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Advances in the Breeding of Tree Peonies and a Cultivar System for the Cultivar Group

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ABSTRACT

Tree peonies native to China have become an internationalized ornamental plant and are widely cultivated in many countries in Asia, America, Europe and Australia. Breeding starting from domestication of wild plants through selection of bud mutations and seedlings to hybridization has been done for about 1500 years. At present a few thousand cultivars are cultivated globally in gardens or propagated in nurseries, all of which genetically originated from infra- and inter-specific, inter-subsectional and -sectional hybridization and are included in different groups with distinct traits from each other. A cultivar system of tree peonies that considers the origin and diversity of cultivars should theoretically be set up at the base of a cultivar group, and would depend on recent advances from botanical studies and breeding achievements in the last few decades. Such a system composed of 17 cultivar-groups first identified by our work will explain the relationship between species and cultivar groups and among cultivar groups and would benefit tree peony breeding as an important reference for ascertaining breeding strategy and objectives, selecting and matching parents in crossing, and exploring and utilizing valuable genes controlling desirable ornamental traits and resistances. Attempts made in *in vitro* tissue culture, ovule and embryo culture, somatic embryogenesis and plantlet regeneration are uncovering a promising use of bio-techniques to overcome problematic issues of tree peony breeding such as cross incompatibility, hybrid abortion and sterility, slow growth to flower and difficulties in propagation. Combined with molecular marker-assisted and transgenic breeding, inter-subsectional and -sectional distant hybridization will effectively promote the breeding of tree peonies and undoubtedly bring about more opportunity to release better cultivars. A scientific strategy and an international cooperation on tree peony breeding are very necessary for effective work and objectives to be achieved in the future.

Keywords: bio-technique, breeding strategy, distant hybridization, hybrid, incompatibility, selection, *Paeonia*
Abbreviations: APS, American Peony Society; **BAP**, N^6-benzylaminopurine; **GA**, gibberellic acid; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **ICNCP**, International Code of Nomenclature for Cultivated Plants; **ISSR**, inter-simple sequence repeats; **MS**, Murashige and Skoog (1962) medium; **1/2MS**, half-strength major salts of MS medium; **PGR**, plant growth regulator; **QTLs**, Quantitative Trait Loci; **RAPD**, random amplified polymorphismic DNA; **RFLP**, restriction fragment length polymorphism; **SPIN**, Species Peony International Network; **TDZ**, thidiazuron

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Invited Review

INTRODUCTION

The tree peony, native to China, and one of the most magnificent, most beautiful, and most interesting of all plants (Wister 1962), is at last coming into its own as an important garden plant in many countries of Asia, America, Europe and Australia. Respected as "the King of Flowers" in China with a special cultural symbol of peace and happiness, prosperity and development, power and wealth over many other common plants, the tree peony has been cultivated since the Tang Dynasty (618-906 AD), and its Chinese name, Mudan, has been translated as Bohtan or Botan in Japanese and Moutan in Western languages. Its present global popularity which resulted from the prosperity of its breeding requires a systematic knowledge to promote further development, which now, even though is very belated, has become possible due to the huge attempts of botanists, breeders and horticulturists in the last few decades. This review is mainly focused on issues relevant to breeding and cultivars which, in fact, are always crucial to tree peonies and its development even if in the past, now or in the future.

The genus *Paeonia* is taxonomically composed of three sections, *Moutan*, *Onaepia* and *Paeon*. Tree peony, belonging to the section Moutan, is composed of the subsection *Vaginatae* and the subsection *Delavayanae* including groups *Suffruticosa* and *Delavayi*, respectively (Stern 1946; **Fig. 1**). All wild species of tree peonies are endemic to China even though a variety of a few thousands cultivars have been selected in different countries. The first species, *P. suffruticosa* Andrews (Andrews 1804), was first described on the basis of a plant which was a hybrid cultivar originated centuries ago in China (Haw and Lauener 1990; Osti 1994; Wang *et al*. 1998; Haw 2001; Cheng *et al*. 2005) and it is believed to be the result of hybridization between *P. jishanensis*, *P. rockii*, and *P. ostii*. An accepted name for it is *P.* ×*suffruticosa* (**Fig. 2**) in this paper, including a complicated cultivar complex that has actually developed into various cultivar-groups at the base of breeding activity of human beings.

Fig. 2 A flowering plant of a common Chinese Mudan (*P.* ×*suffruticosa*) in a garden in China.

The wild plants of tree peonies is identified and accepted as seven species, *P. jishanensis*, *P. rockii*, *P. ostii*, *P. qiui* and *P. decomposita* of the subsection *Vaginatae* group *Suffruticosa*, and *P. delayayi* and *P. ludlowii* of the subsection *Delavayanae* group *Delavayi*. All of these, excepting *P. decomposita* and *P. ludlowii*, have been separated directly into cultivars or cultivar groups to a different degree, which created a foundation and principle for us to discuss the origin and breeding of tree peonies to set up a cultivar system.

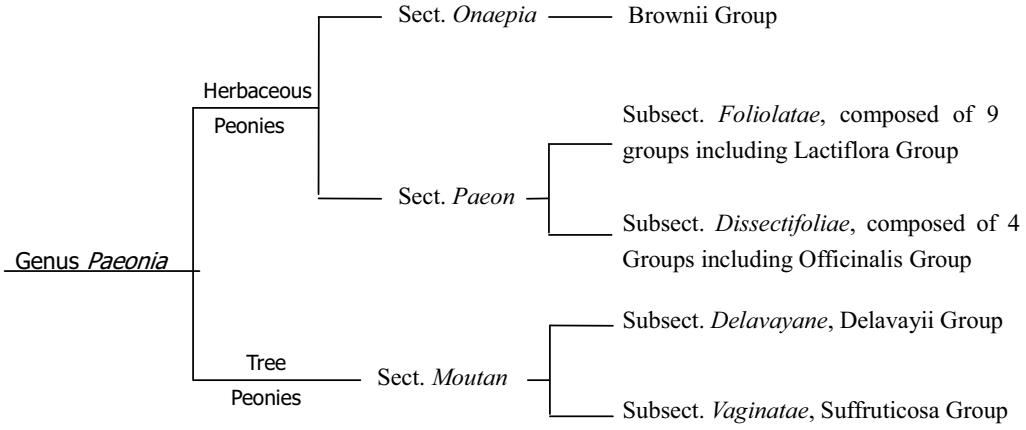

Fig. 1 Composition and classification of *Paeonia*. (based on Stern 1946)

APPROACHES IN THE EVOLUTION OF TREE PEONY BREEDING

Development of a crop is always closely relevant to its breeding, which in many cases was often carried out unconsciously and naturally in the beginning. When its significance and essentiality in agriculture or in other life fields became apparent or un-replaceable with more and more accumulation of knowledge and cultivars, breeding was purposefully pursued to release more and new cultivars that eventually promoted sustained development focused on cultivar improvement and scientific studies. Tree peony, as a special floricultural crop with origins in China, is a very good model through which we can clearly and completely see a historical process of plant breeding evolving from wild to cultivated, simple to complicated and ancient to modern. It is actually the history of tree peony that is its breeding history and is also the history of Mudan culture, representing the most important composition of traditional Chinese floral culture (Li *et al*. 1998). Some events recorded in history are still repeated today in China but it has to be confessed that achievements from modern hybridization and the application of bio-techniques are writing a new history of tree peonies and breeding.

Breeding by domestication of wild plants

Introduction of wild plants into cultivation occurred since very ancient times in China. Peonies cultivated in Chinese gardens are composed of two species, the herbaceous peony or Shaoyao and the tree peony or Mudan, both now well known the world over. From time immemorial Shaoyao has been cultivated in China, the flower being mentioned in the Book of Odes written about in the 5th century BC (Xia dynasty). Its domestication dates back to prehistoric times, over 3900 years ago, so that it is doubtless that the herbaceous peony is in the list of earliest domesticated plants in ancient China. However, no records of the tree peony can be found in the Qin dynasty (221-206 BC). People began to know the tree peony due to its medicinal use from the Qin to the Han dynasty (206 BC-220 AD). Aside from the earliest reliable records that the tree peony was used to treat blood stasis diseases is from a bamboo book dug out from an ancient tomb of the Han dynasty, and many historical literatures definitely show that its introduction into cultivation began since the Qin to Han dynasties, at least 2000 years ago, and originally its root was employed in medicine and not as flowers for gardens (Li 1984; Li *et al*. 1998; Cheng *et al*. 2005).

Under cultivation it seems impossible that anyone does not want to pay attention to the gorgeous and attractive flowers of tree peonies, so that the cultivation for ornamental purposes appeared naturally with a commencement of primary breeding activities, which, commonly accepted now, started in the East Jin dynasty (317-420 AD) about 1600 years ago (Li 1984; Li *et al*. 1998; Cheng *et al*. 2005). By the Sui dynasty (581-618 AD), decades of named cultivars had been collected in imperial gardens. Then the tree peony historically became a leader of plant development in China through the Tang to Song dynasties (619-1279 AD) and became very well-known both because of hundreds of precious cultivars with various colors and forms and because of its special esteem established in the culture and life of Chinese. Ouyang Xiu (1007-1072), the contemporary essayist and great scholar, summarized the advances of tree peony breeding at that time in his work. He recorded that many cultivars were directly selected from wild species and collected together to Luyang, the capital of China at that time, after being domesticated in different regions, and that flowers had showed a very clear direction of evolution from single through semi-double to double in cultivation, which revealed that domestication of wild plants is an important way of tree peony breeding, particularly in the primary stages of cultivar and cultivation development in the Sui, Tang and Song dynasties (Ouyang 1993).

Domestication as a way of forming a tree peony cultivar has never stopped in China since ancient times by digging for wild plants at random for medicinal or ornamental uses, which unceasingly introduced wild plants into gardens or fields or local peoples' yards and directly resulted in new varieties as a base to select new cultivars. As cultivars selected from direct domestication of wild plants normally have smaller single flowers that were not appreciated by most Chinese people who esteemed bigger double flowers, those single flowers – similar to wild species – were neglected and eliminated from gardens along with any chance to become named or pass on through clonal propagation when larger and double cultivars did appear (Cheng 2007a). However, when Chinese people began to feel that single flowers had the same ornamental value as double ones and that there was a need to breed new cultivars with biological traits of resistance to various adversities or more easily cultivated and produced in nursery industry, the importance of plant species with a single flower and their domestication has recovered. Such wild species as *P. rockii*, *P. decomposita*, *P. jishanensis* and so many varieties of *P. delavayi* have themselves great ornamental value worthy to be domesticated into cultivation as named cultivars. Studies on the reproductive characteristics of the species and their domestication (Qian *et al*. 1991; Gong and Wu 1993; Li *et al*. 1995; Cheng *et al*. 1997; Hong *et al*. 1998; Jing and Zhang 1999) supply plenty of references to their domestication for breeding purposes in the future.

Breeding by selection

Breeding by selection was used very early in crops in China and started at first in horticultural crops from fruit trees 1500 year ago (Chen 1953) and found its way practically in tree peony breeding when grafting was incorporated into propagation as of the Tang dynasty (Yao 1982; Li 1984). As it was, formation and establishment of a cultivar is the result of clonally propagating a variation with ornamental value, and propagation by grafting which was set up as early as in the Song Dynasty of ancient China, created a presupposition chiefly for fixing variations from bud mutations and seedlings. Selection breeding is a process induced by the preference of breeders and has played the most important role throughout the history of tree peony breeding. Successive repeated selection from the original category created a floricultural miracle in the breeding of tree peonies and will continue to play a significant role in the future.

Selection from bud mutations and induced polyploids

Bud mutations, a type of gene mutation, has been effectively utilized in tree peony breeding as early as the Song dynasties. Ouyang Xiu (1009-1072) and Lu You (1125-1210) described many samples of releasing known-well cultivars by selection from bud mutations at that time, e.g., 'Qianxi Fei', 'Yu Pao Huang', etc., and showed that good conditions under cultivation produce more chances for mutation and promoted variations or derivation of colors and forms with petalization of anthers, so that "every year taking the changed as news" became common knowledge to peony growers (Yu and Yang 1962; Yao 1982; Lu 1993; Ouyang 1993). In modern times, the typical example of mutation selection is 'Shima-nishiki' (**Fig. 3D**), a Japanese variegated cultivar from a bud mutation in 'Taiyao' released on Daiku island of Shimane, Japan in 1974. The former resembles the latter almost in every respect except for petal variegation (Hashida 1993).

A thing relevant to bud mutation is to induce chromosome mutants. In tree peonies, except for 'Shou An Hong' (Li and Zhang 1982), a natural triploid cultivar from mutation, all species and other cultivars are diploids with 2n = 10. Larger but less chromosomes made tree peonies suitable for inducing polyploids, but there were no successful reports of induction until now. In recent years, our attempts to

Fig. 3 Species of tree peonies and the various ways of cultivar origination. *P. decomposita* (**A**) and *P. ludlowii* (**B**), two valuable species with great potential but not being utilized in breeding. *P.* ×*yananensis* Yan'an Group 'Rui Xiang Zi' (**C**), a natural variety directly domesticated to be cultivated most commonly in the Yan'an area. *P.* ×*suffruticosa* Japan Group 'Shima-nishiki' (**D**), a variegated cultivar selected by bud mutation. Newly-registered cultivar *P. rockii* Gansu Group 'Gao Yuan Sheng Huo' selected from open-pollinated seedlings (**E**). Modern infra-sectional or inter-subsectional hybrid *P.* ×*lemoinei* 'Souvenir de Maxime Cornu' bred in France.

Fig. 4 Doubled chromosomes (2n=20) induced by colchicine solution in the bud of *P. ostii* Fengdan Group 'Feng Dan Bai'.

induce pollen or bud meristem mutants led to the discovery that both buds and anthers of tree peonies were very sensitive to colchicine treatment which could induce an increase of chromosomes in cells (**Fig. 4**) but we are still pursuing a protocol for cultivars with different genetic backgrounds to induce chromosome doubling (Cheng *et al*. unpublished).

Inducing variation by satellite-loaded seeds was done in cultivars of *P.* ×*suffruticosa* Zhongyuan Group and *P. rockii* Gansu Group in China and the seedlings from these loadings were obtained in Heze, Shangdong and Lanzhou, Gansu, respectively but no differences from common seedlings were observed in their morphology and we are now waiting for confirmation with respect to their flowering. As polyploid breeding is effective in improving many trees and horticultural crops, it is, we felt, a very promising theme that should be followed up in tree peonies.

Selection from open-pollinated seedlings

Selection from open-pollinated seedlings greatly contributed to tree peony breeding in the past but has now become the most important way to release cultivars in China since the Song dynasty. It first happened in Luoyang where elite cultivars were collected together from around the country and then become common in Chengdu, Tianpeng, Hangzhou, Bozhou, Caozhou and many other places as the dynasties changed, i.e. when tree peonies were being scattered and distributed more widely. Every leap of tree peony cultivars in China's history was closely related to cultivar collection from different regions or areas, which meant some highly-respected cultivars were introduced to grow together with local plants, generally single and of low ornamental but more suitable to the local climate, so that open pollination occurred naturally among them and plenty of seedlings, used for selecting new varieties leading to new cultivars were eventually obtained from seeds whose gene pool had been much enlarged with gene exchange between plants of various provenances. In the Tianpeng Mudan Record, Lu You (1125-1210 AD) described in Tianpeng (Pengzhou today), Sichuan province that 'Shao Xin Chun' was a new cultivar from the seed of 'Xiang Yun' and that almost all growers planted seeds to get new varieties, not only from 'Xiang Yun' (Lu 1993). In Bozhou, Anhui province in the Ming Dynasty (1368-1644 AD) and Caozhou (Heze today), Shangdong province since the Qin Dynasty (1616-1911 AD), the selection from open-pollinated seedlings was carried out on a greater scale to release a number of cultivars some of which can still be seen growing today. During the Ming and Qin dynasties when the tree peony was almost unknown by most people in the West (Li *et al*. 1998; Cheng *et al*. 2005), the main cultivars and cultivar-groups that originated in China had basically been formed, indicating that selection from open-pollinated seedlings had founded the cultivars of tree peonies.

Similarly, the selection from open-pollinated seedlings played a main role in tree peony breeding in Japan that had been carried on for many hundreds of years since the Edo Period (1600-1867 AD) and had resulted in the release of many very classic cultivars termed Botan, or Japanese tree peonies (*P.* ×*suffruticosa* Japan Group), which seemed more common than Chinese cultivars in European gardens because of more frequent business from Japan with Europe before opening of China to the outside world as of the 1980s (Hashida 1996; Cheng 1997; Cheng and Li 1998).

Variations from open-pollination are very stochastic and not as effective in selecting new cultivars but easily followed up for people with little knowledge of botany and genetics. Based on genetic diversity of the stock population, open-pollination can still play an important role in tree peony breeding today, especially in the case where selection has never been done before or where a distinct germplasm was newly introduced into the population for breeding. A typical instance occurred in flare tree peonies (**Fig. 5**), *P. rockii* hybrids. In the last few decades a number of cultivars were selected in the *P. rockii* Gansu Group from open-pollinated seedlings, during which the chance to select new cultivars increased substantially because of the introduction of traditional *P.* ×*suffruticosa* cultivars in open crosses but the rate of selection was very limited since about 500 clones were selected from about 200,000 seedlings for further observation, some of which were gradually named and released (Cheng *et al*. 1998, 2005). In another instance in Heze, seeds from open-pollinated *P.* ×*suffruticosa* cultivars were sowed in 1956 after many peonies were devastated by big flooding of the Yellow River and 5474 seedlings were obtained from which 252 new cultivars were released after being selected again and again between 1974 and 1975 (Yu

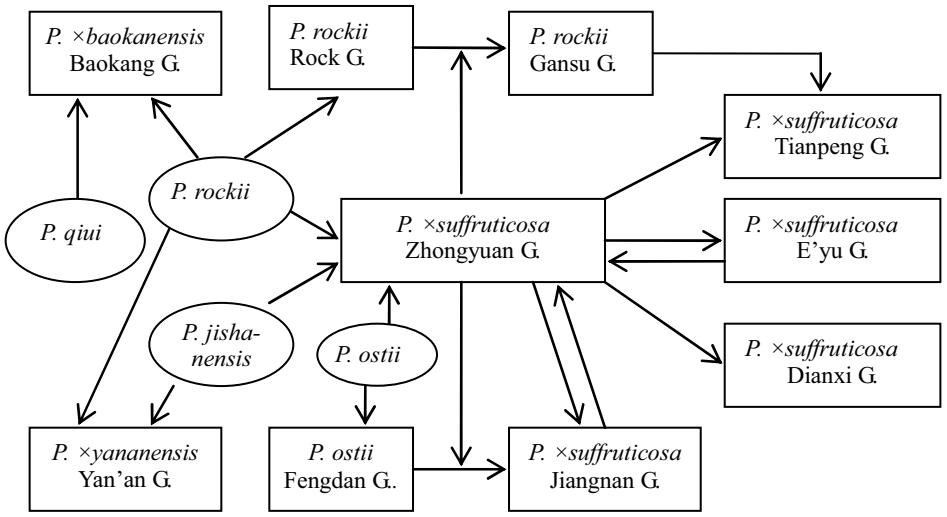

Fig. 5 Relationship between cultivar groups originated in China and species of tree peonies.

1982). These cultivars are the primary focus in the peony industry in China and are popular within and abroad.

Today, more and more people hope that tree peonies could grow normally in colder or damper and warmer regions where no suitable cultivar now grows. Besides introduction and domestication, stress-breeding by seedling selection should be a possible means for this purpose as seedlings are commonly more resistant to various adversities than plants from clonal propagation. The hybrids of *P. rockii* and *P. ostii* should be chosen for this purpose because their genetic traits are more resistant to hardy environments. Totally, the significance of seedling selection in tree peony breeding must wake up to even if other methods, including bio-techniques, would have been developed in the future.

Selection from hand-pollinated seedlings

Selection from hand-pollinated seedlings is an important leap of tree peony breeding and implies a start for breeders to improve cultivars purposefully and directionally by hybridization. This was started later in China only since the 1950s, and some work was done in Heze, Beijing and Lanzhou (Yu 1962, 1982; Zhang 1995; Li *et al*. 1997) but a few cultivars were released from them. However, at present, with an increase of botanical and horticultural studies in the last few decades, hand-pollinated seedling selection is becoming popular in Heze, Luoyan, Lanzhou and Beijing, with joint research by some universities and botanical institutes and gardens. Even though most of this pollination is still confined among cultivars or cultivar-groups of *P. ×suffruticosa*, it is predictable that some new cultivars will be released from them. Similar crosses were done also in Shimane, Japan (Cheng and Aoki 2006) and among breeders of Western countries.

An understanding of the reproductive system of a plant is one of the bases for the cross-breeding of hand-pollinated seedlings and a series of studies on flare tree peonies reported developmental events and the process of sexual reproduction and their developmental time-course, including ontogeny of the anther, pollen, ovule and embryo sac, pollination, fertilization and the development of the embryo and endosperms (Cheng 1996, 1998; Cheng and Aoki 1999a, 1999b; Cheng 2000), which provided a systematic reference for studying the cross-breeding of tree peonies.

Breeding by distant hybridization

Distant hybridization of tree peonies started with the exporting of species of the subsection *delavayanae* from China at the end of the 19th century to the West (Haworth-Booth 1963; Wister 1993; Cheng and Li 1998; Cheng *et al*. 1998), by which the successful introduction of completely new genes into cultivars has created a new period of tree peony breeding different from what happened in China and Japan, as mentioned above. Here the distant hybridization of tree peonies can be divided into two types, the inter-subsectional (or inter-group) between the subsection *Vaginatae* group *Suffruticosa* and the subsection *Delavayanae* group *Delavayi* within the section Moutan, and the inter-sectional between the section *Moutan* and the section *Paeon* of *Paeonia*. Here inter-subsectional hybridization is actually equal to infra-sectional or inter-group hybridization.

Inter-subsectional or infra-sectional hybridization

Except for species of the subsection *Vaginatae*, another subsection *delavayanae* in *Paeonia* had been neglected in breeding in China and Japan until breeders in France, L. Henry of the Paris Museum and V. and E. Lemoines of Nancy first used *P. delavayi* var. *lutea* as female stock to cross with pollen of *P. ×suffruticosa* cultivars from China. Infra-sectional hybrids (*P. delavayi* var. *lutea* × *P. ×suffruticosa*) raised by French breeders such as 'Souvenir de Maxime Cornu' (**Fig. 3F**), 'Le Printemps' and 'Avant Garde', etc. which are still being marketed today (Hashida 1993; Rogers 1995). The first of them was released in about 1900, which should be an important milestone of tree peony breeding having enlarged greatly the gene pool for selecting new varieties of colors.

Afterwards, similar hybridization, in which pollen collected from *P. ×suffruticosa* Japan Group was pollinated on the stigma of *P. delavayi* and *P. delavayi* var. *lutea*, was done by A. Saunders (1869-1953), the most outstanding peony breeder in the US. He started the cross-pollination of peonies including tree peonies at Hamilton College, New York after moving there in 1900. There are some seventy cultivars he registered, "from silver-cream through all the yellows to the color of ripe grain, and from dusty pink through deep strawberry tones to a maroon that is close to 'black', with a scattering of subtle mauves and shadowed rose colors" (Kessenich 1988; Wister 1993).

With the soft stems from *P. delavayi*, the hybrids of Henry, Lemoines and Saunders had flowers hiding or hanging down under the foliage. To breed a new race of hybrid embodying the best qualities of *suffruticosa*, with the strong stems and the well-held flowers, and *delavayi*, with finer foliage, vigor and rare color, in a carefully contrived combination, W. Gratwick (1904-1988) and N. Daphnis continued and extended Saunders's work by using the Saunders hybrids including two rare F_2 seedlings. As the attempts of reverse-cross (*P. ×suffruticosa* × *P. delavayi*) had shown to be unsuccessful, Daphnis had to insist on back-crossing to overcome the incompatibility over 50 years. Generally, the infra-sectional F_1 hybrids are sterile and in a few cases the full seeds can be formed but are very difficult to germinate

into seedlings. Daphnis found that repeated backcrosses obviously improved the fertility and the ornamental value by increasing the composition of the *suffruticosa* gene in his hybrids and, eventually, he registered and released over 50 cultivars which made him known-well globally as well as contributing greatly to peony breeding (Hughes 1985).

Cross incompatibility and hybrid sterility occurring in distant hybridization are problems facing these tree peony infra-sectional hybrids. Recently, abortion of sexual reproduction was reported in 'High Noon', a very promising infra-sectional F_1 cultivar of *P. delavayi* var. *lutea* × *P.* ×*suffruticasa* in further breeding because of its pure yellow and fragrant flowers, vigorous growth and two or three annual flowerings (Smith 1997); this phenomenon was systematically investigated and the results showed that sterility in this cultivar was mainly caused by unviable gametes which were enough for valid pollination, suggesting that using highly viable pollen and appropriate pollination in the best condition of stigmas could overcome abortion in an artificial cross (He and Cheng 2006a). Meanwhile, from the offspring of repeated backcrosses of these infra-sectional hybrids, a group of advanced generation of cultivars has been selected and released into the global market, and are now becoming known to people. As more infra-sectional hybrids are used, crossing will certainly promote the breeding of tree peonies to a new level.

Effects of any breeding always depend on the genetic materials used in crosses. As a very variable species or a complex (Hong *et al*. 1998), *P. delavayi* has a number of varieties in their natural habitats that have puzzled taxonomists for many years but which are in fact attracting the interest of breeders. Very few wild variations of *P. delavayi* were introduced to the West one century ago and have been utilized in infra-sectional crosses. Therefore, newly uncovering the values of so many natural varieties of the *P. delavayi* complex in breeding will supply us with very good genetic resources by which both the infra-sectional hybrids and the hybridization of tree peonies will be much improved.

Inter-sectional hybridization

Inter-sectional hybridization between Sect. *Moutan* and *Paeon* commenced with the remarkable cross by T. Itoh in Tokyo, Japan in 1948. He first raised 6 seedlings from the cross *P. lactiflora* 'Kakoden' × *P.* ×*lemoinei* 'Alice Harding', the former being a white semi-double herbaceous cultivar and the latter an early infra-sectional hybrid bred in France, but he did not see the bloom before he died in 1956. The four of Itoh's seedlings were introduced to the USA by Smirnow to bloom in 1963 and were registered in 1974 as 'Yellow Heaven', 'Yellow Emperor', 'Yellow Crown' and 'Yellow Dream' (Smirnow 1972; Smith 2004). Since then, inter-sectional crossing has been followed up by R. Anderson, D. Hollingsworth, B. Seidl and D. Smith and the hybrids, in common referred to as Itoh's hybrids, have been accepted as a new type of peony to be distributed globally and have become an important field of both peony breeding and cultivation. There are totally about 92 international hybrids named as cultivars, 72 of which were registered in the American Peony Society.

Inter-sectional hybridization of *Paeonia* included three types, *Paeon*×*Moutan* (herbaceous peony × tree peony), *Moutan*×*Paeon* (tree peony × herbaceous peony) and *Moutan* (or *Paeon*)×*Onaepia*, but the named and distributed hybrids almost all came from *Paeon*×*Moutan* in which 'Bartzella', 'Garden Treasure', 'First Arrival' and many other hybrids have been listed as the best peonies and have became popular around the world. The reverse cross of *Moutan*×*Paeon* is very difficult, from which only two cultivars, 'Impossible Dream' and 'Reverse Magic', were selected and registered. From various crosses of *Moutan* (or *Paeon*)×*Onaepia*, it was reported that the hybrid seeds were harvested but did not sprout into seedlings (Cheng *et al*. 2007).

Morphological and biological features of the inter-sectional hybrids are between tree and herbaceous peonies, their stems and foliage die back when entering winter but form buds similar to tree peonies at the base of the bush while the root system resembles that of tree peonies. Having obviously displayed heterosis as vigorous and luxuriant growth, up-right and strong stems, very good bushes, beautifully fragrant flowers with good substance and extended blooming period, inter-sectional hybrids appeared to be better adapted to stress environments than tree peonies and are more ornamental than herbaceous peonies. As more extended species or cultivars including the advanced generation of infra-sectional hybrids are used in crossing, inter-sectional hybridization will be continually developed and bring about more opportunity to release more valuable cultivars. However, such problems as how to overcome cross incompatibility, decrease hybrid sterility and germinate hybrid seeds have to be studied further for more effective breeding.

Breeding by bio-techniques

The traditional breeding of tree peonies is difficult, time-consuming and unpredictable because a long breeding cycle that results from slow growth to flower and difficult propagation, low rate of hybrid seeds or seedlings from cross incompatibility and hybrid abortion in distant hybridization and unpredictable variation from offspring. Therefore, as in other ornamental plants, using bio-techniques has been an urgent issue for tree peony breeding even though such techniques have never been practiced until recently. Many attempts with the commencement of genetically molecular studies were made on the *in vitro* culture of tissues, ovules, embryos and somatic embryos, which will someday make it possible to breed by molecular maker-assisted or transgenic techniques that had received extensive attention and could significantly hasten the breeding cycle and achieve objectives hard to reach by traditional approaches.

Tissue culture in vitro and micro-propagation

Many studies on the micropropagating of tree peonies by *in vitro* tissue culture occurred, not because of the importance of a successful *in vitro* culture protocol as the base for transgenic breeding but because of a huge commercial demand from the nursery industry for tree peony plants which grow slowly and are difficult to propagate. There have been studies that established aseptic cultures through multiplication and rooting to weaning and transplanting plantlets, but more studies are still much required for a feasible micropropagation protocol in the nursery as well as a stable experimental system for breeding studies. These should be established cultivar-by-cultivar as the response to *in vitro* culture is cultivar-dependent.

An aseptic culture is a key step for tree peonies, which is pertinent to explants, inoculation and disinfection. Shoots in spring and winter were reported as suitable explants for culture but it was difficult to determine a procedure of surface disinfection as the concentration of solution and the time for disinfection varied obviously with the explant and culture condition (Albers and Kunneman 1992; Bouza *et al*. 1994a, 1994b; Kong *et al*. 1998; Wang and van Staden 2001; Beruto *et al*. 2004). A method by shoot splitting and division of axillary buds was first described in herbaceous peony (Hosoki *et al*. 1989) and was also used for the multiplication of tree peonies during subcultures on various basal media (Harris and Mantell 1991; Albers and Kunneman 1992; Bouza *et al*. 1992, 1993, 1994a, 1994b, 1994c, 1994d; Kong *et al*. 1998; Wang and van Staden 2001; Beruto *et al*. 2004). A complicated effect of plant growth regulators (PGRs) on shoot multiplication and growth were investigated using cytokinins (benzyl amino purine, or BAP) (Bouza *et al*. 1993), gibberellic acid (GA_3) and its combination with BAP (Bouza *et al*. 1994b), thidiazuron (TDZ) (Gabryszewska 1998) and indole-3-acetic acid (IAA)

or α-naphthalene acetic acid (NAA) (Kong *et al*. 1998).

Rooting is one of the bottlenecks limiting the *in vitro* culture of tree peonies and IBA was demonstrated optimal and commonly used in different ways. Shoots could be cultured continuously by rooting on a low IBA concentration medium (Harris and Mantel 1991; Albers and Kunneman 1992; Bouza *et al*. 1994c; Kong *et al*. 1998; Beruto *et al*. 2004), by quickly dipping in a strong IBA solution (Bouza *et al*. 1994c) or by culturing on medium containing IBA to induce rooting and then cultured on a PGR-free medium for root development (Harris and Mantel 1991; Bouza *et al*. 1992, 1994a, 1994c, 1994d; Beruto *et al*. 2004). The balance of IAA/BA and chilling treatment before root induction was affected by other factors like shoot size, subculture period and cultivar rooting capacity.

Weaning and transplanting was another bottleneck in the *in vitro* culture of tree peonies, as almost all studies reported that, after being transplanted, the plantlets could not establish normal growth naturally, even though a plantlet of *P*. ×*suffruticosa* 'Papaveraceae' flowered after being weaned for two years (Harris and Mantel 1991); I have also personally been able to keep a plantlet alive for two growth years (Cheng *et al*. unpublished). The cause for plants halting their development or for dying was that, during *in vitro* culture, explants lost their capacity to regulate hormone metabolism, and this metabolic perturbation was also maintained after transfer *ex vitro* (Bouza *et al*. 1994a). Considering the similarity in dormancy between seeds and buds (Li *et al*. 1998; Jing and Zhang 1999; Cheng *et al*. 2001), it was suggested that any breakthrough in weaning and transplanting tree peony *in vitro* plants will have to eventually depend on further studies about the mechanism of tree peony dormancy.

Despite such encouraging advances, the micropropagation of tree peonies has not yet conclusively reached the scale of commercial protocols, which equally means a long way for this technique as a platform for transgenic attempts to affect the breeding of tree peonies. Similarly, callus induction and culture *in vitro* from various explants of tree peonies could not result in a protocol which can be effectively used in propagation and breeding (Demoise and Partanen 1969; Gildow and Mitchell 1977; Shoyama *et al*. 1990; Wang and van Staden 2001; Beruto *et al*. 2004).

The in vitro culture of ovules and embryos

Cross incompatibility, embryo abortion and hybrid sterility became the main obstacles to obtain new cultivars effectively since inter-subsectional and -sectional distant hybridization has become an inevitable choice of tree peony breeding in the future (He and Cheng 2004). With complex seed dormancy and very slowly growth, a tree peony takes at least about 10 years to grow from a seed to a mature plant (Buchheim and Meyer 1992; James *et al*. 1996; Cheng and Chen 1998). Several such problems could be overcome with the use of *in vitro* ovule and embryo culture or rescue, which were successfully attempted in *Hydrangea*, *Spiraea*, *Azalea*, *Lilium*, *Lycoris* and some other flowers (Reed 2001; Iizuka 2001; Ayano 2001; Ma 2001; Chi 2002). In tree peonies *P*. *rockii* 'Shu Sheng Peng Mo'and *P*. *ostii* 'Feng Dan Bai', it was reported that young ovules of 66 and 90 days after anthesis (DAA), in which organogenesis occurs, could be cultured into plantlets, but those before 48 DAA failed to do so (He *et al*. 2006). Although the *in vitro* culture of ovules is more difficult because it requires complex media and conditions, when used in combination with *in vitro* fertilization, would rescue the early aborting hybrid embryos so as to obtain seedlings and overcome the incompatibility of a distant hybridization (Zhu 2003). Thus, with a prodigious potential in shortening the breeding period and overcoming embryo abortion or hybrid sterility, *in vitro* ovule culture should be a technique worthy of being pursued for the breeding of tree peonies.

In vitro culture has been widely used to rescue embryos from hybrid seeds usually aborting *in vivo* and overcoming dormancy of recalcitrant seeds (Raghavan 2003). In *Paeonia*, it significantly increased the germination rate of embryos, shortened the germination time and promoted the growth of seedlings (Meyer 1976; Buchheim and Meyer 1992; He 2006; Cheng and Aoki 2007). The *in vitro* culture of tree peony embryos at different development stages indicated that embryos possessing obvious cotyledons (at 60-65 DAA) could be cultured into seedlings but younger globular and torpedo-shaped ones were unsuccessful (He 2006). When embryos from the cross between cultivars of *P*. ×*suffruticosa* were cultured *in vitro* for about 2 weeks before harvest, at harvest and 1~2 week after harvest of seeds, Cheng and Aoki (2007) showed that the culture was clearly related to the physiological property of explants and attached endosperm. Embryos harvested at a different time had a distinct appearance on MS and 1/2MS media and the endosperm significantly inhibited embryo germination. Cultured-embryo seedlings were well established after about 2 months and their growth was much accelerated with the disappearance of epicotyl dormancy, so that 5~6-month-old seedlings could be comparable with two-year-old seedlings from germinated seeds. Therefore, embryo culture *in vitro* should be a versatile technique benefiting the breeding of tree peony (Cheng and Aoki 2007). A protocol for the *in vitro* culture of nearly-mature embryos has been established in *P*. *rockii* hybrids and *P*. *ostii* in our laboratory, but weaning and transferring of the seedlings still failed to permit cultured-embryo seedlings normally growing up to flower as happened with the *in vitro*-plants of tree peonies.

Somatic embryogenesis and plantlet regeneration

Since the first reports in *Daucus carota* cell suspensions by Steward *et al*. (1958), the potential for somatic embryogenesis has been shown in a wide range of plant species, including woody ornamental plants as *Aesculus hippocastanum* (Profumo *et al*. 1991; Čalić *et al*. 2005), *Camellia sinensis* (Mondal *et al*. 2002), *Eucalyptus globulus* (Nugent *et al*. 2001), *Heavea brasliensis* (Etienne *et al*. 1993; Cailloux *et al*. 1996), *Medicago arborea* (Hita *et al*. 2003), *Quercus robur* (Cuenca *et al*. 1999; Toribio *et al*. 2004) and *Rosa hybrida* (Marchant *et al*. 1996; Kim *et al*. 2003), reviewed extensively in Teixeira da Silva (2006). As an important biotechnological tool, somatic embryogenesis demonstrates significant benefits when applied to woody plants and, especially with a prominent role in clonal propagation, provides a valuable tool for increasing the pace of genetic improvement of plants when integrated with conventional breeding programs and molecular and cell biological techniques.

Peonies have a few distinct reproductive features from general angiosperms with zygotic embryogenesis *in vivo* similar to somatic embryogenesis *in vitro* and some dimorphic pollen initiating androgenesis *in vivo* (Yakovlv and Cavey 1957; Li 1982; Li and Zhang 1982; Brukhin and Batygina 1994; Cheng 1996, 1998) that are very predominantly natural for the study and application of embryogenesis. Androgenetic embryoids, embryogenic callus, somatic embryos, and regenerated plantlets have already been obtained in herbaceous peonies (Lin *et al*. 1987; Buchheim and Meyer 1992; Brukhin and Batygina 1994), but actually there have been no attempts to make them feasible in practicable breeding or propagation. In tree peonies there was a report that only a few androgenetic embryoids enclosed by the exine wall and containing about thirty cells with incomplete organogenesis were obtained (Zenkteler *et al*. 1975). Recently in our laboratory, somatic embryogenesis was obtained directly without a callus phase from pre-mature embryos and also secondarily directly from the regenerated plantlets from somatic embryos of *P*. *rockii* 'Shu Sheng Peng Mo', *P*. *ostii* 'Feng Dan Bai' and *P*. ×*suffruticosa* 'Rehkaku' and 'Shima-nishiki', with an 1.7% to 27.3% induction frequency and 12.5% to 23.8% sprouting of somatic embryos. The rate of regenerated plants was 5.8% in 'Shu Sheng Peng Mo' and the plantlets lived 90 days after being

transplanted to the greenhouse. Embryos and somatic embryos of tree peonies could not only keep their ability to form embryos on modified MS or 1/2MS medium over one year but somatic embryos could be proliferated via different ways (He 2006). This study successfully established a primary protocol for tree peony embryogenesis and plant regeneration, which greatly encouraged us to work continuously on it even though many challenges still exist because of low induction rate, poor somatic embryo quality, low regeneration rate and difficult weaning and transplanting.

Plant regeneration through somatic embryogenesis will undoubtedly find its use in the breeding of tree peonies. It could be used in cases where only a few seeds are recovered from distant crosses between infra- and intersectional parents. The embryos could be excised to culture *in vitro* as explants until somatic embryos formed. The latter could be sub-cultured to produce secondary somatic embryos that sprouted into plants after being treated with GA_3 or chilling. Breeders would not only have a plant from the original seed/cross, but also have clones of this plant, and increasing the stock considerably for shortening the period of selecting and naming cultivars (Brukhin and Batygina 1994). Moreover, such a protocol could be employed effectively for transgenic breeding and the scientific studies of *in vitro* culture systems of tree peony are now pending.

Molecular marker-assisted and transgenic breeding

The development of DNA molecular makers and transgenic techniques is providing a valuable means of expanding the plant gene pool so promoting the generation of new cultivars. In tree peonies, increasing the accumulation of DNA molecular data will certainly promote the evolution of breeding approaches even though they are still not enough for such purposes today. Such attempts based on RAPD, RFLP, ISSR and other techniques were related mostly to the phylogenetic and interspecific relationship of species (Pei *et al*. 1995; Sang *et al*. 1997a, 1997b; Zou *et al*. 1999; Lin *et al*. 2004; Meng and Zheng 2004; Zhao *et al*. 2004) and the genetic diversity, relationship, identification and classification of cultivars (Hosoki *et al*. 1997a, 1997b; Chen *et al*. 2002; Meng and Zheng 2004; Suo *et al*. 2005a, 2005b; Su *et al*. 2006), but there are no reports on molecular marker-assisted breeding due to the lack of studies on characterizing genes and QTLs (Quantitative Trait Loci) as well as the difficulty and complexity in tree peony breeding programs. Setting up a genetic map or a linkage map has received great attention in many plants and is becoming an important subject for breeders interested in molecular marker-assisted breeding of tree peonies and, likewise, transgenic breeding has produced some novel cultivars for the global flower industry (Tanaka *et al*. 2005; Teixeira da Silva 2006) but the prerequisite, a feasible system for the transformation based *in vitro* culture protocol, was not met by recent studies in tree peonies. We feel, however, that molecular marker-assisted and transgenic breeding has to be included in the strategy of tree peony breeding in the future, even though so many problems exist at present.

CULTIVAR SYSTEM OF TREE PEONIES ON THE ORIGIN OF CULTURES

According to the International Code of Nomenclature for Cultivated Plants (ICNCP), a cultivar group is a formal category for assembling cultivars, individual plants or assemblages of plants on the basis of defined similarity (Brickell *et al*. 2004). Such a similarity observed in appearance is virtually an expression of genetic similarity of cultivars included in the same group, so that the origin on a similar genetic background should be the most significant in establishing the group. As plenty tree peony cultivars have been distributed globally, a cultivar system on the origin and the group to show the relationship of various cultivars is in great demand for their breeding as well as their cultivation. However, the lack of a clear definition of the cultivar group or with a confused concept about it, the tree peony cultivars originated in China were divided into groups simply but synthetically by geographical distribution (Li *et al*. 1998; Wang *et al*. 1998; Cheng *et al*. 2005; Li *et al*. 2005), and these cultivars were also bred in Japan, American and Europe and grouped differently based on a distinct understanding of them (Wister 1995; Martin 2005). In fact, cultivars presently grown around the world had naturally grouped with their formation and development and it is little difficult to distinguish them as distinct groups. Here, the author firstly named and identified all groups of tree peonies around the world at the basis of cultivar origins by ICNCP and described a cultivar system reflecting the relationship of cultivar groups which will be significant for a global strategy of tree peony breeding.

Cultivars and the group bred in China

All tree peony cultivars originated in China belong to the subsection *Vaginatae* group *Suffruticosa* of the section Moutan in *Paeonia* without any genes of the subsection *Delavayanae* group *Delavayi*. At present in China there are about 1,000 cultivars cultivated in gardens and nurseries. These cultivars are usually divided into four main groups, Zhongyuan (Central Plains), Xibei (Northwest), Jiangnan (Southern Yangtse) and Xinan (Southwest), mainly by geographically cultivated distribution (Wang *et al*. 1998; Li *et al*. 1999; Cheng *et al*. 2005; Li *et al*. 2005), but such a division often brings about confusion because various cultivars with diverse genetic background that should belong to different groups can possibly be included in the same group as they grow in the same region. By their origin and attached biological characters, the cultivars bred in China were firstly named and defined as ten groups, which outlined a breeding system of tree peonies in China.

P. ×suffruticosa Zhongyuan Group

Mainly cultivated in the middle and lower regions of the Yellow River, the Group is called in common as Zhongyuan Mudan (Central Plains Tree Peony), representing or covering traditional Chinese tree peonies over a long period with plenty of cultivars and breeding history. Now, Heze, Shandong Province, and Luoyang, Henan Province, are very well-known for its cultivation. The Group is very diverse in flower colors, forms and other botanical and horticultural characters, which were identical using recent molecular data based on RAPD analysis in the Group (Chen *et al*. 2002; Meng and Zheng 2004). Direct evidence of the when, where and how of the origin are still absent, but a trendy possibility that seems to be commonly accepted is that the cultivars composing the Group originated from the repeated crosses of cultivated and domesticated plants and their main relatives include *P. jishanensis*, *P. rockii*, and *P. ostii* (Li *et al*. 1998; Wang *et al*. 1998; Haw 2001; Yuan and Wang 2002; Zhou *et al*. 2003; Cheng *et al*. 2005; Li *et al*. 2005). The Group consists of 400-500 cultivars and is the largest and the most extensively cultivated in China as well as in the world.

P. ×suffruticosa Jiangnan Group

Mainly cultivated in Shanghai, middle and south Jiangsu, southeast Anhui and north Zhejiang in the middle and lower reaches of the Changjiang (Yangtse) River, the Group is commonly called as Jiangnan Mudan (southern Yangtse tree peony) and is a product of introducing and extending southward of the Zhongyuan Group. When the cultivars of the Zhongyuan Group were historically introduced to the south areas with rainy, moist and warm weather, most of them died away but some that were gradually domesticated were hybridized with *P. ostii* and its cultivars (*P. ostii* Fengdan Group) suitable for growing in the weather of these regions. As early as the Ming and Qing dynasties, there were over

100 cultivars in the Group that grew in Southern China forming a distinct production and cultivation method from the North China Group, from which they were developed (Ji 1809; Li *et al*. 2005). Now the number of cultivars in the Group has decreased much but they should be very valuable for the breeding of new cultivars that may grow normally in large warm and humid areas in the South.

P. ×suffruticosa Tianpeng Group

Originated and cultivated in Chengdu and Pengzhou areas of Sichuan Province, southwest China, often called as Xinan Mudan (southwest tree peony), it is an ancient group developed on the basis of domesticated cultivars from the Zhongyuan Group and *P. rockii* Gansu Group (Tang 1999; Li *et al*. 2005) and the cultivars conserved to date are mostly cultivated around Pengzhou city whose climate is warm and humid. It is believed that the Group is composed of domesticated Zhongyuan and Gansu cultivars and selections from hybrid descendants of them, genetically indirectly relative to *P. jishanensis*, and *P. ostii*, *P. rockii* and a very good genetic resource for breeding cultivars which can grow in warm and humid climates. Morphologically the plants of the Group prefer the Zhongyuan Group to the Gansu Group, having tall bushes with vigorous growth, full-doubled and large flowers with a proliferated structure, shallow distribution but a well-developed root system and resistances to diseases and pests.

P. ×suffruticosa E'yu Group

In the eastward extending regions of Daba Mountains which border the Hubei province (short for E) and Chongqing municipality (short for Yu) and the transitional mountainous areas of Chongqing to the Chengdu plain, tree peonies are grown for their medicinal use on a larger scale along the banks of the Yangtse River chiefly in Enshi, Jianshi and Lichuan of western Hubei and Dianjiang and Wanzhou of Chongqing, and formed a group of cultivars growing in local rainy and humid climate with a warm winter and a hot summer. After being introduced to Heze, Luoyang and Beijing, some cultivars in the Group like 'Jing Pao Hong' and 'Tai Ping Hong' were released, they were widely applied to different climates and conditions and showed great potential to be used in breeding. With very similar plants and flowers to the Zhongyuan Group in morphology, the Group may possibly be derived from the southward introduction of the Zhongyuan Group, but a possibility that there were endemic wild plants waiting to be discovered can not yet be still excluded.

P. ×suffruticosa Dianxi Group

Cultivated mainly traditionally for its medicinal use in Lijiang, Dali and nearby regions of the Jinshan River valley, the west of Yunnan province, the Group is composed of less than 20 cultivars mostly with pink, red and purple double sterile flowers. A new species *P. yunnanenesis* Fang was mistakenly published (Fang 1958), but actually it has been proved to be identical with *P. ×suffruticosa* Zhongyuan Group in morphological characters and should be another group derived from the domestication of the Zhongyuan Group and specific to local mild and humid climate. Totally, there is a shortage of scientific investigation and observation on this Group.

P. rockii Gansu Group

Mainly cultivated and originated in Gansu, eastern Qinghai, southern Ningxia and western Shaanxi, northwest provinces of China, the Group is centralized in Gansu as the main composition of Ziban Mudan (flare tree peony) and Xibei Mudan (Northwest Tree Peony). As an assemblage of cultivars originating mostly from *P. rockii* in genetics, the Group is the result of repeated crossings of the Rock Group joined by the cultivars of *P. ×suffruticosa* Zhongyuan Group introduced to Gansu during a different period, so that *P. jishanensis* and *P. ostii* possibly indirectly affected the Group through crossing with *P. ×suffruticosa* cultivars. To date, 200-300 cultivars in the Group are grown in Gansu and composed of the second largest group of Chinese tree peonies after the Zhongyuan Group (Cheng and Li 1994; Li *et al*. 1998; Wang *et al*. 1998; Cheng *et al*. 2005; Li *et al*. 2005). Having wider ecological adaptability and great resistant to stress environments, plants of the Gansu Group grow tall, vigorous, fragrant, full blossom, are long-lived and very distinct from those of *P. ×suffruticosa* cultivars in many other features. An obvious potential of the Gansu Group in breeding, production and landscape use has become more and more attractive as the plants become available and extensively distributed in China and other countries (Cheng *et al*. 2005; Cheng 2007a).

P. rockii Rock Group

The Group is composed of original cultivars that originated directly from the domestication of *P. rockii* species. So-called 'Rock's Variety' belong to the Group and was collected and introduced to the Western and became very well-known (Stern 1946; Wister 1995). With smaller and single flowers and smaller but hairy leaflets, the plants are similar to wild species in morphological appearance. They are vigorous, fragrant, very fertile and resilient to stress environments but were ignored in the past by Chinese who esteemed large and double flowers so that it was planted only for medicine use in most cases. As tastes for tree peony cultivars changed, some plants with original single flowers were named and propagated as cultivars. Actually, the Group includes varieties of true to species under cultivation and has the same distribution regions as the Gansu Group.

P. ostii Fengdan Group

The Group, composed of cultivars originated directly from *P. ostii* species, has usually been cultivated to harvest roots for Chinese traditional medicine industry for a long time. The most common 'Fengdan Bai' is cultivated widely in China but centralized in Anhui provinces of south China. The Group suitable to grow in the moist and warm climate of southern Yangtse areas (Jiang Nan) was often loosely included into the so-called Jiangnan Mudan (Li *et al*. 2005) but, actually, plants of two groups, *P. ×suffruticosa* Jiangnan Group and *P. ostii* Fengdan Group, are cultivated simultaneously in Jiangnang areas, so that it is very significant and necessary to distinguish them from each other for their scientific utilization in breeding cultivars with heat and moist tolerance.

P. ×yananensis Yan'an Group

Tree peonies had been cultivated in Yan'an area since the North Song Dynasty (Ouyang 1993), but now the cultivars of the Group are almost all localized in Wuahuan (ten thousand flowers) mountain. Having been developed directly from natural hybridization of *P. jishanensis* and *P. rockii* distributed in secondary forest covering the Mountain by the influence of human activities for more than 500 years, the Group includes more than 15 cultivars from single through anemone to crown flower forms that are intermediate in morphological characters between *P. jishanensis* and *P. rockii*, of which only a few cultivars are cultivated in gardens of local people surrounding Yan'an area besides Wuahua mountain (**Fig. 3C**). There were many disputes about if the species *P. yananensis* Hong T et Zhao MR could be accepted (Hong T *et al*. 1992; Zheng *et al*. 1992; Hong and Pan 1999; Haw 2001), but a distinct cultivar group exists actually in Yan'an and was classified into a sub-group of *P. ×suffruticosa* by horticulturists (Li *et al*. 1998, 2005). In any case, even though the cultivars of this Group have lower ornamental value than other cultivars, it

should be very valuable to study the origin of Chinese traditionally cultivated *P.* ×*suffruticosa*, a very significant subject for both botany and breeding practice of tree peonies.

P. ×*baokanensis* Baokang Group

Derived from hybridization of *P. rockii* and *P. qiui*, the Group is younger and composed of about 20 named cultivars bred in recent decades in Baokang, Jingshan Mountain areas of Northwest Hubei, which is a diversity center of tree peony species with wild distribution of *P. rockii*, *P. qiui* and *P. ostii*. Cultivars in this Group were genetically most affected by *P rockii* but obviously *P. qiui* has also joined its formation, so that it is the only group containing *P. qiui* in its cultivar origin.

Cultivars and the group bred in Japan, Europe and Americas

In Japan, Europe and America, the breeding works of tree peonies began with the introduction of cultivars and species from China and passed through different ways to develop various cultivar groups and resulted into diverse cultivars and types, which will be identified next.

P. ×*suffruticosa* Japan Group

The tree peony was introduced to Japan as early as the Nara Period (724-749 AD), grown initially for its medicine use, but became a popular garden plant by the Mumati Period (1393-1573 AD) when almost all kinds of cultivars in China from single through semi-double and double to full double flowers were re-introduced to Japan through peninsular Korea. Since then, on the basis of domesticated Chinese cultivars, breeding by selection commenced by Japanese taste about a good cultivar, so that most full-double cultivars were eliminated but single and semi-double flowers were named and propagated to result in the formation of so-called Japanese tree peonies or *P.* ×*suffruticosa* Japan Group.

According to the literature, the Japan Group was distributed widely in areas around Osaka and Kyoto in the Edo Period (1603-1867) but most cultivars bred at that time were lost and were not maintained to modern times. Modern cultivars growing in Japan like 'Taiyo', 'Hatsu Garasu' and 'Go Dai Shun', etc. were bred again after the Meiji Period (1868-1911 AD) as the cultivation of tree peonies started to transform into an industry production which gained its prosperity at the transition time from the 19th to 20th centuries. Breeding and nursery production stopped during the Wars, were restored in the 1970s and developed much in Niigata and Shimane Prefectures and at present there are about 200 Japanese cultivars cultivated in Japan (Hashida 1993, 1996).

The Japan Group is very distinct from its Chinese originals of the Zhongyuan Group. Besides, most of the cultivars are semi-double or single, purer in color, upright and with a longer stem holding flowers above the foliage and more suitable to the humid climate in Japan; they flower later in the same cultivation condition and require more accumulated temperature for sprouting and flowering than the Zhongyuan Group (Cheng *et al*. 2001). The Japan Group is derived from domestication and seedling selection of the Zhongyuan Group in Japanese climate conditions and by Japanese taste customs for flowers (Cheng and Li 1998) so that it has the same genetic background as the Zhongyuan Group into which no new genes were introduced through breeding.

P. ×*suffruticosa* Europe Group

Tree peonies started to be distributed in Europe, first blooming in 1789. The early introductions from China were *P.* ×*suffruticosa* Zhongyuan Group 'Banksii', 'Rosea', 'Papavaraceae', etc. that enlightened the knowledge and interest of the West in tree peonies. Before new introductions by R. Fortune (1846), seedlings had begun to appear on the continent and some of them were named as early cultivars originated from Europe. Fortune was known well as his several collections of Chinese tree peonies for the Royal Horticultural Society which, undoubtedly, became seeds/seedlings resources to select and name new cultivars among nurseries in England, France, Germany, Belgium and Netherlands (Boyd 1928; Haworth-Booth 1963; Wister 1995). Almost towards the end of the 19th century, a distinct group had been established in Europe and the number should be no less than 110 cultivars listed in English (Harding 1993), which had such flowers full-doubled, downward and hiding in foliage with short and soft stems that were usually identified into the same type as traditional Chinese cultivars (Wyman 1987).

As Europeans has no preference for double flowers like Chinese and since seedlings of *P.* ×*suffruticosa* has a smaller ratio of double than single or semi-double, a reasonable explanation why the Europe Group is composed almost of double cultivars is that the Chinese cultivars domesticated in Europe were renamed and propagated (Cheng and Li 1998). Therefore, the Europe Group was a product of domesticated and renamed Chinese cultivars (maybe included some Japanese ones), keeping similar characters in morphological appearance to the Zhongyuan Group but adapting to grow in the local climate of Europe. With more and more introductions of the Zhongyuan and Japan Groups to Europe during the 20th century, the Europe Group seems to have been submerged and ignored gradually but it should play an appropriate role in breeding tree peony that really fits to European climates. After all there are obvious climatic differences between China and Europe.

P. ×*suffruticosa* Gretwick Group

As a pioneer distributing tree peonies in American, W. Gratwick at Pavilion, New York introduced plants of *P.* ×*suffruticosa* Japan Group directly from Japan since 1946, collected seeds to germinate from them and eventually selected to name some cultivars from many seedlings, like highly prized 'Companion of Serenity' and 'Guardian of the Monastery'. Therefore, the Gretwick Group is a smaller group of *P.* ×*suffruticosa* derived in the US from the Japan Group and has no substantial genetic difference from the latter.

P. ×*lemoinei* France Group

As wild *P. delavayi* and *P. delavayi* var. *lutea* found in Yunnan of China were sent to Europe and American, a new age of tree peony breeding commenced with inter-subsectional crosses between them and *P.* ×*suffruticosa*, which introduced true yellow into cultivars and greatly extended color combinations of tree peonies (Wister 1995). The Franch Group was composed of such hybrids as 'La Lorraine', 'L'Esperance' and others which originated from the crosses between *P. delavayi* var. *lutea* and *P.* ×*suffruticosa* by V. Lemoine (1823-1912) and his son E. Lemoine (1862-1943) in their nursery and L. Henry in the Paris Museum of Natural History in about 1900. With double flowers hanging very downward and hidden among foliage, the cultivars of the Group are very similar to their parentages, the Zhongyuan or the Europe Group, and they have been propagated for a long time and are still popular in global markets today. In comparison with other hybrids of *P.* ×*lemoinei* originated in the US, the France Group still may be used to improve forms of tree peony cultivars and has played a special role in intersectional hybridization of *Paeonia* because of their double form and compatibility in crossing.

P. ×*lemoinei* Saunders Group

After French breeders, Prof. A. Saunders in the US started his wonderfully successful similar crosses in which magenta *P. delavayi* and yellow *P. delavayi* var. *lutea* were

used as female stock and pollen were collected from single and semi-double cultivars of *P.* ×*suffruticosa* Japan Group (Wister 1995; Cheng and Li 1998; Martin 2005). After the first introduction of 'Argosy' in 1928, he registered totally about 75 cultivars with a very wide color range and single and semi-double flowers, which make up the Saunders Group, termed Saunders hybrids by some horticulturists.

P. ×*lemoinei Daphnis Group*

It is at the base of the Saunders Group that, after 1946, N. Daphnis continued the works of Saunders at Pavilion for about 50 years. By continued repeated backcrosses, the genetic make-up of the hybrids was changed in a carefully contrived combination and the ornamental quality was much improved and eventually Daphnis introduced about 50 cultivars including some advanced-generation to form a distinct group, the *P.* ×*lemoinei* Daphnis Group, in which symmetrical, beautifully colored flowers are held well above the foliage and make excellent garden plants superior to the France and Saunders Groups (Martin 2005). These Daphnis and Saunders hybrids are often termed American tree peonies.

P. Itoh Group (P. lactiflora × *P.* ×*lemoinei)*

This Group includes all intersectional hybrids between herbaceous and tree peonies. After first success in Japan in the 1960s, the inter-sectional cross was followed up for half a century in the US and released nearly 92 cultivars to date. With good substance similar to tree peony but the growth habitat being herbaceous, the Group has achieved flowers of unusual beauty of true yellow and various colors and blends and has become an important cultivar type for further peony breeding as mentioned above.

Cultivar system and relationship of the groups

Taxonomical advances in recent decades has allowed for the considerable identification of tree peony species (Haw and Lauener 1990; Hong T *et al*. 1992; Hong and Pan 1999; Haw 2001) and created an opportunity to study cultivar origin and to set up a cultivar system. Commonly-accepted species, *P. decomposita*, *P. delavayi*, *P. jishanensis*, *P. lodlowii*, *P. ostii*, *P. qiui* and *P. rockii*, played an unbalanced role in breeding cultivars and establishing a cultivar system. Even though the complicated polyphyletic and polytopic origin of the cultivars is still pending today, basic species relative to cultivar groups cultivated globally has never been in doubt, so that it has become possible and necessary to suggest a cultivar system of tree peonies by genetic composition of cultivar-groups and their provinces in order to ascertain contributions of each species and the relationships of species and cultivars/cultivar groups as references for further breeding.

Relationship of cultivar groups and the relative species

A considerable knowledge of Chinese cultivars should be a base to understand the breeding of tree peonies. All woody species of *Paeonia* are native to China, but actually only the species of subsection *Vaginatae* group *Suffruticosa* distributed naturally mostly in central China are involved in the origin of cultivated tree peonies while the species of subsection *Delavayanae* group *Delavayi* distributed in Yunnan and Tibet Plateaus were not directly related to the origin of any cultivars with Chinese origin, which can be explained well by the fact that origin and development of any cultivated plants are always closely related to human social and economical activities (Li 1984), since the two main distribution areas of tree peony species were really in different cultural and social conditions at the time when tree peonies were domesticated and breeding first began in China. The distribution areas of group *Suffruticosa* were the center of economy and culture development in ancient China and had relatively developed agriculture and horticulture, which resulted in the domestication of tree peonies and the development of its breeding and cultivation; moreover, the distribution areas of group *Delavayi* were generally underdeveloped in agriculture and horticulture for a long time so that the domestication and breeding of tree peonies was literally impossible. Therefore, social or economical but not scientific or direct breeding activities had a great influence on the origin and formation of tree peony cultivars and cultivar-groups in China, which is very different from other countries.

The *P.* ×*suffruticosa* Zhongyuan Group, representing traditional Chinese tree peonies to a great extent, contains very complicated cultivars extendedly distributed in the north of China. Other cultivar-groups endemic to diverse local climates like the Jiangnan, Tianpeng, E'yu and Dianxi Groups are derived from the Zhongyuan Group by means of domestication and seedling selection. As a hybrid complex, *P.* ×*suffruticosa* contains genes of *P. jishanensis*, *P. rockii* and *P. ostii* and great genetic diversity in its cultivars from molecular data (Hosoki *et al*. 1997a, 1997b; Chen *et al*. 2002; Meng and Zheng 2004; Suo *et al*. 2005a, 2005b; Su *et al*. 2006) indicated the possibility to breed new cultivars in it.

Among species related to cultivars, *P. rockii* is involved in almost all cultivar groups of China directly or indirectly. It did not only result directly into two specific groups, *P. rockii* Rock Group and the Gansu Group, but, as one of

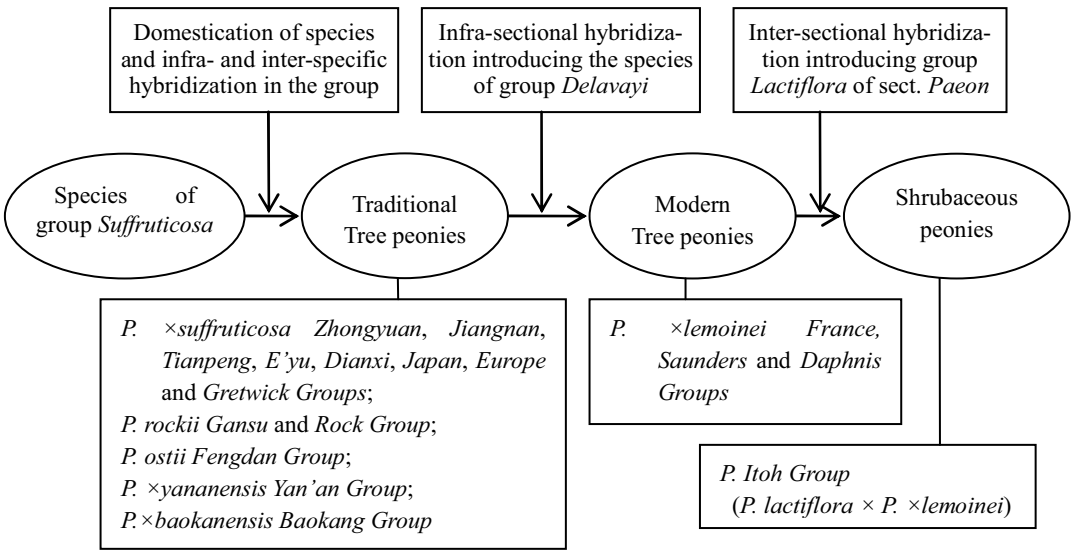

Fig. 6 Evolution of tree peony breeding and the origin and classification of cultivar groups.

main parent in hybridization, was hybridized into *P.* ×*suffruticosa*, *P.* ×*yananensis and P.* ×*baokanensis*. Other species like *P. jishanensis*, *P. ostii* and *P. qiui* also joined a different cultivar group, but *P. decomposita*, a very beautiful transition species between the subsection *Vaginatae* and *Delavayanae* distributing plateaus of northwest Sichuan province and worthy of breeding with red flower and ornamental pinnated leaflets (**Fig. 3A**), does not find its use in any cultivated plants. The relationship of cultivar groups originnated in China and species is summarized as in **Fig. 6**.

No species of the subsection *Delavayae* group *delavayi* were involved in the cultivars originated in China, but *P.* ×*lemoinei* raised in France and the US were the result of hybridization between *P.* ×*suffruticosa* and *P. delavayi* (including *P. delavayi* var. *lutea*). However, *P. ludlowii*, more vigorous than *P. delavayi*, with more spreading petals and larger flowers held by a robust stem, is not utilized in any hybridization. This species (**Fig. 3B**) is confined naturally to a small area of Tibet along the Zangbu gorges but has been successfully introduced to many gardens in the West. It was believed to be a good genetic resource that should be utilized in improving tree peony cultivars through breeding.

Type of cultivars or hybrids and their evolution

Tree peony breeding in other countries started with the introduction from China. Japanese made their selection from open-pollinated seedlings in the same way as Chinese did, but breeders in Europe and American took a very different strategy in which purposeful hand-pollination greatly improved cultivars due to the release of inter-subsectional (or inter-group) hybrids. Cheng (1996) suggested the classification of global tree peonies into traditional and modern types mainly by means of approaches used in breeding and the genetic differences as parentages. Having been bred traditionally by selection from open-pollinated seedlings, traditional tree peonies include infra- and inter-specific hybrids within or between cultivar groups originated genetically from species of the subsection *Vaginatae* group *Suffruticosa* in China and Japan, mostly before 1900. Hybrids bred by purposeful artificial inter-subsectional (or inter-group) hybridization between the subsection *Delavayae* group *Delavayi* and the subsection *Vaginatae* group *Suffruticosa* in France and the US after 1900 can be defined as modern tree peonies. Accordingly, the cultivar groups of *P.* ×*suffruticosa*, *P. ostii*, *P. rockii*, *P.* ×*yananensis* and *P.* ×*baokangensis* correspond to the traditional type and the hybrid groups of *P.* ×*lemoinei* [*P. delavay*i (*P. delavayi* var. *lutea*) × *P.* ×*suffruticosa*] to the modern type. The cultivars of traditional type including infra- and inter-specific hybrids founded the breeding and nursery industry of tree peonies around the world and the cultivars of modern inter-subsectional hybrids are becoming more and more known even though their flower form needs further improvement. Meanwhile, as a completely new type of peony and a great breakthrough of breeding in *Paeonia*, the inter-sectional hybrids or the Itoh's hybrids are greatly extending the field of peony breeding and making peonies more and more attractive in gardens and nursery industry. Therefore the evolution of tree peony breeding or cultivars has passed through three stages from infra- and inter-specific through infra-sectional (inter-subsectional or inter-group) to inter-sectional hybrids, as seen in **Fig. 6**.

STRATEGY AND PROCEDURE OF TREE PEONY BREEDING

A strategy or a systematic program on the basis of breeding objectives is a basic subject that any breeder must face and work out in advance of breeding. For the breeding of tree peonies, determining breeding objectives and selecting parental mating in crosses are two crucial basics and other contents include new methods or techniques application and international or regional cooperation on the relative studies or breeding activities. A breeding strategy will be carried on by a conventional procedure established in breeding practice.

Strategy of tree peony breeding

The first objective for tree peony breeding is still to improve the form and color of flowers, the two main facets for evaluating a cultivar. Other objectives should include: a) to prolong flowering time for a single flower, a plant and a cultivar-group and more flowers for a stem and a bush, which will increase the value of tree peonies as ornamental landscape plants in gardens or as cut flowers to produce in nurseries, since shorter duration of blooming has become the biggest complaint about tree peonies; b) to select specific horticultural traits suitable for growth under cut-flower, contained and forced or retarded cultivation, which will meet the needs of promoting the peony industry; c) to breed vigorous or disease-resistant plants growing in stress (cold, drought or high humidity) environments, which will allow tree peonies to be cultivated and distributed over a wider distribution of various climates around the world.

Since limited collections of genetic resources were used in breeding or due to the lack of purposeful hybridization in the past, the breeding of tree peonies was ineffective, even though such a great diversity of cultivars were released, contrasting to the great potential they should have displayed throughout the evolution of cultivars. With splendid color mixture, the modern cultivars (*P. delavayi* × *P.* ×*suffruticosa*) are rewriting the history of tree peony breeding and promoting the improvement of cultivar quality and production. However, they resulted just from a few gene resources of *P. delavayi* (including *P. delavayi* var. *lutea*), a variable species including many natural varieties (Hong *et al*. 1998) worthy of utilization in horticulture, and *P.* ×*suffruticosa* in which many advanced generation hybrids were still hybridized in infra- and inter-specific but not in inter-subsectional or -sectional breeding. It can be predicted that inter-subsectional and -sectional distant hybridization will create a race of hybrids embodying the best qualities of the traditional and modern cultivars in carefully contrived combinations, because in existing tree peonies the traditional ones have strong stems, well-held flowers of great size and elegant texture, while modern ones are vigorous with pure color and other fantastic color mixtures missing from the former's spectrum.

Since inter-subsectional and -sectional distant hybridizations have become the principle ways of breeding tree peony, selecting parental mating in crosses will become more important than ever before and must be carried on according to the objectives at the base of the collections. As a comprehensive subject requiring a good understanding of genetic resources and the use of approaches available for breeding, it had been greatly limited by confined resources of tree peonies with their biological traits like cross incompatibility and hybrid sterility or divergent blooming date and by methods and techniques applied by breeders. Actually the shortage of studies on the genetic variation and other topics related to breeding made it very difficult to predict results accurately, so that it is urgent to ask institutes, universities and companies interested in peonies to join their breeding activities of tree peonies to overcome many existing problems which cannot be solved by amateurs, as occurred in the past. Because of incompatibility and sterility, parental mating in crosses becomes so crucial, and this will determine the success or failure of breeding to a great extent. Recently evident progress in inter-subsectional and -sectional hybridization has been made in China because such new materials not introgressed into the modern hybrids as hybrids of *P. rockii* Gansu Group, *P. ostii* Fengdan Group and herbaceous peonies *P. veitchii* and *P. lactiflora* were selected as parents in done by my students in the peony collection of Beijing Forestry University (He and Cheng 2005; He 2006; Zhang *et al*. 2007), which indicated that there is a very large space to discover new parental combinations from existing but unused sources for effective

breeding.

Besides traditional approaches for hybridization, new methods or techniques will be utilized in tree peony breeding such as mutation breeding, tissue culture *in vitro*, embryo culture, somatic embryo induction and molecular marker-assisted and transgenic breeding. However, achievements in these aspects have still not established protocols available for the breeding of tree peonies, but are becoming a promising field for studying the breeding of tree peonies.

An international or regional cooperation on research and breeding of tree peonies should become an important part of the strategy of tree peony breeding. Species Peony International Network (SPIN), a group composed of amateurs on peonies, greatly promoted international exchange of peonies and a similar function has been run continuously now by the seed distribution program of the American Peony Society (APS). But eventually, an international cooperation with Chinese breeders and researchers should be more significant in the breeding of tree peony since China has the richest genetic resources in the world, including species and cultivars unavailable in other countries. In the past, breeders and researchers in the West had always to made attempts depending on limited scattered collections or samples in herbaria which were perhaps neither the core genes for breeding nor the typical samples for identifying them. In contrast, Chinese people in many places traditionally repeated the same things in breeding over a long time by selecting varieties from open-pollinated seedlings or made their studies based on Western references, which did not correspond accurately to the facts. A classic example is the so-called 'Rock's Variety' that was a very commonly cultivated plant not receiving any attention in Gansu, China but was highly respected because of its origin and distribution filled with a mysterious legend in the West (Stern 1946; Cheng *et al*. 2005). Therefore, an international cooperation of China and other countries should really be a feasible and fruitful way to study and breed tree peonies (Osti 1999). In addition, it is very necessary to establish a registration system for Chinese cultivars in China, which should be done by the supervision of the International Society for Horticultural Science (ISHS) and ICNCP as APS does and will promote Chinese breeding works to internationalize them so that breeders and gardeners of other countries could correctly and promptly enlarge their gene bank for the parent selection in the breeding of peonies. Some newly-bred cultivars in China started to be registered in APS (Cheng 1994; Cheng 2007b), but in most cases it is impossible for Chinese breeders and gardeners who do not know sufficient English and do not have sufficient knowledge of cultivar nomenclature to register new cultivars through the ICNCP. An international register system covering China will be very important for tree peony breeding and its global development owing to China's leading position in tree peony breeding, cultivation and application in the world.

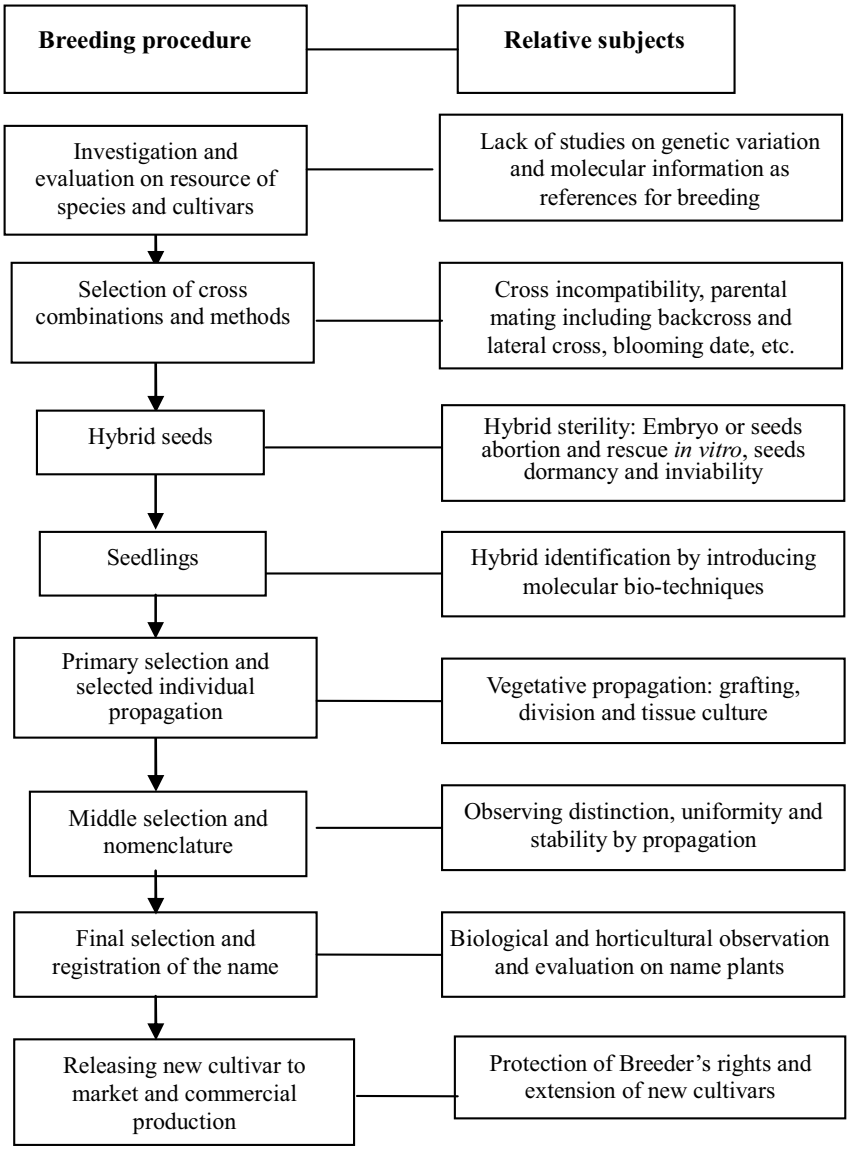

Fig. 7 A conventional procedure for tree peony breeding and relative subjects to study.

Procedure for breeding tree peonies

Any breeding always starts with the collection of resources for crossing and ends with the release of new cultivars, but the procedure for breeding varies according to plants with distinct biological and genetic characters. Breeding that releases many tree peony cultivars has encountered many difficulties and problems. In classic breeding of tree peonies, the seeds can take up to 2 or 3 years naturally to germinate and the seedlings develop extremely slowly to flower after 4~6 years and to give stable and typical blooms after another 3~4 years, so that it takes at least about 10 years to grow from a seed to a mature plant (Cheng and Chen 1998; James *et al*. 1996; Cheng *et al*. 2005). Furthermore, a selected individual has to be propagated vegetatively to evaluate its distinction, uniformity and stability so that it can be named, registered and released eventually as a new cultivar, which will take another 4~5 years. Such a conventional procedure of tree peony breeding is summarized in **Fig. 7**, including basic genetic or physiological or even horticultural topics involved in every step of the breeding program. Obviously, the breeding of tree peonies requires much patience and is a time-consuming process and, in the future, has to depend on the use of modern bio-techniques in order to shorten the cycle. Before establishing a protocol of biotechniques including molecular marker-assisted and transgenic breeding, traditional ways by seedling selection and hybridization will still be the most significant means, but any attempts to use modern techniques will undoubtedly promote more efficient breeding and has become an urgent task for researchers and breeders interested in tree peony breeding and its nursery industry.

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