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Determination of Difference between Herbaceous and Tree Peony Hybrids with SRAP Markers

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ABSTRACT

Turkish flora has approximately 12,000 plant taxons and 35% of them are known to be endemic. The herbaceous and woody forms of peony plants have been commonly used as ornamental plants and for medical purposes, especially in the far East, for many years. Herbaceous species with 12 taxons including 55 populations have been detected and collected from Turkey's flora since 2000. Then breeding programs started with interspecific crosses and some new hybrids were obtained. To achieve targeted results it is especially important to distinguish between the resulting herbaceous or woody structures of individuals. Therefore, the selected 10 SRAP primers were used to separate some woody and herbaceous peony hybrids and also standard peony cultivars from each other. Successful results were obtained with these SCAR primers. As a result of interspecific crosses the obtained herbaceous or tree structure of individuals that were most reliable, especially Me2+Em1 and Me8+Em2 primer pair combinations were derived from testing. Thus, they are the first hybrid plants with leaves and undesirable characteristics can be determined in those forms to be eliminated. Thus, targeted results have been obtained in a much shorter time while the ratio of peony breeding success has increased.

Key words: Peony, breeding, herbaceous, woody, SRAP, difference

INTRODUCTION

Turkey, with critical geographic position, geomorphic structure and quite a large diversity of habitats, is a very important gene centre in terms of biodiversity. However, this genetic diversity has not been adequately assessed. It has been suggested that there are approximately 12,000 plant taxons in Turkey, 35% of them are endemic and among them, around 1000 geophyte taxons are economically important. Geophytes are one of the most important plants in Turkey. 'Geophyte' is the name given to herbs which stay dormant underground for the greater part of the year and have specialised food-storing underground stems such as bulbs, corms, rhizomes and tubers (Ozhatay, 2000).

Paeonia, peonies, do not have bulbs but because they spend their winter dormancy as large storage organs called rhizomes, they are often treated as geophytes and the rhizomes are often sold along with bulbs in autumn, when herbaceous peonies are divided and transplanted. There are both herbaceous and shrubby species, the latter known as "tree" peonies. The genus is widespread in the northern hemisphere, with centres of distribution in the northern Mediterranean rim, the Caucasus and China and one or two species in far Western North America (Halda and Waddick, 2004). Particularly for 9 species of herbaceous peony plant their homeland is reported as Turkey

(Davis, 1982). Also Osti (2004), mentioned that *Paeonia kesrouanensis*, *Paeonia mascula*, *Paeonia mascula* ssp. *mascula*, *Paeonia mascula* ssp. *arietina*, *Paeonia mascula* ssp. *bodurii*, *Paeonia mascula* ssp. *triternata* syn. *Paeonia triternata* syn. *Paeonia daurica*, *Paeonia peregrina* and *Paeonia wittmanniana* species have natural distribution in Turkey's flora and reported their location.

Despite the fact that the majority of herbaceous species are native to Turkey, China is the homeland of tree peony species. Tree peony species have plenty of petals and larger flowers so they are used as parents in breeding studies. Herbaceous and tree peony species have been crossed and some new hybrids have been obtained by Kaya (2010). Hybrids have some superior properties compared with standard varieties. Some hybrids are herbaceous and others are tree peony or semitree peony. While herbaceous species are often preferred because of the different coloured flowers, tree peony species are selected due to the large flowers and plants.

Kaya (2010) has attempted to transfer these two characteristics into the new individuals by cross-species hybridization. These hybrid characteristics of individuals are expected to be observed an average of 5 years later.

Molecular markers are commonly used in order to clarify genetic characteristics of plants nowadays. Without depending on the environmental conditions they represent every point of the genome and also provide more advantages than other morphological and biochemical markers (Tingey and del Tufo, 1993). With marker based selection of new hybrids it is easier to determine the characteristics of individuals and individuals with undesirable properties can be eliminated (Rajapakse, 2003).

For this purpose, different *Paeonia* species have been identified, especially with different RAPD markers (Pei *et al*., 1995; Hosoki *et al*., 1997a, b, c; Meng and Zheng, 2004; Mazeikiene *et al*., 2007). ISSR markers also were used for genetic determination of inter-specific hybrids in different species (Suo *et al*., 2003, 2004).

But in recent years, especially Sequence Related Amplified Polymorphism (SRAP) technology has begun to be used because it is repeatable, provides more stable results and is also less complex (Li and Quiros, 2001; Li *et al*., 2011). SRAP markers have been used successfully with different plants such as *Brassica* (Li and Quiros, 2001), *Cucurbita* (Ferriol *et al*., 2003), turfgrass (Budak *et al*., 2004a), *Nelumbo* (Liu *et al*., 2006) and faba bean (Alghamdi *et al*., 2012). Budak *et al*. (2004b) compared SRAP with SSR, ISSR and RAPD markers in the Buffalograss plant. SRAP yielded more successful results in separation of close relatives. Especially the SRAP markers give quite promising results for separation of herbaceous and tree peony species (Han *et al*., 2008a, b).

In this study, interspecific hybrids obtained from breeding studies of peony species were used. The first leaf of these hybrids was used in order to determine their form, as herbaceous, tree peony or half tree. The aim of this study is to find suitable SRAP primer combinations and to purposefully shorten the duration of the breeding period.

MATERIALS AND METHODS

Plant material: Peonies used in this study were collected from the conservation area of the genetic resources and peony (*Paeonia* L.) experimental breeding garden in Atatürk Horticultural Central Research Institute (AHCRI). A total of 25 peony species, varieties and hybrids were used in this study. The studied peony species and hybrids are shown in Table 1.

Table 1: Peony species and hybrids used in this study

Species or hybrids name	Characteristic
Paeonia suffruticosa hybrid (OD1)	Tree form
Paeonia suffruticosa hybrid (OD2)	Tree form
Paeonia suffruticosa (OD3)	Tree form
Paeonia suffruticosa hybrid (OD4)	Tree form
Paeonia suffruticosa hybrid (OD5)	Tree form
Paeonia suffruticosa hybrid 'High Noon' (OD6)	Half tree form
Paeonia suffruticosa hybrid (OD7)	Tree form
Paeonia suffruticosa hybrid (OD8)	Tree form
Paeonia mascula (2901)×*Paeonia suffruticosa* (OD1)-2005-1	Half tree form
Paeoniadaurica (3301)×*Paeoniatenuifolia* (2201)-03-2005	Herbaceous
Paeonia mascula (2901)×Sarah Bernhardt (SB)-3-2005	Herbaceous
Paeonia peregrina (1701)×Sarah Bernhardt (SB)-13-2007	Herbaceous
Paeonia peregrina (1401)×Sarah Bernhardt (SB)-59-2007	Herbaceous
Paeonia peregrina (1101)×Sarah Bernhardt (SB)-30-2007	Herbaceous
Paeoniadaurica (3301)×OD4-1	Half tree form
Paeonia turcica (0701)×*Paeonia*×*kayae* (1703)-4-2005	Herbaceous
Paeoniatenuifolia (2201)×*Paeonia peregrina* (7701)-1-2005	Herbaceous
Fireball-Alevtopu (*Paeonia peregrina* hybrid)	Herbaceous
Eful (*Paeonia peregrina* hybrid)	Herbaceous
Kaya (*Paeonia*×*kayae* hybrid)	Herbaceous
Tombak (*Paeonia peregrina* hybrid)	Herbaceous
Dancing Water Lily (*Paeonia lactiflora* hybrid)	Herbaceous
Dr. Alexander Fleming (*Paeonia lactiflora* hybrid)	Herbaceous
Duchesse de Nemours (*Paeonia lactiflora* hybrid)	Herbaceous
Sarah Bernhardt (*Paeonia lactiflora* hybrid)	Herbaceous

Genomic DNA isolation: Fresh and young leaves were harvested for DNA extraction and SRAP analysis from the AHCRI experimental garden and ground into a fine powder with liquid nitrogen using a sterile mortar and pestle. Genomic DNA was extracted from young leaves with a Qiagen DNeasy plant mini kit according to the manufacturer's protocol. DNA isolation protocol was completed by making some minor modifications. The quality and concentration of DNA were verified on 1% agarose gels and were measured by spectrophotometry. A dilution test was conducted to determine the 90 ng μL^{-1} DNA for PCR amplification.

PCR application with SCAR primers: The 17 Hybrids and 9 standard varieties were genotyped with SRAP primers. The 17 hybrids and 8 standard cultivars were analysed for the above-mentioned SCAR markers (Table 2). PCR reactions were carried out in 25 μL of 1X reaction buffer that contained $(NH_4)_2SO_4$, 2.5 mM of $MgCl_2$, 200 μM of each dNTP, 0.3 mM of primer, 1 Unit of Taq DNA polymerase (Fermentas) and 90 ng of genomic DNA. The remaining volume was completed to 25 μL with ultrapure water (Fermentas). In the PCR reactions, predenaturation was conducted at 94°C for 3 min and 40 cycles were applied. In each cycle, denaturation was conducted at 94°C for 30 sec, annealing was conducted at 37°C for first 8 cycles and 50°C for last 42 cycles for 45 sec and extension was performed for 90 sec at 72°C. The final extension stage was conducted for 7 min at 72°C. PCR-amplified DNA fragments were separated on a 1.5% agarose gel with 1X TBE buffer and were stained with ethidium bromide. The agarose gels were visualised in a UV transilluminator.

Table 2: Sequence of SRAP primer pairs (Han *et al*., 2008a; Hao *et al*., 2008)

Primers forward/reverse		Base sequence (5'-3')
Me2	Forward (5'-3')	TGA GTC CAA ACC GGA GC
Me4	Forward (5'-3')	TGA GTC CAA ACC GGA CC
Me5	Forward (5'-3')	TGA GTC CAA ACC GGA AG
Me7	Forward (5'-3')	TGA GTC CAA ACC GGA CA
Me8	Forward (5'-3')	TGA GTC CAA ACC GGA AC
Em1	Reverse (5'-3')	GAC TGC GTA CGA ATT AAT
Em2	Reverse (5'-3')	GAC TGC GTA CGA ATT TGC
Em3	Reverse (5'-3')	GAC TGC GTA CGA ATT GAC
Em8	Reverse (5'-3')	GAC TGC GTA CGA ATT CTG
Em10	Reverse (5'-3')	GAC TGC GTA CGA ATT CAG

RESULTS

Firstly, a certain amount of each peony species (herbaceous, tree form and half tree) bulk was added individually to the different tubes. They were tested with 24 different SCAR primer combinations. In these experiments Me2+Em1, Me5+Em1, Me5+Em8, Me8+Em1 and Me8+Em2 primer combinations were selected as the promising combinations in order to seperate herbaceous and tree species (Fig. 1). A total of 25 different peony cultivars/hybrids were amplified with these primer pairs and especially Me2+Em1 and Me8+Em2 primer pairs were determined to be the best primer combinations to identify both herbaceous and tree form peonies (Fig. 2 and 3).

After PCR applications with Me2+EM1 primer combination only 700 bp band was obtained for tree peony cultivars or hybrids; on the other hand both 700 and 800 bp bands were obtained for almost all herbaceous peony cultivars or hybrids. Also 1000 bp bands were obtained with some herbaceous peonies. The 700 bp band was obtained from one of the two hybrids which have semi-tree form, similar to those with tree form while the other provided 650, 700 and 800 bp bands (Fig. 2).

After application of Me8+Em2 primer combination the 950 bp band was amplified in all tree form peonies. In addition to 950 bp band, different bands from 250-300 bp size were obtained from some tree form peonies. However, in almost every individual of herbaceous varieties only 500 bp band was obtained. While 950 bp band was obtained in one of the hybrids which have semi-tree form, similar to tree form peonies, the 500 bp band obtained from the other semi-tree hybrid, similar to herbaceous form peonies (Fig. 3).

DISCUSSION

The molecular analysis showed that semi-tree form hybrid individuals selected can be similar to either tree form or herbaceous form. With morphological characterization studies to be completed later this situation can be more clearly demonstrated.

Herbaceous and tree peony forms used in the study of all hybrids between species vary morphologically from each other. Especially unique common bands from herbaceous forms could be separated from the tree forms. In a similar manner tree forms are separate from the herbaceous forms. Similar studies by different researchers made with different primer systems have obtained similar results.

In one of these studies, the origin and genetic diversity of different types and varieties of wild tree peony forms were investigated with 14 SSR markers by Wang *et al*. (2009). As a result of the study, 20 individuals were different from each other and the ratio of heterozygosity was found from 0.41-0.67.

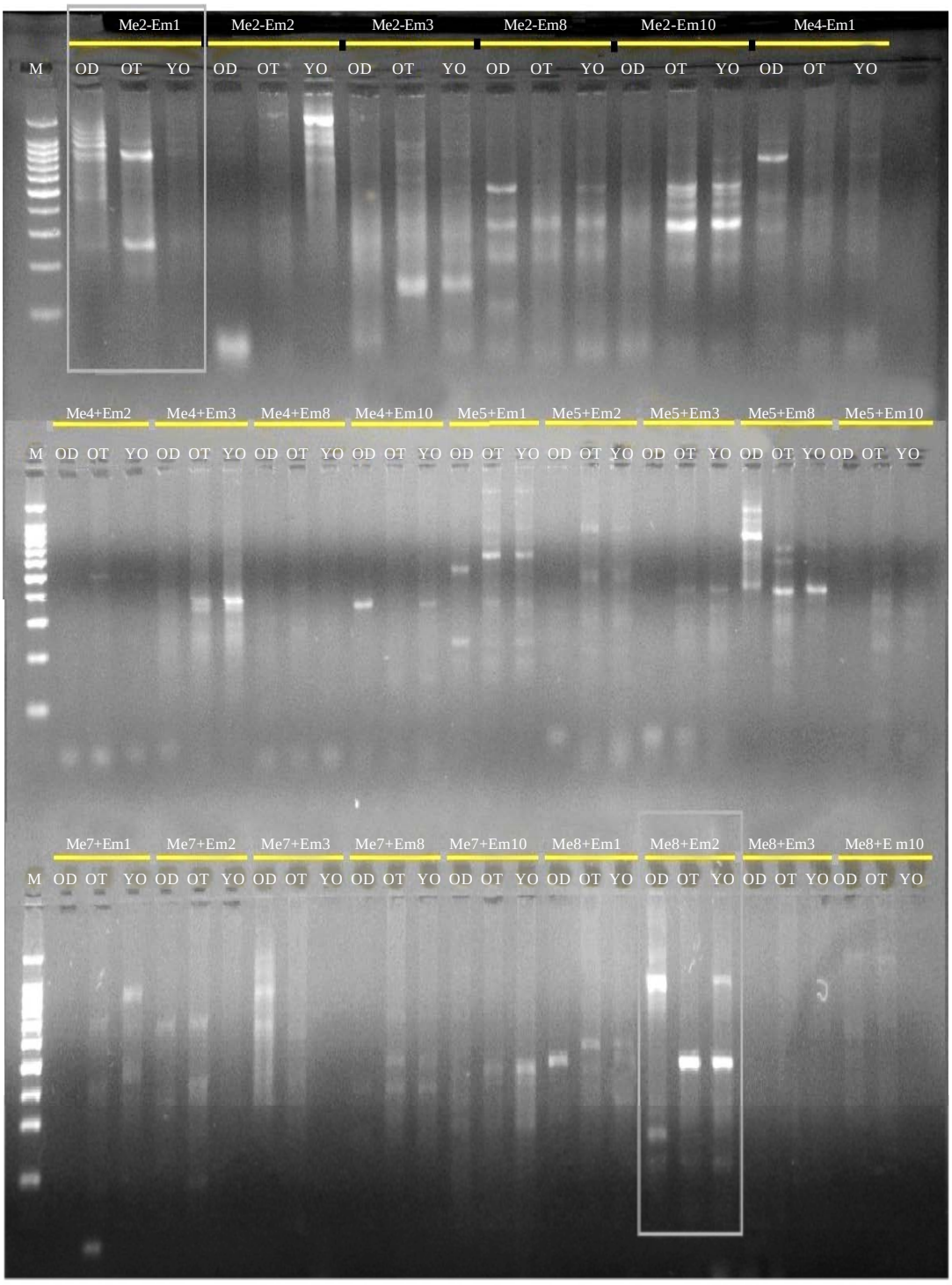

Fig. 1: PCR results of different SRAP primers with herbaceous (OT), tree form (OD) and semi-tree (YO) peony bulks

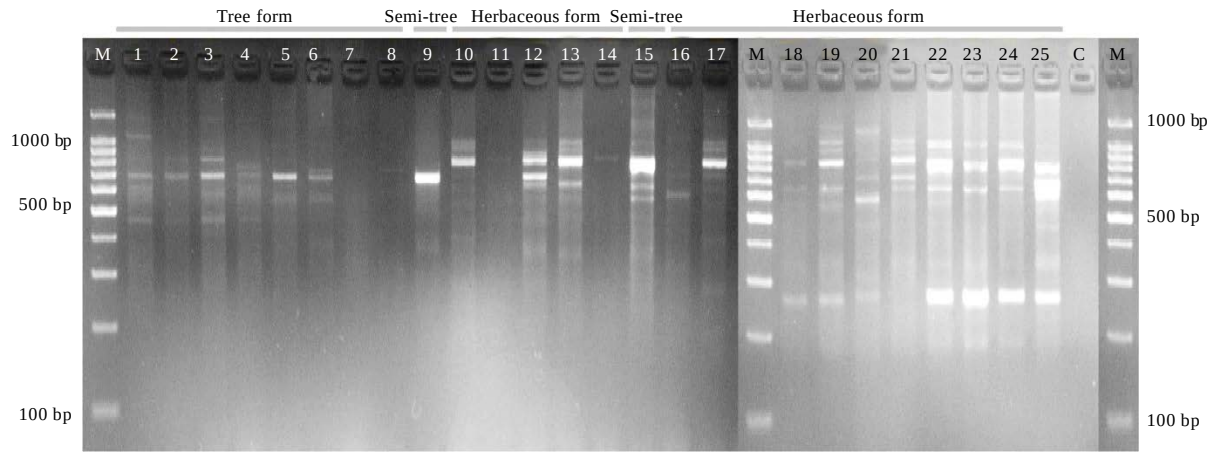

Fig. 2: Me2+EM1 bands obtained by SRAP primers (1-8: Tree form hybrids, 9 and 15: Semi-tree hybrid, 10-14 and 16-25 herbaceous species and herbaceous hybrids, M: 100 bp DNA ladder (Biomatik), C: Control)

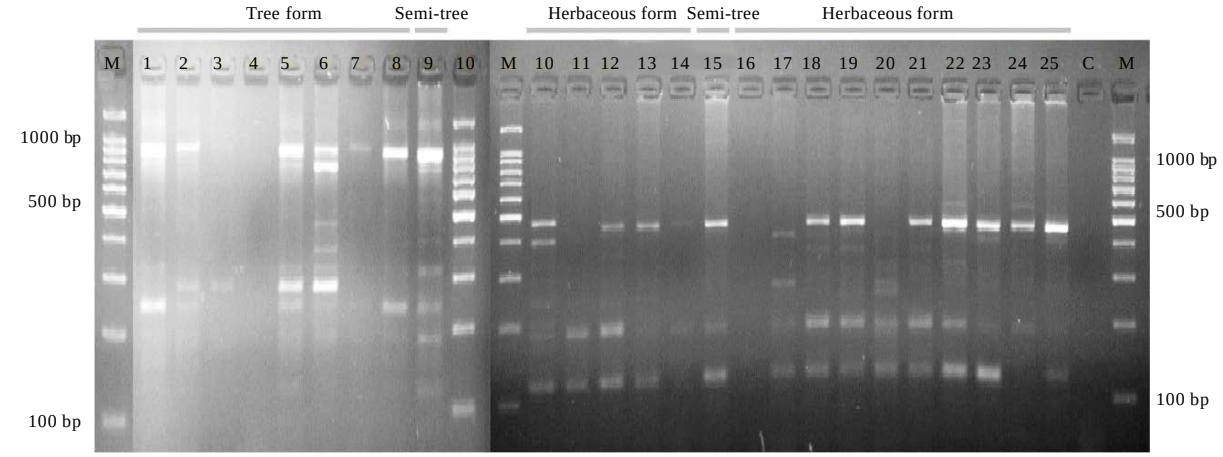

Fig. 3: Me8+Em2 SRAP primer bands obtained from peony species and hybrids (1-8: Woody hybrids-tree form, 9 and 15: Semi-woody hybrid-YO, 10-14 and 16-25 herbaceous species and hybrids-herbaceous, M: 100 bp DNA ladder (Biomatik), C: Control)

Herbaceous, tree form hybrids and inter-species hybrids were distinguished by RAPD primers by Hosoki *et al.* (1997a, b). Similar results to ours were obtained from RAPD primers and they also reported that these primers can be used to identify herbaceous and tree form varieties. In this study, the anthocyanin content of petals, especially in trees, similarities were consistent with the results of morphological characterization, unlike the classification made by the form of hybrids with their parents.

Han *et al.* (2008a) studied 63 tree peony specimens, consisting of 3 wild species and 63 cultivars, using Sequence-Related Amplified Polymorphism (SRAP) markers for the purpose of detecting genomic polymorphisms. Bulk DNA samples from each specimen were evaluated with 23 SRAP

primer pairs. Among the 296 different amplicons, 262 were polymorphic. Han *et al.* (2008b) again used Sequence Related Amplified Polymorphism (SRAP) markers to characterize two tree peony groups with bud sports. 'Er Qiao' and 'Luo Yang Hong' formed group 1 and are derived from Chinese cultivars while 'Shima-nishiki' and 'Taiy' formed group 2 and are related to Japanese cultivars. The results are similar with our studies in that this marker system appears effective in detecting molecular differences between original parents and their bud sports in tree peony. This study provides a technical base for characterizing plants with bud sports which are valuable genetic resources for further breeding.

A similar study was done Hao *et al.* (2008) with SRAP markers. They studied genetic relations and hybrid identification among different sections of *Paeonia* using Sequence Related Amplified Polymorphism (SRAP) markers. A total of 29 cultivars including 2 intersectional hybrids, 13 sect. *Moutan* and 14 from sect. *Paeonia* were used. They obtained a total of 197 bands produced using 24 primer combinations, among which 187 bands showed polymorphism. Owing to the high polymorphism obtained with Em and Me series of SRAP primers, tree and herbaceous form peonies could be separated from each other. Finally they reported that they could identify the peony cultivars using unique SRAP markers and specific primer combinations especially for marker assisted breeding studies, similar to our study.

The phylogeny of the wild tree peony species (section *Moutan*, *Paeonia*, Paeoniaceae), represented by twelve accessions collected from all eight species in the section, was investigated based on the DNA sequence in five DNA fragments from both nuclear (Adh1A, Adh2 and GPAT) and chloroplast (trnS-trnG and rps16-trnQ) genomes, as well as morphological characteristics by Zhao *et al.* (2008). After the study they clarified that the phylogeny of wild tree peony species is in sect. *Moutan*. Also some other studies were done to separate different peony forms, species and hybrids successfully with SRAP primers (Guo *et al.*, 2009; Guo *et al.*, 2011).

Herbaceous and tree peonies belonging to different species and varieties were analysed mostly with SCAR and SSR primer systems. Especially SRAP primer combinations were found to give better results to separate the two different forms, as in our study. SSR primers are preferred to distinguish between varieties and are used successfully in QTL studies (Homolka *et al.*, 2010; Li *et al.*, 2011; Sun *et al.*, 2011; Hou *et al.*, 2011a, b; Zhang *et al.*, 2012; Yu *et al.*, 2013).

There are quite a number of different peony species with different characteristics in the world. These are herbaceous, tree and semi-tree forms. Nowadays, some of these different types are used as parents in breeding studies. Observing the results of these breeding studies takes 3-5 years, particularly in tree forms. In addition, to know the characteristics of new intersectional hybrids is very important for the breeder because they can save time, labor and provide a significant contribution in the financial investment.

CONCLUSION

SRAP markers were used in this study to reduce the time and to achieve targeted results from different marker systems, especially given the positive results reported from peony. In conclusion, SCAR primers can be successfully used to distinguish between herbaceous and tree form peonies in breeding studies. Thus individuals without the selection criteria can be eliminated and the desired result can be achieved more easily.

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