

The conservation and sustainable utilization of the core germplasm of tree peonies

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Tree peonies belong to the section *Moutan* in the genus *Paeonia*, family Paeoniaceae. It is one of the earliest-known and the most famous of horticultural plants in the world. There are eight species and more than 1,500 cultivars of tree peony in the world. The wild species are *Paeonia ludlowii*, *P. delavayi*, *P. decomposita*, *P. qiui*, *P. rockii*, *P. suffruticosa*, *P. jishanensis*, *P. ostii*, while, the cultivars belong to eight cultivar-groups (Zhou 2003). In China, the tree peony has been used as an ornamental plant and cultivated since the Dongjin Dynasty, 1,600 years ago. The root bark of the tree peony, known as 'Dan Pi', which is widely grown, is one of important items of Chinese traditional medicine (Liu 2005). In order to conserve the majority of existing cultivars and wild species, we have spent three years in establishing the Paeonia Germplasm Resource Garden. Till now, we have introduced about 800 cultivars of tree peony from the world. Based on the broad collection of tree peony germplasm resource, we have planned to study their genetic diversity, construct and conserve core germplasm of tree peony and breed new cultivars with high yields, multi-resistance, excellent agricultural characters, and rich in available medicinal components. All these things will be done in order to promote the sustainable development of the tree peony industry. The following part will illustrate the conservation and utilization of it.

Currently, the tree peony is used for its ornamental value as well as Chinese traditional medicine. In China, special gardens of tree peony have been established for visiting. It is widely used as one component of 35 kinds of traditional medicine plants.

In the meantime, based on the collection and conservation of tree peony germplasm, we successfully employed SRAP (sequence related amplified polymorphism, Li and Quiros 2001) markers to analyze the genetic diversity among tree peony cultivars and the genetic differentiations between some cultivars and corresponding bud mutants; the NJ tree was constructed by these markers (Han 2007). Meanwhile, studies on *Paeonia* cultivars and hybrid identification based on SRAP analysis were also carried out. Results



Figure 1 Photographs of tree peonies with different flower colour and flower form (Han 2007).

suggested that cultivars were distinguished from each other used in this study by using totally 35 SRAP markers, which were generated by 16 primer pairs.

Two specific primer pairs can be used to identify cultivars from different sections of *Paeonia*. Flower-form is an important ornamental character for tree peonies; after cultivation and domestication, their flower-form illustrated great variation (Figure 1). Starting from transcriptional factors (MADS-box), which specify flower organ, we try to identify and characterize genes of MADS-box gene family and investigate their role on flower-forms. Now a candidate MADS-box gene from class A was cloned, researches on charactering of its function are undergoing. In order to characterize other MADS-box genes and explore the molecular mechanism of flower-form, we first observed its flower bud developments and differentiation through which we can use as tools for cloning genes and other molecular manipulation. Then a cDNA library using flower buds from different developmental stage was constructed, by randomly sequencing, we obtained 2,300 ESTs among which are important for flower bud differentiation and flower colours. It included genes for vacuolar H⁺/- ATPase proteolipid, gibberellin-responsive, small heat shock protein, programmed cell death, light-inducible protein ATLS1, Cold acclimation, Glutathione S-transferase, putative WD-40 repeat protein,

auxin-repressed protein-like protein, histone 3, metallothionein-like protein, zinc finger protein, YABBY transcription factor, ethylene transcription factor, RAP2.2, DNA binding / transcription factor, TCP transcription factor, MYB family transcription factor. The establishment of cDNA library sets up a milestone for studying the mechanism of flower-form, flower colour and other important characteristics through molecular means.

Flower colour is another important character for its ornamental value. It also aids the differentiation of various cultivars (Figure 1). We have obtained *F3H* and *DFR* genes involved in anthocyanins synthesis pathway, which help us to investigate flower coloration mechanisms. In addition, the distribution of flower colour of tree peonies on CIEL^{*}a^{*}b^{*} scale (included about 500 cultivars) were studied using three-dimensional digital analysis. Research on qualitative and quantitative analysis of flower pigments was carried out using high-performance liquid chromatography (HPLC); results indicated that it contain 6 kinds of anthocyanins in the petals, namely, cyanidin-3,5-di-*O*-glucoside (Cy3G5G), cyanidin-3-*O*-glucoside (Cy3G), peonidin-3,5-di-*O*-glucoside (Pn3G5G), peonidin-3-*O*-glucoside (Pn3G), pelargonidin-3,5-di-*O*-glucoside (Pg3G5G), pelargonidin-3-*O*-glucoside (Pg3G) (Wang 2004, Zhang 2007).

In order to enhance the peony's ornamental value, we are also developing the key techniques of successive secondary flowering of tree peony cultivars so that it can flower successfully during the four seasons; two cultivars were screened for its steady successive secondary flowering. The above technique was patented in China (patent application No., 200610012101.6). The two cultivars can be used widely as materials for forcing cultivation for tree peony production (Jiang 2007).

Since the cortex of the tree peony is a component of Chinese traditional medicine, the medicinal evaluation of different cultivars was investigated using HPLC. We detected eight compounds including three analogues of paeonol, paeoniflorin, two analogues of paeoniflorin and paeonol.

Another of our major objectives is to breed new cultivars. According to the constituents of flower pigments and molecular markers, some hybrids were created, such as *Chinese tree peony cultivars* crossed with *Japanese tree peony cultivars* with purple blotches, or hybrids with big buds, low content of saccharide, vivid red blotches, strong pedicels, suitable as cut-flowers. Especially, from the ESTs, we developed 230 EST-SSR markers for studying their genetic diversity and other characteristics, plus SRAP markers, which can be used for the construction of core germplasm, genetic linkage map, molecular marker assisted selection breeding (MAS) and so on.

In future we need do further work on the followings aspects of the conservation and sustainable utilization of tree peonies: (1) Breeding new cultivars with new flower colours, i.e. blue, black, and multi-coloured for enhancing its ornamental value. (2) Studying further accumulation of active components in the root cortex and increasing its medicinal value. (3) Comprehensive utilization of its petals and natural pigments for human health and functional food.

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