Breeding status of tung tree (*Vernicia* sp.) in China, a multipurpose oilseed crop with industrial uses

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(Received 28th May 2012)

Abstract

As a developing country with the world's largest population, China faces a serious challenge in satisfying its continuously increasing energy demands. Tung trees (*Vernicia* sp., especially *V. fordii* and *V. montana*), are multipurpose, perennial plants belonging to the *Euphorbiaceae* family. The unique chemical properties of tung seed oil make it one of the best known industrial drying oils. In this review, the breeding status of tung trees in China and some factors which limit the development of tung tree breeding will be summarised. Improvements in ecological performance and pathogen resistance, through to improved breeding methods, will help to rapidly expand the development and use of tung trees and their oil products in China. It is essential for tung tree breeding to advance in the future to keep pace with the increased demand.

Key words: Tung tree; *Vernicia* spp.; eleostearic acid; bio-energy; cross-breeding.

1. Introduction

As a developing country, industry in China has developed rapidly since the implementation of reform policies in 1970, and because of this, the demand for energy in China has increased dramatically. The Global Times pointed that in the end of 2009, China exceeds US to become Saudi Arabia's top oil customer (http://www.globaltimes.cn/business/china-economy/2010-02/507404.html) and had become the largest oil market in the world. To meet the increasing demand for petroleum products, more than 200 million tons of crude oil and oil products (approximately 52% of global market) was imported to China in 2008 (FANG et al., 2009). Such a high level of dependence on oil imports could threaten the stability of the Chinese economy and society (YIN and LIU, 2006). Therefore, petroleum substitutes must be developed in China to address this important issue. The government has devoted large amounts of money to develop the bioenergy industry. Bioenergy contributes a significant share of global primary energy consumption and its importance is likely to increase in future world energy scenarios (VASUDEVAN et al., 2005).

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Tung (*Vernicia fordii* H., previously classified as *Aleurites fordii*) oil, extracted from tung tree seeds, contains 80% (w/w) α-eleostearic acid, a conjugated trienoic 18-carbon fatty acid ($18{:}3\triangle^{9cis,\ 11trans,\ 13trans}$) that imparts useful drying and blending properties to the oil (SONNTAG, 1979). Tung oil is currently used in paints, high-quality printing, plasticisers, and in certain types of medicines and chemical reagents (PARK et al., 2008; SHANG et al., 2010; CHEN et al., 2010a).

The tung tree is a multipurpose perennial plant belonging to the genus *Vernicia*. Tung is adaptable to several soil types, provided that proper drainage and aeration conditions are met (POTTER, 1959). Tung begins to flower approximately three years after planting. Most flowers are monoecious, but small percentages are dioecious. Tung trees in China typically flower time from late April to early May, (approximately 15 days). Flower petals range in number from 4–9 and they are white tinged with red and yellow, darker at the base with dark red-branched lines running lengthwise. Individual tung fruits typically contain multiple (usually 4–5) seeds surrounded by a thick verrucose seed coat. Seeds contain approximately 50–70% oil by weight. Seeds are harvested at the end of October, having reached the maximum oil content. Many tung tree species are native to China, which has a long history of tung tree plantations, which collectively produce about 80,000 tons of oil per year, or about 70–80% of the world market (CHEN et al., 2010a). However, the output of tung oil in China still will not meet the projected requirements of the international market in future years. The ultimate objective of the tung tree breeding program in China is to create a new hybrid species or find improved varieties with enhanced oil yield and quality. This paper summarises the current status of tung tree breeding, and provides a critical analysis that will assist in the development of new and improved breeding strategies in China.

2. Breeding status

2.1. Genetic resources

To assist in future breeding plans, comprehensive efforts have been undertaken to collect, analyse and classify the variety of tung tree genetic resources (SHEN, 1994). Tung tree plantations have been developed for thousands of years, dating from the Han dynasty. Over this length of time, China has developed abundant natural tung tree varieties (*Table 1*). In the 1980s, China started to collect and conserve the tung tree germplasm, and constructed 5 gene banks (LING et al., 1991) to protect the species diversity. These gene banks are located

DOI:10.1515/sg-2012-0033
edited by Thünen Institute of Forest Genetics

in Guizhou, Hunan, Zhejiang, Guangxi and Henan provinces to facilitate the provision of these materials to the other provinces as needed. Tung tree cultivars from Zhejiang and Guangxi provinces have been compared recently in Fuzhou of Jiangxi province. The results showed that the cultivars from Guangxi outperformed those from Zhejiang due to higher oil yield, stronger fruiting potential and better overall growth (ZHOU et al., 1993).

2.2. Species selection

Plant traits which directly affect oil production can be simply named superior traits. Oil yield, oil quality and biotic/abiotic stress resistance are the most important superior traits in tung tree breeding (*Table 2*). Improved varieties are those cultivars which have some of these superior traits. The focus of cultivar selection is to find improved varieties from among all available natural germplasm, using certain testing methods. The selection process is quite slow, and achievements in finding improved varieties have been rare. The main tung species used in China today still lack some superior traits, which ultimately results in a loss of oil yield and a failure to fulfill the needs of the tung oil market.

In 1985, researchers constructed a tung tree plantation in Hunan province that contains 50 clones bearing superior traits relative to the indigenous cultivars. Of these clones, four showed a two-fold increase in the yield of individual fruit (HE et al., 1991). Factors such as primary stem and secondary stem quality, fruit yield and oil yield can be used to study relationships between growth and oil output in tung trees. CHEN (1998) measured these factors in 69 clones and classified them into four groups relative to the oil output of the control sample. The average oil output percentages were 238.6%, 187.7%, 112.3% and 56.1% of the control asexual sample, respectively. Other researchers (WANG and SONG, 1992) identified superior clones which average 67% higher yield of fruits than the control, after testing the fruit yield, and fruit, stem and oil quality.

2.3. Asexual Propagation

The degree of genetic variation in tung trees is higher than in other species from the *Vernicia* genus because of increased occurrences of natural hybridization (FANG and HE, 1998). This process results in gene segregation and gene recombination between generations and brings about character segregation. Character segregation greatly reduces the yield of tung oil from earlier generations of previously improved varieties. Asexual propagation is frequently used to minimise this problem (LI and FENG, 2005).

Graft breeding and tissue culture are the main technologies used in asexual propagation of tung tree. Experiments conducted at the Chinese plantations show that interspecies grafts within the same genus are often successful. Grafts among species are not only helpful in improving fruit yield, oil quality and stress resistance,

Table 1. – Comparison of key oil production traits of Chinese tung tree cultivars.

Cultivar*	Annual Oil Ouput (AOO, in kg oil/hectare/year)	Oil Content (% dry weight)	Initial Fruiting Time (F, in years from planting)	Full Fruit Bearing time FBP, in years from planting)	Fruit Bearing Lifetime (in years)
Zhejiang wuzhua	400~450kg/km^2	64.89%	3-4	5-6	20-30
Hubei wuzhua	225~350kg/km^2	59.7%	3	5	30~40
Sichuan dami	200~350kg/km^2	63.7%	3~5	7~8	20~30
Shanxi dami	200~250kg/km^2	61.1%	4	7~22	25~35
Longsheng dapan	200~225kg/km^2	64~68%	4~5	8~9	25~30
Zhejiang mantianxing	300~400kg/km^2	67.06%	4	5~6	10~15
Zhejiang zuo	300~400kg/km^2	67.24%	3~4	6~7	25~30
Henan yelicang	300~400kg/km^2	65.9%	4~5	6~20	30~40
Anhui yelicang	300kg/km^2	60.8%	3~4	5	30~40
Fujian yizhandeng	350kg/km^2	54.0%	3	5~6	30~40
Sichuan xiaomi	250~500kg/km^2	64.0~68.0%	3~4	6~7	30~40
Hunan putao	250~450kg/km^2	53.5%	3	4~5	15
Yunnan aizi	200~250kg/km^2	60.21%	3	8	30
Hubei jiuzi	200~450kg/km^2	60.4%	3	4~5	20~25
Hubei jingyang	200~400kg/km^2	59.1%	3	5~6	30~40
Hunan guzhuaqing	250~300kg/km^2	63.9%	3~4	6~15	30
Shanxi xiaomi	200~300kg/km^2	66.7%	3~4	6~7	25~30
Zhejiang congsheng	250~300kg/km^2	66.17%	3	4~5	25
Fujian chuan	200~250kg/km^2	56.5%	3~4	6~7	20~30
Zhejiang taoxing	200~250kg/km^2	67.01%	3~4	6~7	30
Guangxi duinian	150~180kg/km^2	59.0%	2	3~5	15
Guizhou duinian	150kg/km^2	57.14%	2	4~5	10~15
Chongqingzhaiguan	200~250kg/km^2	60.4%	3	7~8	30~40
Guizhou zhaiguan	200~250kg/km^2	59.72%	3	6~8	30

* All cultivars described belong to *Vernicia fordii.*

DOI:10.1515/sg-2012-0033
edited by Thünen Institute of Forest Genetics

Table 2. – The superior traits desired in an improved tung tree variety.

Considered parts	Characters	References
Stem	The primary stem is straight and the arrangement of secondary branches is amenable to fruit development.	Shi et al. 2010; Zong et al. 2010; Wan et al. 2010; Weng et al. 2010
Fruit	The fruit is big and contains maximum number of seeds per fruit. The fruiting period starts earlier and lasts longer. The annual fruit yield is high ($\geq$500 kg/km^2).	Liu et al. 2011; Wu et al. 2011; Peng et al. 2010; Zhu et al. 2011
Oil	Oil content (as a percentage of dry weight) and oil quality (generally a function of eleostearic acid content) is increased.	Li et al. 2009; Wang et al. 2009
Resistance	Trees demonstrate high adaptability to biotic and abiotic stresses (including temperature and water deficit stress and challenges from diseases and insect pests).	Zhang et al. 2011; Xu et al. 2011; Fang et al. 2011; Ge et al. 2011

but also can reduce age to maturity. Graft compatibility and graft timing are the major factors that affect graft survival. The survival rate is different between different combinations of rootstocks and scions. A previous study compared the survival rates of different combinations of Dami × Xiaomi and *Aleurites montana* × *Aleurites fordii*; results showed that the survival rate in the combination of Dami × Xiaomi was higher (WANG and XIONG, 2006). Graft timing significantly affects the survival rate. The optimal time for grafting in tung trees is March to April (TAN, 1987) due to the sufficient soil hydration and temperatures compatible for cambium cell action. The technology of tissue culture also can be successfully applied to tung tree clone production. The reported survival rate of plants generated by tissue culture has recently surpassed 85% (ZHANG et al., 2009).

2.4. Analysis of potential parental lines for cross-breeding

Currently, the study of tung tree cross-breeding in China is not advanced, and a new objective of cross-breeding studies is the selection of crossing parents. Good combinations of crossing parents are helpful in enhancing the quality and yield of tung oil. WANG (2002) found that tung tree plantations which contained some cross-pollinated trees were more economical than the plantations which did not plant cross-pollinated trees. Twenty cross-pollination combinations were chosen for production after this study. Previous studies analysed the general combining ability (GCA) and special combining ability (SCA) of certain characters, including the ratio of female and male flowers and the fruit yield per individual tree, using progeny derived from cross-bred lines from five local cultivars from Hunan, Hubei, Sichuan and Zhejiang provinces. The results indicated that differences in GCA were significant at the 1% level, while the SCA differences were not significant (LI et al., 1988). These data may be useful in choosing additional parental lines for the next generation of cross-breeding strategies.

2.5. Resistance breeding

Black spot (GUO, 1992), anthracnose (CAO, 1988) and blight (HUA, 1991) are the main diseases in tung trees, and the main insect pests are longicorn beetle, scarab and stinkbug (CHEN et al., 2005). Both disease and insect pests reduce the yield of tung oil. It is necessary to develop resistance breeding programs in tung trees, as another method to improve the yield and export of tung oil. A correlation exists between black spot disease and free amino acid content in tung trees (XU et al., 1998). Trees containing reduced free amino acid content are more easily infected by black spot. Maintenance of high free amino acid levels results in healthy tung trees. This conclusion is supported by studies of other plants by RUDGARD and WHEELER (1985) and now it has become a test index to measure the resistance against black spot at the seedling stage. HUA (1991) isolated two clones with high blight resistance several years of in-depth study. Studies of root rot in tung trees (primarily caused by *Clitocybe tabescens*), recognised that proper maintenance of soil conditions is the most effective way to control this disease. High temperature, excessive soil moisture and alkaline pH were the main critieria that led to increased rates of root rot infections (CHEN and XIAO, 1990).

2.6. Promotion and preservation of improved varieties

After the work of species selection is successful, the improved varieties and superior clones must be planted widely in China to enhance the yield of tung oil. Promoting widespread use of newly developed germplasm is often the most effective method of preserving it. Superior clones named Yu1, Yu2 and Yu3 from Henan province possess high yield, and improved stability and oil quality. The output of tung oil in Henan increased 40%–70% after substantial planting of these clones (LIU et al., 1996). Many of the tung tree plantations in Hunan province consist of improved varieties or superior clones; recent yields from these plantations have increased over

DOI:10.1515/sg-2012-0033
edited by Thünen Institute of Forest Genetics

longer term averages as well. The trees in Guangxi have been particularly successful, having recently returned the highest yields in this provinces' history (LING, 1993). Guangxi province constructed the first cutting orchard which contained 28 well-recognised superior clones in 1983 (LING, 1993).

3. Limiting factors

3.1. The lack of efficient breeding resources

The direct effect of efficient breeding resources on the development of improved varieties of tung trees in China began many years ago. Results indicated that an acceleration of development of efficient breeding resources was a necessity (SHEN, 2010a). The 1960s were a period of great development of the tung tree industry in China, due to the recognition of tung oil as a major agricultural commodity. Many of the programs for developing tung tree stocks that were initiated then made use of breeding resources and practices that were sufficient at that time. But later, as the price of tung oil in the world market decreased and the policy for developing tung trees changed, a great number of tung plantations were cut down by farmers. After years of effort, traditional breeding methods have led to an increase in tung tree germplasm diversity again, but modern tools and resources for efficient breeding are still scarce. It is therefore necessary to accelerate the development and implementation of efficient breeding resources in tung trees.

3.2. The low breeding efficiency

In past years, the selection of improved varieties and superior clones had focused on the selection of economically-valuable traits by traditional breeding methods. Traditional breeding programs have historically only emphasised the maximisation of economic benefits, without considering ecological benefits. Like many other trees, tung plantations can provide protect and enhance the local environment (such as by reducing hillside erosion and providing habitats for wildlife), if the cultivars are carefully chosen to be well-suited for the typical climate and soil (CATER et al., 1998). The use of gene variations and the interaction between genotype and environment were often ignored in breeding programs of the past. These traditional breeding methods have fallen behind because breeding methods do not make full use of the power and flexibility of modern biotechnology (HU et al., 2004). Collectively, these challenges have lead to low tung tree breeding efficiency despite the 40 year history of this project.

4. Some suggestions for improvement

4.1. Enlarging the breeding resources

New tools and genetic information are the bases for the development of efficient breeding resources (SHEN, 2010a). The level of effectiveness of these resources directly impacts the breeding result. The lack of effective breeding resources is the primary cause for the industry-wide gaps between theoretical and actual yields in tung oil quantity and quality. The promotion of improved varieties must abide by laws that require matching tree species with the proposed growing site, which allows the improved varieties to make full use of its potential production, while reducing the risk of negative unintended ecological outcomes (CATER et al., 1998; SHEN, 2010b). New breeding resources, including the creation of artificial breeding resources (MA, 1993; XIAO et al., 1996), are now under development.

4.2. Development of ecological breeding strategies

Trees can reach their potential production only if optimally suited to their environmental conditions (SHEN, 2010b). In the past, general comparative geographical analyses were the major determinants for estimation of environmental adaptability and subsequent species selection. Overlooking local environmental characters can cause serious misinterpretation of the data from these comparative experiments. Therefore, understanding and development of ecological breeding characters has since become a major point of emphasis in various forestry projects, including those dedicated to tung (LIU, 2007). The basis of ecological breeding is similar to the selective pressure from nature. The selective pressure helps to select species or cultivars with the best adaptability to environmental challeges. Specifically, tung plantations must consist of species with the best adaptability not only for maximal oil production, but also for best protection of the local soil ecology and least amount of soil erosion. Ecological benefits and economic benefits should be considered to the same degree.

4.3. Continued development of resistant clones and cultivars

China observes frequent breakouts of tung tree disease and insect pests, resulting in large economic losses. At present, the achievements in resistance breeding are insufficient to reliably protect against such losses. Improvements in resistance have been achieved for only a few insect pests and diseases, and better resistance is necessary to reduce and stabilise the loss potential. Enlarging the number of breeding resources and other related tools can solve this problem. Secondly, the genetics of the trees' resistance to diseases and insect pests should be studied more intensively (XIAO and CHYE, 2011).

4.4. Development of improved breeding methods

Distant hybridization is an effective method to generate a new genetic variation. The oil content of *Ricinus* is higher than in *Jatropha curcas* (SUJATHA et al., 2008; SUNIL et al., 2008), however, these two plants are closely related in the Euphorbiaceae family. Thus, it is possible that a new genetic variation of *Jatropha curcas* will be generated by distant hybridization with *Ricinus*. Additionally, marker-assisted selection breeding is another effective method can help us to improve the breeding efficiency. The genetic relationships of *Jatropha curcas* from various countries have been analysed by random amplification of polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR). The results indicated the Mexico contains a rich diversity of *J. curcas* germplasm (BASHA et al., 2009), suggesting

DOI:10.1515/sg-2012-0033
edited by Thünen Institute of Forest Genetics

that other breeding resources of *Jatropha curcas* can be improved by germplasm penetration. Expressed sequence tag (EST) sequencing technology was used to identify important genes controlling fatty acid biosynthesis in *J. curcas*; these genes were cloned and will be used to improve germplasm quality and diversity by genetic transformation (LIN et al., 2003). Also, the advancement of modern biotechnology provides a good opportunity for tung tree breeding. Biotechnology, especially transgenic technology (LI et al., 2011; WANG and ZHANG, 2011), radiation-mutation breeding (YI et al., 2010), and space breeding (OUYANG and GUO, 2010) could help researchers gain access to new gene variations. Essential tung oil biosynthetic genes have been identified and cloned successfully (XU et al., 2011a; CHEN et al., 2010b; LI et al., 2008a; SHOCKEY et al., 2006). In addition, the genetic relationships among the different tung cultivars in China were analysed by ISSR (LI et al., 2008b) and microsatellite markers (XU et al., 2011b). Such basic research on the molecular level of tung tree will lay a solid foundation for molecular breeding development. Improving the breeding methods and production will very likely require combining traditional breeding methods with modern biotechnology.

5. Conclusion

We summarised here the breeding status of tung trees in China including the species resources, the status of asexual propagation, cross-breeding and resistance breeding. In this review, we also provided analysis of some factors that have limited the development of breeding and gave some suggestions for strategies that may enlarge the breeding resource diversity.

6. Acknowledgements

This work was supported financially by Special Fund for Forestry-scientific Research in the Public Interest (200904042).

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DOI:10.1515/sg-2012-0033
edited by Thünen Institute of Forest Genetics