Variation in Ploidy and Karyological Diversity in Different Herbaceous Peony Cultivar Groups

Liuhui Yang and Jianjun Zhang

College of Landscape Architecture, Beijing Forestry University, Beijing, 100083, P.R. China

Jaime A. Teixeira da Silva

P.O. Box 7, Miki-cho post office, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan

Xiaonan Yu¹

College of Landscape Architecture, Beijing Forestry University, Beijing, 100083, P.R. China; and Beijing Key Laboratory of Ornamental Plants Germplasm Innovation and Molecular Breeding, National Engineering Research Center for Floriculture, Beijing, 100083, P.R. China

ADDITIONAL INDEX WORDS. chromosome numbers, cytological data, *Paeonia*, symmetry

ABSTRACT. The karyotypes of 21 herbaceous peony (*Paeonia*) cultivars were studied using root tip squashes revealing a wide variety of ploidy levels. There were three tetraploid ($2n = 4x = 20$), 11 triploid ($2n = 3x = 15$), and one diploid ($2n = 2x = 10$) cultivars in the hybrid group; five triploid ($2n = 3x = 15$) cultivars in the Itoh group; and one diploid ($2n = 2x = 10$) cultivar in the lactiflora group (LG). The asymmetry index (AI) ranged from 59.61% ('Cytherea') to 64.03% ('Little Red Gem'). The karyotypes of all peony cultivars were 2A with 60% metacentric (m), 20% submetacentric (sm), and 20% subtelocentric (st) chromosomes. The karyotypic background of these 21 herbaceous peony cultivars is discussed in the context of the evolution of ploidy and the three cultivar groups. These results provide cytological information that would assist in a peony hybridization program.

Herbaceous peonies are perennial flowers belonging to section *Paeonia* of the genus *Paeonia* in the Paeoniaceae. Herbaceous peonies usually blossom from April to June and have a variety of flower patterns and flower colors, making them famous traditional flowers in China and offering them an extremely important position in the global cut-flower market (Yang et al., 2015; Yu et al., 2011). According to a new classification, section *Paeonia* has 25 species and more than 1000 cultivars around the world (Hong et al., 2010; Sang et al., 2004). The American Peony Society has divided herbaceous peony into three groups: lactiflora, hybrid, and Itoh. The LG uses *Paeonia lactiflora* as the parent ($2n = 2x = 10$) (Hong et al., 2010), and the hybrid group (HG) uses *P. lactiflora*, *P. officinalis*, *P. macrophylla*, and other species as the parents, whereas members of the Itoh group (IG) are the product of distant hybridization in which peony (section *Paeonia*) served as the female parent and species or cultivars of tree peony (section *Moutan*) served as the male parent (Qin, 2004; Yang and Yu, 2016).

Herbaceous peonies have rich germplasm diversity in terms of external morphology and chromosome ploidy (Hao et al., 2016; Ji et al., 2014). Species and cultivars have been used in the hybridization of different cultivar groups resulting in complex genetic backgrounds. Members of the LG are all diploid (Hong et al., 2010). The HG consists of a variety of species involved in hybridization. There are many natural tetraploids such as *P. officinalis* ($2n = 4x = 20$) and *P. macrophylla* ($2n = 4x = 20$). Cultivars of the HG usually have

either of these species as their parents, so most cultivars in this group are triploid or tetraploid (Sang et al., 2004). Cultivars of the IG have characters that are intermediate to herbaceous and tree peony (Wu et al., 2011).

Much cytological work has been carried out on *Paeonia*. Dark (1936) found that the basic chromosome number was $x = 5$. Ding and Liu (1991), Hong et al. (1988), La Cour (1952), and Yang and Zhu (1989) studied the ploidy of *P. mairei* and *P. obovata*, finding that diploid ($2n = 2x = 10$) and tetraploid ($2n = 4x = 20$) species exist in China. Hong et al. (1988) and Sang et al. (2004) found that *P. veitchii* and *P. anomala* are both diploid ($2n = 2x = 10$). According to Zhang and Shao (2000), *P. lactiflora* and *P. obovata* are diploid ($2n = 2x = 10$), and Li and Xu (1990) found that 'Huang Jin Lun' and 'He Bao', two cultivars of the LG, are diploid ($2n = 2x = 10$). Few researchers have focused on the study of different peony cultivar groups. Only Ma and Yu (2013) reported two triploid ($2n = 3x = 15$) cultivars, Buckeye Belle and Red Red Rose, which belong to the HG.

There is some research on the karyotype of peony and tree peony species or cultivars. When the arm ratio is between 0.1 and 0.5, using the ratio of the longest chromosome to the shortest one, the karyotype of a cultivar can be judged. When the ratio is less than 2:1, it is referred to as 2A, but when the ratio is between 2:1 and 4:1, it is referred to as 2B (Stebbins, 1971). The karyotype of peony is $2n = 2x = 6m + 2sm + 2st$; i.e., most peonies belong to 2A (Sang et al., 2004), whereas only Hong et al. (1988) and Liu (2016) found 2B among a population of *P. obovata* and a hybrid of 'Zhu Sha Pan' and 'Cream Delight', respectively. All tree peony species are diploid (Zhao, 2014), but Li and Zhang (1982) found that one cultivar, Shou'an Hong, is triploid ($2n = 3x = 15$). The karyotype of tree peony is usually $2n = 2x = 10 = 6m + 2sm + 2st$, $2n = 2x = 10 = 8m + 2st$, $2n = 2x = 10 = 8m + 2sm$, or $2n = 2x = 10 = 5m + 2sm + 3st$ but all are 2A (Hou et al., 2006).

Received for publication 4 Jan. 2017. Accepted for publication 15 Apr. 2017. This work was financially supported by the National Natural Science Foundation of China (31400591) and the Co-building Graduate Training and Development Program of the Beijing Municipal Commission of Education (BLCXY201615).

¹Corresponding author. E-mail: yuxiaonan626@126.com.

Table 1. Information about the group, flower color, and parents of the 21 herbaceous peony cultivars that were used in the study of variation in ploidy and karyological diversity.

Code	Group	Cultivar	Flower color	Parents
A	Lactiflora	Henry Sass	Pure white	Unknown
B	Itoh	Border Charm	Yellow	<i>Paeonia lactiflora</i> ‘Carr East’ #2 × <i>Paeonia</i> ‘Alice Harding’ (<i>P. lactiflora</i> ‘Yaso-okina’ × <i>P. lutea</i>)
C	Itoh	Going Bananas	Yellow	Unknown
D	Itoh	Old Rose Dandy	Dark yellow with dark red	Unknown
E	Itoh	Prairie Charm	Yellow	<i>P. lactiflora</i> ‘Miss America’ × <i>Paeonia</i> ‘Alice Harding’ (<i>P. lactiflora</i> ‘Yaso-okina’ × <i>P. lutea</i>)
F	Itoh	Lemon Dream	Yellow	<i>P. lactiflora</i> ‘Martha Washington’ × <i>Paeonia</i> ‘Golden Era’
G	Hybrid	Carina	Brilliant scarlet	<i>P. lactiflora</i> × <i>P. peregrina</i>
H	Hybrid	Chalice	White	<i>P. lactiflora</i> × <i>P. macrophylla</i>
I	Hybrid	Command Performance	Cardinal red	<i>P. lactiflora</i> × <i>Paeonia</i> ‘Good Cheer’ (<i>P. officinalis</i> ‘Alba Plena’ × <i>P. peregrina</i>)
J	Hybrid	Cytherea	Bright rose pink	<i>P. lactiflora</i> × <i>P. peregrina</i>
K	Hybrid	Etched Salmon	Pink	Unknown
L	Hybrid	Fairy Princess	Red	Unknown
M	Hybrid	Henry Bockstoe	Dark red	<i>P. officinalis</i> × <i>P. lactiflora</i>
N	Hybrid	Joker	Pink	Unknown
O	Hybrid	Lovely Rose	Pink	<i>P. lactiflora</i> × <i>P. peregrina</i>
P	Hybrid	Little red gem	Light red	Unknown
Q	Hybrid	Many Happy Returns	Red	<i>P. lactiflora</i> ‘Nippon Splendor’ × <i>Paeonia</i> ‘Good Cheer’ (<i>P. officinalis</i> × <i>P. peregrina</i>)
R	Hybrid	Old Faithful	Red	Unknown
S	Hybrid	Prairie Moon	Pale yellow	<i>P. lactiflora</i> ‘Laura Magnuson’ × <i>Paeonia</i> ‘Archangel’ (<i>P. lactiflora</i> × <i>P. macrophylla</i>)
T	Hybrid	Roy Pehrson’s Best Yellow	Soft yellow	Unknown
U	Hybrid	Scarlet O’Hara	Red	<i>P. officinalis</i> × <i>P. lactiflora</i>

Table 2. Classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971) and relative chromosome length of 21 herbaceous peony cultivars.

Lt/St ^z	Chromosome arm ratio > 2:1 (%) ^y				IRL ^x	IRL type ^w
	0.0	0.01–0.50	0.51–0.99	1.00		
<2:1	1A	2A	3A	4A		
2:1–4:1	1B	2B	3B	4B		
>4:1	1C	2C	3C	4C		
					≥1.26	L
					1.01 ≤ IRL ≤ 1.25	M2
					0.76 ≤ IRL ≤ 1.00	M1
					0.67 ≤ IRL ≤ 0.76	S

^zRatio of longest chromosome to shortest chromosome.

^yLong arm/short arm of each chromosome.

^xIndex of relative length.

^wL = long chromosomes, M2 = medium chromosomes 2, M1 = medium chromosomes 1, S = short chromosomes.

Karyotype analysis, which is the comparison of chromosome measurements, is a traditional cytogenetic step to compare genomes among related species. In this study, a karyotypic analysis of the chromosomes of 21 non-Chinese cultivars of herbaceous peony is reported. The characteristics and variation in chromosome number and karyotype have also been analyzed. Cytological data of these cultivars will allow better guidance of future breeding work.

Materials and Methods

PLANT MATERIALS. The herbaceous peonies used in this study (Table 1) were planted at the Xiaotangshan cultivation base,

Beijing Forestry University, National Engineer Research Center for Floriculture, Beijing, China.

METHODS. Actively growing root tips (1–3 cm) were cut and pretreated with cycloheximide for 6–8 h in the dark, fixed in Carnoy’s solution (100% ethanol:acetic acid = 3:1) for 24 h, and then stored in ethanol at 4 °C until use. Root tips were hydrolyzed in 1 M HCl at 60 °C for 10 min, washed with distilled water three times and stained by improved carbol fuchsin for 5–10 min. Karyokinetic observations were made and documented by Leica Application Suite version 3 (Leica Microsystems, Wetzlar,

Germany). All chemicals (100% ethanol, acetic acid, HCl, and carbol fuchsin) were purchased from Lanyi Co. (Beijing, China). Chromosome measurements were made using five well-spread metaphase plates per population. Karyotype analysis was performed using the criteria described by Chen et al. (2003) and Li and Zhang (1982). Karyotype symmetry was classified according to Stebbins (1971) as shown in Table 2 in this article. The AI percent (long arm length/total genome length × 100%) was determined according to the calculation defined by Arano (1963). The index of relative length [IRL (chromosome length/mean genome length)] and composition of the relative length of the genome [CRL (chromosome length/genome length)] were

calculated according to Kuo et al. (1972). The resulting classification is compiled in Table 2. Chromosome morphology was determined using arm ratio (long arm/short arm). Accordingly, chromosomes were classified as either m (1.01–1.70), sm (1.71–3.00), or st (3.01–7.00) (Levan et al., 1964). The ratio of longest to shortest chromosome (Lt/St) and centromere index percent (short arm length/chromosome length $\times$ 100%) were also calculated. Another AI to measure karyotype asymmetry proposed by Paszko (2006) was also used in this study. The AI is defined as the product of a component expressing the relative variation in chromosome length (CV_{CL}) and a component expressing the relative variation in centromeric index (CV_{CI}). Relationships between these parameters are summarized by the following equation: $AI = CV_{CL} \times CV_{CI}/100$.

Results

CHROMOSOME NUMBER OF 21 CULTIVARS. The metaphase chromosomes and karyotypes of each studied species are shown in Figs. 1 and 2, respectively. Karyotypes of the studied species are compared in Table 3. No aneuploidy was observed in any species. The basic chromosome number, which is reported for the first time for these 21 cultivars, is $x = 5$ although they have different ploidy levels. The cultivars in the LG were diploid with 10 chromosomes ($2n = 2x = 10$), the IG cultivars were triploid with 15 chromosomes ($2n = 3x = 15$), and the HG had diploid, triploid, and tetraploid cultivars. ‘Little Red Gem’ was diploid with 10 chromosomes ($2n = 2x = 10$), ‘Old Faithful’, ‘Roy Pehrson’s Best Yellow’ and ‘Scarlet O’Hara’ were tetraploids with 20 chromosomes ($2n = 4x = 20$), whereas the remaining HG cultivars were triploid with 15 chromosomes ($2n = 3x = 15$).

KARYOTYPE PARAMETERS OF 21 CULTIVARS. The karyotypes of the studied taxa are reported here for the first time. The chromosomes of all cultivars were composed of three

Fig. 1. Chromosome karyotypes of 21 peony cultivars that were used in a study of variation in ploidy and karyological diversity. The ploidy of 21 herbaceous peony cultivars can be clearly seen in each individual figure. The order of letters correspond to the letters in Table 1 (A–U); scale = 10 μ m; (A) karyotype of the lactiflora group cultivars; (B–F) karyotype of the itoh group cultivars; (G–U) karyotype of the hybrid group cultivars; (A, P) diploid karyotype; (B–O, Q, S) triploid karyotype; and (R, T, U) tetraploid karyotype.

Fig. 2. Chromosome ideograms of 21 peony cultivars that were used in the study of variation in ploidy and karyological diversity. The relative length of the short and long arm of the chromosome and classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971) are clearly shown. The order of letters corresponds to the letters in Table 1 (A–U); scale = 10 μm. x-axis: Five pairs of chromosome ideograms of 21 peony cultivars. The first, second, and third pairs of chromosome ideograms of each peony cultivar are metacentric (m), and the arm ratio of each chromosome is from 1.01 to 1.70; the fourth pair of chromosome ideograms of each peony cultivar is submetacentric (sm), and the arm ratio of each chromosome is from 1.71 to 3.00; the fifth pair of chromosome ideograms of each peony cultivar is subtelocentric (st), and the arm ratio of each chromosome is from 3.01 to 7.00. y-axis: chromosome relative length (%) of 21 peony cultivars. Zero on the y-axis represents the location of the centromere, with zero as the boundary. Greater than zero on the y-axis is the relative length (%) of the short arm of the chromosome. Less than zero on the y-axis is the relative length (%) of the long arm of the chromosome.

Table 3. Comparison of the range of six indexes of chromosomes parameters, and three classifications according to ploidy, index of relative length (IRL), and karyotype among 21 herbaceous peony cultivars numbered from A to U (Table 1) that were used in a study of variation in ploidy and karyological diversity.

Code	Cultivar	Ploidy	Relative length (%) ^z	Mean arm		IRL	Karyotype ^v	Avg centromere index (%) ^u	CV _{CL} ^t	CV _{CL} ^s	AI ^r
				ratio ^y	Lt/St ^{*s}						
A	Henry Sass	2n = 2x = 10	16.98–23.67	2.03	1.39	62.26	4M2 + 6M1	35.61	12.20	25.11	3.06
B	Border Charm	2n = 3x = 15	15.79–24.28	1.83	1.54	61.02	9M2 + 6M1	37.76	16.60	24.16	4.01
C	Going Bananas	2n = 3x = 15	15.19–24.71	1.87	1.63	61.93	9M2 + 6M1	36.86	17.17	21.69	3.72
D	Old Rose Dandy	2n = 3x = 15	15.75–24.75	1.71	1.57	61.98	6M2 + 9M1	38.99	16.02	20.90	3.35
E	Prairie Charm	2n = 3x = 15	16.04–25.11	1.88	1.57	61.71	3L + 3M2 + 9M1	37.35	15.57	22.87	3.56
F	Lemon Dream	2n = 3x = 15	14.84–25.19	2.17	1.70	62.41	9M2 + 6M1	35.89	18.03	28.94	5.22
G	Carina	2n = 3x = 15	15.83–25.27	1.90	1.60	61.60	3L + 3M2 + 9M1	37.03	17.83	25.66	4.58
H	Chalice	2n = 3x = 15	16.30–24.25	1.95	1.49	62.81	6M2 + 9M1	36.27	14.16	23.30	3.30
I	Command Performance	2n = 3x = 15	15.21–25.55	1.96	1.68	60.80	3L + 6M2 + 6M1	37.55	19.60	27.39	5.37
J	Cytherea	2n = 3x = 15	14.13–26.19	1.92	1.85	59.61	3L + 6M2 + 3M1 + 3S	38.31	20.84	29.48	6.14
K	Etched Salmon	2n = 3x = 15	15.97–23.69	1.90	1.48	61.80	6M2 + 9M1	37.22	13.47	23.21	3.13
L	Fairy Princess	2n = 3x = 15	16.22–24.72	1.88	1.52	61.57	6M2 + 9M1	37.39	15.14	24.94	3.78
M	Henry Bockstoe	2n = 3x = 15	15.81–24.87	1.94	1.57	60.75	6M2 + 9M1	37.89	16.03	27.92	4.48
N	Joker	2n = 3x = 15	16.32–24.94	1.86	1.53	61.05	6M2 + 9M1	37.74	15.39	25.31	3.90
O	Lovely Rose	2n = 3x = 15	15.39–23.00	1.78	1.49	60.05	3L + 3M2 + 6M1 + 3S	38.81	13.21	23.37	3.09
P	Little Red Gem	2n = 2x = 10	14.78–23.59	2.00	1.60	64.03	6M2 + 2M1 + 2S	34.87	16.69	19.34	3.23
Q	Many Happy Returns	2n = 3x = 15	15.07–24.62	1.82	1.63	60.59	6M2 + 6M1 + 3S	38.11	17.16	24.38	4.18
R	Old Faithful	2n = 4x = 20	14.44–25.40	1.82	1.76	60.90	4L + 4M2 + 8M1 + 4S	38.00	19.18	22.77	4.37
S	Prairie Moon	2n = 3x = 15	14.96–23.68	1.92	1.58	61.16	9M2 + 3M1 + 3S	37.47	16.95	24.73	4.19
T	Roy Pehrson's Best Yellow	2n = 4x = 20	15.46–24.63	1.86	1.59	59.96	12M2 + 8M1	38.47	17.80	26.31	4.68
U	Scarlet O'Hara	2n = 4x = 20	15.74–24.08	1.98	1.53	62.88	12M2 + 8M1	36.01	14.75	23.42	3.45

^zChromosome length/total chromosome length.

^yLong arm/short arm.

^{*}Ratio of longest chromosome to shortest chromosome.

^wClassification of karyotype in relation to their degree of asymmetry according to Stebbins (1971).

^vLong arm length/total genome length × 100.

^uShort arm length/chromosome length × 100.

^tRelative variation in chromosome length.

^sRelative variation in centromeric index.

^rIndex to measure karyotype asymmetry proposed by Paszko (2006), where AI = (CV_{CL} × CV_{CL})/100.

sections of m chromosomes, one section of sm chromosomes, and one section of st chromosomes. As Figs. 1 and 2 and Table 2 show, the IRL of the studied cultivars varied considerably. The first pair of chromosomes from 'Cytherea' (HG) had the longest relative total length of 26.29%, while that of 'Lovely Rose' (HG) had the shortest relative total length of 23.00%. With a relative total length of 16.98%, the last pair of chromosomes from 'Henry Sass' (HG) was the longest and that from 'Cytherea' (HG) was the shortest with a relative total length of 14.13% among all the studied species. The genomes of 'Henry Sass' (HG), 'Border Charm' (IG), 'Going Bananas' (IG), 'Old Rose Dandy' (IG), 'Lemon Dream' (IG), 'Chalice' (HG), 'Etched Salmon' (HG), 'Fairy Princess' (HG), 'Henry Bockstoce' (HG), 'Joker' (HG), 'Roy Pehrson's Best Yellow' (HG), and 'Scarlet O'Hara' (HG) were one of two chromosome types, a medium long chromosome (M1) or a medium short chromosome (M2). The genomes of 'Prairie Charm' (IG), 'Carina' (HG), 'Command Performance' (HG), 'Little Red Gem' (HG), 'Many Happy Returns' (HG), and 'Prairie Moon' (HG) were composed of three chromosome types: long chromosomes (L), M1, and M2. The genomes of 'Cytherea' (HG), 'Lovely Rose' (HG), and 'Old Faithful' (HG) were composed of four types of chromosomes: L, M1, M2, and short (S).

Only 2A symmetry was found, and the karyotypes of the studied species were somewhat symmetric. The AI varied from 59.61% ['Cytherea' (HG)] to 64.03% ['Little Red Gem' (HG)]. The mean arm ratio ranged from 1.71 ['Old Rose Dandy' (IG)] to 2.17 ['Lemon Dream' (IG)]. The AI values varied from 3.06 ['Henry Sass' (HG)] to 6.14 ['Cytherea' (HG)].

Discussion

Since the 1960s, studies related to chromosome number, karyotype, and genome composition have been widely used in biosystematics research and studies on the phylogenetic and evolutionary relationships among biological taxa (Yang et al., 2014). The basic chromosome number of *Paeonia* is $x = 5$, all of them being euploid (Wang, 2010). Polyploidy is an important aspect of evolutionary diversification and is one of the most important cytogenetic mechanisms in plant evolution and rapid speciation (Grant, 1981; Jian et al., 2013; Levin, 2002; Stebbins, 1971). The parent of the LG is *P. lactiflora* ($2n = 2x = 10$), so the cultivars of this group are usually diploid; e.g., 'Henry Sass'. Because there is only one triploid tree peony, 'Shou' An Hong' (Li and Zhang, 1982), due to its triploid status in the IG, the female parent may be tetraploid herbaceous peony, whereas the male parent may be diploid tree peony, but this should be confirmed in a future study. The ploidy range of the HG is very wide, with diploid, triploid, and tetraploid species having been found in this study. For example, 'Little Red Gem' is the self-cross of 'Gwenda F2', which itself is the cross of *P. tenuifolia* ($2n = 2x = 10$) and *P. mlokosewitschii* ($2n = 2x = 10$). In this study, 'Little Red Gem' was verified to be a diploid. The female parent of 'Chalice' is *P. lactiflora* ($2n = 2x = 10$), whereas its male parent is *P. macrophylla* ($2n = 4x = 20$), and 'Henry Bockstoce' is the cross of *P. officinalis* ($2n = 4x = 20$) and *P. lactiflora* ($2n = 2x = 10$), which were confirmed as triploid in this study.

Except for five cultivars of the IG, the remaining 16 cultivars are the hybrid of herbaceous peony. The parents of the IG usually belong to *P. lactiflora* and *P. lutea* (Sun, 2007). The karyotype of herbaceous peony is $2n = 2x = 10 = 6m + 2sm + 2st$,

and the karyotype of *P. lutea* is usually $2n = 2x = 10 = 6m + 2sm + 2st$, but Gong found that a Ludian population is $2n = 10 = 5m + 2sm + 3st$ (Gong et al., 1991, 1999; Hou et al., 2006). Most herbaceous and tree peonies belong to 2A (Sang et al., 2004), whereas Hong et al. (1988) and Liu (2016) found cases of 2B among a population of *P. obovata* and the hybrid of 'Zhu Sha Pan' and 'Cream Delight', respectively. In this study, however, the karyotypes of all 21 herbaceous peony cultivars were $2n = 2x = 10 = 6m + 2sm + 2st$, $2n = 3x = 15 = 9m + 3sm + 3st$, $2n = 4x = 20 = 12m + 4sm + 4st$, and 2A.

According to Stebbins (1971), the evolutionary trend of karyotypes in the plant kingdom is from symmetry to asymmetry. Thus, relatively primitive plants in systematic evolution have relatively symmetric karyotypes, whereas derivative or more advanced plants may have an asymmetric karyotype (Deng et al., 2009, 2011; Stebbins, 1971). In our study, we also used the AI index, a new AI that was developed by Paszko (2006) to give a single value that assesses karyotype asymmetry. The AI index has the advantage of allowing a high degree of precision and sensitivity to assess karyotype asymmetry, and higher values of the AI index are considered to indicate higher levels of karyotypic heterogeneity (Paszko, 2006; Zhang et al., 2013). Generally, the mean of cultivars of the HG (4.12) had higher asymmetry karyotypes than cultivars of the IG (3.97) and the LG (3.06). A cultivar of the LG, 'Henry Sass', had the lowest AI index (3.06). Unfortunately, only a single cultivar was used in the LG in this study, so additional studies on a larger pool of cultivars are needed to validate our findings. Only *P. lactiflora* is involved in the hybridization of cultivars of the LG. However, in the HG and IG, especially the former, different species or cultivars of different groups have been used in the hybridization of these cultivars, such as *P. lutea*, *P. tenuifolia*, *P. mlokosewitschii*, *P. macrophylla*, *P. officinalis*, and others. The AI index can indicate an evolutionary trend, and primitive species or cultivars usually have a lower AI index, a plant having a higher AI value would indicate that it is more advanced (Deng et al., 2011; Gao, et al., 2012; Zhang et al., 2013).

Differences among karyotypes and chromosomes can reflect genetic, morphological, and cytological diversity, as shown for studies on *Vernonia* (Angulo and Massimiliano, 2009), *Rosa odorata* (Jian et al., 2010), wild *Rosa* (Jian et al., 2013), and *Forsythia* (Shen et al., 2015), which have used karyotypes and chromosomes to reflect the genetic diversity of these species or cultivars. The karyotypes of all 21 herbaceous peony cultivars in this study are similar, but their ploidies differ. To establish a future study on evolutionary trends and genetic diversity, chromosome banding, in situ hybridization, and molecular markers should be combined to provide more useful information for a breeding program.

Literature Cited

- Angulo, M.B. and D. Massimiliano. 2009. Karyotype analysis in eight species of *Vernonia* (Vernonieae, Asteraceae) from South America. *Caryologia* 62:81–88.
- Arano, H. 1963. Cytological studies in subfamily Cardioideae (Compositae) of Japan XIII. *Bot. Mag. Tokyo* 76:419–427.
- Chen, R.Y., W.Q. Song, X.L. Li, M.X. Li, G.L. Liang, and C.B. Chen. 2003. Chromosome atlas of major economic plants genome in China (Tomus III): Chromosome atlas of garden flowering plants in China (translation). Science Press, Beijing, China (in Chinese).
- Dark, S. 1936. Meiosis in diploid and tetraploid *Paeonia* species. *Genetics* 32:353–372.

- Deng, T., Y. Meng, H. Sun, and Z.L. Nie. 2011. Chromosome counts and karyotypes in *Chaetoseris* and *Stenosoris* (Asteraceae-Cichorieae) from the Hengduan Mountains of SW China. *J. Syst. Evol.* 49:339–346.
- Deng, X.L., X.J. He, W.L. He, Y.D. Gao, H.Y. Liu, and Y.C. Zhang. 2009. Karyotype and cytogeography of the genus *Heracleum* (Apiaceae) in the Hengduan Mountains. *Plant Syst. Evol.* 47: 273–285.
- Ding, K.Y. and M.Y. Liu. 1991. Taxonomic studies on the so-called *Paeonia obvata* from northeast China. *Bull. Bot. Res.* 112:85–90 (in Chinese with English abstract).
- Gao, Y.D., S.D. Zhou, X.J. He, and J. Wan. 2012. Chromosome diversity and evolution in tribe Lilieae (Liliaceae) with emphasis on Chinese species. *Plant Res.* 125:55–69.
- Gong, X., Q.J. Gu, and Q.A. Wu. 1991. Cytological study of seven populations in *Paeonia delavayi* var. *lutea*. *Acta Bot. Yunnan* 13: 402–410 (in Chinese with English abstract).
- Gong, X., D.J. Xiao, Z.J. Gu, and Y.X. Lu. 1999. Giemsa C - banding patterns in 8 populations of *Paeonia delavayi* var. *lutea*. *Acta Bot. Yunnan* 21:477–482 (in Chinese with English abstract).
- Grant, V. 1981. *Plant speciation*. Columbia Univ. Press, New York, NY.
- Hao, L.H., H. Ma, J.A. Teixeira da Silva, and X.N. Yu. 2016. Pollen morphology of herbaceous peonies with different ploidy levels. *J. Amer. Soc. Hort. Sci.* 141:275–284.
- Hong, D.Y., K.Y. Pan, S.L. Zhou, X.Q. Wang, D.M. Zhang, Y.B. Luo, G.Y. Rao, and L.M. Ma. 2010. *Peonies of the world*. Royal Botanic Gardens Kew, Richmond, UK.
- Hong, D.Y., Z.K. Zhang, and X.Y. Zhu. 1988. The study of *Paeonia* (1) - Karyotypic study of several wild species in *Paeonia*. *J. Syst. Evol.* 26:33–43 (in Chinese with English abstract).
- Hou, X.G., C.Y. Duan, S.Y. Liu, J.X. Lv, Y.B. Zhang, and J.J. Li. 2006. Advances on chromosome study of Chinese tree peony. *Chinese Agr. Sci. Bul.* 22:307–309 (in Chinese with English abstract).
- Ji, L.J., J.A. Teixeira da Silva, J.J. Zhang, Z.J. Tang, and X.N. Yu. 2014. Development and application of 15 novel polymorphic micro-satellite markers for sect. *Paeonia* (*Paeonia* L.). *Biochem. Syst. Ecol.* 54:257–266.
- Jian, H.Y., H. Zhang, T. Zhang, S.F. Li, Q.G. Wang, H.J. Yan, X.Q. Qiu, and K.X. Tang. 2010. Karyotypes analysis of different varieties on *Rosa odorata* sweet. *J. Plant Genet. Resources* 11:457–461 (in Chinese with English abstract).
- Jian, H.Y., T. Zhang, Q.G. Wang, S.B. Li, H. Zhang, and K.X. Tang. 2013. Karyological diversity of wild *Rosa* in Yunnan, southwestern China. *Genet. Resources Crop Evol.* 60:115–127.
- Kuo, S.R., T.T. Wang, and T.C. Huang. 1972. Karyotype analysis of some Formosan gymnosperms. *Taiwania* 17:66–80.
- La Cour, L.F. 1952. Chromosome counts of species and varieties of garden plants. *J. Ann. Rpt. John Innes Hort. Inst.* 23:83–110.
- Levan, A., K. Fredga, and A.A. Sandberg. 1964. Nomenclature centromeric position on chromosomes. *Hereditas* 52:201–220.
- Levin, D.A. 2002. *The role of chromosomal change in plant evolution*. Oxford Univ. Press, New York, NY.
- Li, M.X. and X.F. Zhang. 1982. The study of cytogenetic triploid tree peony (translation). *Hereditas* 4:19–21 (in Chinese).
- Li, Y.Z. and J.L. Xu. 1990. A comparative karyotypic study of three cultivars in *Paeonia*. *J. Anhui Univ. (Natural Sci.)* 14:90–94 (in Chinese with English abstract).
- Liu, J.X. 2016. Compatibility of distant hybridization in *Paeonia* L. and identification of F1 hybrid progenies. MSc Diss., Beijing Forestry Univ., Beijing, China (in Chinese with English abstract).
- Ma, H. and X.N. Yu. 2013. Studies on karyotypes of four cultivars in herbaceous peony. *Agr. Univ. Hebei* 36:61–65 (in Chinese with English abstract).
- Paszko, B. 2006. A critical review and a new proposal of karyotype asymmetry indices. *Plant Syst. Evol.* 258:39–48.
- Qin, K.J. 2004. *Paeonia*. China Forestry Publ. House, Beijing, China (in Chinese with English abstract).
- Sang, T., J. Pan, D.M. Zhang, F. Diane, C. Wang, K.Y. Pan, and D.Y. Hong. 2004. Origins of polyploids: An example from peonies (*Paeonia*) and a model for angiosperms. *Biol. J. Linn. Soc. Lond.* 82:561–571.
- Shen, J.S., Y.J. Ye, H.T. Pan, and Q.X. Zhang. 2015. Karyotype analysis of twelve *Forsythia* species and cultivars. *Plant Genet. Resour.* 16:178–184 (in Chinese with English abstract).
- Stebbins, G.L. 1971. *Chromosomal evolution in higher plant*. Edward Arnold, London, UK.
- Sun, J.F. 2007. Preliminary report on the introduction of intersectional hybrids between tree and herbaceous peonies. *Chinese Landscape Architecture* 23:51–54 (in Chinese with English abstract).
- Wang, S.Q. 2010. Advances on chromosome study of section *Paeonia* in China. *North Hort.* 16:217–219 (in Chinese with English abstract).
- Wu, G.X., L.H. Cui, S.H. Liu, F. Yao, S.X. Si, and S.H. Ren. 2011. Preliminary studying report of cultivating and demonstrating Itoh hybrids introduced from abroad. *North. Hort.* 24:67–71 (in Chinese with English abstract).
- Yang, C.T., X. Fan, Y. Wang, L.N. Sha, H.Q. Zhang, H.Y. Kang, Q. Chen, D.D. Wu, and H. Zhou. 2014. Karyotype studies on 12 populations of *Leymus secalinus* (Georgi) Tzvelev (Poaceae: Triticeae). *Caryologia* 67:110–115.
- Yang, D.Q. and X.F. Zhu. 1989. Karyotypic studies of *Paeonia obvata*, *P. delavayi* and *P. delavayi* var. *lutea*. *Plant Diversity Evol.* 11:139–144 (in Chinese with English abstract).
- Yang, L.H. and X.N. Yu. 2016. Phylogenetic relationship analysis of different cultivars groups of herbaceous peony according morphological characteristics. *J. Plant Genet. Resources* 17:209–216 (in Chinese with English abstract).
- Yang, L.H., Y.B. Yuan, and X.N. Yu. 2015. Study on the introduction and adaptability of herbaceous peony of hybrid group, p. 168–175. In: *Advances in ornamental horticulture of China*. China Forestry Publ. House, Beijing, China (in Chinese with English abstract).
- Yu, X.N., Q.L. Yuan, and H.Z. Song. 2011. Comparison on the cultivation and application histories of herbaceous peony and its flower cultures in China and Western Countries. *J. Chinese Landscape Architecture* 27:77–81 (in Chinese with English abstract).
- Zhang, D.C. and J.Z. Shao. 2000. Karyotypes analysis of four species of *Paeonia* L. from Anhui. *J. Anhui Normal Univ. (Natural Sci.)* 23:327–330 (in Chinese with English abstract).
- Zhang, Y., M.L. Zhu, and S.L. Dai. 2013. Analysis of karyotype diversity of 40 Chinese chrysanthemum cultivars. *J. Syst. Evol.* 51:335–352.
- Zhao, N. 2014. Study on morphology and chromosome karyotype of distant hybridization progenies and their parents of tree peony. MSc Diss., Beijing Forestry Univ., Beijing, China (in Chinese with English abstract).