J. Plant Biol. 39(3) : 203~207 (1996)

Intraspecific Polymorphism and Classification of *Paeonia lactiflora* Based on the Giemsa C-banding Patterns

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On the basis of karyotypic analysis performed by conventional staining and Giemsa C-banding technique, cytological relationship was inferred for 21 lines of *Paeonia lactiflora* Pal. cultivated in Korea. It was very difficult to infer their organized karyotypic classification system using the composition of somatic chromosomes involving sat-chromosomes, relative length of chromosomes, arm ratio and karyotypic formulae by conventional staining. From the distribution and number of Giemsa C-bands on the chromosomes b and c, 21 lines can be subclassified into 5 groups. It seems that the karyotypic polymorphism is observed in 21 lines of cultivated *P. lactiflora* because peony mainly propagates by outbreeding.

Keywords : *Paeonia lactiflora*, Giemsa C-band, karyotypic polymorphism, outbreeding

Peony is a perennial herb belonging to genus *Paeonia*, and 33 species have been reported in this genus (Heywood, 1991; Son, 1993). *Paeonia* originated from Siberia and China is distibuted to Korea, Japan, South and Middle Europe, North America and so on (Park and Chung, 1971; Harn and Lee, 1976; Yamamoto, 1988). This plants have colorful, large and beautiful flower, so that it has been widely cultivated for ornament. Particularly, since the roots contain paeonin, paeonoflorin, albiflorin, oxypaeoniflorin and paeonol which have spasmolytic, analgesic, astrictive, antipyretic and diuretic efficacy, it has been widely used as a chinese herb medicine materials in the Orient (Kim, 1987; Leu, 1988; Park *et al.*, 1988; Chung *et al.*, 1993 and 1994). Thus it is necessary to subclassify this cultivars to select the line having more efficacious constituents.

Many authors have performed the cytological studies in genus *Paeonia* (Dark, 1936; Saunders and Stebbins, 1938; Sinoto, 1938; Walters, 1952: Harn and Lee, 1976; Nakamura and Nomoto, 1982; Lee, 1988) since karyotypic analysis was done by Miyaji (1927). Giemsa C-banding technique is very useful for identification of individual chromosome or homologous chromosomes (Kalkman, 1984; Ramachandran and Seshadri, 1986), for investigation of polymorphism of chromosome (Suzuki and Yoshimura, 1986; Miyamoto and Kurita, 1990), for recognition of the parental genomes in interspecific or intergeneric hybrids (Xu and Snape, 1988) and for elucidation of interspecific relationships (Shang *et al.*, 1989; Zheng *et al.*, 1991).

Karyotypic analysis in *Paeonia* using Giemsa C-banding method was reported by Nakamura and Nomoto (1982). In this paper, Giemsa C-banded karyotypes on 21 lines of the cultivated *Paeonia lactiflora* showing identical or very similar karyotypes by conventional staining were investigated and inferred their karyotypic classification based on the polymorphism of C-banding patterns.

MATERIALS AND METHODS

Twenty-one lines of cultivated *Paeonia lactiflora* Pal.-Dowon pyojun, Milyang 1, Milyang 3, Milyang 4, Milyang 6, Milyang 7, Milyang 8, Punggi pyojun, Punggi 6, Punggi 9, Punggi 20, Punggi 34, Sangju 2, Sangju 4, Sangju 7, Uisung pyojun, Uisung 3, Uisung 7, Ulreung 1, Wonkwang 6 and Yongchun- which have been collected and preserved for several

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years at the experimental gardens of Kyungpook Provincial Rural Development Administration and Youngnam Experimental Station were examined. Because most of them have not been classified yet, their names has been called as the geographical designation where were collected from and the number. Therefore, the same designation were quoted in this paper, representing "line" for each.

For conventional karyotype, fresh root tips were pretreated in 2 mM 8-hydroxyquinoline for 3 hours at 16°C, and followed by fixation in acetic-ethanol (1 : 3) at 4°C for overnight. Root tips were hydrolysed in 1 N HCl for 10 seconds at 60°C, and the preparation were conducted with 1% aceto-orcein by squash method.

Giemsa C-bands were obtained the following procedures. Root tips hydrolysed were squashed with a drop of 45% acetic acid, and cover slips were removed using dry ice method. The slides were air dried for about one week, treated with 10% barium hydroxide solution for 10 minutes at 50°C, washed, and then incubated in series of 2XSSC for 10 minutes at room temperature and one hour in 60°C. After rinsing, slides were stained in 3% Giemsa solution diluted with M/15 Sörensen phosphate buffer (pH 6.8) for 10 minutes.

The metaphase cells were observed about 5 cells per each line and karyotypic analysis was applied the criteria proposed by Levan *et al.* (1965). Relative length of C-band (%) was estimated as percentage of C-band length to total length of diploid chromosomes.

RESULTS

Somatic chromosome number of all lines were 2n= 10. The chromosome lengths were ranged from 8.5 to 20.3 μm showing a little deviation in the intra-lines as well as among the lines. Most of chromosomes had little difference between homologous pair, but considerable difference was observed in chromosome c in lines of Uisung 7, Milyang 1, Punggi 6 and Yongchun (not shown in Figures). Chromosome compositions in most of lines consisted of three pairs of metacentric, a pair of submetacentric and a pair of subtelocentric chromosomes. Those of Punggi 6 and Uisung 7 consisted of 5 metacentric, 3 submetacentric and a pair of subtelocentric chromosomes. All chromosomes except chromosome c had satellites at the terminal of short arm. There were polymorphisms in chromosome morphology but it was very difficult to classify the lines with only this criteria.

Table 1. Relative length (%) of C-banded region in each homologus chromosome pairs

Line	a	a'	b	b'	c	c'	d	d'	e	e'
Dowon pyojun	1.1	1.5	1.7	1.5	0.7	0.7	0.9	0.9	0.6	0.7
Milyang 1	1.2	1.4	1.6	1.8	0.8	1.4	1.0	1.1	0.5	0.6
Milyang 3	1.3	1.5	1.7	1.8	0.6	0.7	0.9	1.0	0.7	0.5
Milyang 4	1.5	1.7	1.7	1.9	0.5	0.7	1.0	1.3	0.5	0.6
Milyang 6	1.4	1.6	1.7	1.8	0.9	0.9	1.1	1.4	0.3	0.9
Milyang 7	1.1	1.3	1.8	1.8	0.5	0.5	0.9	1.3	0.4	0.7
Milyang 8	1.3	1.3	1.9	1.8	0.8	0.8	1.1	1.4	0.5	0.9
Punggi pyojun	1.3	1.5	1.7	1.8	0.9	1.0	1.1	1.2	0.7	0.8
Punggi 6	1.2	1.1	1.4	1.7	0.7	1.3	1.2	1.1	0.7	0.7
Punggi 9	1.3	1.4	1.6	1.8	0.8	0.8	1.3	1.3	0.8	0.9
Punggi 20	1.0	1.4	1.7	1.6	0.6	0.6	0.9	1.5	0.7	1.0
Punggi 34	1.5	1.4	1.8	1.7	0.5	0.6	0.8	1.2	0.3	0.6
Sangju 2	1.2	1.0	1.5	1.7	0.6	0.6	1.1	1.0	0.6	0.6
Sangju 4	1.4	1.2	1.9	1.9	0.6	0.8	1.3	1.2	0.6	0.7
Sangju 7	1.3	1.3	1.8	2.2	0.8	0.9	1.3	1.3	0.7	1.0
Uisong pyojun	1.2	1.4	1.7	1.7	0.8	0.8	1.4	1.5	0.7	0.8
Uisong 3	1.1	1.2	1.8	1.8	0.8	0.9	1.2	1.2	0.6	0.7
Uisong 7	1.0	1.3	1.8	2.2	0.7	1.3	1.1	1.3	0.6	0.7
Ulreung 1	1.4	1.4	1.8	2.2	0.8	0.9	1.0	1.1	0.7	0.8
Wonkwang 6	1.4	1.5	1.9	1.6	0.6	0.6	1.0	1.4	0.8	0.8
Yongchun	1.3	1.3	1.7	1.9	0.7	1.3	1.0	1.2	0.8	0.9

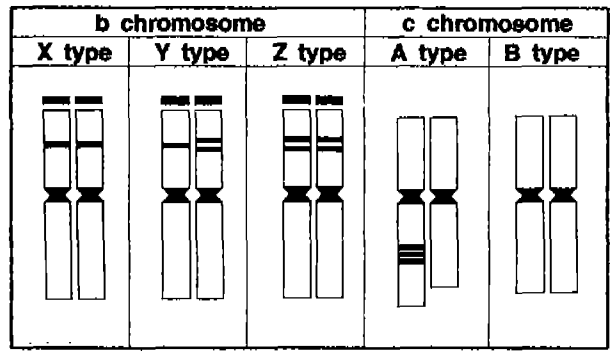

Fig. 1. Diagrammatic representation showing different C-banding patterns in homologous chromosomes b and c.

Clear C-bands were observed at the centromeric regions, satellites and interstitial region in the short arm of chromosome b in all lines (Figs. 1 and 2). C-band sizes were ranged from 0.5 to 2.7 μm showing more or less difference between homologous pairs and inter-lines. However, there was no distinguishable difference in the relative C-band lengths (Table 1). In lines of Milyang 1, Punggi 6, Uisung 7, and Yongchun, distinct C-bands were observed in the interstitial region of the long arm in only one chromosome of c (Figs. 1, 2 and Table 2).

The C-banding patterns were polymorphic among the lines in two sets of chromosomes, b and c. In chromosome b, three types of C-banding patterns

Table 2. Classification of cultivated lines of *P. lactiflora* by the C-band polymorphism of chromosome b and c

Chromosome	Type	Lines
b	X	Dowon pyojun, Milyang 1, Milyang 3, Milyang 4, Milyang 6, Milyang 8, Punggi 9, Punggi 20, Punggi 34, Uisong pyojun, Ulreung 1, Wonkwang 6, Yongchun
	Y	Punggi 6, Sangju 2, Sangju 4, Sangju 7, Uisong 7
	Z	Z Milyang 7, Punggi pyojun, Uisong 3
c	A	Milyang 1, Punggi 6, Uisong 7, Yongchun
	B	Dowon pyojun, Milyang 3, Milyang 4, Milyang 6, Milyang 7, Milyang 8, Punggi pyojun, Punggi 9,Punggi 20, Punggi 34, Sangju 2, Sangju 4, Sangju 7, Uisong pyojun, Uisong 3, Ulreung 1, Wonkwang 6

were identified according to the number of C-bands in the interstitial region of short arm (Figs. 1, 2 and Table 2). That is, one C-band were shown in homologous chromosomes (type X), one band in homolog 1 and two bands in homolog 2 (type Y), and two bands in both chromosomes (type Z). In chromosome c, two types of banding pattern were appeared according to the chromosome size, relative length of C-band and the presence or absence of C-band (Fig. 1). The lines which have difference in size between homologous chromosomes and conspicuous bands in the interstitial regions of the long arm of a large chromosome were designated as "type A". The lines belonging to "type B" have the same morphology between homologous chromosomes and no interstitial band in the long arm. The lines were classified into 5 groups depending on the type of chromosome b and c (Table 2). Thus, combining the types of chromosome b and c showing polymorphic C-banding patterns, 21 lines of cultivated *P. lactiflora* were classified into 5 groups-type I, II, III, IV and V (Fig. 2 and Table 3).

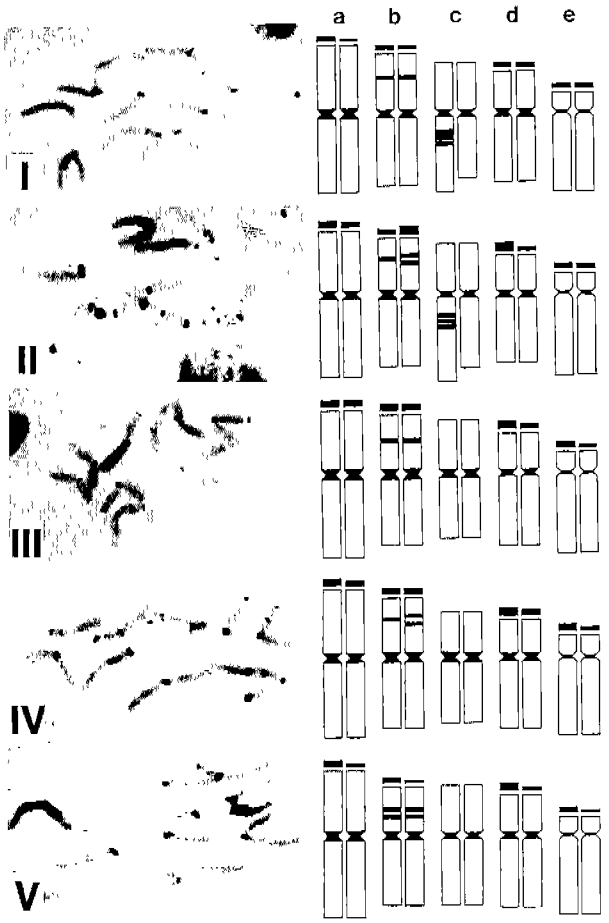

Fig. 2. C-banded metaphases and representative representation of C-banding patterns of the classified five types in *P. lactiflora*.

DISCUSSION

Harn and Lee (1976) reported that chromosome number and size are same or similar but numbers and kinds of sat-chromosomes, arm ratio and karyotypic formulae are different among the lines in *Paeonia*. In this paper, we proved the number of sat-chromosomes is eight in somatic chromosomes of all lines and C-banding patterns are heteromorphic in size and distribution. The lines of Milyang 1, Punggi 6, Uisung 7 and Yongchun showed remarkably different C-banding patterns between homologs in chromosome c. One homolog in chromosome c which has no C-band in the long arm is expected to originated from one of the other lines showing no C-band in the interstitial region of the long arm, but the origin of the other one having distinctive three adjacent bands in the long arm has not been known yet because the species which has the same C-band pattern in both homologs has not been reported. Nakamura and Nomoto (1982) reported one large C-band in the long arm of chromosome c,

Table 3. Classification of the lines of *P. lactiflora* by the combination of chromosome b and c showing polymorphic C-banding patterns

Karyotype formula	b-c chromosome type	Combined type	Lines
6m+2sm+2st	X-A	I	Milyang 1, Yongchun
5m+3sm+2st	Y-A	II	Uisong 7, Punggi 6
6m+2sm+2st	X-B	III	Dowon pyojun, Milyang 3, Milyang 4, Milyang 6, Milyang 8, Punggi 9, Punggi 20, Punggi 34, Uisong pyojun, Ulreung 1, Wonkwang 6
6m+2sm+2st	Y-B	IV	Sangju 2, Sangju 4, Sangju 7
6m+2sm+2st	Z-B	V	Milyang 7, Punggi pyojun, Uisong 3

while we identified at least three minor bands in the same region.

Twenty-one lines of *P. lactiflora* showed polymorphism according to the presence or absence, number and size of C-bands. Although all lines belong to same species, there are polymorphic in morphological characters. There are some papers that tried to combine the morphological polymorphism with C-band patterns in *Paris tetraphulla* by Suzuki and Yoshimura (1986) and Miyamoto and Kurita (1990), and in *Allium* species by Cai and Chinnappa (1987). But they could not find any correlation between morphological characters and karyotypic polymorphisms because of similar C-banding patterns. Ehrendorfer (1986) reported it is impossible to use the band as marker because most of plants have small amount of constitutive heterochromatin in the interstitial regions. Unlike these reports, C-banding patterns of chromosome b and c in this study were very distinct so that it was enough to grouping each line into five types. But it was impossible to explain the morphological differences by these karyotypic polymorphisms. It seems that various morphological and karyotypic lines can be produced outbreeding in this species.

More studies should be carried out to know the relation between morphological characters and karyotypic polymorphisms in the cultivated *P. lactiflora*.

ACKNOWLEDGEMENTS

The authors thank Kyungpook Provincial Rural Development Administration and Youngnam Crop Experimental Station for sampling of plant materials. This work was supported by grants from Provincial Rural Development Administration of Korea.

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(Received August 23, 1996)