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ORIGINAL PAPER

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GENETIC DIVERSITY AND RELATIONSHIP OF HUNAN PROVINCE OF CHINA LOCAL TREE PEONIES BASED ON SSR MARKERS

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ABSTRACT

Paeonia sect. *Moutan* is a wide known ornamental plant in the world. The objective of this study was to provide the theoretical basis for scientific preservation and utilization of tree peony resources of Hunan province of China. Simple sequence repeat (SSR) markers were applied to reveal the genetic diversity and relationship of 21 tree peony resources and 45 domestic and foreign tree peony cultivars. Clear bands, the size of which ranged from 115 bp to 379 bp, were detected with 14 primers. In total, 90 alleles were detected and the number of alleles detected with one primer varied between 5 and 13; the number of effective alleles ranged from 1.183 to 2.070; the polymorphism ratio of each locus was 100%. The observed heterozygosity, which ranged from 0.120 to 0.851 with an average of 0.532, was larger than the expected one, which ranged from 0.090 to 0.470 with an average of 0.300. Shannon index ranged from 0.137 to 0.695 and fixation index ranged from −0.332 to −0.869. The results show abundant genetic diversity in tree peony of Hunan province and SSR markers distinguishing homonymous tree peony resources successfully.

Key words: tree peony, genetic diversity, genetic relationship, SSR markers

INTRODUCTION

Tree peony (*Paeonia* sect. *Moutan*) is a deciduous shrub native to China featured with its unique cultural symbolism, striking ornamental value, important medicinal and oil use. However, due to the long cultivation history and complex genetic background, many problems remains obscure [Li et al. 2011, Shi et al. 2012, Ai et al. 2013], such as the origin of wild tree peony species, genetic relationship between wild species and cultivars, and classification system of tree peony groups, that cause a lot of limitations on breeding of tree peony [Cheng 2007, Du et al. 2013, Yu et al. 2013a]. With the breeding technology developed and global planting area increased, those questions urgently need to be answered by reliable molecular markers [Boucket al. 2007].

Due to its extensive distribution, high polymorphism, high mutation rate, good reproducibility, codominance, easy operation, etc., simple sequence repeat (SSR) markers are widely applied in genetic research of various ornamental plants, crops and trees [Cai 2015]. In recent years, molecular markers for tree peony have been widely used, mainly focusing on the study of classification and genetic relationship between cultivars. That provides new information on genetic background for the choice of breeding programs [Li 2007, Atak et al. 2014, Liu et al. 2015].

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Tree peony has a long cultivation history in Hunan, but there are only few cultivars. Currently, peony cultivars are subdivided into five groups, mainly for medicinal purposes. With the discovery of a large number of ancient wild tree peony in Xiangxi, Hunan, we have a new understanding of tree peonies originated in Hunan. With high degree of horticulture, high percentages in double petal, large flower, bright color, extensive landscape application prospects, and strong adaptability in hot and humid climate during summer in the south of China, the tree peony cultivars in Xiangxi are very precious. In addition, our research group also found wild tree peony in Cili, Xiangxi. However, due to dispersive cultivations, less application in landscape and unclear genetic background, the promotion of cultivars, choice of breeding parents and cultivation plots, are limited [Kumar et al. 2015]. In this study, using SSR markers, genetic diversity and genetic relationship were revealed between Hunan tree peonies and others from different habitats, which provided the theoretical basis to rational utilization, fine protection and molecular marker assisted breeding of wet-heat resistant tree peony [Mega 1983, Liu et al. 2013].

MATERIALS AND METRODS

Plant material and DNA extraction. Sampling was performed during March 2014–May 2015. Only young and healthy leaves of tree peony were collected, and quickly frozen with liquid nitrogen and stored in –80°C until DNA extraction [Guo et al. 2015]. In total, 66 accessions were analyzed, including 7 wild species, 19 cultivars from Hunan, 13 cultivars of *Paeonia suffruticosa* Zhongyuan Group, 14 cultivars of *P. suffruticosa* Jiangnan Group, 7 cultivars of *P. suffruticosa* Tianpeng Group, 5 cultivars of *P. suffruticosa* Japan Group and 1 cultivar from France. The main morphological characteristics of all tested accessions are presented in Table 1. Genomic DNA of each sample was extracted by modified CTAB method [Budak et al. 2004a, 2004b, Yu 2013b].

Some new mutations (cultivars) were found in this study, and in this paper, they are presented with serial numbers.

SSR markers and PCR amplification. Fourteen polymorphic microsatellite loci, described by Cheng [Cai et al. 2015], were selected through screening and pre-experimentation [Zhang et al. 2014] for this study. Fluorescently labelled primers were synthesized by Beijing Dingguo Changsheng Biotechnology Co. LTD. The characteristics of 14 pairs of SSR primers are listed in Table 2. The PCR reactions, 25 μL in volume for each sample consisted of 2.5 μL of 10 × PCR buffer (containing Mg^{2+}), 0.5 μL of Taq enzyme (2 U/μL), 0.5 μL of dNTPs (10 mmol/L), 0.5 μL of each forward and reverse primer (10 mmol/L), 2 μL of DNA template (50 ng/μL), and 18.5 μL dd H_2O. The PCR reaction was carried out on Gene Amp PCR System 9600 (Perkin Elmer, USA). The PCR cycle parameters consisted of an initial step of denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 49–56°C for 30 s and extension at 72°C for 30 s, followed by a final extension step at 72°C for 10 min.

For each primer pair, forward (F) and reverse (R) sequences (Sequence), size of the original fragment (Size), optional annealing temperature (*Ta*).

The amplified products were isolated by ABI3730X Genetic Analyzer (Applied Biosystems, USA), and ROX500 was used as internal standard for molecular weight analysis, and GeneMapper v 4.0 was used software analysis to obtain the length of different sample amplification fragment.

Data analysis. One site was detected for every 1 pair of SSR primers. The number of alleles per loci (*Na*), the effective numbers of alleles (*Ne*) and the observed and expected heterozygosities (*Ho* and *He*) were calculated using GenAlEx software version 6.51 [Peakallet al. 2006a]. Firstly, we calculated the genetic distance [Cai et al. 2015] (D) between populations (its value can be from 0 to infinity). More than 20 different graphs summarize data and aid exploration. Secondly, the cluster graph is drawn using POPTREE version 2 and unweighted pair-group method with arithmetic means (UPGMA) [Zhang et al. 2015]. Finally, genetic distance and germplasm relationship was analyzed.

RESULTS

Genetic diversity of tested accessions. The polymorphisms of 66 peony germplasm resources were analyzed with 14 SSR primers. The high polymor-

Table 1. Origin and flower characteristics of 66 accessions of tree peony species and cultivars used in this study

No.	Accession	Provenance	Flower form	Flower color
1	2	3	4	5
1	*P. jishanensis*	Yichuan, Shanxi, China	single form	pink
2	*P. decomposita*	Chengdu, Sichuan,China	single form	fuchsia
3	*P. rockii*	Baokang, Hubei,China	single form	white
4	*P. ludlowii*	Xizang, China	single form	yellow
5	*P. lutea*	Yunnan, China	single form	yellow
6	*P.qiui*	Hubei, China	single form	pink
7	*P.ostii*	Cili, Hunan, China	single form	white
8	*P. ostia* ‘Feng Dan Fen’	Changsha, Hunan, China	single form	pink
9	*P. ostia* ‘Feng Dan Zi’	Shaoyang, Hunan, China	single form	fuchsia
10	NX109	Shaoyang, Hunan, China	globular form	white
11	NX104	Yongshun, Hunan, China	globular form	white
12	NX110	Yongshun, Hunan, China	single form	white
13	NX102	Yongshun, Hunan, China	single form	white
14	Ci Li Hong	Cili, Hunan, China	single form	red
15	SY-B14	Yongshun, Hunan, China	globular form	white
16	SY-Fen	Yongshun, Hunan, China	globular form	pink
17	SY-B9	Shaoyang, Hunan, China	single form	white
18	SY-B6	Shaoyang, Hunan, China	single form	white
19	SY-B13	Shaoyang, Hunan, China	single form	white
20	SY-B8	Shaoyang, Hunan, China	single form	white
21	*P. suffruticosa* ‘Yong Shun Fen’	Yongshun, Hunan, China	globular form	pink
22	*P. ostia* ‘Feng Dan’	Shaoyang, Hunan, China	single form	white
23	*P. suffruticosa* ‘Yong Shun Hong’	Yongshun, Hunan, China	single form	red
24	SY-B7	Shaoyang, Hunan, China	single form	white
25	*P. suffruticosa* ‘Xiang Dan’	Shaoyang, Hunan, China	single form	rose
26	*P. suffruticosa* ‘Yong Shun Zi’	Yongshun, Hunan, China	globular form	fuchsia
27	*P. suffruticosa* ‘Shou An Hong’	Luoyang, Henan, China	crown form	purple
28	*P. suffruticosa* ‘Rou Fu Rong’	Luoyang, Henan, China	chrysanthemum form	fuchsia
29	*P. suffruticosa* ‘Bai Xue Ta’	Luoyang, Henan, China	crown form	white
30	*P. suffruticosa* ‘Zhu Sha Lei’	Luoyang, Henan, China	lotus form	rose
31	*P. suffruticosa* ‘Wu Long Peng Sheng’	Luoyang, Henan, China	globular form	fuchsia
32	*P. suffruticosa* ‘Hu Hong’	Luoyang, Henan, China	chrysanthemum form	fuchsia
33	*P. suffruticosa* ‘Shi Ba Hao’	Luoyang, Henan, China	crown-proliferation form	fuchsia

Table 1. cont.

1	2	3	4	5
34	*P. suffruticosa* 'Ying Ri Hong'	Luoyang, Henan, China	crown-proliferation form	red
35	*P. suffruticosa* 'Zhao Fen'	Luoyang, Henan, China	lotus form	pink
36	*P. suffruticosa* 'Zhi Hong'	Luoyang, Henan, China	crown-proliferation form	purple
37	*P. suffruticosa* 'Tai Ping Hong'	Luoyang, Henan, China	anemone form	fuchsia
38	*P. suffruticosa* 'Luo Yang Hong'	Luoyang, Henan, China	rose form	fuchsia
39	*P. suffruticosa* 'Jin Pao Hong'	Luoyang, Henan, China	rose form	fuchsia
40	*P. suffruticosa* 'Fen Lian'	Ningguo, Anhui, China	rose-proliferation form	pink
41	*P. suffruticosa* 'Hu Hong'	Ningguo, Anhui, China	crown form	purple
42	*P. suffruticosa* 'Yu Hong'	Ningguo, Anhui, China	chrysanthemum form	fuchsia
43	*P. suffruticosa* 'Chang Hong'	Ningguo, Anhui, China	chrysanthemum form	fuchsia
44	*P. suffruticosa* 'Si Xuan'	Ningguo, Anhui, China	rose form	fuchsia
45	*P. suffruticosa* 'Mei Hong'	Ningguo, Anhui, China	rose form	fuchsia
46	*P. suffruticosa* 'Qing Luo'	Ningguo, Anhui, China	rose-proliferation form	fuchsia
47	*P. suffruticosa* 'Que Hao'	Ningguo, Anhui, China	rose-proliferation form	fuchsia
48	*P. suffruticosa* 'Xi Shi'	Ningguo, Anhui, China	chrysanthemum-proliferate form	pink
49	*P. suffruticosa* 'Zhi Yu'	Tongling Anhui, China	single form	pink
50	*P. suffruticosa* 'Xue Yuan Hong'	Tongling Anhui, China	rose form	red
51	*P. suffruticosa* 'Tao Hua Fei Xue'	Tongling Anhui, China	chrysanthemum form	pink
52	*P. suffruticosa* 'Xiang Yu'	Tongling Anhui, China	crown form	white
53	*P. suffruticosa* 'Chuan Hong'	Tongling Anhui, China	chrysanthemum form	red
54	*P. suffruticosa* 'Peng Zhou Zi'	Pengzhou, Sichuan, China	lotus-proliferate form	fuchsia
55	*P. suffruticosa* 'Dian Jiang Hong'	Pengzhou, Sichuan, China	single form	red
56	*P. suffruticosa* 'Yu Lou Zi'	Pengzhou, Sichuan, China	chrysanthemum form	purple
57	*P. suffruticosa* 'Jin Yao Lou'	Pengzhou, Sichuan, China	crown-proliferation form	pink
58	*P. suffruticosa* 'Po Mo Xiang'	Pengzhou, Sichuan, China	chrysanthemum form	purple
59	Tian peng tree peony	Pengzhou, Sichuan, China	double	fuchsia
60	*P. suffruticosa* 'Dan Jing Hong'	Chengdu, Sichuan, China	crown-proliferation form	pink
61	*P. suffruticosa* 'Hua Wang'	Japan	chrysanthemum form	rose
62	*P. suffruticosa* 'Tai Yang'	Japan	lotus form	red
63	*P. suffruticosa* 'Chu Wu'	Japan	single form	purple
64	*P. suffruticosa* 'Dao Jin'	Japan	rose form	red and white
65	*P. suffruticosa* 'Hua Jing'	Japan	chrysanthemum form	pink
66	*P. suffruticosa* 'Jin Huang'	France	rose form	yellow

Table 2. Characteristics of 14 pairs of SSR primers used in this study

No.	Primer name	Primer sequence (5′~3′)	Expected product size (bp)	Annealing temperature (°C)
1	PS002	F: TCGGTGACCTGGCTGTTG	241	56
		R: CCCTCCTCCCACTTTGTT		
2	PS047	F:AGACGACGAGCAAAGATAT	126	50
		R:AAAGGGCAAGATTGGAAAT		
3	PS106	F:GGCTGAACTTCTATCACTTT	202	52
		R: TCTTTACTTGTACTTGCTCC		
4	PS119	F: GCAAAGACAACAGCCTCG	289	55
		R: CTCACCATCCAATCCCAC		
5	PS149	F: AGTCGCCTCCTACACCTC	173	54
		R: TCCGTAAAGCCCACAATAC		
6	PS153	F: ATGTCCAAACTGGCAATA	260	50
		R: CCCTCCCTCAACACTTAC		
7	PS184	F: TGACCACAAGAAGCCAACT	180	52
		R: AGGAAGCGACGAGAAACT		
8	PS219	F: TGACATTGGCATTCCTTG	211	53
		R: CAGACCCTACCCTCTTGG		
9	PS244	F: CGGCAAAACAAAGAGGAT	291	53
		R:ATGGGAGCGTAAGGAACATG		
10	PS004	F: GTGCTTAGCCTCTAATCTG	274	51
		R: CTTTGCTCCAAGTCTGTC		
11	PS076	F: ATGCCACCTTTTCCTAAT	258	49
		R: TTCTTGTTCCCCTTGTTTC		
12	PS290	F: TTCTTTCACCTCCACTTCA	245	51
		R: CGTCGTTTCGTTTACTCTT		
13	PS265	F: TTTTATGGGTCCTGTTGC	290	51
		R: GAAGAGTAAGCCTTTGTCG		
14	PS273	F: CCCTCAGATGGGATGGAA	314	55
		R: CGGTGGTGGTACAACGAAC		

Table 3. Genetic diversity of tree peony populations

Locus	*Na*	*Ne*	*I*	*Ho*	*He*	*F*
PS002	1.485	1.410	0.284	0.287	0.192	–0.461
PS047	1.803	1.731	0.522	0.683	0.370	–0.824
PS106	1.424	1.283	0.212	0.220	0.137	–0.568
PS289	1.621	1.533	0.379	0.495	0.264	–0.835
PS149	1.439	1.320	0.235	0.248	0.158	–0.550
PS153	2.197	2.070	0.695	0.856	0.470	–0.845
PS184	1.712	1.657	0.456	0.573	0.319	–0.789
PS219	1.894	1.836	0.564	0.716	0.389	–0.860
PS224	1.818	1.678	0.480	0.572	0.329	–0.750
PS004	2.091	1.956	0.660	0.851	0.456	–0.869
PS076	1.742	1.670	0.463	0.551	0.321	–0.709
PS290	1.258	1.183	0.137	0.120	0.090	–0.332
PS265	1.788	1.710	0.506	0.638	0.355	–0.777
PS273	1.879	1.720	0.508	0.633	0.347	–0.826
Mean	1.725	1.626	0.436	0.532	0.300	–0.766

Parameters shown for each locus: the mean number of alleles (*Na*), the effective number of alleles (*Ne*), Shannon index (*I*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and fixation index (*F*)

phisms of the selected SSR primers also indicated that the tested peony varieties had high genetic diversity and high variability. The results are shown in Table 3. Clear bands the size of which ranged from 115 bp to 379 bp, were amplified in all samples, with good reproducibility and polymorphism. For the polymorphic loci, a total of 90 alleles were generated across 66 tree peony accessions and the number of alleles ranged from 5 to 13 with an average of 7 per locus. The number of alleles detected by most primers was between 5 and 9. Most of the primer on the number of alleles detected variation between 5 and 9, including PS149, PS002, PS224, PS004 PS153 and the number of alleles is more, respectively, 8, 8, 8, 9 and 13. The number of effective alleles ranged from 1.183 to 2.070 with an average of 1.725 per locus. The number of effective allele of PS153 was the largest, followed by PS219, which was 1.836. The polymorphism ratio of each locus was 100%. The observed and expected heterozygosity per locus ranged from 0.120 to 0.851 (an average of 0.532) and from 0.090 to 0.470 (an average of 0.300), respectively. In addition, the Shannon index ranged from 0.137 to 0.695 and the fixation index ranged from –0.869 to –0.332. The results of capillary electrophoresis detection (Fig. 1) indicated that each band had a high resolution. In Table 3, the polymorphisms of amplified fragments of different primers in all accessions are presented, which indicates that Hunan peonie trees have abundant genetic diversity.

Genetic relationship of germplasm resources. Based on genetic distance and using UPGMA clustering method a dendrogram of 66 analyzed accessions were generated (Fig. 2). *P. ludlowii* and *P. lutea* were clustered into a large group and far from other accessions, which was consistent with previous researches [Yu et al. 1962, Li 1999]. In other words, there was a distant genetic relationship between succulent disk and the peony of leathery disk, and the subgroup of succulent disk had nothing to do with the origin of Chinese cultivated peony. At the similarity coefficient of 0.692, *P. sporetanea* was clustered separately into one class, which showed that the relationship between *P. sporetanea* and the cultivated varieties of China was relatively far, which was consistent with the view put forward by Hong [Hong et al. 1999]. Hunan province local tree peonies were clustered with different accessions from home and abroad. The series of an-

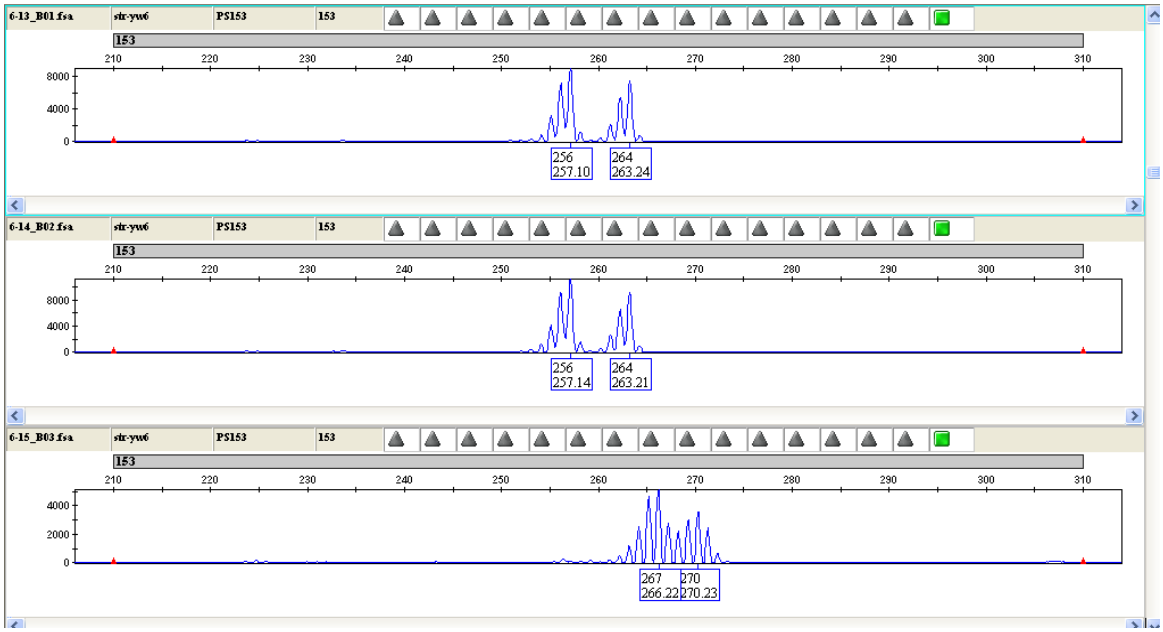

Fig. 1. Capillary electrophoresis detection obtained by PS1153

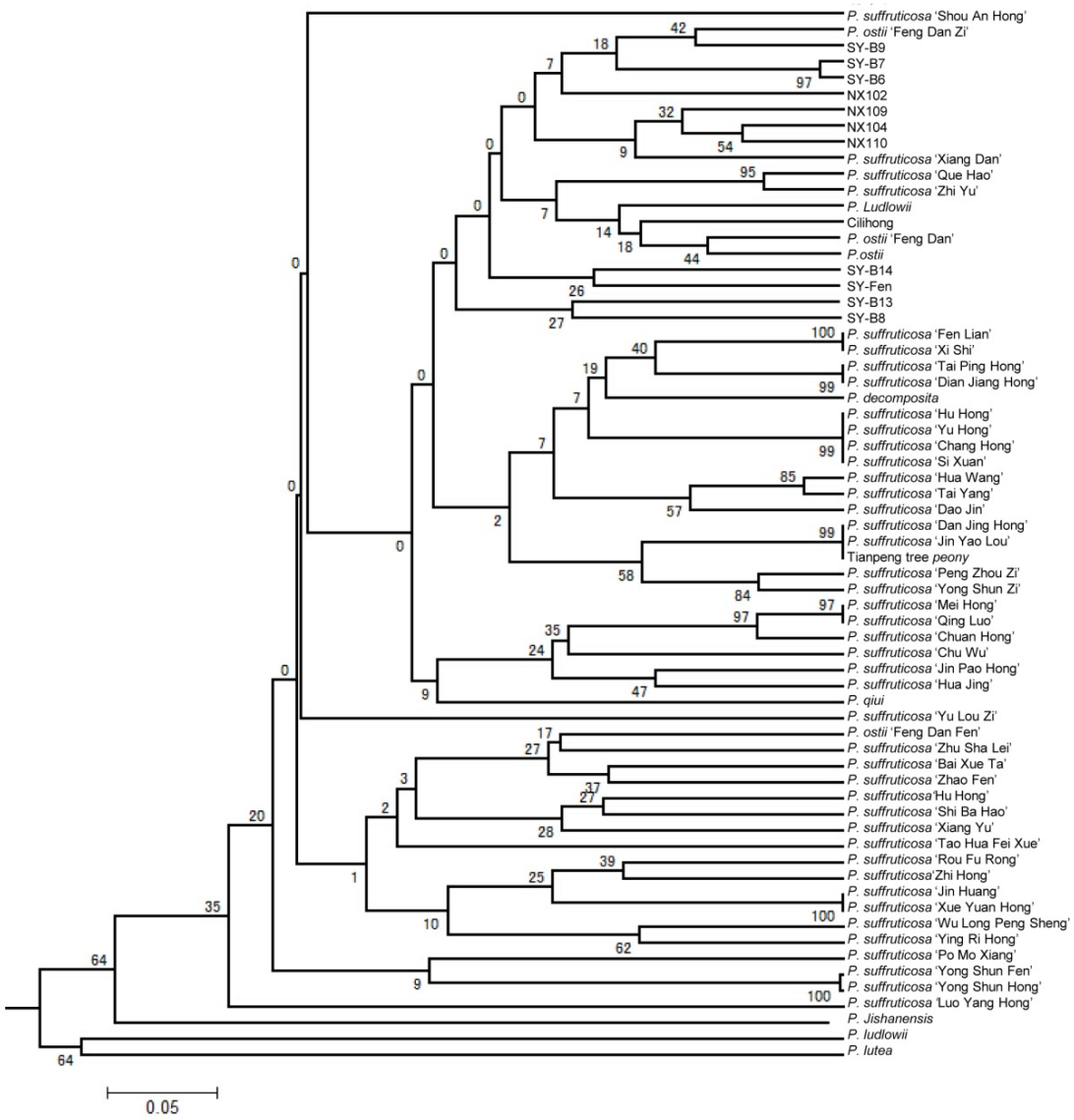

Fig. 2. Genetic clustering tree of 66 tested accessions

cient double purple cultivars from Xiangxi had a very close genetic relationship with 'Pengzhouzi' [Merrell et al. 1981, Cordeiro et al. 1999, Morgante et al. 2002, Rota et al. 2005, Cai 2015], and were clustered with Tianpeng tree peony, 'Jinyaolou' and 'Danjinghong', which proved the local farmers' claim that these purple cultivars originated from Pengzhou Sichuan and belong to 'Pengzhouzi'. Among cultivars from Xiangxi, the series of ancient double pink flower cultivars were clustered with red single cultivars, but had a far genetic relationship with the series of ancient double purple cultivars, which indicated that these two series of ancient tree peony originate from different habitats. The series of double pink cultivars and local red single cultivars likely originated from Xiangxi Hunan. The series of pink double cultivars came from its long-term natural evolution in Xiangxi. *P. ostii*, 'Xiangdan', *P. rockii* ', Cilihong, and 'Fengdan', which were all native to Hunan, were clustered together with some of mutations in Hunan, which molecularly certified that the 'Xiangdan' and Cilihong originated in Hunan and there was a close genetic relationship between Cili wild tree peony, *P. ostii* and *P. rockii*. 'Xiangdan', 'Fengdan' and *P. ostii* were gathered together with 'Fengdanzi' and some other local cultivars, indicating that these local cultivars were derived from perennial natural hybrid in Hunan. *P. ostii*, 'Fengdan', Cilihong and *P. rockii*, had a more close genetic relationship [Li 2007, Hou et al. 2011, Zhang et al. 2011, Liu et al. 2015]. According to further geographical analysis, Xiangxi located at the border of Hubei Province, which was one of the places of origin of *P. rockii* [Cheng 2005].

DISCUSSION

Origin of Hunan tree peonies. Tree peonies of Hunan province of China belong to the *P. suffruticosa* Jiangnan group. The results showed that the five wild species belonging to subsection *Vaginatae* were close to the cultivated tree peony. Besides, *P. ostii*, *P. rockii*, and other tree peony cultivars had a close relationship, which have further identified a complex origin of Hunan Province tree peonies [Rogers 1995, Sarker et al. 1999]. Based on the results of previous studies, this paper selected peony from some other regions as the control, confirming that there were significant genetic differences in Hunan peony [Zhang et al. 2015]. Therefore, according to the experimental results, Tree peonies of Hunan province was not only affected by *P. ostii*, *P. rockii* and *P. decomposita*, but was also affected by *P. qiui*. All tested accessions could be differentiated by DNA marker technology. The genetic relationship and morphological characteristics demonstrated that series of double purple cultivars in Xiangxi belong to 'Pengzhouzi'. The clustering results showed that most of purple series and pink series were genetically different from *P. ostii* 'Fengdan' and 'Xiangdan', which indicated that those ancient tree peony cultivars could be introduced from field to local garden. Five ancient tree peony cultivars, which had a close genetic relationship with *P. ostii*, 'Fengdan' and 'Xiangdan', were likely to be local cultivars in West of Hunan province, and introduced from mountains by local farmers in the past. Due to the distribution of wild *P. ostii* in Hunan, these cultivars, especially four purple cultivars, had very close genetic relationship with *P. ostii*. 'Xianggdan' with *P. ostii* and other five double cultivars were clustered together, indicating that 'Xiangdan' can be originated from Hunan province [Qin et al. 2013]. 'Xiangdan' also had close genetic relationship with *P. ostii* and 'Fengdan'.

Genetic relationship among tree peony varieties. The result also confirms that Chinese tree peonies made huge contribute to the history of international tree peony breeding. The results of genetic lineage and SSR markers were highly consistent, indicating that SSR markers were reliable for the research of genetic relationship between tree peony cultivars and species. There was a large genetic distance between tested accessions with different origins, such as *P. ludlowii*, *P. lutea* and *P. spontanea* clustered as different classes. Although some accessions were distributed in the same cluster, but the genetic similarity between them was small, which indicated that the genetic diversity of tree peony was abundant.

According to the distribution areas of germplasms and the average genetic similarity coefficient, the native tree peonies of Hunan province showed a close genetic relationship among the same germplasm resources, and the genetic difference between accessions that from different regions was relatively large, materials with huge genetic diversity can be applied for

breeding excellent cultivar. Finally, the DNA fingerprint database of tree peony can be established based on the authorized and known tree peony cultivars in China, and the information management and application of new cultivars can be implemented.

The application of Hunan tree peonies. Wild resources plays an important role in plant genetic breeding, but existing study results was rarely included, so our group carried out numerous in-deep investigations about tree peony resource of Hunan province from 2013 to 2015. The result showed that the original tree peony cultivars, bred by Hou [Deng et al. 2010], had been lost or dead. At the same time, some new mutations were discovered, and we intend to name them. In this paper, we distinguished cultivars in purple series and pink series still by serial number, because those cultivars haven't been officialy published [Li et al. 2011, Shi et al. 2012, Ai et al. 2013].

Although the native peony germplasm resources in Hunan are abundant and diverse, some precious resources are mostly planted before and after the farmer's fields and houses, and they are allowed to grow naturally [Hall et al. 2007, Homolka et al. 2010]. In terms of variety naming, it is very confusing. In most cases, local farmers randomly name the flowers, colors and patterns of the peonies, resulting in serious homonymy or homonymy phenomenon, which greatly impedes the in-depth study of resources [Andrieu et al. 2007, Gilmore et al. 2013]. The research group strengthened the collection and preservation of native peony resources in Hunan province, and conducted genetic identification and related research, which is not only an important supplement to the genetic information of peony, but also of great significance to the hybridization and breeding of peony.

CONCLUSIONS

The results indicated that abundant genetic diversity in tree peony of Hunan province and SSR markers distinguish homonymous tree peony resources successfully. And it could provide good results for further studies. Using SSR molecular marker, this study discusses the genetic diversity and genetic relationship between peony of Hunan province varieties, the results show that genetic diversity of peony in Hunan province is abundant, and SSR markers could well distinguish the same species of tree peony resources. This provides more scientific basis for further understanding the relationship between peony species and varieties, and also provides some theoretical basis for the selection of hybrid parents. At the same time, it provides a basis for cultivating peony suitable for planting in hot and humid areas of southern China.

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