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# Chromosomal Localization of rDNA Genes in the Korean Endemic *Lycoris flavescens* M. Kim et S. Lee and Its Related Species (Amaryllidaceae)

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## ABSTRACT

**The 18S - 5.8S - 26S ribosomal RNA gene (18S - 26S rDNA) loci were examined directly on mitotic chromosomes in the Korean endemic *Lycoris flavescens* and its related species by fluorescent in situ hybridization (FISH) method. The prominent yellow signals of biotin labeled rDNA probes were detected on three chromosomes in *Lycoris sanguinea* var. *koreana*, six in *L. flavescens*, and eight in *L. chinensis*. All of their signals were located at the distal end of rod shaped chromosomes. The other weak signals were associated with centromeric regions of metacentric chromosomes. One or two nucleolus organizing regions (NORs) were also found in all three specis. This result is reported for the first time and offers new cytological markers in *Lycoris*.**

**Key words : FISH, *Lycoris flavescens*, 18S - 26S rDNA.**

## INTRODUCTION

*Lycoris*, a small genus of Amaryllidaceae, consists of about twenty species and is distributed in moist warm temperate woodlands of China, Korea and Japan. On the basis of the morphological and cytological studies on *Lycoris*, the chromosome shape and numbers are very variable in interspecific and intraspecific population (Nishikawa *et al.*, 1979; Kurita, 1986, 1988a, 1988b, 1989; Kim and Lee, 1991; Kurita *et al.*, 1992; Hsu *et al.*, 1994; Tae and Ko, 1996). However, it is still unsolved whether a successive decrease in chromosome numbers as a result of chromosomal fusion or a gradual increase by centric fission for explaining the karyotype evolution and speciation in *Lycoris* (Nishikawa *et al.*, 1979; Kurita, 1989; Liu and Hsu, 1989; Tae and Ko, 1996). This genus is easily hybridized each other in nature and in cultivation irrespectively of their morphological and ecological differences (Nishikawa *et al.*, 1979; Kurita, 1988a, 1988b; Hsu *et al.*, 1994). The properties of hybrids can be maintained by means of vegetative propagation. *Lycoris flavescens* M. Kim et S. Lee is endemic and distributed only in the south-western regions of Korea. From the taxonomical studies on *L. flavescens*, the floral color, the waviness of tepals and its odd chromosome number strongly suggested that this species is the interspecific hybrid

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between *L. chinensis* Traub and *L. sanguinea* var. *koreana* (Nakai) Koyama (Kim and Lee, 1991) in its origin. While such studies reported useful insights on the interspecific relationships in *Lycoris*, it provided little information on actual donor species.

Recent rapid development of molecular cytogenetic techniques such as in situ hybridization have been successfully applied to reveal new insight for genomic analysis and chromosomal locations of specific and repetitive DNA sequences in many plants (Mukai *et al.*, 1991; Leitch and Heslop-Harrison, 1992; Kim *et al.*, 1993; Fukui *et al.*, 1994; Hanson *et al.*, 1996; Seo *et al.*, 1997).

In the present study, we used a fluorescent in situ hybridization analysis to determine the distribution of 18S-26S rDNA sites of natural hybrid *L. flavescens* and its related species. A variable number of hybridization loci of pTa71 probe presented the information on genome differentiation in the genus *Lycoris*.

## MATERIALS AND METHODS

### Plant Materials and Chromosome Preparations

Bulbs of *Lycoris sanguinea* var. *koreana* (Nakai) Koyama, *L. chinensis* Traub, and *L. flavescens* M. Kim et S. Lee were collected from Baekyangsa, Mt. Naejang, and Seonunsa on March 1997, respectively. Root-tips were used for in situ hybridization. Cell walls were digested in enzyme mixtures according to Fukui *et al.* (1994).

### Fluorescence in situ Hybridization (FISH)

The probe pTa71 contains 5.8S, 18S, 26S, and nontranscribed spacer sequences (Gerlach and Bedbrook, 1979), and was kindly provided by Dr. NS Kim (Kangwon National University). The probe DNA was labeled with biotin-14-dATP (Gibco BRL) by nick translation.

Slides of chromosomal spreads were treated with 100 μg/ml of DNase-free RNase (Sigma) in 2×SSC at 37℃ for 1 h, washed in 2×SSC, fixed in fixative solution for 30 min, then dehydrated in ethanol series (70%, 90%, absolute), and air dried. The chromosomal DNA on the slides was denatured by immersion in 70% formamide in 2×SSC at 70℃ for 2 min, then rapidly dehydrated in ethanol series at −20℃ (Leitch *et al.*, 1994). The hybridization mixture consists of 1 μg/ml biotinylated probe DNA, 50% deionized formamide, 10% dextran sulfate, 500 μg/ml sonicated salmon sperm in 2×SSC. This mixture was denatured at 95℃ for 10 min and quickly cooled on ice. DNA-DNA in situ hybridization was carried out overnight in a humid chamber at 37℃. After this hybridization, slides were dipped into in 2×SSC twice 5 min at 40℃, and then stringently washed in 50% deionized formamide in 2×SSC for 10 min at 42℃. Slides were washed in fresh 2×SSC at 42℃, and in 2×SSC twice 10 min at room temperature.

The biotinylated DNA probes were incubated with 5 μg/ml fluorescein avidin DN (Vector Lab.) at 37℃ for 1 h and amplified once with 25 μg/ml biotinylated anti-avidin D followed by 10 μg/ml fluorescein avidin DN as described by Leitch *et al.* (1994). Preparations were counterstained with 0.5 μg/ml of the nonspecific fluorochrome propidium iodide (PI) in PBS, pH 7.4 in the dark for 10 min, and then 2 μg/ml of the differentiating fluorochrome 4',6-diamidino-2-phenylindole (DAPI) in SDW, and then mounted in antifade solution (Vectashield, Vector Lab.).