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Genetic Diversity and Geographic Differentiation of Tung Tree, *Vernicia Fordii* (Euphorbiaceae), A Potential Biodiesel Plant Species with Low Invasion Risk

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Received: 4 June 2019; Accepted: 16 July 2019; Published: 20 July 2019

Abstract: The tung tree, *Vernicia fordii* (Herbert Kenneth Airy Shaw), is a woody species native to South-East Asia (from Central and Southwest China to North Vietnam), which is also cultivated in China for the production of industrial oil. It is listed as a Category II invasive plant species in Florida, USA. During the introduction period of the tung tree from China to other countries in the last century, its low invasion feature led to its successful establishment in only a few countries. However, the genetic consideration for the population in its widespread native environment remains lacking. In this study, a set of 95 accessions covering most of the tung tree distribution areas in China were collected. Fifty simple sequence repeat (SSR) primer pairs were selected for the genotyping of the germplasm. Population genetics analysis indicated a medium level of genetic variation within the collected samples. The genetic diversity of the tung tree from the main production region was obviously higher than those from the marginal regions. A significant genetic differentiation occurred between the two regions, as well as among the 12 regional groups of administration. The dendrogram based on Nei's gene diversity showed that the clustering pattern for the germplasm collections basically coincided with their geographic distribution. In their native environment, human activities have had a significant impact on the gene flow via seed movement among the production areas of the tung tree in history. This study will be helpful for molecular breeding and germplasm preservation of the tung tree, and for understanding the tung tree as a biodiesel plant species with a low invasion risk when introduced into foreign countries.

Keywords: genetic diversity; woody plant; tung tree (*Vernicia fordii*); geographic differentiation; simple sequence repeat (SSR) marker; invasion risk

1. Introduction

The tung tree, *Vernicia fordii* (Hemsl) Airy Shaw (Euphorbiaceae), is a woody plant species used in production of industrial oil. The oil extracted from its fruits is a non-edible superior drying oil, an important feedstock for industrial goods and biodiesel [1,2]. The tung tree is also a plant with medicinal value in its roots, shoots, leaves, flowers and fruit against viruses and various illness such as skin conditions and constipation. At the same time, most parts of the tung tree are poisonous, especially the seeds, and accidental ingestion by humans is fatal [3]

(http://plants.ifas.ufl.edu/plant-directory/aleurites-fordii/). The tung tree is native to China, and is mainly distributed in the Sichuan, Hubei, Guizhou, and Hunan provinces, and the Chongqing municipality. These areas were historically the main production base of tung oil in China [3]. The tung tree was also distributed in the surrounding areas of the main production base, including the southern region of Henan province, the southern region of Shaanxi province, and the following provinces or autonomous region —Anhui, Jiangsu, Jiangxi, Zhejiang, Fujian, Guangdong, Guangxi, Yunnan, Hainan and Taiwan. It has been reported that the distribution pattern was due to the invasion of the tung tree from its center of origin to the surrounding areas during long-term cultivation [3]. The tung tree is a northern subtropical deciduous species. Its range of distribution is limited to the temperate zones. Due to the high industrial value of its oil, the tung tree was once introduced from China to over 40 countries in the middle of the last century, leading to the widespread of tung trees in the world [4]. However, because of the low adaptability of the tung tree to new environments that are different from its native habitats, tung tree populations were only successfully established in a few countries, such as Paraguay and Argentina [4]. As an exotic species successfully growing in non-native places, tung tree is reported to be able to survive in a wide array of environmental conditions, but is slow invasive. Thus, it is listed as a Category II invasive plant species in Florida, USA [5].

The tung tree has been cultivated in China for over one thousand years [6]. To date, wild tung tree species have not been found and the semi-wild individual tung trees collected have all belonged to the cultivated varieties. As an important industrial feedstock, tung oil was once irreplaceable, mainly used in painting. During this period in China, many local varieties with high yield and good oil quality were developed, for instance, the well-known Jinsi Youtong variety was developed in the Enshi Prefecture of Hubei province [7]. These cultivars of the tung tree generally evolved into some characteristics for the specific cultivation environments during long-term cultivation. For example, in its native environment, it needs a large space to grow normally and its branches will die if they are placed in heavy shade conditions. The yield of the tung tree is very low, and its lifespan is sharply shortened if planted in poor cultivation environments. Therefore, the tung tree possesses a low risk of invasion and should not significantly alter plant communities in new environments.

In China, tung tree germplasm was once widely collected in 1980s to establish both the national and local germplasm repositories [3]. In this era of tung tree industry, high-yield cultivars were frequently introduced from one place to another for commercial production. For example, in order to enhance the production in north China, cold-resistant tung tree cultivars were once screened from the germplasm repositories and subsequently introduced to the Shaanxi province [8]. However, this golden era for tung oil production ended when many competitive chemical substitutes for tung oil-derived products appeared in the late 1980s. Consequently, the replacement of tung oil resulted in a series of negative impacts—the sharp price decrease of tung oil, large-scale cutting of tung trees, the withdrawal of scientific research funds and, finally, the abandonment of the germplasm repository [9]. Currently, the tung tree landraces have largely disappeared with cutting or natural death due to the desertion of tung tree plantations since the late 1980s. Tung trees are now basically in a semi-wild status. Thus, germplasm collection and genetic diversity evaluation could facilitate the conservation of germplasm and also the detection of a genetic basis for the low invasion risk.

Genotyping using molecular markers is an efficient approach for the evaluation of genetic diversity. In the last two decades, there has been an enormous increase worldwide in the use of molecular markers to assess genetic variation in trees. The molecular analyses can provide significant insights into features of different varieties and the information may be used to define appropriate management strategies for tree species [10–12]. However, molecular marker application is very limited in tung trees [13–15]. Microsatellite or simple sequence repeat (SSR) markers have become one of the preferred types of molecular markers in recent decades due to the high polymorphism, high reproducibility and ubiquitous distribution in genome, and thus have been widely applied to evaluate genetic diversity [16–18]. Traditionally, SSR development was based on genomic DNA libraries [18] or published sequence information [19]. An alternative strategy based on comparative genomics is now

widely used to develop SSR markers, and the developed SSR is called a cross-species SSR [14,20]. In this study, our objectives are to detect the genetic diversity of tung tree germplasm in China, understand the genetic basis for the low invasion risk, and to facilitate our future work on gene discovery and molecular breeding in tung trees, an important industrial feedstock and biodiesel plant species.

2. Materials and Methods

2.1. Collection of Tung Tree Germplasm

Ninety-five accessions were collected (Table S1) from 11 provinces and one municipality in China (Figure 1). All the accessions were collected in the semi-wild areas of the 12 administration regions. Of the 95 accessions, 56 were collected from the main production regions including Sichuan, Hubei, Hunan, Guizhou and Chongqing, and 39 from the marginal production regions including Shaanxi, Anhui, Zhejiang, Fujian, Guangdong, Guangxi and Jiangxi. The collected accessions were also divided into 12 groups corresponding to their administration regions (Figure 1). Genomic DNA was isolated using a modified CTAB isolation method [21].

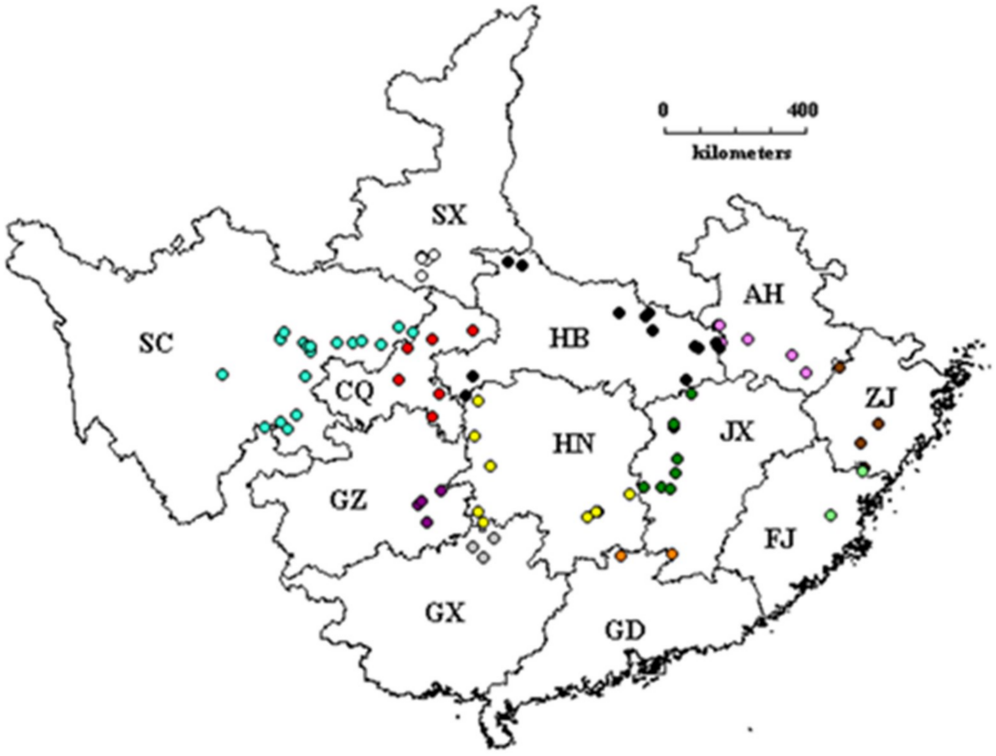

Figure 1. Geographical distribution of the 95 tung tree accessions shown in an incomplete map of China. SC, Sichuan Province with light blue dots; SX, Shaanxi Province with colorless or hollow dots; HB, Hubei Province with black dots; AH, Anhui Province with pink dots; GZ, Guizhou Province with purple dots; HN, Hunan Province with yellow dots; JX, Jiangxi Province with green dots; ZJ, Zhejiang Province with brown dots; FJ, Fujian Province with light green dots; GD, Guangdong Province with orange dots; GX, Guangxi Zhuangzu Autonomous Region with grey dots; CQ, Chongqing Municipality with red dots.

2.2. SSR Genotyping and Data Analysis

A subset of 50 SSR markers were randomly selected from the pool of SSR markers available in our lab, including 25 tung tree genomic SSR [22] and 25 cross-species SSR developed from genomic sequences of cassava [14] (Table 1). Little genomic information is available and the whole genome sequencing is still not completed in tung tree. Both cassava and tung tree belong to the Euphorbiaceae

family. Therefore, based on the principle of comparative genomics, we took advantage of the available whole genome sequence of cassava to develop SSR markers for tung tree [14]. In this study, in order to evaluate the genetic diversity of tung tree, we used the two types of SSR markers to genotype the collected germplasm accessions. The PCR procedure, and the method to separate and visualize the amplification products were the same as used by Zhang et al. [14]. The amplified fragments were treated as dominant markers and were scored in terms of a binary code (1/0). POPGENE software (University of Alberta, Edmonton, AB, Canada) [23] was used to estimate the genetic parameters, including the number of observed alleles, the number of effective alleles, Shannon's information index, the average Nei's gene diversity and Nei's genetic distance. Based on Nei's genetic distance [24], the dendrogram was drawn using the un-weighted pair–group method with arithmetic average (UPGMA). The analysis of molecular variance (AMOVA) was used to estimate variance components and to test the differentiation between and within the groups using the software GenAlEx ver. 6.4 [25].

We would like to point out that the genotyping error rate was not determined and is thus unknown, and its effect on the results cannot be determined in the present study.

Table 1. Simple sequence repeat (SSR) primer pairs selected to genotype the germplasm collections of tung trees.

Primer Code	Forward Primer	Reverse Primer	Primer Code	Forward Primer	Reverse Primer
Xwtm4	GGAAACTGCTTGCACAAAGA	CAGCAAGACCATCACCAGTTT	*vfSSR9*	GATCGAGTGCTTCATGTGCT	TGACTAGGAAATCTCACTTTAG
Xwtm27	GCCAGCAAGGTTTGCTACAT	AACTGTCAAACCATTCTACTTGC	*vfSSR10*	TGAAAGTAGGGGCACAGCTT	TTCACACTCATGGCACTGCT
Xwtm50	TTCTATAGACCAGGGGCCAA	TTTATCCCCAAAGTTGCAGG	*vfSSR12*	CATCCCATGTCCTTTTCTGG	CTTCATAGGCATGGCCACAT
Xwtm51	TGGCATCTCAAGACTGGTTG	ATTGCGCCTCTGTCATCTCT	*vfSSR15*	TGGGTATACAAGAGGCTAGGTT	CTTGACCTCTTGCTCTGTGCT
Xwtm56	TACCCAATTGACTTGAGGGG	AATGGGTTTTAACTTGGGGC	*vfSSR16*	GAAGATCACCCTTCCGACAA	CTTCTATCAAGGTTTTCATGCT
Xwtm59	ATCACATCCCATGGGTCACT	CCGCCAACATGGTGTAAGTT	*vfSSR20*	CCATCATCTTTTCTCATTTCAC	CCATATTGGCCAAACATCAA
Xwtm63	GTACCAGCAGGTCCCACAAT	ACTTCCTTTTCGCCTGTTGT	*vfSSR22*	TTCCTAGAAAAGGGGCGTCT	GCATCATTTGGAGGTCTGGT
Xwtm64	ACTGGCTGTATGGGGTCACT	TGTCAGAGGTGATTTGGGTTC	*vfSSR25*	GCCTACAGTCTACAGTTCCAAAAA	CAAAAATTGAGACAACACATGACA
Xwtm76	CAGACAGGAAGTCCAGGAGC	CGTGTCACCACATAGTCCCA	*vfSSR26*	AATGAAAGAGCACTGCATGG	TCCAAACACCAAAGCCCTAC
Xwtm89	CGTCAAATTAATGGCGAGGT	ATTGGCAGTGTCTTTGACCC	*vfSSR35*	AATGTATGATTGCATGAGAA	CTGGCCATCCATTGATATT
Xwtm99	CCCAACGGCCATATTTAAGA	TTCTTTGCTCAATGCCACAC	*vfSSR36*	GACCCACTAACACAAATTGC	TGGATCTAGCATGTGCTCACT
Xwtm102	GAGCTTCAGCCAGAGGTGAG	GTAGAATGGGCGTCACTGGT	*vfSSR40*	CGGAGTTAGTGGCATGT	CCTTCAAAAACAAAACAGAAGC
Xwtm104	CGGGTCACATGCTAATGGAG	TCCACTGCCCTTATGTCTCA	*vfSSR41*	AAGACCGGCGAAAGCTAAC	CAAGCCCAACATTTCTACC
Xwtm113	TTCTGCATTCAGATTCTCCAAA	TTCTTCTTCTTCTTCTTCCTCC	*vfSSR44*	GGGGAGCTCAAAGAAAAGA	CTTTATATGCACAATCATTGAC
Xwtm116	CTGCCATTTGCAAGGAAGAT	ATCCAAGAAGCACATCAGGC	*vfSSR45*	GTTGGAAACGGAGGTAGAA	AAGCAGAAAAGGAGAGACAAAA
Xwtm124	TTTGATTTGGTTTAGTTGATTTTGA	CAAGCCTTTCTCTTGGTTCG	*vfSSR49*	ATTACATGAATGTTCGGGATCT	AAGCTGTAGGCGTCGGATA
Xwtm125	AAATAAGCGCCGAGTTTTGA	TAAGGAAAAGTGGATGGGCA	*vfSSR50*	TGAACCAGAGAAACAAACG	AACCAGAACTCTTCTTCTTTTT
Xwtm152	GTGAGGATTCGCACTTAGCC	TCCAAAGTCACCTCCAAACA	*vfSSR56*	CAAACTGTAATACCCTAAGGA	CAGTGGCAGCATCTCTTTT
Xwtm160	TGCGATTAAGTGTGAAGGCA	TACGCAAGCCCTTAATCTGG	*vfSSR57*	GTAATTTTACATGCTGGTG	AGAATGCATGTGCTGTTGC
Xwtm168	CCTACGCTCCTCCATGAAAA	TAAACAACACCGTTCCGTCA	*vfSSR58*	AAAATAACCGTATAAGACA	TCCCAAGTTTCTTTGGACATT
Xwtm181	AACCGCAACAATGACCTTTC	CGGTAAACTCGAAGCTGAGG	*vfSSR61*	GGTGAATACTTCGTTGGTCTT	CTCAACACTATGCACATAACCA
Xwtm183	TATTGGTGGCCATGTCTTCA	ACCGGATGCACCATAAACAT	*vfSSR63*	TGTTTGTTTCTATCTTCCCTCTTTT	GCGTAACGTTTCACTCTCC
Xwtm207	CAGCTAGAGTTGGTCCGAGG	GGTTCGATTCGGTTTCTGAC	*vfSSR65*	TTGGGAGATAGCCAAAGCA	AGAGAGGTGGGTACTGAAGTG
Xwtm222	TTTAAACGTGTTATGGGGGC	CGGTTCTACTTCACACCCAAA	*vfSSR73*	ACAACAAAACTAGAGAAAC	CTTCGGAGCGTCACTTCTT
Xwtm227	TTAGGTGAATAGGGCGATGG	TAACTTGGGATGGACCTTGC	*vfSSR76*	TGCGGAACAGAGAACTAAGAGA	CCCCTAATATGGTTGCCTACTTT

The detailed information for the 25 pairs of transferable SSRs in the left part of table refers to Zhang et al. [14], and the detailed information for the 25 pairs of tung tree native SSRs in the right part of table refers to Pan et al. [22].

3. Results

3.1. Genetic Diversity Revealed by SSR Markers in Tung Tree Germplasm

In this study, a total of 188 bands were amplified across the entire germplasm accessions using the 50 SSR markers, and 131 (69.68%) of those were polymorphic (Table 2). The average observed and effective alleles per locus were 1.6968 and 1.3134, respectively. The Shannon information index (*I*) was 0.2927 and the average Nei's gene diversity (*H*) was 0.1891 for the population. Thus, the tung tree germplasm showed a medium level of genetic diversity.

Table 2. Population genetic parameters for the geographical groups of tung tree germplasm.

SSR Type	Group	GS	NPL	PR	*Na*	*Ne*	*He*	*I*
All SSR	Anhui	9	65	34.57	1.3457	1.2287	0.1314	0.1941
	Shaanxi	6	46	24.47	1.2447	1.1525	0.0910	0.1359
	Hubei	15	88	46.81	1.4681	1.2367	0.1453	0.2237
	Hunan	9	77	40.96	1.4096	1.2572	0.1516	0.2259
	Chongqing	6	65	34.57	1.3457	1.2292	0.1334	0.1972
	Guizhou	5	72	38.30	1.3830	1.2700	0.1543	0.2259
	Sichuan	21	83	44.15	1.4415	1.2289	0.1383	0.2117
	Jiangxi	9	76	40.43	1.4043	1.2433	0.1435	0.2154
	Zhejiang	4	47	25.00	1.2500	1.1830	0.1042	0.1516
	Fujian	4	41	21.81	1.2181	1.1596	0.0909	0.1322
	Guangdong	4	57	30.32	1.3032	1.2170	0.1247	0.1820
	Guangxi	3	47	25.00	0.0125	1.2021	0.1117	0.1597
Cassava SSR	Marginal region	39	60	56.07	1.5607	1.2595	0.157	0.243
Tung tree SSR		39	40	56.34	1.5634	1.2481	0.1554	0.2435
All SSR		39	108	57.45	1.5745	1.2586	0.1590	0.2476
Cassava SSR	Main production region	56	72	67.29	1.6729	1.3414	0.2024	0.3092
Tung tree SSR		56	48	67.61	1.6761	1.297	0.1807	0.2806
All SSR		56	128	68.09	1.6809	1.3279	0.1959	0.3007
Cassava SSR	Whole region	95	74	69.16	1.6916	1.3177	0.1904	0.2943
Tung tree SSR		95	49	69.01	1.6901	1.2961	0.1806	0.2809
All SSR		95	131	69.68	1.6968	1.3134	0.1891	0.2927

GS, group size; NPL, number of polymorphic loci; PR, polymorphism rate (%); *Na*, observed number of alleles; *Ne*, effective number of alleles; *I*, Shannon's information index; *He*, average Nei's gene diversity.

Out of the 188 loci, 107 were amplified by the 25 cross-species SSR markers derived from the cassava genomic sequence, and the other 81 were amplified by the 25 tung tree genomic SSR markers. As shown in Table 2, genetic diversity revealed by the cassava SSR markers was basically similar to that detected by the tung tree genomic SSR markers. Thus, the transferable cassava SSR is effective as the tung tree SSR in the detection of the genetic variation in tung tree.

3.2. Group-Wise Genetic Diversity and Geographical Differentiation

As shown in Table 2, the polymorphism rate of the main production region was 68.09% as revealed by the 50 SSR markers, slightly lower than 69.68% for the whole population, but obviously higher than 57.45% for the marginal production region. The *Na* had a similar pattern as the polymorphism rate. The Shannon information index (*I*) was 0.1959 in the main production region, obviously higher than that in the marginal production region (0.1590), and even slightly higher than that for the whole population (0.1891). Two important indexes, *Ne* and *He* showed a similar pattern to *I* (Table 2). Also, similar results were obtained by either 25 cassava SSR or 25 tung tree SSR. In short, these results demonstrated that the main region had a higher genetic diversity than the marginal region of tung trees.

To investigate the within- and between-group/region genetic variation, an AMOVA analysis was performed [25]. The results showed that the within- and inter-group variation accounted for 93.64%

and 6.35% of the total population variation, respectively (Table 3). The genetic variation in the whole population was highly significant and can mainly be attributed to the within-group/region variation. The genetic differentiation (*Fst* = 0.064) between the two groups or regions was relatively small but highly significant ($p = 0.010$) as indicated by a permutation test (Table 3). Therefore, significant genetic differentiation between the main and marginal production regions exists in tung tree germplasm.

Table 3. Analysis of molecular variance (AMOVA) based on SSR markers for the main and marginal production regions.

Source of Variation	d.f.	Sum of Squares	Variance Component	Percentage of Variation
Between-region	1	73.209	1.206	6.35%
Within-region	93	1653.191	17.776	93.64%
Total	94	1726.400	18.982	100%
Fixation index, *Fst* = 0.064, $p = 0.010$; Genetic distance, GD = 0.0185				

3.3. Genetic Diversity and Geographical Differentiation among the Administration Regions

As shown in Table 2, the polymorphism rates detected by the 50 SSR markers varied from 21.81% to 46.81% in the 12 groups of provinces, and were obviously lower than that of the whole population. Other genetic parameters showed a similar tendency as the polymorphism rate. AMOVA analysis revealed 21.24% and 78.76% of genetic variation for the among- and within-groups/regions, respectively (Table 4). The fixation index (*Fst* = 0.212) was highly significant ($p = 0.001$), indicating that the genetic differentiation among the administration regions should have occurred. Besides, we presented the fixation index (*Fst*) for each pair of the 12 groups (Supplementary Table S2) and genetic distance (GD) for each pair of the 12 administration groups (Supplementary Table S3). The between-group differentiation was significant ($p < 0.05$) for over 95% of the possible pairs (63/66 = 0.9545), and highly significant ($p < 0.01$) for over 75% of the possible pairs (50/66 = 0.7576).

Table 4. Analysis of molecular variance (AMOVA) based on SSR markers for the twelve administrative groups.

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among groups	11	497.534	3.993	21.24%
Within groups	83	1228.866	14.806	78.76%
Total	94	1726.400	18.799	100%
Fixation index, *Fst* = 0.212, $p = 0.001$				

The *Fst* and genetic distance for each pair of the 12 groups were shown in the Supplementary Tables S2 and S3, respectively.

3.4. Genetic Distance and Phylogenetic Analysis

With the aid of the POPGENE computer program [23], Nei's genetic distance (GD) [24] was estimated for all possible pairs of the tung tree accessions. Based on Nei's GD, cluster analysis was carried out using the UPGMA method, which resulted in a phylogenetic dendrogram. As shown in Figure 2, 90 of the 95 accessions were clustered into two mega paraphyletic groups. One mega group included 18 accessions that could be classified into three distinct groups (Ib, III and IV). All the 18 accessions were collected from the marginal production regions (Guangdong, Guangxi, Anhui, Fujian and Zhejiang provinces), relatively far from the main production region. As shown in Figure 2, the 18 accessions from the same or neighboring province was clustered into the same group, primarily in agreement with their geographic distribution.

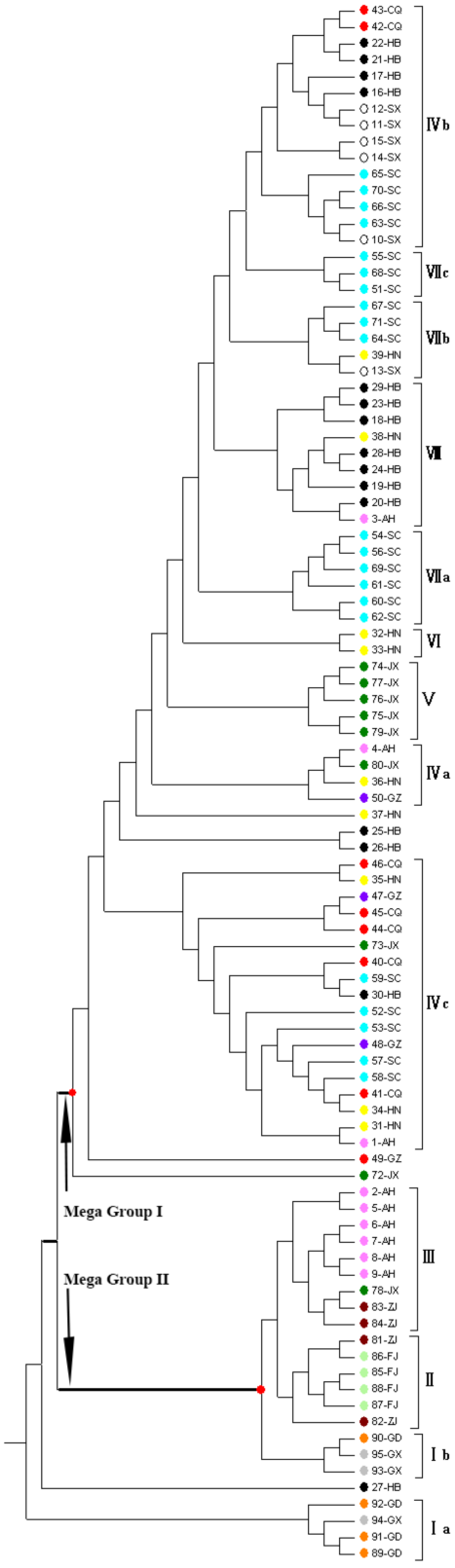

Figure 2. Dendrogram of 95 tung tree accessions generated by using the UPGMA method. The clustering groups are described in the section of results and discussion. The dot color for each germplasm accession is correspondent with that color representing the place of origin in Figure 1. SC, Sichuan Province; SX, Shaanxi Province; HB, Hubei Province; AH, Anhui Province; GZ, Guizhou Province; HN, Hunan Province; JX, Jiangxi Province; ZJ, Zhejiang Province; FJ, Fujian Province; GD, Guangdong Province; GX, Guangxi Zhuangzu Autonomous Region; CQ, Chongqing Municipality.

The other mega group included 72 accessions that could be classified into 14 distinct groups (VIIIa, VIIIb, VIIIc, etc.). The 55 of 56 accessions from the main production region and 17 of 39 accessions from the marginal production region were included in this mega group. The 17 marginal accessions were from Anhui, Jiangxi and Shaanxi provinces, neighboring to the main production regions, indicating a coincidence relationship between the geographic distance and the genetic distance. The majority (>98%) of the accessions from the main production region were classified into this mega group. Some accessions from the same province of the main production region were clustered together in this mega group. For example, seven of the nine accessions in Group VIIIb were from Hubei province. However, the cluster pattern of some accessions was not consistent to its geographic distribution. For instance, there were 18 accessions in group VIIa, which were from seven provinces—5 accessions from Chongqing, 5 from Sichuan, 3 from Hunan, 2 from Guizhou, 1 from Jiangxi, 1 from Hubei, and 1 from Anhui.

4. Discussion

The tung tree is a woody oil-bearing plant species, and the oil extracted from its fruit is an inedible superior drying oil. The tree was cultivated in China for over one thousand years, and many endemic varieties were cultivated. However, these endemic varieties were severely destroyed in the late of the last century due to the competitive substitutes of tung oil-derived products. Wild species always have a relatively high genetic diversity, giving them more potential in genetic adaptability to a new environment [26,27], and thus they may have a high risk of invasion compared to their cultivars. For the cultivar, many genes of agronomical importance were artificially selected while a body of other genes were also lost during the process of improvement [28]. For example, intensive breeding has reduced the most resistant gene diversity in crops, rendering them more vulnerable to disease outbreak [29]. However, wild tung tree species have not been found to date, although tung tree germplasm were collected from the semi-wild. In this study, we collected tung tree cultivars, and found that the tung tree is spottily distributed in the semi-wild of its native environments.

In the early to middle of the last century, tung trees were introduced to over 40 countries from China. During this tung tree introduction rush, many studies were conducted on how to improve the survival conditions after transplanting so that the trees could grow well and produce normal tung fruits under good care [30–32]. That might be one of the reasons why the introduction of tung tree cultivars into other countries resulted in population establishment only in a few countries, such as Paraguay and Argentina [4]. It is known that the genetic diversity of crop plants generally decays, relative to the wild progenitors, during the process of domestication and breeding [33]. In the long-term (>1000 years) process of cultivation and artificial selection of tung tree, the gradual loss of many "wild" alleles resulted in the reduction of genetic diversity and also the relatively poor adaptability to new environments. This may explain the low rate of population establishment of tung tree when introduced to other countries in early 1900s [4]. As an exotic species successfully growing in non-native places, tung trees in Florida are reported to be able to survive in a wide array of environmental conditions, but is slow invasive. Thus, the tung tree is only listed as a Category II invasive species in Florida because it has not altered plant communities to such an extent as to be ranked as Category I invasive [5].

Overall, given the above examples, the tung tree has a low invasive potential unless under the right environmental conditions. Therefore, good agronomical practices and monitoring should be used when cultivating tung tree for oil or biodiesel in new environments.

Concerning the tung tree germplasm distributed in China, our study showed that genetic differentiation occurred between the main and marginal production regions (Table 3). It might be the results of geographic isolation and low ability of invasion of the tung tree during the long-term cultivation. Besides, the genetic differentiation also occurred among the 12 administration groups (Table 4, Table S2). Zhang et al. [15] reported that genetic differentiation occurred between two tung tree populations from Sichuan and Hubei provinces. In the long-term cultivation, many landraces were formed based on the local tung tree germplasm, and cultivated mainly in the same region/province,

such as Aijiaotong in Yunnan province, Jiuzitong in Hubei province, Yelicang in Henan province and so on [7]. Thus, it is understandable that genetic differentiation occurred among the administration groups of tung tree germplasm in China.

Phylogenetic analysis showed a coincidence between the geographic distance and genetic distance for part of accessions. Accessions from multiple provinces, especially from the main production region were also clustered into the same group, for example of group VIIa, VIIb and VIIc. Tung oil was once the main export good, like silk, in China. In the history of the tung industry, the cross-region introduction events of tung tree cultivars occurred frequently in order to improve tung oil yield and other traits [3,7,34]. These activities accelerated the exchange of tung tree germplasm resources, especially in its main product region, and could account for the inconsistency of geographic and genetic distance for part of the germplasm accessions in the phylogenetic dendrogram (Figure 2). Thus, human activity has played an important role in the spread of different cultivars in China. But the tung tree is still a marginally cultivated plant species, and cannot grow well without proper agronomic care in China, the center of origin for tung trees. Given its past introduction history, except in Florida, USA, the tung tree is likely to not be very invasive in many environments. Therefore, in the case of tung trees introduced into foreign countries as a new industrial/biodiesel plant species, the invasion risk is limited to a large extent and can be controlled by human activity.

5. Conclusions

In the present study, we characterized the genetic diversity of tung trees in China using SSR markers. The results showed that the germplasm collection of tung tree has a medium level of genetic diversity. Tung tree germplasm from the main production region has obviously higher genetic diversity than those from the marginal region. The clustering pattern for the main production region basically coincides with the geographic distribution. In its native environment, human activities has had a significant impact on the gene flow via seed movement among the main production region of tung trees in history. However, tung trees in China, the center of its origin, is a marginally cultivated plant species, and cannot grow well without proper agronomic care. Given its past introduction history, except in Florida, USA, the tung tree is likely to not be very invasive in many environments. Therefore, the invasion risk is low and, to a large extent, may be controlled by proper human measures for tung trees when introduced as a new industrial/biodiesel plant species into foreign countries.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/7/402/s1, respectively. Table S1: The place of origin of 95 tung tree accessions, Table S2: Fixation index for each pair of the 12 tung tree groups. Lower triangular matrix, Fst; Upper triangular matrix, Probability values based on 999 permutations, Table S3: Genetic distance of each pair of the 12 tung tree groups. Lower triangular matrix, Genetic distance.

Author Contributions: J.P. initiated the research project in the tung tree. L.Z. and J.P. designed this study. L.Z. carried out the experiment, analyzed the data, and drafted the manuscript. X.L. participated in analyzing the data, proofed and revised the manuscript. J.P. revised and finalized the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (NSFC) (Grant Numbers: 31030055 and 30870233 to J.P., and 31500267 to L.Z.) and Natural Science Foundation of Hubei Province (Grant Number: 631631k0231 to L.Z.).

Acknowledgments: We sincerely thank Robin Permut, English editor in the Institute of Evolution at University of Haifa, for her professionally editing the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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