

Systematic Study of Genus *Porphyra* Using ITS Sequences

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Introduction

ITS (nuclear ribosomal DNA internal transcribed spacer sequences) and *rbcL* gene (the gene encoding a large subunit of ribulose-1,5-bisphosphate carboxylase/oxidase) have been widely used in plant molecular phylogenetics of genera, subfamilial taxa and families (Martins et al., 2003). Although it is important to gain knowledge of the genetic variation for conservation purposes, detailed information on the levels and distribution of this variation, as well as population structure, are not available for most woody taxa in Korea. Therefore, the objectives of this study were to estimate how much genetic diversity is maintained in genus *Acanthopanax* and to describe how species-specific markers, which may be useful in germ-plasm classification are distributed among species.

Materials and Methods

All of the five species were collected from Korea. The genomic DNA of the samples including outgroup was extracted from fresh leaves using the plant DNA Zol Kit (Life Technologies Inc., Grand Island, New York, U.S.A.) according to the manufacturer's protocol. Primer sets of about 20 bases in length (ITS1 and ITS4) (White et al., 1990) were used for PCR analysis. These primers were based on well-characterised DNA Sequences and were designed making use of conserved regions of the 18S and 28S rRNA genes to amplify the noncoding regions between the (ITS1 and ITS2) and 5.8S rRNA gene. After the amplification, PCR products were separated on 1.5% agarose gels and purified using the QIAquick Gel Extraction Kit

(QIAGEN). Multiple alignments of the nucleotide sequences were obtained by using the MALIGN program in the ODEN package (Ina, 1994), with manual modifications to minimize the number of gaps. The phylogenetic relationships were inferred by parsimony analysis with PAUP ver. 4.0b8 and the NEIGHBOR program in PHYLIP version 3.57, as mentioned previous.

Results and Summary

Genus *Acanthopanax* is a long-lived woody species primarily distributed throughout Asia. Many species of this genus are regarded as medically and ecologically important in the world. We evaluated a representative sample of the nine taxa with nuclear ribosomal DNA internal transcribed spacer sequences (ITS) to estimate genetic relationships within genus. As some Korean populations were isolated and patchily distributed, they exhibited a low level of genetic diversity. *Acanthopanax seoulense* was similar to *A. sessiliflorus*, while *A. rufinerve* and Chinese and Russian *Acanthopanax* taxa were more distinct. *A. senticosus* is closed related to *A. seoulense* and *A. sessiliflorus*, whereas other species (*A. koreanum*) are more diverse from the Korean accessions. The phylogenetic tree clearly distinguished three clades, the Korean, Chinese, and Russian clades. Species-specific markers, which may be useful in germ-plasm classification and agricultural process.

References

- White, T.J.T., S. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. Innis, D. Gelafand, J. Sninsky, and T. White (eds.), PCR protocols: a guide to methods and applications, 315-322. Academic Press, San Diego, California, USA.