Advances in Genetic Improvement of Teaoil Camellia

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Abstract

The efforts to genetically improve teaoil camellia, *Camellia oleifera* Abel, started more than two thousand years ago when forest dwellers chose to use seeds to extract table oil and later selectively planted teaoil camellia trees. Since the 1970s, China has sponsored many national-level projects on genetics studies and selective breeding of this taxon. So far, over 200 superior cultivars, families, clones, and hybrids have been selected, and those varieties have markedly improved the productivity of teaoil by renovation of low-yielding plantations and application of superior new varieties. The analysis of present production status and the situation of genetic improvement in teaoil indicate that mutation breeding and biotechnological improvement should be emphasized based on conventional breeding methods. Rich germplasm resources should benefit a molecular breeding program.

INTRODUCTION

Teaoil (*Camellia oleifera* Abel.) is a unique edible-oil tree in China. Combining with oil palm, olive and coconut trees, teaoil is considered as one of the world's four best woody plants for producing edible oil. Teaoil is distributed mainly in southern China. Among them, Hunan, Jiangxi and Guangxi are the main production areas (He, 1997; Li, 1980; Yang, 1992; Zhuang, 1988) and virtually all parts are useable. In addition to high-quality cooking oil, teaoil also has many other uses (Zhang et al., 1996). In the recent 50 years, Chinese teaoil scientists have performed extensive research, resulting in a great quantity of valuable information. However, many problems remain in regard to genetic improvement of teaoil. For example, annual production of this plant is still low and unstable (Chen et al., 2002; Zhang, 1996). In order to supply sufficient cooking oil, it is imperative to improve teaoil varieties as well as its production (Tan and Chen, 2004; Zhang et al., 2004; Zhu, 1978).

PRESENT SITUATION OF TEAOIL BREEDING RESEARCH

Present Situation of Conventional Breeding Research

The production of teaoil relies on natural growth directly from seed reproduction. With such a rudimentary management style for a very long time, the nation's tea oil output is very low. Since the 1970s, efforts have been put into studies of comparison and appraisal, superior tree screening, fine pedigree appraisal, vegetative reproduction, fine clone appraisal among cultivars (types), and scionwood garden establishment. Scientists have used selection breeding for teaoil varieties and single batch selection breeding with successive single pedigrees and clones. Such work has resulted in greatly improved varieties and has laid a solid foundation for the improvement of Chinese teaoil output (Chen et al., 2005; Lei et al., 2003; Wu, 1996).

Investigations on Teaoil Germplasm Resources

The linear model (from protection to appraisal to use) is generally regarded as plant idioplasmic resource management (Berthaud, 1997). The purpose of management is to retain gene resources in the gene bank. Therefore, a clear understanding of management of plant idioplasmic resources is necessary. To track various local teaoil idioplasmic resources is one of the most important methods of teaoil idioplasmic resource management. In this regard, local government-funded research has produced some significant results (Deng et al., 2002; Xiong, 1994; Zhuang et al., 1991).

There are two traditional methods in preserving gene resources. One is native gene bank. This refers to production seeds in the original ecological environment. The typical cases are the establishment of natural protection areas or natural parks that protect wild and closely-related plant species. The second method is non-native gene bank, which refers to preservation of seeds at low temperatures or in countryside plantations (idioplasmic garden, botanical garden, etc.). It also includes adult plant preservation and test-tube seedlings as well as tissue culture preservation. In China, the idioplasmic gene preservation of teaoil generally utilizes the non-native gene bank method. Provinces or local governments adopted their own gene bank methods that are suitable for local development of teaoil fine idioplasmic resources (Li, 1983; Xue and Li, 1996; Yao, 2005).

Selection of Superior Teaoil Trees and Establishment of Scionwood Collections

Teaoil requires insect cross-pollination. For a long period, production was based on wind pollination, which is strongly influenced by the poor management and difference among natural trees. A six-year observation by the Hunan Academy of Forestry showed that more than 9.2% of strains were high-yielding, producing 1.25 kg of fruit per square meter of crown, and 59.3% of strains are low-yielding, producing only 0.75 kg per square meter (Chen et al., 2005). A selection directly from the system is most likely to achieve the aim of improving breeding and genetic gain. From the beginning of the 20th century to mid-1960s, teaoil selection was carried out everywhere. Through the investigation, pilot selection, check selection and final selection stages, more than 16,000 strains of teaoil trees were selected nationwide in the late 1970s and early 1980s. As a result, 153 ha nurseries of selected teaoil trees were established, which laid a solid foundation for a natural teaoil breeding effect (Deng et al., 2002; Zhuang et al., 1991).

Selection of Superior Teaoil Cultivars

As a result of natural cross-pollination, teaoil formed its own diversity in terms of generation, phenology, productivity and pest resistance. He et al. (1984) used cluster analysis and selected 38 excellent teaoil types from more than 1,000 sample areas in 56 counties.

Many cultivar selections are breeding types between varieties of species and breeding groups, which have been selected by farmers through production practices. Some excellent selections are from mixed groups or individual forms that have adapted to local climate and environment and become regional cultivars. These groups are rather complex, and the improvement of these groups can only be achieved by continued breeding. More than 20 good farm cultivars were selected through a breeding survey in the 1970s, and then 1,680 ha seed production stands and 404 ha of seedling seed orchards were established. Families currently used for selection of superior clones are mostly from these fine cultivars. In 1980, breeding experiments were conducted on 12 cultivar types of teaoil species in 26 experimental sites (established by the Association of National Teaoil Varietal Propagation), which were located in Hunan, Jiangxi, Guangxi, Guangdong, Fujian, Zhejiang, Anhui, Hubei, and Guizhou. After more than 10 years of systematic studies, 'Hendong Big Boll' and five other excellent cultivars were selected and suitable regional areas of production were also recommended. Currently, good cultivars of teaoil include 'Yongxing Medium Red Ball', 'Cenxi Soft-branch', 'Baling Seed', 'Hendong Big Boll', 'Shishi Red Pell', 'Erdong Big Red Fruit', and 'Wangmo Teaoil' (Wu et al., 2001; Ouyang, 1991; Wang DB et al., 1985; Qin, 1980).

Selective Breeding of Fine Teaoil Families

Based on tree breeding and variety comparison, excellent families and future generations are often 15% or higher in gain compared to natural populations. After the selection of superior trees, seeds are propagated and compared to one another in field plantings. The varieties selected from these experiments on the filial generation group are called superior family varieties. This method uses seeding reproduction. Despite phenotypic diversity due to progeny and economic traits, it is unlike the traditional natural population method of breeding. Moreover, because good families are reproduced from nursery seedlings, the technical requirements of afforestation are relatively low and easy to learn, thus making the process more acceptable to the farmers.

Research Institute of Subtropical Forestry, Chinese Academy of Forestry (CAF), selectively bred 'Yalin #1' and other two fine families in 1986. Its main economic traits of genetics are high, and its heritability of crown width, clear length, branching angle and branch quantity are more than 55% (Ouyang, 1991). The Forestry Science Institute in Chenzhou in Hunan Province selected family 71-2 through an eight-year test. Mean oil production reached 1187.55 kg per ha, 32.6% higher than the other four families tested (Qin, 1980). The broad heritability of the main economic traits is high, and kernel oil rate is as high as 86.6%. Hunan Academy of Forestry selected three fine families 'Xiang #5', 'Xiang #7' and 'Xiang 39' with mean oil production of 552.0-592.5 kg per ha. These families are planted in Hendong County, Hunan and Fuan City, Fujian. That academy again selected two good families, 'XLJ2' and 'XLC14'. The oil production per ha was 512.20 kg and 490.98 kg, an increase of 38.5% and 32.8% respectively (Chen et al., 2004).

Selective Breeding of Superior Teaoil Clones

Based on conventional methods, breeding of superior teaoil clones was started with cloning experiments in the 1980s. After more than 10 years of unremitting efforts, according to the National Teaoil Collaborative Research Group who developed the criteria and procedures for the selection, more than 200 excellent clones had been selected. Currently, superior clones of teaoil have become the most important germplasm resource. Those clones include Xianglin series from Hunan, Ganwu series from Jiangxi Province, Changlin series from China Subtropical Forestry Research Center, and etc. These superior clones were developed from the systematic breeding process of elite tree selection, scionwood orchard observation and detailed comparative tests. Clonal reproduction can fully maintain the good characters of the parent trees, such as early-fruiting and high-yielding. Hunan Academy of Forestry established an intermediate experimental field for teaoil superior clones in Youxian County, Hunan in 1987. The average oil production in 1991 was 607.5 kg per ha (Chen et al., 2004). Central South Forestry University, collaborated with Lingling Forest Research Institute of Hunan, used superior clones to establish an 85 ha plantation in 1986 and fruiting started 3-4 years afterwards. In 1990, the yearly oil output was 70.4 kg per ha. From the 5th year after planting, the production of the new plantation surpassed the yield of the original forest and oil production amounted to 477.15 kg per ha in the 9th year (Wei et al., 1993). According to incomplete statistics, with the help of the Upgrading Projects of Non-productive Teaoil Plantations sponsored by the former State Ministry of Forestry and the various provincial governments, the acreage in which superior teaoil clones are used to improve production has reached some 2×10^5 ha. More broad extension has been applied to different provinces and regions to promote the good performance after regional tests (Han, 2000; Wang and Chen, 1991).

Teaoil Crossbreeding

In the 1970s, hybrid breeding was studied within teaoil species and among *Camellia* species, such as pollen collection and germination culture experimentation. After teaoil pollen was stored in dry and low temperature conditions for one year, the germination rate still reached 40%. Teaoil used to be considered non-self-fertile. However, in superior clones, it has been found that many are self-fertile at a relatively high rate of self-pollination (Chen et al., 2001; Xi et al., 2005; Zhou et al., 2001).

In early 1990s, Hunan Academy of Forestry began to study teaoil hybridization. After ten years of effort, the first selection, 'XLH13', and other four good crosses were propagated. The oil production was 450.76-660.65 kg per ha, an increase of 37.0%-100.8%. Mean oil production of the selected hybrid offspring was 207% that of the inbreeding combinations (averaging 167.6 kg per ha). The main economic traits of the hybrids are better than their parent clones and these traits include growth habit, strong heterosis, indicating the improvement of these hybrids. Biological characteristics during pollination process showed no self-pollination, resulting in the discovery of a "male sterile line" of the superior clones owing to pollen sterility (Chen et al., 2005). The process of propagating seedlings with hybrid seeds is simple, and the seedlings are ready for transplanting after one-year in the nursery, thus shortening the breeding time and reducing the cost and difficulty of reforestation. Thereby, it has stimulated enthusiasm from the field and greatly accelerated the extension process of using improved planting material.

Other Aspects of Breeding

The conventional breeding for phenotypic characteristics of teaoil requires a wealth of practical experience and is long and can take up to several decades. For 30 years starting with superior tree selection, teaoil breeding has experienced numerous difficulties. In the selective breeding process for good cultivars, families, and clones, many attempts have been made in the improvement of teaoil's resistance to diseases and insects (Chen et al., 2005).

1. Resistant Breeding. Breeding efforts focus mainly on the disease-resistance. Dong et al. (1990), as well as the Teaoil Anthrax Group in the Disease and Pest Control Division of the Research Institute of Subtropical Forestry, CAF (1997), have conducted various disease-resistant studies on different teaoil species. The studies on teaoil anthracnose (*Colletotrichum theae-sinensis*) have been reported the most (Xiao et al., 2005; Zhang, 1996). Zhuang (1973) conducted disease-resistant research and selectively bred four fine clones. Wu et al. (1997) selectively bred 8 resistant trees in 162 teaoil clones, whose natural susceptibility was 0.2%-2.5%. After six years of continuous research, the Forest Institute of Chaling City, Hunan Province, selectively bred plus trees whose anthracnose susceptibility was only 0.23%, while fruit yield was as high as 0.115 kg per square meter of crown (Huang, 1981). The Forest Institute of Lianyuan, Hunan Province, bred 77-1 and 4 other strongly disease-resistant plus trees in 1981 (Zhang, 1996). There are few reports on other related resistance aspects.

2. Radiation Breeding. Radiation breeding has the advantage of short-timing, while maintaining the fine characteristics and even improving one or two undesirable characteristics of the varieties. In practice, few teaoil varieties can fully satisfy all the ideal economic characters, such as a high production of fruit, seed, and oil content. Thus, we must create new types or improve certain existing poor characters through radiation. Reports of teaoil radiation experiments are few. In 1972, Co60- γ ray was used to teaoil seed, cutting as well as pollen. Different studies were conducted on the choice of radiation material, dosage and radiation-induced descendant processing, and the effect of the radiation on resulting plants (Zhuang, 1988).

Biotechnological Breeding and Research at the Molecular Level

1. Teaoil Tissue Culture. Tissue culture teaoil is the same as for other *Camellia* species and its cells are not easy to induce splits to produce the regeneration of adult plants, thus causing great difficulty in teaoil tissue culture. However, studies have shown that certain species can be tissue-cultured, such *C. sinensis* (tea), a few ornamental *Camellia* species and teaoil camellia (Fan et al., 2005; Que et al., 2006; Wen et al., 2005). From 1979, teaoil rapid propagation through tissue culture with the axillary shoot by means of producing embryo-like mass and pseudo-bulbils was creatively studied and the Forest Institute of Guangxi Zhuang Autonomous Region (1975) successfully induced teaoil embryo-like mass through in vitro culture in 1980. Long (1981) and Lu (1982) successfully induced well-formed teaoil regenerated plantlets from cotyledons. Zhang (2002) induced plantlets from the axillary bud of clonal trees, thus establishing a practical foundation for reproduction of fine teaoil clones. With the development of the teaoil tissue culture protocols, mass production of teaoil plant material through asexual reproduction has been made possible.

2. Research on Teaoil at Molecular Level. Along with the present swift advancement of biological technology, traditional research on teaoil and its applications cannot satisfy the current demand on this resource. The classification of teaoil was kind of chaotic, unsystematic and without varieties of true significance because it only divided the seeds into Cold-Dew seeds, Frost-Descent seeds and the Beginning-of-Winter seeds based on production experience and growth seasons. The other classification included selective breeds from family-based practices. This introduced some blindness in seed selection and posed enormous difficulties in promotion of teaoil. Therefore, it is imperative to better understand the molecular genetics of teaoil (Chen et al., 2005; Tan XF et al., 2004; Zhang et al., 2001).

3. Teaoil Molecular Markers. The purpose of performing molecular marking on teaoil is to establish a molecular identification system for its varieties. This allows the screens of gene sequences that are related to its economic characteristics, and has advantages in molecular-marker-assisted breeding selection (Huang et al., 2006; Lei, 2004; Zhang et al., 2003). As pioneers in this area, Tan and Chen (2002) established CTAB total DNA extract technique for *Camellia* species. Zhang et al. (2003) further improved this method for simultaneous extraction of DNA and RNA and improved the reaction system of teaoil RAPD. Zhang et al. (2003) investigated the stochastically expansive status of DNA in teaoil. Zhang (2002) conducted RAPD fingerprinting of 12 teaoil elite clones based on 22 primers, obtained more than 141 polymorphic bands, and distinguished elite clones from natural adult plants through direct primers. Lei (2004) performed RAPD markers for 90 superior teaoil clones using 18 primers and detected more than 569 polymorphic bands. They not only achieved a 94.7% polymorphic ratio, but also found 32 specific bands. Such research provided comprehensive technology and a rich knowledge for the foundation of superior teaoil clone classification system at the molecular level.

4. Teaoil Genetic Mapping and Gene Isolation. In late 1990s, DNA library and EST library were constructed for *Camellia* based on the molecular identification technology available at that time. cDNA library was obtained with 10^6 storehouse-capacity for teaoil seeds for the fat transformation peak period. Based on the constructs from the cDNA library, they determined the DNA sequences for more than 2,000 clones, and isolated key genes controlling the synthesis and metabolism of fatty acid and wax, including *FatB*1, *SAD*, *FAD*2, *FAD*8, and *ACP* (Shi, 2003). These genes were submitted to Gene Bank, establishing solid foundation for further studies on teaoil gene transgenic breeding and directive cultivation.

5. Determination of Functional Expression Genes of Teaoil and Transgenic Breeding. The main objective of teaoil breeding is to enhance the oil production. In order to further explore the functions of the genes responsible for fat conversion, Chen and Tan (2003) measured the functional genes of teaoil fruit using gene chip technology and built an illustration of various functional genes in the process of fat conversion. This early exploratory research pioneered the effort of further research on new high-oil-content varieties and directive breeding of teaoil.

DIRECTION AND STRATEGIES OF TEAOIL BREEDING RESEARCH AND DEVELOPMENT

Present Problems in Breeding Research

1. Low Degree of Intensive Farming of Teaoil and the Serious Loss of the Improved Variety Idioplasmic Resources. For a very long time, the practice and management of teaoil production were un-effective and without appropriate extension of improved varieties due to misunderstanding of the teaoil crop. In addition, lack of awareness to improve teaoil among teaoil farmers, who adopted seeds or seedlings whenever they find them, resulted in contamination of good varieties of teaoil, and seriously degraded the library of good seeds.

After many years of pilot study, a group of teaoil cultivars, families and high-yielding clones have been successfully bred. Moreover, the establishment of breeding bases and scionwood orchards played a significant economic role in the promotion of these resources. However, because of various reasons, many breeding bases and scionwood orchards could not be maintained, expanded or utilized, resulting in great loss of many excellent genetic resources and seriously hindering the process of mass utilization of genetically superior teaoil planting material.

2. Flaws of the Conventional Method of Teaoil Seedling Hybridization. Like many other woody plant species, the improvement of teaoil varieties is never easy. First, teaoil has a long reproduction rotation, which requires a longer time to improve its varieties. Secondly, teaoil is a plant of cross-pollination and the reproduction of teaoil is often in a highly heterozygous state. Therefore, the breeding characteristics of teaoil are complex and unstable. Finally, long-term breeding depletes favorable gene mutatins. For many years, conventional breeding has played an important role in the teaoil improvement. However, the conventional breeding has largely depressed the development of improved varieties because of the inherent limitations of the conventional breeding technology. Thus, it is imperative to reform the conventional breeding technology.

3. A Concern on the Breeding Using General Vegetative Reproduction Method. Clone technology solves many problems in seedling reproductive breeding. For example, this technology can shorten the breeding rotation, maintain the fundamental integrity of good characters of the maternal plant, carry heterosis, and strengthen heritability. Thereby, selection is likely to yield significant results. However, there are some drawbacks in cloning woody plants for breeding (Ni and Zhan, 2002). For example, different parts of woody plants are undergoing different physiological states. These physiological differences along with the genetic differences are even more mystified by the climatic effects in addition to the age difference of those trees, causing great difficulties in identification. Because the basis of genetics is relatively simple, reforestation with clones may reduce the adaptation of forest to environmental conditions, and enhance the possibility of wide-spread of pests and diseases.

Direction and Strategies in Teaoil Breeding Research and Development

China has an abundant teaoil germplasm resource with many valuable breeding materials. Despite great progress in the improvement of teaoil breeding in China, it is relatively late in utilization of the newest technology. Teaoil breeding is currently facing many challenges. For example, expanding genetic variation resources and continuous introduction of new genes will benefit genetic composition and screening of excellent genotypes, accelerate the promotion and propagation of fine breeds, and effectively preserve germplasm resources. From a long-term point of view, the future teaoil breeding efforts should be among the following aspects.

1. On the Basis of Conventional Breeding, Modern Biotechnology Assisted Selection Should Reduce the Breeding Rotation and Enhance Breeding Efficiency. Like numerous other woody trees, the conventional breeding rotation of teaoil requires more than 10 years. Phenotype breeding may also cause differences due to the variation in management and environmental factors. Molecular marker-assisted selection is not influenced by external factors such as those of the environment. Using molecular markers of genetic linkage to find the markers associated with major economic traits can not only raise the efficiency of breeding, but also eliminate the time limit, and possibly shorten the breeding rotation.

Because of the wide distribution and large regional differences of the teaoil crop, conventional breeding techniques in combination with modern biological technology will be the most effective method. By seedling choice and clonal selection, superior clones along with superior family selections have achieved remarkable results in production. In particular, a number of excellent half-sib family selections have demonstrated better characteristics in growth, production, stability, and disease-resistance, as compared to superior clones from cloning technology. Along with the advances in biotechnology in crop breeding, biotechnological breeding characterized by genetic mapping and molecular markers can further unfold teaoil molecular marker-assisted breeding and target gene positioning cloning. Combining teaoil major economic traits with the expression mechanism of the functional genes and using gene recombination technology, it is possible to reveal the resistance genes and their expression mechanism to develop high-yielding and pest-resistant transgenic varieties. The control of the process of oil formation through genes and their expression of fatty acid dehydrogenase may be helpful to cultivate new varieties of high oleic acid and high-linoleic acid with an increased productivity of oil formation and quality (Chen et al., 2005; Wen et al., 2004). Modern biotechnology-assisted breeding on the basis of conventional breeding has provided tremendous opportunities in teaoil breeding.

2. Tissue Culture Technology and Industrialization of Teaoil Tissue Culture. Currently, most teaoil planting material in production is improved clones. Except for a small number of cuttings, the majority of them are realized using graft propagation, which requires a two-year rotation. This technology makes it easy to mix strains and is not conducive to production. Tissue culture can not only guarantee the purity of teaoil strains, but also greatly improve the propagation efficiency, shorten the cycle of nursery stock production, and is absolutely conducive to the extension of elite and improved teaoil varieties. Teaoil tissue culture is also the necessary step to build the teaoil regeneration system using transgenic technology. The current tissue culture techniques are quite reliable. The only issues include several key technologies for industrialized mass production and the expansion of market scale. As long as these key constraints can be resolved, great progress can be anticipated in the industrialization of teaoil tissue culture (Tan and Chen, 2004; Zhang et al., 2004).

3. Further Development of Hybridization and Enrichment of Teaoil Breeding Populations. Among the selected 200 teaoil superior clones and the 1,000 plus trees, the main economic characters are not all the same. Those characters include maturation, oil content, flowering period and disease resistance. On the other hand, some strains that are not selected may actually possess some special quality traits. Meanwhile, among *Camellia* plants other than the teaoil camellia plants there are many other species and cultivars with great economic traits and these can be good materials for crossbreeding. In hybridization, special attention must be paid to distant hybridization and the accumulation of economic traits or complementary strengths so that the hybrids can gain the greatest advantage (Chen et al., 2005; Li, 1987; Wu, 1996).

4. Attacking the Technical Difficulty in Developing Teaoil Anther Culture and Further Developing Polyploidy Breeding. The use of hybrid vigor can be achieved from the homodiploid generated from gametophyte culture and chromosome doubling. In gametophyte culture, anther culture is the main pathway to cultivate haploid plants. Anther culture is a breeding method to foster haploid plants from pollen, and, via natural or artificial chromosome doubling to form homozygous diploids. Its essence is an in vitro anther culture, which induces androgenesis and change in the normal pathway of gametophyte development by developing spores through callus or embryo-like structure to express totipotency (Wen et al., 2004, 2005; Zhang et al., 2004). From the 1970s, this kind of research has been started in this area, but there is no success report yet (Li et al., 2005). Therefore, to overcome the bottleneck in teaoil anther culture, the development of an androgenesis system is the key and crucial point.

5. Strengthening the National Collaboration of Genetic Improvement in Teaoil and Adopting the Combined Strategy of Selection, Introduction, Breeding and Propagation. In the current teaoil production system, individual provinces or regions may not have enough good teaoil genetic resources. Especially, superior clones are far from enough to meet the need of production. Long-tem clonal propagation from the same or a few clones can result in recession of vitality, reduction of disease resistance, weakening of high yield strains, etc. To prevent the degradation of teaoil varieties, stabilize the good characters, and meet the needs of new strains in mass production and extension of fine teaoil planting material, it is imperative to vigorously strengthen national collaboration through the combination of selection, introduction, breeding, and propagation in the future breeding activities. From the surrounding production areas, improved varieties can be introduced to speed up the process of teaoil genetic improvement.

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