

DAPI-bands characterizing certain chromosomes in *Chloranthus japonicus*, Chloranthaceae

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**ABSTRACT:** The chromosomes of three species of *Chloranthus*, *C. japonicus*, *C. serratus*, and *C. spinulosa* were investigated by fluorescent banding method. The chromosome number of 2n=30 and the karyotypes observed in three species were supported previous studies. The karyotype of *C. japonicus* was different from that of the other two species according to the symmetry of chromosomes. To reveal mechanism of this karyotype variation a fluorescent banding using DAPI was adapted to the chromosomes of the three species. In *C. japonicus* having long metacentric chromosomes had ten bright DAPI-bands at the short arms of the subtelocentric chromosomes and numerous DAPI-bands were also observed at the terminal and proximal regions in the long metacentric and short chromosomes. The chromosomes of *C. serratus* and *C. spinulosa* showed a homogeneous DAPI fluorescence and were not stained differentially as a DAPI-band. An addition or generation of the AT-rich DAPI segments might cause karyotype change from asymmetric to symmetric long chromosomes in *Chloranthus* species. This result is discussed with taxonomical and molecular phylogenetic treatments.

**KEYWORDS:** AT-rich segment, *Chloranthus japonicus*, Chromosomes, DAPI-band

In *Chloranthus* 14 species and three species of related genus *Sarcandra* are recognized and most species distribute in East Asia. On the basis of morphology of a stamen attaching on a pistil, *Sarcandra* was separated from *Chloranthus* having stamen separated from a pistil (Li et al. 2005). The chromosome number of 2n=30 had been reported in four *Chloranthus* species including *S. glabra* (Matsuura and Suto 1935), 2n=60 was reported in *C. fortunei* (Shinagawa and Tanaka 1964) and 2n=90 in *C. henry* (Kong 2000). A basic chromosome number is accepted x=15 in this group (Okada 1995). The karyotypes were reported in nine species of *Chloranthus* and two species of *Sarcandra* (Shinagawa and Tanaka 1964, Hizume and Tanaka 1982, 1988, Kong 2000). The karyotypes are separate two groups in respect to centromere positon of eight long chromosomes in all diploid species (Hizume and Tanaka 1982, Kong 2000) and the karyotypic feature maintains in polyploid species (Shinagawa and Tanaka 1964, Kong 2000). Two karyotypes were recognized in respect to shape of larger chromosomes. *Chloranthus glabra* is put in *Sarcandra* but there is no doubt about close relation between these genera. The karyotype of *S. glabra* was very similar to several *Chloranthus* species (Hizume and Tanaka 1982, Kong 2000). In some plants chromosome segment of tandemly repeated DNA add to change chromosome morphology (Shibata et al. 2000). There is a possibility of change of chromosome morphology by addition of heterochromatin segment to chromosomes. In this report aimed to obtain an information on a mechanism of change of chromosome morphology from asymmetric to symmetric in these species, chromosomes of three species were analyzed by a banding technique with DAPI.

**MATERIALS AND METHODS**

*Chloranthus serratus* (Thunb.) Roem. et Schult. and *C. japonicus* Siebold were collected in natural habitat of Matsuyama city and *C. spinatus* (Thunb.) Makino was cultivated in our experimental garden. Root tips were collected and treated with 0.05% colchicine for 2 h. The roots were fixed and stored in acetic alcohol (1:3). After treatment with 45% acetic acid at 60°C for 8 min the root tips were squashed. The cover slip was removed from a frozen preparation and the glass slide was air-dried overnight. The preparation was soaked in McIlvaine buffer (pH7.0) for 30 min, then treated with 0.1 mg/ml actinomycin D for 15 min and rinsed with the buffer for 5 min. The glass slide was dipped into 0.1 µg/ml DAPI for 5 min, then rinsed and mounted with a mixture of the buffer and glycerin (1:1). The preparations were observed under an epifluorescence microscope with an UV filter set and fluorescence images were taken on a high sensitive film.

**RESULTS AND DISCUSSION**

All three species had 2n=30 chromosome number and their karyotypes were similar to previous reports (Hizume and Tanaka1982, 1988, Kong et al. 2000). *C. serratus* and *C. spinatus* showed asymmetric karyotype having long subtelocentric chromosomes and all chromosome arms fluoresced homogeneously after DAPI stain (Fig. 1B, C). *C. japonicus* having a symmetric karyotype composed of eight long metacentric chromosomes and several subtelocentric short chromosomes. In chromosome compliment of *C. japonicus* 10 bright and more 20 weak DAPI-bands appeared (Fig. 1A). The bright DAPI-bands located at short arm and proximal region of short subtelocentric chromosomes. The long metacentric chromosomes had large weak DAPI-band on both...
The size and brightness of DAPI-bands varied among chromosome pairs. The short chromosomes also have at end of long arm. Therefore DAPI-banding patterns were quite different between the species of symmetric karyotype and of asymmetric karyotype. DAPI-band pattern composed of bright and less bright band indicates presence of two or more kinds of DAPI-bands or AT-rich repetitive sequences in *C. japonicus*.

The DAPI-bands seem involve change of karyotype among *Chloranthus* species. The simple hypothesis of karyotype variation is that DAPI-band pattern of *C. japonicus* did not support the first hypothesis of simply addition of chromosome segment at end short arm in metacentric and. More complex phenomena might occur during karyotype change from a symmetric to symmetric.

The speculation should be examined by DAPI-banding to chromosomes of in *C. angustijiliius* and *C. fortunei* having karyotypes like *C. japonicus* (Shinagawa and Tanaka 1964, Kong 2000). If the suggestion is true, genome size should be large in the asymmetric species than a symmetric karyotype species. Unfortunately genome size was known only in *C. spicatus* (Leitch and Hanson 2002). Genome size should be analyzed later.

Molecular phylogenetic analysis using ITS and trnL-F sequences (Kong et al. 2002) revealed relationships among *S. glabra* and 10 *Chloranthus* species. *Sarcandra* and *Chloranthus* separated into sister clades. *Chloranthus* species segregated into two clades of six species and four species. *Sarcandra* and the group of six *Chloranthus* species had an asymmetric karyotype and another group of four species had a symmetric karyotype (Hizume and Tanaka 1984, 1988, Kong 2000). The molecular phylogenetic relationships conform well to the grouping of karyotype. The phenomenon indicates that in these genera an asymmetric karyotype is primitive and a symmetric karyotype is advanced. The karyotype variation might be caused by DAPI-segments. The most feasible possibility is that a certain species getting DAPI-band by unknown mechanism occurred at once and then differentiates other species of this group having a metacentric karyotype.

It is desired an identification of AT-rich repetitive DNA correspond to DAPI and a usage of the identified AT-rich repetitive DNA as a probe in a fluorescence *in situ* hybridization for a understand of chromosome structure. A Southern blot hybridization and PCR for this sequence revealed the hypothesis of karyotype variation from asymmetric to symmetric karyotypes in *Chloranthus*.

**Fig. 1.** Fluorescent chromosomes stained with DAPI in *C. japonicus* (A), *C. serratus* (B), and *C. spicatus* (C). In figure A, arrows indicate large metacentric chromosomes with DAPI-band at both ends, triangles show short subtelocentric chromosomes with bright DAPI-bands at their short arm, circles show subtelocentric chromosomes with DAPI-bands at their long arms end. Bar=10µm.

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