Chromosome banding in the genus Pinus
V. Fluorescent banding patterns in 16 diploxyln pines

Masahiro Hizume1,3, Motonobu Ara1, Yoko Yamasaki1, Satomi Fujii1, Kaoru M. Takeda1, Koizue N. Ohtaka1 and Katsuhiko Kondo2

1Faculty of Education, Ehime University, Matsuyama, Ehime 790-8577, Japan; 2Research Institute of Evolutionary Biology, Setagaya, Tokyo 158-0098, Japan
3Author for correspondence: (masahiro.hizume.mx@ehime-u.ac.jp)
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ABSTRACT: In Pinus. Subgenus Pinus 16 species were investigated on their somatic chromosomes by a fluorescent banding technique using chromomycin A3 (CMA) and 4',6-diamidino-2-phenylindole (DAPI). The chromosome number of 2n=24 was commonly counted in all the species studied. Their karyotypes were composed of many long metacentric chromosomes and a few short submetacentric chromosomes, and two karyotypes were recognized in respect to number of short chromosomes in the subgenus. CMA-bands appeared at an interstitial and/or proximal region of chromosomes and DAPI-band did at a proximal region. Thin DAPI-bands appeared at interstitial regions and DAPI-dots appeared at a centromeric region in most chromosomes. In a chromosome complement each homologous chromosome was identified on the base of its shape and fluorescent banding pattern. The typical banding patterns were compared among the species studied. Many interstitial CMA-bands at the secondary constrictions appeared on many metacentric chromosomes. Proximal fluorescent bands varied in fluorescent nature and number and divided into several groups. Section Pinus short three chromosome pairs were two or three patterns of fluorescent banding. The section Trifolae species had many proximal DAPI-bands and less proximal CMA-bands than section Pinus. The shortest chromosome had proximal bands indicating two groups on the kinds of fluorescent band.

KEYWORDS: Chromosome, Diploxyln pine, Fluorescent band, Karyotype analysis, Subgenus Pinus

Traditional cytological studies in Pinaceae have revealed same basic chromosome number of x=12 excepting for Pseudolarix and that karyotype is very stable in each genus excepting for Pseudotsuga menziesii. The karyotype of genus Pinus have many long metacentric chromosome and a few short submetacentric chromosomes, and then is a symmetric and homogeneous karyotype. Among species in respect to the short chromosomes two karyotypes were recognized in Pinus (Saylors 1972, 1983; Hizume 1988). For more detail understands of chromosomes excellent techniques need to be used. In Pinus several techniques have been applied to chromosome analyses such as observation of small constrictions, C-banding, G-banding, fluorescent banding and in situ hybridization (Tanaka and Hizume 1980; Hizume et al. 1983, 1989, 1990, 2002; Doudrick et al. 1995; Lubaretz et al. 1996; Jacobs et al. 2000; Shibata et al. 2016; Bogunić et al. 2011a,b). An effectiveness of fluorescent banding method and fluorescent in situ hybridization (FISH) using several proves is demonstrated in several pine species (Hizume et al. 1983, 1989, 1990, 2002; Bogunić et al. 2011a, b). The multi-probe FISH supplies the most information of chromosome structure in large scale and of comparative analysis of species among techniques (Hizume et al. 2002; Islam-Faridi et al. 2007; Shibata et al. 2016), but need expensive reagents and several instruments. The fluorescent band method sequentially using two base-specific fluorochromes is more easy and simple technique by just staining and supplies comparable results for FISH. Fluorescent band patterns were reported in four and eight taxa of subgenera Pinus and Strobus, respectively (Hizume et al. 1983, 1989, 1990, 2016; Bogunić et al. 2006). In more species chromosomal information is need for understand of species relationships in respect to chromosomes in a large genus Pinus. On the other hand the genome studies are progressing in several pine species of P. radiata (Kuang et al. 1999), P. thumbergii (Kondo et al. 2000), P. contorta (Li and Yeh 2001), P. elliottii (Brown et al. 2001), P. pinaster (Chagné et al. 2003), P. sylvestris (Hurn et al. 2000; Komulainen et al. 2003), P. densiflora (Kim et al. 2005), P. taeda (Echt et al. 2011; Martínez-García et al. 2013), P. lambertiana (Jermstad et al. 2011) and P. balfouriana (Friedline et al. 2015) and their genome maps are constructed by various molecular markers such as AFLPs, RFLPs, RAPDs, ESTPs, cDNA and SSRs and compared among species in respect to synteny (see Jermstad et al. 2011). In pine genome study correspondence between individual chromosomes and linkage groups of genetic map is not established in Pinus. The easy and reproducible karyotype analysis defined in each homologous chromosome is desired for pine genome study.

In this study the fluorescent banding patterns of chromosomes in 16 species of subgenus Pinus are deposited as basic information of chromosome for phylogenetic, cytogenetic and future genome study in important forest trees of Pinus.
Seeds were collected in natural stands and/or supplied from some institutions as shown in Table 1. Seeds were sterilized with 5% \( \text{H}_2\text{O}_2 \) for 10 min then rinsed in sterilized water. Then seeds were put on wet filter paper in a Petri dish or sowed in sterilized sand in a pot. About one to two weeks after sowing seeds were germinated and grew primary roots to 2-3 cm. The root tips were collected for a chromosome observation, and fixed in acetic alcohol (acetic acid : ethanol=1:3) and stored in a freezer. The chromosome preparation and fluorescent banding procedures using CMA and DAPI should be refer to previous reports (Hizume et al. 1983, 1989).

### RESULTS

All plants of 16 species belonging subgenus *Pinus* examined had \( 2n=24 \) somatic chromosomes and the chromosome number supported previous counts. Their karyotypes were composed of many long metacentric chromosomes and a few short submetacentric chromosomes, and were not found distinct differences to previous reports (cf. Saylor 1972; Hizume 1988). After fluorescent banding with CMA subsequent with DAPI staining CMA-bands appeared at interstitial regions and/or proximal regions of many chromosomes. DAPI-bands appeared at proximal region and thin DAPI-bands did at interstitial region. DAPI-dots were observed at centromeric region as previous reports (Hizume et al. 1983, 1989, 1990; Bogunić et al. 2006). Number of interstitial CMA-bands, and proximal bands of CMA and DAPI varied among species. In some species a few plants showed some variations in fluorescent band pattern but typical fluorescent band pattern of each species was analyzed. Fluorescent band pattern of each species is described briefly below.

1. *P. canariensis* The karyotype was composed of ten long metacentric chromosomes and two short submetacentric chromosomes. Interstitial CMA-bands appeared on 16 long metacentric chromosomes and their locations helped to identify homologous chromosomes (Fig. 1A). The proximal CMA-bands appeared on eight long metacentric chromosomes. Six chromosomes had CMA-bands on both regions. Two metacentric and two short chromosomes had only proximal CMA-band. Two long chromosomes had no CMA-band. The proximal region of two chromosomes with CMA-band showed parallel dots of CMA- and DAPI-bands. After DAPI-staining clear DAPI-bands appeared on proximal region of eight long metacentric chromosomes and other

### MATERIALS AND METHODS

Seeds were collected in natural stands and/or supplied from some institutions as shown in Table 1. Seeds were sterilized with 5% \( \text{H}_2\text{O}_2 \) for 10 min then rinsed in sterilized water. Then seeds were put on wet filter paper in a Petri dish or sowed in sterilized sand in a pot. About one to two weeks after sowing seeds were germinated and grew primary roots to 2-3 cm. The root tips were collected for a chromosome observation, and fixed in acetic alcohol (acetic acid : ethanol=1:3) and stored in a freezer. The chromosome preparation and fluorescent banding procedures using CMA and DAPI should be refer to previous reports (Hizume et al. 1983, 1989).
chromosomes showed a pair of DAPI-dots at centromeric regions (Fig. 1B). The short submetacentric chromosomes had an interstitial thin DAPI-band on the long arm in addition to proximal CMA-band. Many thin DAPI-bands appeared at interstitial region of nearly all chromosomes. All homologous chromosomes were identified by fluorescent banding.

2. *P. caribaea* The karyotype was composed of 22 long metacentric chromosomes and two short submetacentric
chromosomes. Interstitial CMA-bands appeared on 14 long metacentric chromosomes and weak CMA-bands did on two long chromosomes (Fig. 2A). The proximal CMA-bands appeared on six long metacentric chromosomes. Two proximal CMA-bands were larger than other bands. Four chromosomes had CMA-bands both interstitial and proximal regions. Two chromosomes with interstitial CMA-bands had thin CMA-band. The short chromosomes had no CMA-band. After DAPI-stain clear DAPI-bands appeared at proximal regions of 18 long metacentric chromosomes and two short chromosomes. Other chromosomes showed a pair of DAPI-dots at centromeric regions (Fig. 2B). Many thin DAPI-bands appeared at interstitial region of most chromosomes and were useful to chromosome identification.

3. **P. clausa**  The karyotype was composed of 22 long metacentric chromosomes and two short submetacentric chromosomes. Interstitial CMA-bands appeared on 14 long metacentric chromosomes (Fig. 3A). Four chromosomes had two CMA-bands at proximal and interstitial regions. The short submetacentric chromosomes had proximal CMA-band. The ten metacentric chromosomes had not any CMA-band. After DAPI-stain clear DAPI-bands appeared on proximal regions of 18 long metacentric chromosomes (Fig. 3B). The shortest submetacentric chromosomes had no proximal DAPI-band. Many thin DAPI-bands appeared at interstitial region of most chromosomes.

4. **P. glabra**  The karyotype was composed of 22 long metacentric chromosomes and two short submetacentric chromosomes. The CMA-bands located at interstitial region of 16 long metacentric chromosomes and at proximal region of six chromosomes (Fig. 4A). All chromosomes having proximal CMA-band had interstitial CMA-bands and four chromosomes had at both regions. Six metacentric chromosomes and two short submetacentric chromosomes had no CMA-bands. After DAPI-staining clear DAPI-bands appeared at proximal regions of 18 long metacentric chromosomes and two short submetacentric chromosomes (Fig. 4B). In a pair of chromosomes having interstitial and proximal CMA-bands the proximal DAPI-band appeared both distal sides of proximal CMA-band. Many thin DAPI-bands appeared at interstitial regions of most chromosomes.

5. **P. kesiya**  The karyotype was composed of 20 long metacentric chromosomes and four short submetacentric chromosomes. CMA-bands appeared at interstitial region of 12 long metacentric chromosomes and at proximal regions of eight chromosomes. Four metacentric chromosomes had CMA-bands in both regions and two metacentric chromosomes had no CMA-band (Fig. 5A). The short submetacentric chromosomes (No. 11) had no CMA-band and the shortest chromosomes had proximal CMA-bands. After DAPI-staining DAPI-bands appeared at proximal regions of six metacentric chromosomes and two short chromosomes (No. 11) (Fig. 5B). Many thin interstitial DAPI-bands appeared on most chromosomes. All chromosomes excepting for having proximal DAPI-bands appeared a pair of DAPI-dots at centromeric region.

6. **P. oocarpa**  The karyotype was composed of 22 long metacentric chromosomes and two short submetacentric chromosomes. Interstitial CMA-bands appeared on 14 long metacentric chromosomes and weak CMA-bands did on two long chromosomes (Fig. 2A). The proximal CMA-bands appeared on six long metacentric chromosomes. Two proximal CMA-bands were larger than other bands. Four chromosomes had CMA-bands both interstitial and proximal regions. Two chromosomes with interstitial CMA-bands had thin CMA-band. The short chromosomes had no CMA-band. After DAPI-stain clear DAPI-bands appeared at proximal regions of 18 long metacentric chromosomes and two short chromosomes. Other chromosomes showed a pair of DAPI-dots at centromeric regions (Fig. 2B). Many thin DAPI-bands appeared at interstitial region of most chromosomes and were useful to chromosome identification.
chromosomes. CMA-bands appeared at interstitial region of 14 long metacentric chromosomes and at proximal region of six long chromosomes (Fig. 6A). Two pairs of long metacentric chromosomes had CMA-bands at both regions. After DAPI-staining nearly all chromosomes appeared DAPI-bands at their proximal regions (Fig. 6B). The size of proximal DAPI-bands varied among chromosome pairs and few DAPI-bands appeared as pair of dots. Four long metacentric chromosomes having interstitial and proximal CMA-bands showed proximal DAPI-bands. Also many interstitial DAPI-bands appeared at interstitial region and their locations depending on chromosome pairs. The short submetacentric chromosomes had proximal DAPI-band and interstitial thin DAPI-band on the long arm.

7. *P. pinea*  
Karyotype of the species was composed of 22 long metacentric chromosomes and two short
submetacentric chromosomes. CMA-bands appeared at interstitial regions of 12 long chromosomes and proximal region of four chromosomes (Fig. 7A). A pair of long chromosomes had both proximal and interstitial CMA-bands. The short chromosomes had a proximal CMA-band. After DAPI-staining, proximal DAPI-bands appeared on nearly all chromosomes (Fig. 7B) and some of them might be DAPI-dots. Also many clear interstitial DAPI-bands.
Fig. 8 Fluorescent banded chromosomes of *P. ponderosa*. A: CMA-banding, B: DAPI-banding. Bar=10μm.

Fig. 9 Fluorescent banded chromosomes of *P. pungens*. A: CMA-banding, B: DAPI-banding. Bar=10μm.
appeared on most chromosome arms and their locations depending on chromosome pairs. The short submetacentric chromosomes had an interstitial thin DAPI-band on the long arm and no proximal DAPI-band.

8. *P. ponderosa* Karyotype of the species was composed of 22 long metacentric chromosomes and two short submetacentric chromosomes. CMA-bands appeared at interstitial regions of 14 long metacentric chromosomes and at proximal region of one arm in four long chromosomes (Fig. 8A). Two long chromosomes having interstitial CMA-bands have thin CMA-band on another arm. Short submetacentric chromosomes had proximal CMA-band. After DAPI-banding all chromosomes had proximal DAPI-bands varying in size (Fig. 8B). 16 CMA-bands were larger than other DAPI-bands. Six ones were DAPI-dots. Also many interstitial DAPI-bands appeared at interstitial regions and their locations depending on chromosome pair. The short submetacentric chromosomes had proximal DAPI-band and an interstitial thin DAPI-band on the long arm.

9. *P. pungens* Karyotype of the species was composed of 22 long metacentric chromosomes and two short metacentric chromosomes. CMA-bands appeared at interstitial regions of 14 long metacentric chromosomes and proximal region of one arm in ten long chromosomes (Fig. 9A). Six chromosomes had CMA-bands at both regions and short chromosomes of No. 12 had no CMA-band. After DAPI-staining the proximal DAPI-bands appeared on 18 chromosomes (Fig. 9B). Six chromosomes have weak DAPI-band or DAPI-dots at the proximal region. A pair of chromosomes having interstitial CMA-band had not any proximal band. Also several thin DAPI-bands appeared at interstitial regions and their locations were different among on chromosome pairs. The short chromosome was centromere at medium position than other species and had a proximal DAPI-band and an interstitial thin DAPI-band.

10. *P. resinosa* The karyotype was composed of 20 long metacentric chromosomes and four short submetacentric chromosomes. CMA-bands appeared at interstitial regions of 16 long metacentric chromosomes and at proximal regions of 10 long metacentric chromosomes. Six long metacentric chromosomes had CMA-bands in both regions (Fig. 10A). Two chromosomes of them had additionally two weak CMA-bands at interstitial region of both arms. Two short submetacentric chromosomes had no CMA-band and the shortest chromosomes had a proximal CMA-band. After DAPI banding DAPI-bands appeared at the proximal regions of six long chromosomes and two short...
chromosomes and faint interstitial DAPI-bands appeared on most chromosome arms (Fig. 10B). All chromosomes showed a pair of DAPI-dots at centromeric region excepting for having proximal DAPI-bands. The short chromosomes had a proximal DAPI-band and interstitial DAPI-band on long arm. The shortest chromosomes showed just centromeric DAPI-dots.

11. *P. rigida*   
Chromosome complement was composed of 22 long metacentric chromosomes and two short submetacentric chromosomes. Interstitial CMA-bands appeared on 12 long metacentric chromosomes and at proximal CMA-bands did two long chromosomes (Fig. 11A). The chromosome having CMA-bands at both regions was not observed. Six long metacentric and two
short submetacentric chromosomes had no CMA-band. After DAPI-banding clear DAPI-bands appeared at the proximal regions of 16 long metacentric chromosomes and two short submetacentric chromosomes (Fig. 11B). Four metacentric chromosomes had no proximal DAPI-band. Thin DAPI-bands appeared at the interstitial region of most chromosomes but less clear than other species. The shortest pair of submetacentric chromosomes had a proximal DAPI-band.

12. *P. roxburghii*  
Chromosome complement of the species was composed of 22 long metacentric chromosomes and two short submetacentric chromosomes. CMA-bands appeared at the interstitial region of 14 long chromosomes and at the proximal region of eight chromosomes (Fig. 12A). Four long chromosomes out of them had CMA-bands on both interstitial and proximal regions and four chromosomes had no CMA-band. Short submetacentric chromosomes had a CMA-band at a half side of proximal region. After DAPI-staining, DAPI-bands appeared at the proximal region of eight long metacentric chromosomes (Fig. 12B). Other ones had DAPI-dots at the centromeric region. Also many thin DAPI-bands appeared at interstitial regions of most chromosome arms. The short submetacentric chromosomes had the combined CMA- and DAPI-band at the proximal region and an interstitial thin DAPI-band on the long arm.

13. *P. serotina*  
The chromosome complement of the species was composed of 22 long metacentric chromosomes and two short submetacentric chromosomes. CMA-bands appeared at interstitial regions of 14 long metacentric chromosomes and proximal region of eight long chromosomes (Fig. 13A). Six chromosomes having a proximal CMA-band had also an interstitial CMA-band. Eight long chromosomes and two short chromosomes had no CMA-band. After DAPI-staining, DAPI-bands appeared at the proximal region of 18 long metacentric chromosomes (Fig. 13B). Six chromosomes with proximal CMA-band had DAPI-dots or weak DAPI-band at the proximal region. Interstitial weak DAPI-bands appeared at interstitial regions of most chromosomes. The short submetacentric chromosomes had DAPI-band at proximal region and thin DAPI-band on the long arm.

14. *P. taburaeformis*  
The karyotype was composed of 20 long metacentric chromosomes and four short submetacentric chromosomes. CMA-bands appeared at the interstitial regions of 12 long metacentric chromosomes and at proximal regions of 12 long metacentric chromosomes (Fig. 14A). Eight metacentric chromosomes had CMA-bands in both regions. All four short submetacentric chromosomes had an proximal CMA-band. Only two long chromosomes had no CMA-band. DAPI-bands appeared at the proximal regions of six long metacentric chromosomes and faint proximal DAPI-bands appeared on few chromosomes (Fig.14B). Other chromosomes had DAPI-dots at centromeric region. Many interstitial DAPI-bands appeared in all chromosomes excepting for the shortest chromosomes.

15. *P. taeda*  
A chromosome complement was composed of 22 long metacentric chromosomes and two short submetacentric chromosomes. Interstitial CMA-bands appeared on 14 long metacentric chromosomes and proximal CMA-bands appeared on ten chromosomes (Fig. 15A). The chromosomes having CMA-bands at both regions were six. Two short submetacentric chromosomes had no CMA-band. DAPI-bands appeared at proximal regions of 16 long metacentric chromosomes and two

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Fig. 13. Fluorescent banded chromosomes of *P. serotina*. A: CMA-banding, B: DAPI-banding. Bar=10μm.
Fig. 14. Fluorescent banded chromosomes of *P. tabraeformis*. A: CMA-banding, B: DAPI-banding. Bar=10μm.

Fig. 15. Fluorescent banded chromosomes of *P. taeda*. A. CMA-banding. B. DAPI-banding. Bar=10μm.
short submetacentric chromosomes (Fig. 15B). Four metacentric chromosomes had not proximal DAPI-band. Many thin DAPI-bands appeared at interstitial region of most chromosomes. The proximal regions of four long chromosomes having interstitial CMA-band showed CMA and DAPI fluorescence.

16. *P. yunnanensis* The karyotype was composed of 20 long metacentric chromosomes and four short submetacentric chromosomes. CMA-bands appeared at interstitial regions of 12 long metacentric chromosomes and at proximal regions of 12 long metacentric chromosomes (Fig. 16A). Eight metacentric chromosomes had CMA-bands in both regions. Four chromosomes had no CMA-band. All four short submetacentric chromosomes had proximal CMA-bands. DAPI-bands appeared at the proximal regions of six long metacentric chromosomes (Fig.16B). Other chromosomes had DAPI-dots at centromeric region. The longer submetacentric chromosomes show CMA-band in most plants and in some plants DAPI-band appeared at proximal region of long arm. Many interstitial DAPI-bands appeared in all chromosomes excepting for the shortest chromosomes.

The fluorescent banding pattern of each species showed some variation of mainly in interstitial CMA-bands and fluorescence of proximal bands as previous reports (Hizume et al. 1989; Bogunić et al. 2006). Intraspecific variations of fluorescent bands in each species were not described this time. In each species all or nearly all homologous chromosomes were identified by the CMA- and DAPI-band pattern but were not described in detail by limit of space. We indicate that the fluorescent banding can use for chromosome identification of homologous chromosomes in each *Pinus* species.

**DISCUSSION**

Stebbins (1971) had described that a homogeneous and symmetric karyotypes are more primitive than an asymmetric and heterogeneous karyotypes. In the Pinaceae it is well known that the genus *Pinus* has the most symmetric and homogeneous karyotype among other Pinaceae genera by many old studies (see Hizume 1988). In the *Pinus* karyotypes based on length of chromosome arms two types differing in number of short chromosomes are recognized (Saylor 1972, 1983; Hizume 1988). Species of subgenus *Strobus* and a part of species of subgenus *Pinus* has one pair of short chromosomes. The species of subgenus *Pinus*, section *Pinus*, subsection *Pinus* had two pairs of short chromosomes. This phenomenon might suggest the karyotype of subgenus *Strobus* and a part of subgenus *Pinus* is a primitive and basic one. The karyotype having two short chromosome pairs will be generated in

![Fig. 16 Fluorescent banded chromosomes of *P. yunnanensis*. A. CMA-banding. B. DAPI-banding. Bar=10μm.](image-url)
subject Pinus by unknown structural change of chromosomes and then this karyotype may be advanced and divorced recently in pine species. This speculation should be considered with taxonomic treatments (Mirov 1967; Little and Critchfield 1969; Farjon and Styles 1977; Farjon 2005) and molecular phylogeny (Price et al. 1998; Liston et al. 1999; López et al. 2002; Zhang and Li 2004; Eckert and Hall 2006; Gernandt et al. 2009; Hernández-León et al. 2013; Wang and Wang 2014).

In several pine species conventional karyotypes were reported after identification of homologous chromosomes by chromosome shape and location of secondary constrictions (e.g. Natarajan et al. 1961; Cesca and Peruzzi 2002) and C-bands (Borzan and Papê 1978; MacPherson and Filion 1981) but they seem less reproducible and difficult to compare among studies or species. The difficulty of karyotype analysis in Pinus may be caused by their large genome size (Ohri and Khosho 1986; O'Brien et al. 1996; Morse et al. 2009) or long chromosomes. For karyotype analysis in species of Pinus having large genome, long-term pretreatment of the chemicals are required to get well contract chromosomes suitable for karyotype analysis. The pretreatment operates well contraction of chromosome arms but causes to obfuscate secondary constrictions on chromosomes. This problem was solved by CMA-banding which had detected all secondary constrictions as interstitial bright CMA-bands demonstrated in several Pinus species (Hizume et al. 1983, 1989, 1990, 1992, 2016). In addition to interstitial CMA-bands at secondary constrictions, DAPI-bands also appeared at the proximal and interstitial regions of most chromosomes. The fluorescent bandings applied sequentially to supply many fluorescent bands which contribute to identification of homologous chromosomes and then comparative karyotype analysis among populations and species (Hizume et al. 1983, 1989, 1990, Bogumić et al. 2006).

In this study the fluorescence banded chromosomes were observed in 16 species of subgenus Pinus. In each species homologous chromosomes were identified in a chromosome complement but the lengthy description of banded karyotype of each species is not performed. We intend to summarize to tendency of variation in

| Section    | Subsection  | Species     | No. of interstitial CMA-bands | No. of proximal bands |
|------------|-------------|-------------|------------------------------|-----------------------|
| Pinus      | Pinus       | P. densiflora* | 7                            | 10 2                   |
|            |             | P. yunnanensis | 6                            | 8 3                    |
|            |             | P. tabuliformis | 6                            | 8 3                    |
|            |             | P. thunbergii* | 6                            | 7 4                    |
|            |             | P. luchiensis* | 6                            | 8 4                    |
|            |             | P. resinosa   | 8                            | 6 4                    |
|            |             | P. nigra*     | 8                            | 4 4                    |
|            |             | P. kesiya     | 6                            | 4 4                    |
| Pinaster   |             | P. canariensis | 8                            | 4 4                    |
|            |             | P. pinea      | 7                            | 2 6                    |
|            |             | P. roxburghii | 7                            | 4 4                    |
| Trifolae   | Contrortae  | P. clausa     | 7                            | 3 8                    |
| Ponderosae |             | P. ponderosa  | 7                            | 4 8                    |
| Australis  |             | P. caribaea   | 7                            | 3 10                   |
|            |             | P. glabra     | 8                            | 3 10                   |
|            |             | P. pungens    | 7                            | 5 9                    |
|            |             | P. serotina   | 7                            | 4 10                   |
|            |             | P. taeda      | 7                            | 5 9                    |
|            |             | P. oocarpa    | 7                            | 3 10                   |
|            |             | P. radiata    | 8                            | 2 8                    |
|            |             | P. rigida     | 6                            | 1 9                    |

* Hizume et al (1993, 1989, 1990)

Taxonomic treatment was followed Farjon (2005)
fluorescent band pattern among species and overview on diversity of fluorescent band pattern in subgenus Pinus. Previous our reports of fluorescent band pattern of diploxylon pines (Hizume et al. 1983, 1989, 1990) and observations of this time shows many interstitial CMA-bands locates at all secondary constrictions and thin DAPI-bands, and the proximal bands with CMA or DAPI in chromosome complement of each species. The alternative appearances of proximal fluorescent bands with CMA or DAPI might depend on species or species groups. This characteristic of proximal fluorescent bands is effective to demonstrate interspecific hybrids of P. thunbergii and P. densiflora (Hizume et al. 1989). This time number of interstitial CMA-bands, that of proximal CMA- and DAPI-bands and characteristics of the proximal region of easily identifiable short chromosomes are compared among species (Table 2).

Interstitial CMA-bands locating at secondary constriction containing rDNA are six to eight in karyotypes of 16 Pinus species examined as previous study (Hizume et al. 1983, 1989, 1990, 1992, Jacobs et al. 2000, Bogunić et al. 2006) and the number of CMA-bands is not found relation with any taxonomic ranks. Six species of subsection Pinus had six to ten proximal CMA-bands and other species had few (four or six) proximal CMA-bands. P. rigida, P. radiate, P. oocarpa and P. pinea had small number of proximal CMA-bands from other species. Number of proximal DAPI-bands is two to six in all 11 species of section Pinus and eight to 11 bands in all 10 species of section Trifolae (Table 2).

The karyotype having two short chromosome pairs (No. 11 and 12) appeared in species of subsection Pinus and another karyotype having one pair of short chromosomes (No. 12) did in species of section Pinus, subsection Pinaster and section Trifolae. These short chromosomes are identified distinctly and homoeologous among species, and are compared their characteristics of proximal fluorescent band. In subsection Pinus the shortest metacentric chromosome pair (No. 10) is also easily identifiable by chromosome shape and interstitial CMA- and DAPI-band pattern. The No. 12 chromosomes of subsection Pinus have proximal CMA-band in all species examined. The fluorescent band patterns of proximal region in No. 10 and 11 chromosomes varied in fluorescent banding (Table 2). In No 10 and 11 chromosomes the proximal fluorescent bands varied in base composition in subsection Pinus. P. densiflora and P. yunnanensis had proximal CMA-band, and other species of P. resinosa, P. thunbergii, P. nigra and P. kesiya had proximal DAPI-bands. P. tabulaeformis shows an intermediate type of CMA-band on No. 11 and DAPI-band on No. 11 chromosomes (Fig. 14). In some plants of P. yunnanensis the No. 10 chromosome had bands of CMA and DAPI in proximal region of each arm. These patterns might be generated by hybridization and recombination at centromeric region between chromosomes with variation of proximal fluorescent bands.

In another karyotype having a pair of the shortest chromosomes (No. 12) the chromosomes have an interstitial DAPI-band on the long arm and might correspond to No. 11 chromosomes of subsection Pinus. This karyotype appeared in several species of section Pinus, subsection Pinaster, and section Trifolae, subsection Contrortae, Ponderosae, and Australis. Three species of subsection Pinaster had CMA-band on the proximal region of No. 12 chromosomes. Nine all species of section Trifolae had the proximal DAPI-band in No. 12 chromosomes (Table 2). This might suggest relationships of species and position of P. clausa in the section Trifolae.

The results of number of interstitial CMA-bands, proximal CMA- and DAPI-band, and flourochemical nature of proximal region of short chromosome pairs are distinguished several species groups (Table 2). The grouping seems to match with a taxonomic treatment of Farjon (2005) and some molecular phylogeny (Liston et al. 1999; López et al. 2002; Zhang and Li 2004; Eckert and Hall 2006; Gerndt et al. 2009; Hernández-León et al. 2013; Wang and Wang 2014). In this observation fluorescent band patterns of chromosomes are performed in moderate number of Pinus species. More species should be analyzed on their fluorescent band patterns to clearly the correspondence of characteristics of fluorescent band patterns and taxonomic treatments, and how chromosomes structure changes according to species differentiation of genus Pinus.

The multi-probe FISH seems the most successful technique for identification of homologous chromosomes and comparative analysis in Pinus species (Hizume et al. 2002; Shibata et al. 2016). The probes used in the FISH are repetitive sequences and their FISH signals appear at fluorescent bands, and there is a limit in usable number of probes. In going genetic and genomics studies of several pine species the linkage maps are constructed and BAC clone library are established (Eckert and Hall 2006; Bautista et al. 2007). FISH using probes of BAC clones will be applied on the chromosomes landmarked with fluorescent bands and then integrated genome map between physical and genetic maps will be obtained. It is expect to understand of phylogeny in Pinus on the basis of whole genome structure and to supply basic information for improvement of pine breeding.

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