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ASSESSMENT OF GENETIC DIVERSITY IN 55 HERBACEOUS PEONY CULTIVARS WITH SSR MARKERS

Yongming FAN[1, 2], Liuhui YANG[1, 2], Qi WANG[1, 2], Jaime A. TEIXEIRA DA SILVA[3], Xiaonan YU[1, 2*]

[1]College of Landscape Architecture, Beijing Forestry University, Beijing, 100083, P.R. China
[2]Beijing Key Laboratory of Ornamental Plants Germplasm Innovation & Molecular Breeding, National Engineering Research Center for Floriculture, Beijing Laboratory of Urban and Rural Ecological Environment, Beijing, 100083, P.R. China
[3]Independent researcher, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan

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Using SSR, a molecular marker, this study aimed to evaluate the genetic diversity and genetic relationship among 55 herbaceous peony (*Paeonia lactiflora* Pall.) cultivars belonging to the Lactiflora, Hybrid and Itoh Groups to lay a foundation for the molecular classification of peony cultivars, as well as for genetic background analysis and breeding of new cultivars. Our results show that 86 alleles, 76 of which were polymorphic, were obtained by screening 13 pairs of polymorphic primers from 100 initially tested primers, each of them having an average of 6.615 alleles. The range of variation of the effective alleles was 2.243–7.800, PIC content was 0.554–0.872 and Shannon's genetic diversity index was 1.701–3.126. The 55 herbaceous peony cultivars were divided into nine groups based on UPGMA. This analysis indicates that the 55 cultivars have rich genetic diversity

Corresponding author: Xiaonan Yu, College of Landscape Architecture, Beijing Forestry University, Beijing, 100083, China, E-mail: yuxiaonan626@126.com

while a phylogenetic relationship of most cultivars was identified. This molecular-based classification is a useful and simple reference molecular method to differentiate peony cultivars in crossbreeding programs.

Keywords: Cluster analysis, Genetic diversity, Herbaceous peony, SSR molecular marker

INTRODUCTION

Paeonia lactiflora Pall. (*Paeonia* Sect. *Paeonia*), which belongs to the Paeoniaceae, is a perennial plant (LI, 1999; QIN, 2004). The genus *Paeonia* is divided into three sections, *Onaepia*, *Moutan* and *Paeonia*, while section *Paeonia* includes the Lactiflora, Hybrid and Itoh groups (WANG *et al*., 2014a; HONG *et al*., 2017). Herbaceous peony has a cultivation history of more than 3900 years in Chinese history and has the reputation of 'Prime Minister of flowers' (LI, 1999; QIN, 2004). Given the assortment of flower patterns and flower colors, herbaceous peonies are commonly used as plant material in landscaping while their proportion in the cut flower market has also increased annually (YU *et al*., 2011).

Although herbaceous peony has a long history of cultivation in China, its breeding status lags behind countries in Europe and the Americas (ZHANG *et al*., 2015). The cultivars are mostly of the Lactiflora group in China, and compared with the Hybrid and Itoh groups, there are certain defects in flower color, flower pattern, plant shape and adaptability (YU *et al*., 2010). In recent years, China has introduced some cultivars of herbaceous peony from countries in Europe and the Americas. Due to changes in their living environment after introduction, there may be problems such as unstable growth characteristics, and since most cultivars were bred by horticultural plant enthusiasts, many cultivars have been used for crossbreeding of different cultivar groups resulting in complex genetic backgrounds (HONG *et al*., 2017). Hybridization between cultivars reduced the genetic differences between them and it is increasingly difficult to identify cultivars based exclusively on their morphology (ZHANG *et al*., 2015). At the same time, due to the poor reliability of morphological identification, cultivars are easily affected by environmental and subjective factors, which may lead to more problems underlying species confusion (YU *et al*., 2012). Over time, parents of most cultivars often go missing or may even be incorrect, and their genetic background and kinship are often unclear, which seriously restricts their application and breeding value (JI *et al*., 2013).

Molecular marker technology can be applied to the study of genetic diversity and genetic relationship in plants (PENG *et al*., 2017). Simple sequence repeats (SSRs) are one type of molecular marker that is based on PCR, using specific primers, also known as microsatellite DNA. SSRs have repeating sequences consisting of several nucleotides (generally 1 to 6) as repeating units (LITT *et al*., 1989). The sequences flanking each SSR are generally relatively conserved single copy sequences, usually present in the protein-coding and non-coding regions of the genome (KALIA *et al*., 2011; LI *et al*., 2011). At present, molecular markers in *Paeonia* research have been used extensively in an applied form to study the scope of molecular genetic diversity (SUN *et al*., 2011), the classification and identification of species, cultivars and hybrids (LI *et al*., 2018), the origin and relationship of cultivated germplasm (LI *et al*., 2015), and the construction of genetic linkage maps (GUO *et al*., 2017b).

In *Paeonia* L., JI *et al*. (2014) used 15 polymorphic SSR markers developed by magnetic bead enrichment to analyze the genetic diversity and genetic relationships of 89 herbaceous

peony cultivars, and found that both cluster analysis and principal component analysis based on 15 polymorphic primer pairs were able to reflect the genetic relationships between all individuals. GUO *et al*. (2017a) detected a total of 8663 SSRs by means of a microsatellite search of unigene sequences identified from the *de novo* assembly of sequence data from different genotypes of tree peony (*Paeonia suffruticosa*) from the Zhongyuan group. Among 100 randomly selected SSR markers, 25 were successfully amplified and showed polymorphism in 31 tree peony accessions. WAN *et al*. (2018) used 21 pairs of primers to establish molecular identifiers of 268 herbaceous peony cultivars by SSR markers, which provided assistance for the identification of herbaceous peony cultivars, breeding of new cultivars and resource conservation.

In this study, 55 herbaceous peony cultivars belonging to Lactiflora, Hybrid and Itoh groups were used as research material. A world-leading society on peonies, the American Peony Society (https://americanpeonysociety.org

), doubts the accuracy of parental information of herbaceous peonies introduced to China from Europe and the Americas. Consequently, we decided in this study to use SSRs to analyze the genetic diversity of 55 cultivars with an unclear genetic background and to explore their genetic backgrounds and relationships as a way to resolve this genetic material. Using SSR, we were able not only to effectively differentiate the 55 cultivars, but could also group them phylogenetically. These findings provide a simple yet useful molecular tool to classify herbaceous peony cultivars from countries in Europe and the Americas, and to resolve their genetic background that would allow their confident use, either in the cultivation of new cultivars, or as material in breeding programs.

MATERIALS AND METHODS

Plant material and DNA extraction

The peony cultivars that were tested were planted in the research field of the Germplasm Resources Nursery of the National Engineering Research Center for Floriculture (116°39′E, 40°17′N; Beijing, China). Their number, name and group are listed in Table 1. From October to November, three plants of each cultivar were randomly selected, and one underground bud from each plant was sampled. After mixing buds in a plastic bag, samples were brought back to the laboratory in an ice box with silica gel to keep samples cool and dry, then stored in a refrigerator at -80°C.

Total DNA was extracted from herbaceous peony underground buds using DNAsecure Plant Kit (TianGen Biotech Co. Ltd., Beijing, China). The extracted DNA samples were detected by 1% agarose gel electrophoresis, and the integrity of the DNA and the clarity of the electrophoresis bands were observed on an ultraviolet gel imager (ChemiDoc MP, Bio-Rad Laboratories Inc., Hercules, CA, USA). The DNA molecular weight standard marker used was purchased from Beijing Aileide Biotechnology Co., Ltd. (Beijing, China). For each DNA sample, 1 μL was used to measure the $A_{260/280}$ and $A_{260/230}$ values, DNA concentration and quality by a Thermo Scientific Nanodrop 2000 micro-ultraviolet spectrophotometer. Qualified DNA samples were diluted to 20 ng/μL and stored at -20°C until use.

Table 1. Information pertaining to the 55 herbaceous peony (Paeonia lactiflora) cultivars used in this research

No.	Cultivars	Group	No.	Cultivars	Group
1	'Dafugui'	Lactiflora group	29	'Chalice'	Hybrid group
2	'Dongfanghong'	Lactiflora group	30	'Etched Salmon'	Hybrid group
3	'Duoyezi'	Lactiflora group	31	'Garden Peace'	Hybrid group
4	'Fenyunu'	Lactiflora group	32	'Charlie's White'	Hybrid group
5	'Guifeichacui'	Lactiflora group	33	'Lovely Rose'	Hybrid group
6	'Hongpantuojin'	Lactiflora group	34	'Red Charm'	Hybrid group
7	'Hongyanzhenghui'	Lactiflora group	35	'Laddie'	Hybrid group
8	'Liantai'	Lactiflora group	36	'May Lilac'	Hybrid group
9	'Qihuanushuang'	Lactiflora group	37	'Coral Sunset'	Hybrid group
10	'Qingwen'	Lactiflora group	38	'Command Performance'	Hybrid group
11	'Shengtaohua'	Lactiflora group	39	'Fairy Princess'	Hybrid group
12	'Tianshanhongxing'	Lactiflora group	40	'Cytherea'	Hybrid group
13	'Tuanyehong'	Lactiflora group	41	'Buckeye Belle'	Hybrid group
14	'Zhongshengfen'	Lactiflora group	42	'John Havrard'	Hybrid group
15	'Zhushapan'	Lactiflora group	43	'Prairie Moon'	Hybrid group
16	'Zifengchaoyang'	Lactiflora group	44	'Going Bananas'	Itoh group
17	'Zifengyu'	Lactiflora group	45	'Lemon Dream'	Itoh group
18	'Duchesse de Nemours'	Lactiflora group	46	'Old Rose Dandy'	Itoh group
18	'Gold Mine'	Lactiflora group	47	'Prairie Charm'	Itoh group
20	'Henry Sass'	Lactiflora group	48	'Border Charm'	Itoh group
21	'Kansas'	Lactiflora group	49	'Little Red Gem'	Hybrid group
22	'Karl Rosenfield'	Lactiflora group	50	'White Innocence'	Hybrid group
23	'Monsieur Jules Elie'	Lactiflora group	51	'Athena'	Hybrid group
24	'Red Magic'	Lactiflora group	52	'Old Faithful'	Hybrid group
25	'Taff'	Lactiflora group	53	'Cream Delight'	Hybrid group
26	'Joker'	Hybrid group	54	'Roy Pehrson's Best Yellow'	Hybrid group
27	'Carina'	Hybrid group	55	'Scarlett O'Hara'	Hybrid group
28	'Many Happy Returns'	Hybrid group			

Primer selection and labelling

Using 'Dafugui', 'Zifengchaoyang', 'Cytherea', 'Command Performance', 'Lemon Dream', 'Old Rose Dandy', 'Old Faithful' and 'Cream Delight' as templates, PCR products were detected by polyacrylamide gel electrophoresis to screen polymorphic primers. The 5' end of the primer was fluorescently modified and sorted according to the approximate size of the target fragment, and each of the two pairs was labeled with FAM and HEX fluorescent probe labeling groups, respectively, and synthesized by Beijing Ruibo Xingke Biotechnology Co. Ltd. (Beijing, China).

SSR-PCR amplification and capillary electrophoresis

The 10 μL reaction system in this experiment was as follows: template DNA: 30-40 ng, 2×Taq PCR Master Mix (Beijing Aidelai Biotechnology Co. Ltd., Beijing, China) 5 μL, upstream primer 0.5 μL, downstream primer 0.5 μL, ddH_2O 3 μL. The PCR amplification procedure was as follows: pre-denaturation at 94°C for 4 min; denaturation at 94°C for 30 s, annealing for 30 s, extension at 72°C for 30 s, 30 cycles; extension at 72°C for 5 min; storage at 4°C.

The PCR product was detected by 1% agarose gel electrophoresis. After the quality of the PCR products were tested, the ABI3730 DNA automatic sequencer was used for short tandem repeat (STR) detection and analysis. Detection and analysis were completed by Beijing Ruibo

Xingke Biotechnology Co. Ltd.

Data analysis

Using GeneMarker 2.2.0 software (SoftGenetics, State College, PA, USA), fluorescence detection data was interpreted and manually judged based on a unified standard (Chen et al., 2017). The allele locus amplified by each pair of primers was interpreted as: "1" indicates that the sample has a band at the allele locus, and "0" indicates that the sample has no band at the allele locus, forming a "0, 1" matrix.

Polymorphism data analysis involved four steps: (1) Polymorphism ratio P=(k/n)×100%; (2) The number of effective alleles $Ne=1/\sum Pi^2$, where Pi is the frequency at which the i-th polymorphic site of each pair of primers appears; (3) Polymorphism information content $PIC=1-\sum Pi^2$; (4) Shannon's diversity index H'=-∑PilnPi (GAO, 2010).

Using UPGMA (Unweighted Pair Group Method with Arithmetic Mean), cluster analysis of the 55 cultivars was performed using NTSYS 2.10 (ROHLF, 1997; ZHAO *et al*., 2008).

RESULTS

Analysis of DNA concentration and purity

The DNA bands of each herbaceous peony cultivar in this study were clear and non-degraded. The purity, concentration and quality of the extracted DNA can meeting the requirements of the next PCR analysis SSR primer screening.

SSR primer screening

Using 'Dafugui', 'Zifengchaoyang', 'Cytherea', 'Command Performance', 'Lemon Dream', 'Old Rose Dandy', 'Old Faithful' and 'Cream Delight' as templates, 13 pairs of polymorphic primers were screened from 100 pairs of primers, and the ratio of polymorphic primers was 13%. Table 2 shows the screening of 13 pairs of polymorphic primer information, and Figure 1 shows the PAGE diagram of partial primer amplification.

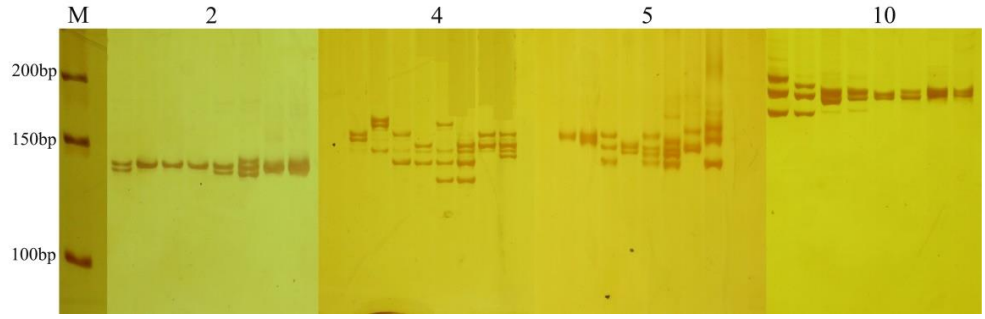

Fig. 1 PCR amplification results of primers 2, 4, 5 and 10. M: DNA marker, 2: primer A2, 4: primer A4, 5: primer A5, 10: primer A10 (details of primers can be found in Table 2).

Table 2. The 13 SSR primers used to assess the 55 herbaceous peony (Paeonia lactiflora) cultivars used in this research

Primers	Primer sequence: 5′-3′	Repeat motif
A1	F: GGGTGCTGAAGGATGTGTTT	$(GA)_9$
	R: AATAACATTCCCATGCCCAA	
A2	F: CGGAAATAGACAAGGTGGGA	$(TCA)_7$
	R: TGATCTTGGTTGTGGGTTCA	
A3	F: TTTCAAAGTACGCCCATCAA	$(CT)_9$
	R: CGGGCAAGTAGTTGGAATGT	
A4	F: ACGCAATTGAAATCCAATCC	$(CTG)_7$
	R: GGTTCTTCTCCGTAGGGACC	
A6	F: TAAGGTATTGGTGGCGGAAG	$(AGC)_7$
	R: ATTGATCCAACCCCAAATGA	
A7	F: TGACGCAGATGAAGAGGATG	$(CT)_9$
	R: TTTAAACCAAACGGCCAAAC	
A9	F: GGTGGCACTTTTGGTTTTGT	$(AG)_9$
	R: ACCCATTCAGCCAACACTTC	
A10	F: TTTGCAATCGCATAGAGGAC	$(GA)_9$
	R: TCCGAGAATATCGAAATGGC	
A11	F: CTCAAGCTTCCTGCCTCTGT	$(TC)_{10}$
	R: CTCCAAAACCCACCTTTCAA	
A13	F: ACATCAAGACAGTGAGGGGG	$(TC)_9$
	R: AAGAATTTTGCGAGCGAGAA	
A14	F: GGGATGAAGACGAACCACAT	$(TGA)_7$
	R: GAACCATTGCCAGACTGGAT	
A15	F: TGCCCAATAAAATCTGAGGG	$(GA)_{10}$
	R: TATTGCGGTAGGTTTGCCTT	
A16	F: GCTTCGTGTTGAGTGGACAA	$(TGG)_7$
	R: TTGTACCCTCCAATGCCCTA	

F, forward; R, reverse

Fluorescent primer detection and analysis

A total of 55 pairs of fluorescent primers were synthesized and amplified by capillary electrophoresis. The results showed that a clear band was amplified by capillary electrophoresis (Figure 2). This indicates that the 13 pairs of polymorphic markers can be used to evaluate the genetic diversity of 55 cultivars.

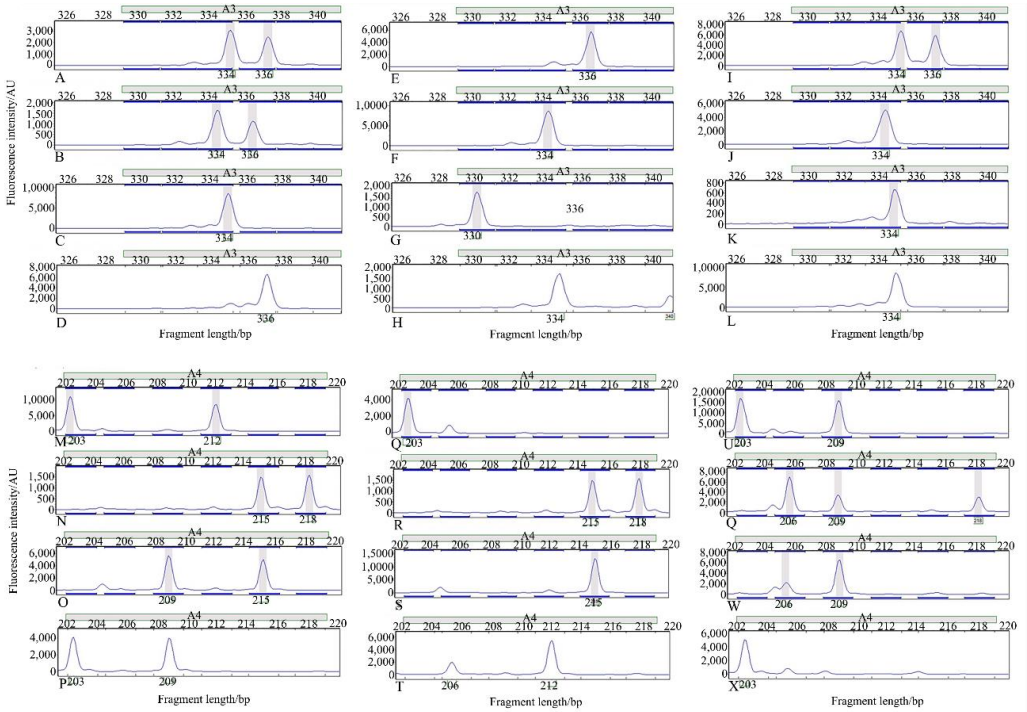

Figure 2. Fluorescence detection of primer A3 and A4 in several herbaceous peony cultivars (A~L: Fluorescence detection of primer A3 in the cultivars of 'Kansas', 'Karl Rosenfield', 'Monsieur Jules Elie', 'Red Magic', 'Taff', 'Joker', 'Carina' and 'Many Happy Returns'; M~X: Fluorescence detection of primer A4 in the cultivars of 'Kansas', 'Karl Rosenfield', 'Monsieur Jules Elie', 'Red Magic', 'Taff', 'Joker', 'Carina' and 'Many Happy Returns').

Genetic diversity analysis based on SSR markers

Thirteen pairs of polymorphic primers were applied to 55 herbaceous peony cultivars belonging to three different cultivar groups. The results of primer diversity parameters are shown in Table 3. The amplification products of 13 pairs of primers in 55 samples ranged from 156 bp to 362 bp; 86 alleles has been detected, including 76 polymorphic alleles. The polymorphic allele locus rate was 88.37%. A2 had the least number of alleles, and only 4 alleles were amplified, while 9 alleles were amplified by A9, A14 and A15. The average number of polymorphic loci was 5.8. Among the 13 pairs of polymorphic primers, A9 had the most effective alleles, with an average of 7.800, and A2 had the least number of effective alleles (average = 2.243). The range of PIC varied from 0.554 to 0.872 with an average of 0.764. A2 had the lowest PIC value (0.554) while A9 had the highest value (0.872). The average PIC value was 0.764, and the PIC values were all above 0.5. Shannon's genetic diversity index ranged from 1.701 to 3.126 with an average of 2.526, with A9 being the highest (3.126) and A2 being the lowest (1.701).

Table 3 Genetic diversity parameters associated with the SSR loci

Locus name	*Na*	Polymorphic alleles (#)	Polymorphic loci (%)	*Ne*	PIC	Shannon's Diversity Index
A1	8	8	1	7.088	0.859	2.868
A2	4	3	0.75	2.243	0.554	1.701
A3	5	4	0.8	3.449	0.710	2.086
A4	6	6	1	5.524	0.819	2.558
A6	6	4	0.667	3.266	0.694	2.217
A7	6	6	1	5.864	0.829	2.766
A9	9	9	0.884	7.800	0.872	3.126
A10	7	6	0.803	5.088	0.803	2.671
A11	5	4	0.8	3.374	0.704	2.072
A13	6	5	0.833	4.364	0.771	2.536
A14	9	8	0.889	6.750	0.852	3.074
A15	9	7	0.778	6.308	0.841	2.676
A16	6	5	0.883	2.651	0.623	2.483
Average	6.615	5.769	0.853	4.905	0.764	2.526

Genetic distance and cluster analysis between cultivars

Based on the SSR data, UPGMA clustering was performed on 55 peony cultivars using NTSYS 2.10 software. The results are shown in Figure 3 (information about the cultivars' parents indicated next in this section was obtained from The American Peony Society). With a genetic similarity coefficient of 0.764, the 55 cultivars were divided into 9 groups using 13 primer pairs.

Group A is composed of 25 cultivars, mainly from the Lactiflora group: 'Qihuanushuang', 'Dongfanghong', 'Zifengchaoyang', 'Dafugui', 'Qingwen', 'Zifengyu', 'Hongyanzhenghui', 'Shengtaohua', 'Duoyezi', 'Tuanyehong', 'Hongpantuojin', 'Fenyunu', 'Zhongshengfen', 'Zhushapan', 'Tianshanhongxing', 'Guifeichuyu', 'Liantai', 'Gold Mine', 'Taff', 'Monsieur Jules Elie', 'Duchesse de Nemrous', 'Henry Sass', 'Red Magic', 'Karl Rosenfield' and 'Kansas'. The parents of the cultivars 'Gold Mine', 'Taff', 'Monsieur Jules Elie', 'Duchesse de Nemrous', 'Henry Sass', 'Red Magic', 'Karl Rosenfield' and 'Kansas' introduced from Europe and the Americas may have the participation of *P. lactiflora*.

Group B includes five cultivars ('Carina', 'Cytherea', 'Lovely Rose', 'Command Performance' and 'Coral Sunset'), of which 'Carina', 'Cytherea', 'Lovely Rose' and 'Coral Sunset' were obtained by crossing *P. lactiflora* as the female parent. The male parent of both 'Cytherea' and 'Carina' is *P. peregrina*, and the male parent of 'Coral Sunset' is *P. officinalis* 'Otto Froebel'. The genetic background of 'Command Performance' is not clear.

Group C consists of two cultivars, 'Joker' and 'Laddy'. Their parents are not known.

Group D consists of five cultivars ('Chalice', 'May Lilac', 'Etched Salmon', 'Red Charm' and 'Garden Peace'). Among them, 'Chalice', 'May Lilac' and 'Garden Peace' were obtained by *P. lactiflora* × *P. macrophylla*, 'Red Charm' was obtained by *P. officinalis* × *P. lactiflora*. The genetic background of 'Etched Salmon' is unknown, but its morphology has certain similarities with other members of the same group.

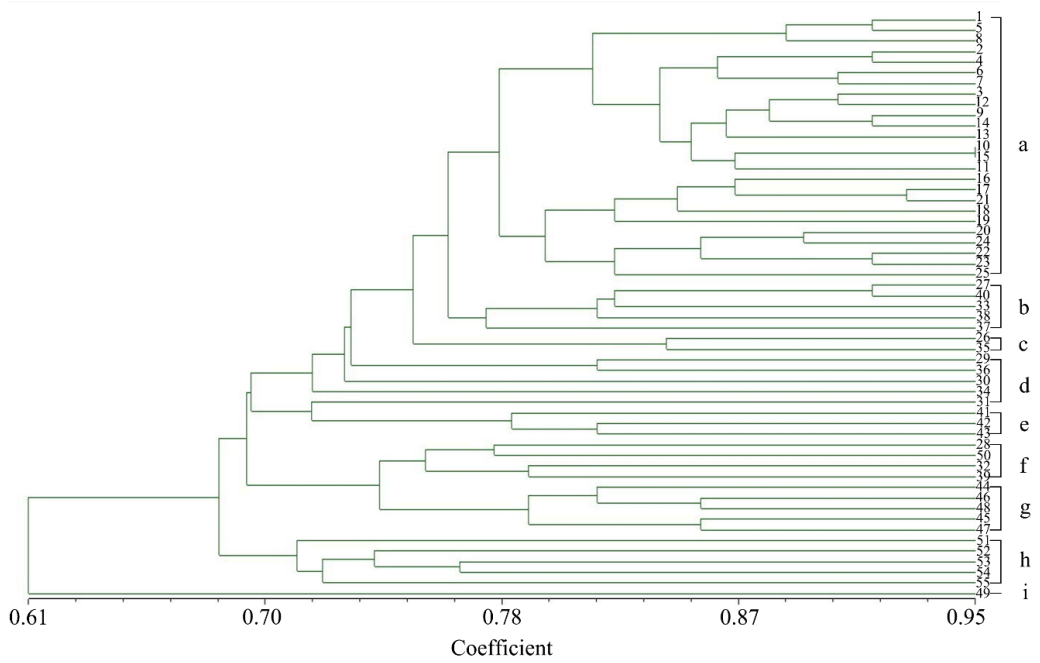

Figure 3. Dendrogram showing the phylogenetic relationship between 55 herbaceous peony (*Paeonia lactiflora*) cultivars. The analysis was performed using UPGMA. Letters A-I indicate the nine groups into which the 55 cultivars could be separated.

Group E consists of three cultivars (‘Buckeye Belle’, ‘John Harvard’ and ‘Prairie Moon’). The parents of ‘Buckeye Belle’ and ‘John Harvard’ had the participation of *P. officinalis* and ‘Prairie Moon’ was obtained by *P. lactiflora* × *P. macrophylla*.

Group F consists of four cultivars, ‘Many Happy Returns’, ‘White Innocence’, ‘Charlie's White’, and ‘Fairy Princess’. Among them, the female parents of ‘Many Happy Returns’ and ‘Charlie's White’ had the participation of *P. lactiflora*, but the male parents of ‘Many Happy Returns’ and ‘Charlie's White’ are not clear. ‘White Innocence’ is a hybrid of *P. lactiflora* and *P. emodi*, and the genetic background of ‘Fairy Princess’ is unknown.

Group G includes ‘Going Bananas’, ‘Old Rose Dandy’, ‘Border Charm’, ‘Lemon Dream’ and ‘Prairie Charm’, all of which were obtained by distant crossing between *P. lactiflora* and *P. suffruticosa,* belonging to the Itoh group.

Group H consists of ‘Athena’, ‘Old Faithful’, ‘Cream Delight’, ‘Roy Pehrson's Best Yellow’ and ‘Scarlett O'Hara’. Among them, ‘Scarlett O'Hara’ was obtained by *P. officinalis* × *P. lactiflora*, and the parents of ‘Athena’ had the participation of *P. lactiflora, officinalis* or *P. macrophylla*. Although the parents of ‘Cream Delight’, ‘Old Faithful’ and ‘Roy Pehrson's Best Yellow’ are not clear, they are tetraploid (YANG *et al*., 2017). Tetraploid parents such as *P. officinalis* may be involved in the hybridization process.

In Group I there is only one cultivar, 'Little Red Gem', whose male parent is *P. tenuifolia*. Thus, nine groups reflected the genetic relationship between the 55 herbaceous peony cultivars. The genetic backgrounds of 'Command Performance', 'Etched Salmon', and 'Fairy Princess' are unknown, but according to the clustering results, the parents of 'Command Performance' may be cultivars belonging to *P. peregrina*, *P. lactiflora* or cultivars closely related to them. 'Etched Salmon' parents may have involved *P. albiflora* or *P. macrophylla*. It is hard to speculate about the parents of 'Joker', 'Laddy' and 'Fairy Princess'.

DISCUSSION

With the development of molecular marker technology, some scholars have carried out genetic diversity analysis and identification of phylogenetic relationships among different cultivar groups of *Paeonia* and different geographic origins (WANG *et al*., 2014b; XU *et al*., 2016; LIU *et al*., 2017). SSR molecular marker technology is widely used in the study of *Paeonia* by virtue of its rich polymorphism, high reproducibility, and ease of operation (CAI *et al*., 2016; WAN *et al*., 2020). It is a useful molecular marker for studies of genetic diversity and genetic relationships among *Paeonia* (WU *et al*., 2014; ZHANG *et al*., 2018; FAN *et al*., 2020).

In this study, 13 pairs of primers were used to assess genetic diversity in the 55 cultivars. A total of 86 alleles were detected, and 88.37% of the loci were polymorphic. The PIC value ranged from 0.554 to 0.872, and Shannon's diversity index ranged from 1.701 to3.126. There is a slight difference in the genetic diversity of different *Paeonia* cultivar groups. JI *et al*. (2014) analyzed the genetic diversity of 89 herbaceous peonies and the PIC value ranged from 0.362 to 0.825 while Shannon's diversity index ranged from 1.082 to2.194. ZHANG *et al*. (2016) analyzed the genetic diversity of 261 herbaceous peonies belonging to the Lactiflora group in which the PIC value ranged from 0.188 to 0.846 and Shannon's diversity index ranged from 2.201 to 0.387. These findings suggest that there are differences in the genetic diversity of different herbaceous peony cultivars, but that the results may differ depending on the primers used.

JI *et al*. (2013) analyzed the genetic diversity of 89 European and American herbaceous peony cultivars and some important parents, dividing them into two categories: wild species and cultivars. The cultivars were divided into six groups according to their provenances. ZHANG (2016) divided 261 domestic cultivars into two groups of wild herbaceous peony and cultivars by SSR polymorphism, indicating a distant relationship between wild herbaceous peonies and cultivars. This study focused on the genetic diversity of cultivars from the Lactiflora, Hybrid and Itoh groups and the genetic background of 38 European and American cultivars. According to our clustering results, 55 herbaceous peonies were divided into nine groups A-I. The 25 cultivars of the Lactiflora group were clustered into a single group. Other cultivars with *P. lactiflora* as the female parent, such as 'Cytherea', have a close genetic distance with the Lactiflora group, and most cultivars of different groups originate from different species. Since the cultivars of the Itoh group in this study are triploid (YANG *et al*., 2017), they are supposedly hybrids of tetraploid herbaceous peony and diploid tree peony. It is necessary to study further whether the genetic relationship is close between cultivars with *P. officinalis* and *P. macrophylla* parents. According to the American Peony Society, the parents of 'Little Red Gem' have the participation of *P. tenuifolia*, and the phenotypic traits are fine leaves. Our clustering results (Figure 3) suggest that 'Little Red Gem' is genetically distant from other cultivars.

CONCLUSION

SSR is an accurate, stable, and efficient molecular markers, and is useful for the study of genetic diversity and phylogenetic relationships among members of the *Paeonia*. In this study, SSRs were used to comprehensively understand the genetic diversity and genetic relationship of 55 peony cultivars. However, in the process of screening polymorphic primers, in order to save costs, polyacrylamide gel electrophoresis was used in early screening. This method requires manual reading of electrophoretic bands, which involves a certain amount of subjectivity. At the same time, the efficiency of screening polymorphic primers was low in this experiment. Only 13 pairs of qualified polymorphic primers were screened from 100 pairs of primers, suggesting that the protocol needs further improvement to improve the efficiency of polymorphic primer screening.

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PROCENA GENETSKOG DIVERZITETA KOD 55 KULTIVARA ZELJASTOG BOŽURA SA SSR MARKERIMA

Yongming FAN[1,2], Liuhui YANG[1,2], Qi WANG[1,2], Jaime A. TEIXEIRA DA SILVA[3], Xiaonan YU[1,2]

[1]Koledž pejzažne arhitekture, Pekinški univerzitet za šumarstvo, Peking, 100083, P.R. Kina
[2]Pekinška ključna laboratorija za inovacije i molekularnu plazmu ukrasnog bilja, Nacionalni inženjerski istraživački centar za cvećarstvo, Pekinška laboratorija za urbano i ruralno ekološko okruženje, Peking, 100083, P.R. Kina
[3]Nezavisni istraživač, Ikenobe 3011-2, Kagava-ken, 761-0799, Japan

Izvod

Koristeći SSR markere, ovaj rad je imao za cilj da proceni genetičku raznolikost i genetski odnos između 55 sorti zeljastog božura (*Paeonia lactiflora* Pall.) koje pripadaju grupama Lactiflora, Hibrid i Itoh kako bi se postavila osnova za molekularnu klasifikaciju sorti božura, kao i za analizu genetičke pozadine i oplemenjivanje novih sorti. Naši rezultati pokazuju da je 86 alela, od kojih je 76 polimorfnih, dobijeno skriningom 13 parova polimorfnih prajmera iz 100 prvobitno testiranih prajmera, od kojih svaki ima u proseku 6.615 alela. Opseg varijacije efektivnih alela bio je 2,243–7,800, sadržaj PIC-a je bio 0,554–0,872, a Šenonov indeks genetske raznovrsnosti bio je 1,701–3,126. 55 zeljastih sorti božura podeljeno je u devet grupa na osnovu UPGMA. Ova analiza pokazuje da 55 sorti ima bogatu genetsku raznolikost, dok je filogenetski odnos većine sorti identifikovan. Ova molekularna klasifikacija je korisna i jednostavna referentna molekularna metoda za razlikovanje sorti božura u programima ukrštanja.

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