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# Development and application of 15 novel polymorphic microsatellite markers for sect. *Paeonia* (*Paeonia* L.)

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ABSTRACT

Herbaceous peony, which belongs to sect. *Paeonia*, *Paeonia* L., Paeoniaceae, is not only a famous traditional flower in China; it is also highly regarded as an ornamental in Europe and the USA. On account of the abundance in germplasm resources and wide distribution ranges, herbaceous peony can be divided into three cultivar groups, the Chinese Peony Cultivar Group, the European Peony Cultivar Group and the Hybrid Peony Cultivar Group. However, most studies on genetic relationships in *Paeonia* are limited to the first group. This study used 15 polymorphic microsatellite markers that were developed by magnetic bead enrichment to explore the genetic diversity of 89 genotypes collected from Beijing, the USA and Canada and introduced to China. From 145 allelic loci that were detected from 15 primers, 140 (96.6%) were polymorphic. The number of allelic loci ranged from 4 to 16 with an average of 9.3, and the polymorphic index content ranged from 0.362 to 0.825 with an average of 0.678. Both cluster analysis and principal component analysis based on the 15 polymorphic primer pairs could distinguish the species from cultivars although the cluster analysis was better able to reflect the genetic relationships between all individuals. Some new insights about *Paeonia* L. are suggested. Furthermore, the 15 SSR markers have great significance as they will allow for furthering the studies on genetic diversity, genetic relationships and even the molecular marker-assisted breeding of *Paeonia* L.



## 1. Introduction

Herbaceous peony is a perennial flower belonging to sect. *Paeonia*, *Paeonia* L., Paeoniaceae (Yu et al., 2012). It is not only a famous traditional flower in China, but is also highly regarded in Europe and the USA as an ornamental. Since herbaceous peony has abundant germplasm resources and wide distribution ranges (Ji et al., 2012), it can be divided into three cultivar groups: the Chinese Peony Cultivar Group, the European Peony Cultivar Group and the Hybrid Peony Cultivar Group (Qin, 2004). According to a new classification, sect. *Paeonia* (*Paeonia* L.) originally had 22 species and more than 1000 cultivars

*Abbreviations:* AFLP, amplified fragment length polymorphism; $H'$, Shannon's diversity index; ISSR, inter-simple sequence repeat; $N_a$, number of alleles; $N_e$, effective number of alleles; $P$, polymorphism ratio; PCA, principal component analysis; PIC, polymorphism index content; RAPD, random amplified polymorphic DNA; SSR, simple sequence repeat.

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around the world (Hong et al., 2010). *Paeonia* germplasm is rich in diversity in terms of external morphology as well as chromosome ploidy. Among the main parents, there are many natural polyploids, all of which are tetraploid (Hong et al., 2010).

New peonies cultivated in Europe and North America form a pool of rich germplasm resources and parents participate significantly in the breeding of peony cultivars around the world. Despite this, there still exist many problems in the application to landscaping in China, which can restrict the extensive application of new cultivars. Moreover, research on the genetic diversity of these new cultivars lags behind that of Chinese peony cultivars (Guo and Wang, 2007; Pan et al., 2007; Li, 2010), and most collectors who own such cultivars are normally nursery lovers that lack advanced scientific breeding principles. In addition, most people have a limited understanding of the Chinese Peony Cultivar Group, or even of the European and Hybrid Peony Cultivar Groups. Over time, this is bound to restrict the process of peony breeding in China and will further influence sustainable development around the world. Above all, it is a fundamental principle to understand the genetic backgrounds of such cultivars if China wishes to absorb and use these new western peony germplasm resources. China can avoid waste caused by blind introduction of 'synonym' peony cultivars and can also provide a scientific basis for parental selections in cross breeding.

To date, microsatellite markers (syn. simple sequence repeats or SSRs) are generally recognized as an ideal molecular marker technology with significant advantages, including codominant inheritance, requirement of little DNA, good experimental repeatability, and high reliability (Kalia et al., 2011). Furthermore, SSR markers have a wide range of applications in ornamentals, including peony (Parks et al., 2006; Tang et al., 2008; Gilmore et al., 2013). Researchers have applied SSR markers to better understand and advance studies on genetic diversity, target gene calibration, the construction of genetic maps and fingerprinting in *Paeonia* L. (Smulders et al., 1997; Lesur et al., 2001; Zhang et al., 2009; Moghaddam et al., 2012). However, no study on the genetic diversity of new peony cultivars in Europe and the USA exists, possibly because of the large genome of *Paeonia* L. and because loci provided when developing markers are always limited and random (Cheng et al., 2011; Li et al., 2011; Sun et al., 2011). There are limited studies also because gene cloning and sequencing are complicated and time-consuming processes, and because most developed markers have been aimed at individuals of the Chinese Peony Cultivar Group.

In this paper, we report on the genetic diversity and genetic relationships of 89 plant materials, including the original species and new cultivars introduced and collected from Europe and North America. These relationships were established by using 15 polymorphic SSR markers developed by magnetic bead enrichment. This study will provide important technical support and a theoretical basis for comprehensive research on the genetic diversity and phylogenic development of *Paeonia* L.

## 2. Materials and methods

### 2.1. Plant materials and DNA extraction

A total of 89 germplasms were collected from Beijing, China, nursery of 'Hidden Springs Flower Farm' in Minnesota, USA and from a Canadian personal breeder, Ms. P. Woodward (Table 1). All the introduced material was grown in Xiao Tangshan *Paeonia* horticulture fields of Beijing Forestry University, Beijing, China (40°2′N, 115°50′E). *Paeonia lactiflora* 'Charlie's White' was chosen as the material for SSR primer development since this cultivar has high ornamental value, it has long been popular in the international flower market and has historically been used in many breeding programs in the USA and Europe. For example, it was used by the Canada Peony Society (CPS) as a male parent of the cultivar 'Pink Hawaiian Coral'. DNA (200 ng/μl) was extracted from fresh leaves in the terminal part of peony plants of all 89 accessions in early April using the Plant DNeasy kit (Qiagen, Shanghai, China) according to the manufacturer's instructions.