Localization of 45S and 5S rDNA on Chromosomes of *Nigella damascena*, Ranunculaceae

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**Summary**  *Nigella damascena* has 2n=12 chromosomes composed of five long metacentric chromosome pairs and a short telocentric chromosome pair. FISH using rDNA probes was applied on the chromosomes of this species. 45S rDNA were located at the terminal region of three pairs of long metacentric chromosomes and a pair of short chromosomes. 5S rDNA appeared at the interstitial regions of the short arm of two long metacentric chromosomes.

**Key words**  Chromosome, FISH, *Nigella damascena*, 45S and 5S rDNA.

*Nigella damascena* is one of the 14 species that belong to the genus *Nigella*. It is planted in gardens all over Japan as an ornamental plant. The *Nigella* species are diploid and its genome (2n=12) has five pairs of long metacentric chromosomes and a short telocentric pair. Therefore, these species were used preferentially in early studies for the early stages of comparative karyotype analysis to study plant chromosome structures and the effects of mutagens or irradiation on chromosomes (Gregory 1941, Kurita 1956, 1959, Moutschen-Dahmen and Moutschen-Dahmen 1965, Moutschen 1968, Moutschen *et al.* 1969, Marks 1975). Previous studies showed the karyotype, localization of heterochromatin or C-bands (Marks 1975), NORs by silver staining (Hizume *et al.* 1982), and fluorescent bands by base-specific fluorochromes (Hizume *et al.* 1989). Modern methods have not been applied to the chromosomes of this species at all. The location of rRNA genes has not been reported until now. We report the locations of 45S rDNA and 5S rDNA on the chromosomes by using FISH, and compare them with previous chromosome studies.

**Materials and methods**

Seeds of *Nigella damascena* cv. Persian Jewel were obtained commercially from Sakata Seed Company. The seeds were germinated on wet filter paper in the dark at 20°C. The primary root tips were collected and treated in 0.05% colchicine for 3 h then fixed in acetic alcohol (1:3). Chromosomes were prepared by enzymatic maceration in 1% Pectolyase Y-23 and 4% Cellulase R10 at 37°C for 30–40 min, followed by air or flame drying. rDNAs were amplified by the CTAB method using genomic DNA extracted from young leaves of the species as the template. DNAs were labeled with biotin or digoxigenin and used as probes for two-color FISH. The PCR, labeling, and FISH procedures are conducted according to a previous report (Hizume *et al.* 2002). Chromosome images and FISH signal features were captured by a cooled CCD camera, and the images were processed using IPLab software.

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Results and discussion

All of the seedling roots had the same chromosome number (2n=12) and similar or the same karyotype, supporting previous reports (Fig. 1). FISH signals of 45S rDNA appeared on four pairs of chromosomes (Fig. 1A). They were localized at the terminal regions of the long metacentric chromosome pairs 2, 3, 4. The signal for chromosome 4 appeared somewhat towards the inside from the terminal region. Other 45S rDNA signal appeared at the terminal region of the short telocentric chromosome. By the silver staining method, 1–6 nucleoli with various size at interphase nucleus were stained (figure not shown, see figure of Hizume et al. 1982), and at metaphase, large NORs at the terminal region in 4–5 metacentric chromosomes and 2–1 small NORs on one or two long chromosomes were stained as found in a previous study (Hizume et al. 1980) (Fig. 1A). Because silver staining did not stain the short arm of short telocentric chromosomes, the 45S rDNA site of the telocentric chromosomes would be suppressed by unknown mechanisms or be localized at the pseudogene of 45S rDNA. To solve this problem, more investigations are needed because intraspecific variation of NOR activity might be present among individuals or populations.

FISH signals of 5S rDNA appeared at the interstitial region of the short arm of two pairs of long metacentric chromosomes 4 and 5. Chromosome 4 has both rDNAs on the short arm, and chromosome 5 only has a 5S rDNA site. The 5S rDNA sites appear as two dots on each chromatid, indicating two loci localized very close to each other (Fig. 1B).

After FISH with two rDNA probes, all chromosomes except for chromosomes 2 and 3 were easily distinguished (Fig. 1C). Applying this FISH technique to other Nigella species may produce valuable information on the chromosomal relationships among the species.

References

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