA in the Genus *Oryza*

Lijuan CHEN, Hak Soo SUH, Dongsun LEE

Dept. of Bio-resources, College of Natural Resources, Yeungnam University, Kyongsan 712-749

Objectives

This study was aimed to determine the potential utility of PCR-RFLP approach for detecting genetic variation of mitochondria DNA (mtDNA) 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 mitochondrial genome were amplified with the plant mtDNA universal primer pairs including *nad*1B-*nad*1C, *nad*4(2)-*nad*4(1), *rps*14-*cob*, *coxII*-*coxII*, and 18S rRNA-15S rRNA (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 then separated on 1.8-2.8% agarose gels (or on 6% denaturing polyacrymide gels) in 1X TBE buffer.

Results and Discussions

1. Except from *coxII*, the primer pairs used in present study successfully amplified the corresponding regions of mtDNA in all the materials investigated, supporting the utility of PCR-RFLP approach for detecting variations of mtDNA in the genus *Oryza*
2. Among four primer pairs, only one primer pair, 18SrDNA-15SrRNA, directly generated polymorphic markers. However, for these primer pairs, although the amplified fragments of mtDNA were same in size, they showed the clear differences in number of copy (Figure 1).

Tel : 053-810-2912 E-mail : chenlijuan@hotmail.com

3. *Zizania* and *Leerisia* were clearly diverged from the genus *Oryza* in the gene regions of *nad*4(2)-*nad*4(1).
4. The genetic differentiation of mtDNA in all regions investigated was not obtained in present study, indicating that most regions of mitochondria genome were highly conserved in the genus *Oryza*. It is, therefore, revealed the very slow evolutionary rate of mtDNA in the genus *Oryza*.
5. In fact, only the point mutation might occur in mitochondria genome during the evolutionary process in the genus *Oryza*, the incomplete cleavage of amplified fragment in region of *nad*4-*nad*4 (Figure 2) might be an evidence for this point mutation process in this study.
6. This study on mtDNA aroused some interesting doubts for origin of *Oryza sativa*. It is likely that further studies including the new primer design and/or more powerful methods for detection of mtDNA variations, will reveal additional new insights into the genus *Oryza*.

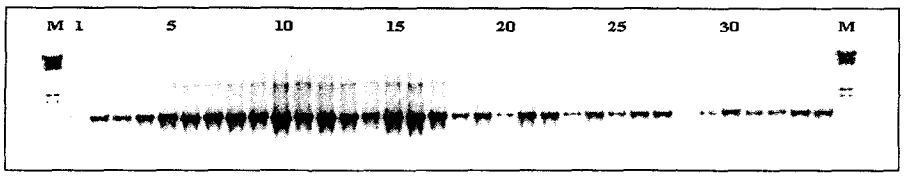

Fig1. Amplified fragment patterns of mtDNA in region of 18SrDNA-15SrRNA.
M, size marker (λ/*Hind III*) Lane 1, weak type; Lane 2, media type; Lane 5, strong type

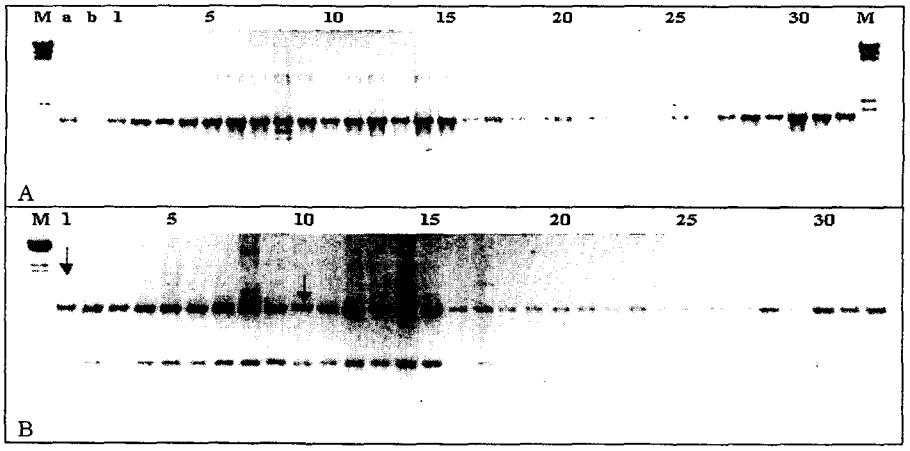

Fig 2. Length of the original undigested fragments (Fig A) and the restriction digested fragments obtained in *nad*4(2)-*nad*4(1)/*Cfo I*. (Fig B). In Figure B, lane 1 shows an example of incomplete digestion which kept the same size as the original undigested fragment. Lane 10, *Zinania latifolia*; Lane 13, *Leerisia hexandra*.