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Chapter

Research Progress on Iron-Heart *Cunninghamia lanceolate*

Ninghua Zhu, Xiaowei Yang, Zhiqiang Han and Xiao Can

Abstract

Cunninghamia lanceolate (Lambert.) Hooker is one of the main fast-growing timber forest species in southern China which has a long history of cultivation and spreads across 28 provinces, cities, and regions. Recently, a variant of fir was discovered in the Xiaoxi National Nature Reserve in Hunan Province. The heartwood is hard as iron and its ratio is more than 80%, with the especial character of anti-corruption. It is a natural germplasm resource, called Iron-heart *Cunninghamia lanceolate*. Study on it is still in the stage of data accumulation. In this paper, we studied it from three points as follows: (1) Plus tree selection and construction of germplasm resources nursery. (2) Study on cone and seed quality. (3) Genetic structure analysis of natural population. The research of Iron-heart *Cunninghamia lanceolate* lays a theoretical foundation for the protection, development, and utilization of the black-heart wood germplasm resources of Iron-heart *Cunninghamia lanceolate* in the future.

Keywords: germplasm collection, plus tree selection, seed and cone quality, genetic diversity

1. Introduction

The Chinese fir, *Cunninghamia lanceolate* (Lambert) Hooker, belongs to the *Cupressaceae* family, which is the family with the largest number of genera among Gymnospermae and includes a number of other significant species in particular, Taiwania Hayata, Cryptomeria D. Don, Glyptostrobus Endl, etc. [1, 2]. As an evergreen coniferous tree species, *C. lanceolate* is native to northern Vietnam and southern China. Because of its desirable wood properties, fast growth, and high disease resistance, *C. lanceolate* has been widely grown in China for 3000 years [3–5]. Recently, a unique natural wild variety of Chinese fir with a high ratio of heartwood and high wood quality was inadvertently found in provenance. Importantly, this Chinese fir has a high corrosion prevention property compared to other species, its wood is dark, and native people use it to make furniture, buildings, and even coffins [6, 7].

The study of cone and seed morphological characteristics of Iron-heart *Cunninghamia lanceolate* is helpful to master phenotypic diversity and formulate population protection strategies [8]. Selecting the best family for seed collection and seedling breeding has a key impact on improving the quality of Iron-heart China fir seedlings [9, 10]. Wild plants are important gene resources for breeding excellent varieties, so it is more important to study the genetic diversity and variation of wild populations [11].

For the germplasm resources of *Cunninghamia lanceolate*, the most common way is to preserve them by ex-situ conservation [12], and the establishment of germplasm collection area of *Cunninghamia lanceolate* is usually realized by grafting. Combining the conservation and application of germplasm resources in the nursery, on the one hand, the improved varieties were screened and preserved by selecting the best in the experimental area, on the other hand, the germplasm resources bank was enriched and high-quality breeding materials were provided. At present, in the field of science, the conservation and application of germplasm resources have been adopted by seed banks and gene banks in most countries, which can be summarized as "two less and one rich", with less use area, less funds, and rich germplasm resources [13]. In addition, the rapid development of modern biotechnology makes it possible to use tissue culture in vitro preservation of *Cunninghamia lanceolate*. In a word, we can take a variety of forms to achieve the preservation of Chinese fir germplasm resources, but we should consider different places, depending on the situation, choose the best way to collect and preserve high-quality resources.

Determining genetic diversity and population structure, which are important for characterizing germplasm under investigation, constitute important steps in plant breeding [14, 15]. However, due to the impact of agricultural climate change, morphological characteristics provide limited genetic information [16]. Therefore, molecular markers unaffected by environmental changes are necessary to estimate genetic diversity and population structure [17, 18]. Based on molecular markers, genetic diversity analysis, germplasm characterization and evolution studies have been possible in the last 30 years [19, 20]. Molecular markers, such as restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR) and simple sequence repeat, microsatellite (SSR), have previously been used to study the genetic diversity and population structure of cultivated and natural breeding populations of many conifers [21]. SSR markers, which are relatively abundant, inexpensive, and provide more informative than bi-allelic markers, have been used to detect the genetic diversity, population structure, and even genetic relationships among landraces and cultivars of *Cunninghamia lanceolate* [22–24]. Single-nucleotide polymorphisms (SNPs), as a type of third-generation molecular marker with high stability and diversity, are expensive to analyze compared with SSR and AFLP markers [25–27].

2. Research contents

2.1 Area sampled

Xiaoxi National Nature Reserve is located in Yongshun County, Western Hunan Province, at the western end of Wuling Mountain. It is located at 110°6′50″–110° 21′35″ E and 28°42′15″–28°53′55″ N. The annual average temperature is between 11 and 12°C, the frost-free period is 250 days, the annual precipitation is between 1300 and 1400 mm, the parent material of soil is sand shale, the soil fertility is high, the total forest storage is 2,223,500 cubic meters, the area has high mountains, dense forests, crisscross valleys, a wide variety of rare plants, rare birds and wild animals, with more than 1000 species of plants in 94 families. There are nearly 200 species of wild animals in the original secondary forest, including 68 species of national key protected animals such as leopard, clouded leopard, and white-necked long-tailed pheasant [28], which are rare in the world and unique in China. The only surviving evergreen broad-leaved primary secondary forest in the 13 provinces of central and southern China is protected from Quaternary glaciers.

2.2 Sampling design

The Iron-heart *Cunninghamia lanceolate* was listed and numbered. According to the principle of uniform dispersion and a random selection, 33 cones of mother trees (52 years old) were collected in the mother forest in mid-October 2019 and were brought back to Central South University of Forestry and Technology to dry naturally for later use in 10, 2019.

About 35 plus trees of Iron-heart *Cunninghamia lanceolate* were selected. Fresh cuttings are collected and used as materials for the establishment of a germplasm resource nursery. See Appendix **Table S1** for the basic information. The germplasm resource nursery is set up in the Chinese fir test demonstration forest base in Xichong Village, Majiang Town, Chaling County, Zhuzhou City, Hunan Province. It has red soil and good site conditions. The demonstration forest has Guangxi provenance seedlings (Guangxi-2.5) and Fujian provenance vegetative Line cutting seedlings-020 (Fujian-020) and Fujian clone Zhongyuan cutting seedlings-061 (Fujian-061) pure forest of young Chinese fir, and grow well.

In total, 548 Iron-heart *Cunninghamia lanceolate* from nine plots (CTY, JZW-1, JZW-2, JZW-3, LYP-1, LYP-2, LYP-3, LYP-4, and XNC) were collected, covering the entire range of Iron-heart *Cunninghamia lanceolate* from (Appendix **Table S2**) (According to the natural distribution of the natural population of iron-heart Cunn*inghamia lanceolate*, we found that it is concentrated in 9 mountains. Therefore, we divided it into 9 plots for population genetic structure analysis). Growth indexes and morphological parameters were considered as selection criteria for the sampled trees, which were chosen by a dominant comparative and comprehensive evaluation method in typical natural forests. The longitude, latitude, and altitude of each sample were determined using a handheld GPS (WGS-84) (Garmin eTrex Handheld GPS; Garmin). Fresh leaves of each voucher sample were collected in a 10 ml freezing tube, transported back to the laboratory in a liquid nitrogen tank, and deposited at −80°C.

2.3 Data sampled

2.3.1 Quality determination of cones and seeds

1. Take 10 kg fresh cones from each plant and dry them. Test the quality of cones and seeds. Repeat for 3 times in each family. The cone length, cone width, seed length, and seed width are measured with a vernier caliper. The total cone quality and seed quality are weighed with an electronic balance (accurate to 0.01 g) to calculate the cone seed extracting percentage. Cone seed extracting percentage = total seed quality ÷ total cone quality × 100%.

2. About 1000 seeds were randomly selected, and the quality of 1000 seeds (g) was measured in the air-dry state, repeated 3 times.

3. Seed goodness test. Seed goodness = real number of good seeds ÷ real number of tested seeds.

4. The seed germination rate was determined by the standard germination method. Take 150 seeds from each plant family, sterilize with 10% antifomin for 15 min, wash 3 times with sterile water, and soak in sterile water at 25°C for 24 h. Take a sterile petri dish, spread it with sterile filter paper, and moisten it with sterile water; spread the soaked seeds on the filter paper, and place them in a 25°C light incubator for cultivation. Repeat 3 times each, observe and

count the germination situation once every 5 days, and count the real number of germinated species after 15 days to calculate the germination rate (%). Germination rate = real number of germinated seeds ÷ real number of initial seeds × 100%.

2.3.2 DNA extraction, amplification, and microsatellite genotyping

A Plant Genomic DNA Kit (TIANGEN Biotech, Beijing, China) was used to extract total genomic DNA. Genomic-SSR polymerase chain reaction (PCR) was performed in a 20 μl reaction volume containing 4.0 μl double-distilled water, 4.0 μl genomic DNA, 10.0 μl 2× Taq Plus PCR MasterMix (TIANGEN, Beijing, China), 1.0 μl forward primer and 1.0 μl reverse primer. The PCR conditions included denaturation for 5 min at 95°C, 30 cycles of 30 s at 94°C, 90 s at the annealing temperature for each SSR marker in the reaction, 1 min at 72°C, and 10 min at 60°C for a final extension. In total, 15 primer pairs with highly polymorphic loci (**Table 1**), for which the clarity and reproducibility of the DNA fragments were amplified, were selected from published papers [29–31].

The forward primer had a universal M13 primer tail and a universal M13 primer fluorescently labeled with 6-carboxy-x-rhodamine, tetramethyl-6-carboxyrhodamine, 6-carboxy-fluorescein, or 5-hexachlorofluorescein. The final PCR products were separated based on capillary electrophoresis fluorescence using an ABI3730xl DNA Analyzer (Genewiz Inc., Beijing, China). The results were analyzed using GeneMarker 1.75 software (SoftGenetics LLC, State College, PA, USA).

2.4 Statistical analyses

Excel 2019 and R4.0.3, Rstudio software were used for summary processing and nested analysis of variance, Pearson correlation analysis, and principal component analysis. Among them, R4.0.3 calculates the mean value, standard deviation, and coefficient of variation of the seed and cone traits; based on the nest's linear model variance analysis between and within groups differences, and Tukey HSD test; using R package Hmisc 4.4.2 [32] to calculate Pearson Correlation coefficient and p-value, use corrplot 0.84 [33] to draw the correlation graph.

The polymorphism information content (PIC) was used to estimate the allelic variation of SSR by applying the formula $PIC = 1-\sum_{i=0}^{n} Pi^2$, where Pi is the frequency of the ith allele and n is the number of alleles detected for given SSR markers. GenALEx 6.5 [34] was used to estimate the genetic diversity indices of each locus and population.

The genetic diversity and population structure of the accessions were further investigated by analysis of molecular variance (AMOVA) using GenAlEx 6.5. The program STRUCTURE v2.3.4 [35] was used to analyze the genetic structure by employing Bayesian clustering analysis with the admixture model of independent allele frequencies. STRUCTURE HARVESTER (http://taylor0.biology.ucla.edu/structureHarvester/) was used to evaluate the most likely number (K) of genetic clusters. The data derived from the STRUCTURE analysis were visualized as bar charts and pie charts using ArcMap v10.0 and DISTRUCT v1.1 software [36, 37]. Interpolation of ArcGIS was used to forecast the expected heterozygosity (He) and the private allele frequency (Fp) of all Chinese firs included. The ArcGIS (Esri) program was used to map the distribution of the He of populations and Fp by employing a kriging spherical interpolation method.

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Loci	Forward primer	Reverse primer	Repetitive unit	Product size (bp)	Ta°C
contig476_526D	TTTGGGACCTTATGGAGGTGGAG	AAACCACCAGGTTGAGAAGCAGC	(GGA)9	153–159	57.10
contig7616_683B	GAGCCGTGAAGAACGAAGGTCTC	ACGATCGGATTGTCTCAGAAACG	(GAA)12	260–281	57.05
contig4728_384B	ATTATCCGAGGCAGATACGCAC	CTTCTCCGTATTTGATCCATCGC	(GGA)10	336–354	55.05
contig5410_1886A	GGCTCGAGTTTGCATCTCACAC	CACATCCAATCCATACAGGAGGG	(TC)9	210–320	56.70
contig16181_1285C	GGTACTGCGAATCTTCAAATCC	TGTTCAAGAAAGGAAGCAAACGG	(TC)9	293–297	53.25
contig406_1209C	TCATCAGCCTCAGTTTGTACTTGC	GCAATCATGGGCTCTCTGCAC	(AT)9	348–384	56.00
Unigene685	CCTTTCTTTTCTGCACCAGC	CTGTGCCTGATGGCTAAACA	(GGT)5	190–284	56.90
Unigene754	AGACGGTCGTTGACGAAAAA	CTCTTTTCCACACACGCAAA	(GCA)4	124–298	55.35
Unigene840	CAGGACGCCTGAGAATTGTT	TCATCGGTAGAAGGAATGGC	(AAG)5	162–169	56.65
Unigene1061	GAAACAAACAAGGGAGGCAA	AGGTCCAAATCCACCTGGTC	(AGG)9	150–276	57.70
Unigene491	TGGAAATGGCTGTAAAGGAG	TGTGCTGAGCCATATTCACA	(GAAG)3	120–168	55.30
contig6319_250C	GCGGCCATTTATATCATCTTC	CACGCCTGTAATTCATCTCCGTC	(GAA)9	126–135	57.30
contig1560_1789D	TTTCGGCTCTCCGACTCCTTAAC	AGAATCGCGTCCAGAACACAGAG	(CT)11	129–147	59.45
CLSSR6	ATTTCAAACACCTCTCCTTTC	GGAATTCCTAGACAAAGATGG	(CTTC)4	136–268	52.35
CLSSR8	ATCGTTGCTTTCAATCTTATG	ATCCAACTGACACACAAAATC	(CTTT)3	143–165	51.80
Ta°C represent annealing temperature of PCR cycles					

Table 1.
Primary simple sequence repeat primers used in the study.

3. Results

3.1 Cone and seed quality

In this study, we use 12 traits (germination rate, seed quality, seed length-width ratio, seed length, cone seed extracting percentage, seed width, total cone quality, goodness, cone length, Seed quality (1000), cone length-to-width ratio, cone width) to assess the of quality of cones and of seeds.

3.1.1 Differences in seed quality of different families

The results in **Table 2** (code is the number of different mother trees) show that the variation range of the cone length of Iron-heart *Cunninghamia lanceolate* is 3.15–6.13 cm, the average is 4.66 cm, and the coefficient of variation is 18.97%; the variation range of cone width is 3.56–2.15 cm, the coefficient of variation is 12.18%, and the average is 2.95 cm; the variation range of the total quality of the cone is 1.15–2.40 kg, with an average value of 1.66 kg. The variation range of seed quality is large, between 0.09–0.33 kg, the coefficient of variation is 36.50%, and the average value is 0.20 kg; the cone seed extracting percentage is 5.59–19.02%, and the coefficient of variation is 24.42%, but the overall cone seed extracting percentage is low. Seed quality of Iron-heart *Cunninghamia lanceolate* from different families is quite different. The largest seed length, seed width, and seed length-to-width ratio are 9.77 mm, 5.75 mm, and 3.03 respectively; the smallest ones are 4.36 mm, 2.05 mm, and 1.16 mm, respectively; the average value are 6.31 mm, 2.35 mm, and 2.69 mm; the coefficients of variation are 26.02%, 22.15%, and 26.91%, respectively. The average seed quality of 1000 seeds is 7.06 g, the maximum is 10.54 g; the average of goodness is 67.65%, and the coefficient of variation is 20.53%. The variation range of seed germination rate is 5.33% ∼ 63.00%, the coefficient of variation is 52.34%; the seed germination rate of TXS-256 and TXS-234 families is the highest, TXS-29 and TXS -30 is the next; TXS-205, TXS-265, TXS-16 germination rates are all lower than 10%. The quality of cones and seeds of families is different in different traits, so it is impossible to evaluate the quality of cones and seeds from a single character.

The coefficient of variation is the comprehensive performance of the discrete characteristics of phenotypic traits. The greater the coefficient of variation, the greater the degree of dispersion of traits. The coefficient of variation of seed traits of 33 families is between 12.18% and 51.34%, and the coefficient of variation of each trait has a certain difference. From large to small, it is germination rate > seed quality >seed length-width ratio > seed length > cone seed extracting percentage > seed width > total cone quality> goodness> cone length > seed quality(1000) > cone length-to-width ratio > cone width (**Tables 2** and **3**).

The P value associated with total cone quality, seed quality, seed germination rate, seed goodness, seed quality (1000), seed width, and cone length-width ratio was less than 0.001 (see the **Table 4**, variance analysis of 33 iron-heart *Cunninghamia lanceolate*), indicating that these traits varied greatly among families; the P value associated with of the cone-length factor is less than 0.01, and the P values associated with other factors of other characteristics were less than 0.1. There are minor differences, and differences mainly exist between individuals. The F value of 12 seed characteristics varies from 0.757 to 965.1 between families, and the order of size is cone seed extracting percentage (0.757) < cone width (1.591) < seed length to width ratio (1.704) < seed length (1.91) < germination rate (2.87) < cone length (2.885) < seed

Code	Cone length (cm)	Cone width (cm)	Length/ width	Cone quality (kg)	Seed quality (kg)	Cone seed extracting percentage (%)
TXS-16	3.29 ± 0.03	2.66 ± 0.02	1.24 ± 0.01	1.34 ± 0.01	0.12 ± 0.02	9.22 ± 1.34
TXS-17	4.08 ± 0.05	2.54 ± 0.02	1.60 ± 0.03	1.93 ± 0.07	0.22 ± 0.02	11.25 ± 1.43
TXS-18	3.77 ± 0.02	3.07 ± 0.03	1.23 ± 0.03	1.32 ± 0.08	0.14 ± 0.01	10.63 ± 0.62
TXS-19	5.49 ± 0.02	3.09 ± 0.01	1.77 ± 0.03	1.86 ± 0.03	0.21 ± 0.01	11.12 ± 0.52
TXS-29	5.76 ± 0.03	3.17 ± 0.02	1.82 ± 0.02	2.21 ± 0.02	0.32 ± 0.05	14.33 ± 0.13
TXS-30	5.76 ± 0.03	3.17 ± 0.02	1.82 ± 0.02	2.21 ± 0.02	0.32 ± 0.08	14.33 ± 0.13
TXS-202	4.12 ± 0.02	2.36 ± 0.01	1.74 ± 0.01	1.56 ± 0.01	0.17 ± 0.01	10.89 ± 0.55
TXS-205	3.15 ± 0.00	2.15 ± 0.03	1.46 ± 0.02	1.55 ± 0.01	0.09 ± 0.08	5.59 ± 0.31
TXS-217	5.24 ± 0.01	2.98 ± 0.05	1.76 ± 0.01	1.61 ± 0.01	0.21 ± 0.07	13.04 ± 0.07
TXS-219	5.95 ± 0.04	3.25 ± 0.01	1.83 ± 0.02	1.75 ± 0.04	0.33 ± 0.01	19.02 ± 0.74
TXS-224	4.57 ± 0.05	3.46 ± 0.01	1.32 ± 0.04	1.22 ± 0.06	0.19 ± 0.08	15.34 ± 0.42
TXS-228	5.85 ± 0.04	2.97 ± 0.01	1.97 ± 0.01	1.93 ± 0.05	0.25 ± 0.03	13.08 ± 1.19
TXS-234	5.84 ± 0.03	3.09 ± 0.06	1.89 ± 0.05	2.16 ± 0.04	0.33 ± 0.01	15.42 ± 0.84
TXS-236	5.02 ± 0.02	3.56 ± 0.02	1.41 ± 0.01	1.81 ± 0.02	0.22 ± 0.04	11.95 ± 0.31
TXS-237	3.66 ± 0.01	2.88 ± 0.01	1.27 ± 0.01	1.45 ± 0.01	0.15 ± 0.01	10.12 ± 0.37
TXS-238	4.61 ± 0.01	3.18 ± 0.01	1.45 ± 0.02	1.77 ± 0.01	0.19 ± 0.01	10.71 ± 0.39
TXS-239	4.35 ± 0.03	2.47 ± 0.01	1.76 ± 0.01	1.15 ± 0.01	0.15 ± 0.09	12.76 ± 0.34
TXS-256	5.89 ± 0.01	3.56 ± 0.00	1.66 ± 0.01	2.40 ± 0.01	0.31 ± 0.02	13.00 ± 0.76
TXS-259	5.64 ± 0.02	3.24 ± 0.04	1.74 ± 0.01	1.56 ± 0.01	0.28 ± 0.01	17.74 ± 0.38
TXS-264	3.27 ± 0.01	2.35 ± 0.01	1.39 ± 0.06	1.18 ± 0.01	0.09 ± 0.07	7.34 ± 0.36
TXS-265	3.45 ± 0.03	2.79 ± 0.03	1.24 ± 0.02	1.16 ± 0.01	0.12 ± 0.07	10.06 ± 0.35
TXS-267	3.89 ± 0.02	2.89 ± 0.05	1.35 ± 0.04	1.34 ± 0.01	0.16 ± 0.06	11.94 ± 0.07
TXS-268	5.10 ± 0.02	3.16 ± 0.01	1.62 ± 0.01	2.14 ± 0.01	0.25 ± 0.04	11.53 ± 1.74
TXS-270	4.03 ± 0.02	2.91 ± 0.02	1.39 ± 0.01	1.39 ± 0.04	0.13 ± 0.09	9.38 ± 0.03
TXS-276	3.91 ± 0.01	2.69 ± 0.02	1.45 ± 0.01	1.57 ± 0.05	0.13 ± 0.02	8.05 ± 0.29
TXS-349	4.99 ± 0.00	2.98 ± 0.01	1.67 ± 0.00	1.84 ± 0.03	0.23 ± 0.02	12.32 ± 1.29
TXS-363	3.88 ± 0.00	2.34 ± 0.02	1.66 ± 0.02	1.62 ± 0.01	0.17 ± 0.01	10.29 ± 0.25
TXS-365	5.03 ± 0.01	2.92 ± 0.45	1.77 ± 0.03	1.18 ± 0.01	0.18 ± 0.05	15.54 ± 0.5
TXS-366	3.94 ± 0.06	2.68 ± 0.01	1.47 ± 0.03	1.24 ± 0.04	0.13 ± 0.02	10.17 ± 1.58
TXS-370	4.87 ± 0.02	3.12 ± 0.01	1.56 ± 0.01	1.64 ± 0.03	0.18 ± 0.01	11.22 ± 1.00
TXS-400	6.03 ± 0.11	3.23 ± 0.01	1.87 ± 0.03	2.40 ± 0.16	0.27 ± 0.02	11.40 ± 0.37
TXS-578	4.64 ± 0.02	3.06 ± 0.02	1.52 ± 0.01	1.67 ± 0.04	0.19 ± 0.01	11.58 ± 0.75
TXS-666	4.75 ± 0.03	3.06 ± 0.01	1.55 ± 0.01	1.94 ± 0.02	0.21 ± 0.01	10.80 ± 0.36
CV/%	18.97	12.18	13.59	21.62	36.50	24.42
SD	0.88	0.36	0.21	0.36	0.07	0.03
Range	3.00	1.48	0.98	1.42	0.3	0.14
Mean	4.66	2.95	1.58	1.66	0.2	11.91

Table 2.
Cone characteristics (average ± standard deviation value) of different iron-heart Cunninghamia lanceolate.

code	Seed length (mm)	Seed width (mm)	Length/ width	Seed quality (1000) (g)	Goodness (%)	Germination rate (%)
TXS-16	4.36 ± 0.04	3.75 ± 0.01	1.16 ± 0.01	7.65 ± 0.05	76.67 ± 1.25	8.67 ± 1.25
TXS-17	6.11 ± 0.50	4.90 ± 0.09	1.25 ± 0.08	8.57 ± 0.01	77.67 ± 2.36	24.67 ± 2.05
TXS-18	5.41 ± 0.03	3.46 ± 0.01	1.56 ± 0.05	6.47 ± 0.03	62.67 ± 0.47	18.67 ± 0.47
TXS-19	7.86 ± 0.08	3.33 ± 0.03	2.36 ± 0.03	6.82 ± 0.01	73.33 ± 3.68	45.67 ± 4.11
TXS-29	9.77 ± 0.02	3.78 ± 0.03	2.59 ± 0.02	7.81 ± 0.02	75.67 ± 3.4	61.33 ± 1.25
TXS-30	9.77 ± 0.02	3.78 ± 0.03	2.59 ± 0.02	7.81 ± 0.03	75.67 ± 3.4	61.33 ± 1.25
TXS-202	4.41 ± 0.03	3.56 ± 0.01	1.24 ± 0.01	6.12 ± 0.05	48.67 ± 0.47	14.33 ± 0.47
TXS-205	4.44 ± 0.01	2.53 ± 0.03	1.76 ± 0.02	5.67 ± 0.07	47.33 ± 4.11	5.33 ± 0.47
TXS-217	8.09 ± 0.04	3.24 ± 0.01	2.50 ± 0.01	6.75 ± 0.02	74.33 ± 3.09	50.33 ± 0.47
TXS-219	9.47 ± 0.06	4.34 ± 0.01	2.18 ± 0.01	8.1 ± 0.02	75.67 ± 1.25	57.67 ± 2.62
TXS-224	6.49 ± 0.02	3.12 ± 0.05	2.08 ± 0.01	6.68 ± 0.05	69.33 ± 1.25	35.00 ± 0.82
TXS-228	8.42 ± 0.02	3.47 ± 0.01	2.43 ± 0.01	6.85 ± 0.03	76.67 ± 3.68	56.00 ± 2.16
TXS-234	9.43 ± 0.02	3.67 ± 0.02	2.57 ± 0.01	7.24 ± 0.03	75.00 ± 4.97	63.00 ± 2.83
TXS-236	6.45 ± 0.08	3.56 ± 0.03	1.81 ± 0.02	6.60 ± 0.02	66.33 ± 1.89	41.00 ± 0.82
TXS-237	5.39 ± 0.02	2.05 ± 0.06	2.63 ± 0.08	4.91 ± 0.05	42.67 ± 0.47	16.00 ± 0.01
TXS-238	6.31 ± 0.03	2.35 ± 0.01	2.69 ± 0.01	4.83 ± 0.07	33.67 ± 0.94	38.00 ± 0.82
TXS-239	5.35 ± 0.02	3.25 ± 0.04	1.64 ± 0.07	6.61 ± 0.05	72.00 ± 0.82	18.33 ± 0.47
TXS-256	9.68 ± 0.02	4.87 ± 0.03	1.99 ± 0.02	8.83 ± 0.01	81.00 ± 1.41	63.00 ± 5.10
TXS-259	8.74 ± 0.10	2.89 ± 0.01	3.03 ± 0.03	6.11 ± 0.05	47.33 ± 0.47	54.33 ± 0.47
TXS-264	4.60 ± 0.07	3.67 ± 0.06	1.25 ± 0.02	6.40 ± 0.03	63.33 ± 0.47	10.67 ± 5.91
TXS-265	4.74 ± 0.05	3.08 ± 0.01	1.54 ± 0.02	6.15 ± 0.03	53.33 ± 0.47	8.67 ± 1.25
TXS-267	6.06 ± 0.04	3.25 ± 0.01	1.86 ± 0.01	6.53 ± 0.05	64.67 ± 0.47	25.67 ± 0.47
TXS-268	6.21 ± 0.02	4.84 ± 0.06	1.28 ± 0.02	8.72 ± 0.01	83.33 ± 3.68	44.67 ± 3.09
TXS-270	5.02 ± 0.05	2.98 ± 0.05	1.68 ± 0.01	6.32 ± 0.05	58.67 ± 0.47	18.00 ± 0.82
TXS-276	4.78 ± 0.01	2.77 ± 0.02	1.73 ± 0.02	5.94 ± 0.04	51.33 ± 0.47	13.33 ± 0.47
TXS-349	6.77 ± 0.01	4.33 ± 0.01	1.56 ± 0.00	8.32 ± 0.01	81.00 ± 2.94	34.00 ± 2.16
TXS-363	4.88 ± 0.02	3.04 ± 0.04	1.60 ± 0.02	5.35 ± 0.05	50.00 ± 0.82	28.67 ± 0.47
TXS-365	6.23 ± 0.02	3.56 ± 0.03	1.75 ± 0.01	6.63 ± 0.05	66.00 ± 0.82	31.67 ± 0.47
TXS-366	5.74 ± 0.18	3.56 ± 0.05	1.62 ± 0.07	7.13 ± 0.05	74.00 ± 3.56	28.67 ± 0.47
TXS-370	6.36 ± 0.02	4.88 ± 0.00	1.30 ± 0.00	8.21 ± 0.00	81.33 ± 3.30	35.33 ± 0.94
TXS-400	8.83 ± 0.04	5.75 ± 0.04	1.54 ± 0.01	10.54 ± 0.02	87.67 ± 1.89	59.33 ± 1.25
TXS-578	6.67 ± 0.02	4.68 ± 0.02	1.43 ± 0.05	8.31 ± 0.01	85.33 ± 2.05	38.00 ± 2.45
TXS-666	5.96 ± 0.04	3.80 ± 0.02	1.57 ± 0.02	7.76 ± 0.04	78.00 ± 2.16	35.33 ± 0.47
CV/%	26.02	22.15	26.91	17.23	20.53	52.34
SD	1.72	0.81	0.5	1.22	0.14	0.18
Range	5.48	3.78	1.92	5.74	0.56	0.63
Mean	6.62	3.64	1.87	7.06	67.65%	34.80%

Table 3.
Seed characteristics (average ± standard deviation value) of different iron-heart Cunninghamia lanceolate.

Source of variation	Df	SS	MS	F
Total cone quality	61	8078	132.42	5.454 ***
Total seed quality	26	4827	185.64	3.221 ***
Cone seed extracting percentage	87	7692	88.41	0.757
Germination rate	45	6364	141.42	2.87 ***
Goodness	39	8408	215.59	22.39 ***
Seed quality(1000)	93	8976	96.51	965.1 ***
Seed width	72	8762	121.7	14.8 ***
Seed length	80	8030	100.38	1.91.
Length/width(seed)	68	7130	104.85	1.704.
Cone length	70	7883	112.61	2.885 **
Cone width	59	6341	107.47	1.591.
Length/width(cone)	53	7353	138.73	3.845 ***

*Note: "***": P < 0.001; "**": P < 0.01; "*": P < 0.05; ".": P < 0.1; "": P < 1.*

Table 4.
Variance analysis of 33 iron-heart Cunninghamia lanceolate*.

quality (3.221) < cone length-to-width ratio (3.845) < total cone quality (5.454) < seed width (14.8) < goodness (22.39) < seed quality (1000, 965.1).

3.1.2 Correlation analysis of seed traits of iron-heart Cunninghamia lanceolate

It can be seen from the **Figure 1** that the seed germination rate of iron-heart *Cunninghamia lanceolate* is positively correlated with the other 8 characteristics except for the total cone quality, seed quality, and cone seed extracting percentage. Among them, the germination rate is positively correlated with the cone length, seed quality, seed length-to-width ratio, and seed length are extremely significantly positively correlated at the level of $P < 0.001$, and are more correlated with seed width, seed length-to-width ratio, seed quality (1000), and goodness at $P < 0.01$; There was a very significant negative correlation ($r = -0.56$, $P < 0.001$) between cone seed extracting percentage and total cone quality, and a very significant

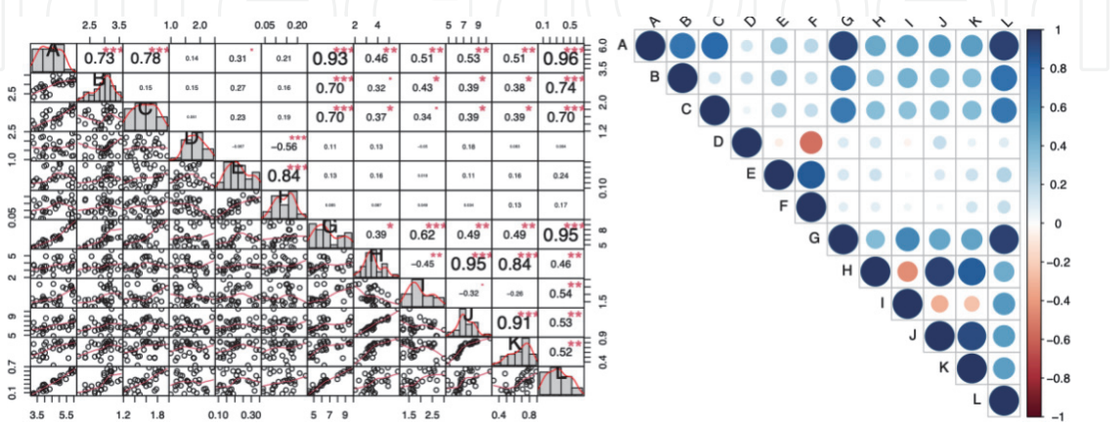


Figure 1.
Correlation analysis of seed and cone characters of iron-heart Cunninghamia lanceolate*. Note: A–L are: cone length, cone width, cone length-width ratio, total cone quality, seed quality, cone seed extracting percentage, seed length, seed width, seed length-width ratio, seed quality (1000), goodness, and germination rate.*

positive correlation ($r = 0.84$, $P < 0.001$) with seed quality, and the correlation between these three traits and the other eight traits was not significant.

3.1.3 Comprehensive evaluation of seed quality of iron-heart Cunninghamia lanceolate

Analyzing the various characteristics that affect the quality of the cones and seeds of the Iron-heart *Cunninghamia lanceolate*, it can be seen from the figure that principal components 1 and 2 can explain 65.8% of the variation (**Figure 2**). Among them, traits A, B, C, G, L has a greater contribution rate to principal component 1, and most of them are cone traits; traits H, J, K, I has a large contribution rate to principal component 2, and most of them are seed traits. The principal component dimensionality reduction method is used to comprehensively evaluate the 12 cones and seed traits of iron-heart *Cunninghamia lanceolate*. It can be seen from the **Table 5** that the cumulative variance contribution rate of the first three main factors can reach 82.30%, which can satisfy the traits of each half-sibling progeny. Therefore, the first three main factors are selected to make a comprehensive evaluation score for iron-heart *Cunninghamia lanceolate*. Take the characteristic value of the main factor as the weight of each index, and multiply each index to obtain the calculation formula of the main factor comprehensive evaluation score:

$$F_1 = (0.408X_1 + 0.308X_2 + 0.308X_3 + 0.145X_5 + 0.106X_6 + 0.389X_7 + 0.286X_8 + 0.135X_9 + 0.315X_{10} + 0.310X_{11} + 0.403X_{12}) \times \sqrt{2.334} \quad (1)$$

$$F_2 = (0.156X_1 + 0.142X_2 + 0.104X_3 - 0.124X_4 + 0.130X_6 + 0.191X_7 - 0.453X_8 + 0.573X_9 - 0.417X_{10} - 0.369X_{11} + 0.160X_{12}) \times \sqrt{1.564} \quad (2)$$

$$F_3 = (0.441X_4 - 0.541X_5 - 0.668X_6 + 0.152X_7 + 0.161X_9) \times \sqrt{1.408} \quad (3)$$

The variance contribution rates of the first three main factors are different. In the comprehensive evaluation of growth traits, the focus of each factor needs to be coordinated. The contribution rates of the three factors are 45.4%, 20.4%, and 16.5% as weights, combined with 3 common factors. The contribution rate and

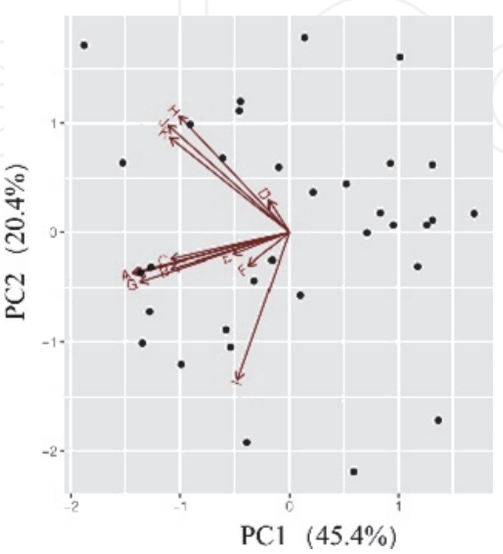


Figure 2.
PCA analysis. Note: A–L are: cone length, cone width, cone length-width ratio, total cone quality, seed quality, cone seed extracting percentage, seed length, seed width, seed length-width ratio, seed quality (1000), goodness and germination rate.

factor score Fi, refer to the calculation formula of the comprehensive score, the mathematical model of the comprehensive score of seed traits of iron-heart *Cunninghamia lanceolate* can be established:

$$D_n = F_1 \times 45.4\% + F_2 \times 20.4\% + F_3 \times 16.5\% \quad (4)$$

Using the comprehensive ranking as an indicator, a total of 14 excellent Iron-heart Cunninghamia lanceolate were selected with a 40% selection rate (**Table 6**).

3.2 Seed garden construction

3.2.1 Grafting and management of the germplasm resource nursery of Iron-heart Cunninghamia lanceolate

3.2.1.1 Seedling grafting

Before grafting, we selected high-quality rootstocks to mark and hang tags. The height of the rootstocks was uniformly about 15.6 cm. The rows are 2 m × 2 m, and at least 10 plants should be planted for each clone. After grafting, apply an appropriate amount of organic fertilizer according to the standard of 30–60 t per hectare to promote the growth and development of Iron-heart *Cunninghamia lanceolate* and improve the survival rate, stress resistance, cold resistance, and adaptability of grafted seedlings. The trails are set up in Iron-heart *Cunninghamia lanceolate* germplasm resource nursery, which is mainly used for convenient work such as planting, cultivation, observation, management, and protection. At present, 35 genotypes of superior trees selected from nature reserves are still preserved in the germplasm resource nursery (**Table 7**). In May of the same year, the research team conducted statistics and surveys on the survival rate of grafting.

Traits	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5
A	0.408	0.156			
B	0.308	0.142		−0.456	−0.511
C	0.308	0.104		0.407	0.598
D		−0.124	0.441	−0.622	0.486
E	0.145		−0.541	−0.459	0.313
F	0.106	0.130	−0.668		
G	0.389	0.191	0.152	0.105	
H	0.286	−0.453			
I	0.135	0.573	0.161		
J	0.315	−0.417			
K	0.310	−0.369			−0.135
L	0.403	0.160			
λ	2.334	1.564	1.408	0.972	0.896
Contribution rate	0.454	0.204	0.165	0.079	0.067
Total contribution rate	0.454	0.658	0.823	0.902	0.969

Table 5.
PCA analysis of iron-heart Cunninghamia lanceolate.

Code	F1	F2	F3	Score	Ranking
TXS-16	13.878	−3.074	1.581	5.934	28
TXS-17	15.059	−4.192	2.067	6.323	22
TXS-18	13.916	−1.64	1.8	6.28	23
TXS-19	15.962	−0.623	2.64	7.555	10
TXS-29	17.937	−0.83	3.098	8.485	3
TXS-30	17.846	−1.248	2.976	8.339	4
TXS-202	12.952	−2.023	1.646	5.739	32
TXS-205	12.394	−0.859	1.517	5.702	33
TXS-217	15.963	−0.385	2.523	7.585	9
TXS-219	18.056	−1.591	2.678	8.315	5
TXS-224	14.879	−0.851	1.957	6.904	16
TXS-228	16.393	−0.56	2.721	7.777	8
TXS-234	17.425	−0.59	3.006	8.286	6
TXS-236	15.077	−1.269	2.257	6.959	14
TXS-237	12.626	0.83	2.081	6.245	24
TXS-238	13.447	1.004	2.373	6.701	19
TXS-239	13.838	−1.612	1.685	6.232	25
TXS-256	19.568	−2.255	3.082	8.932	1
TXS-259	15.982	0.817	2.626	7.856	7
TXS-264	13.25	−2.174	1.579	5.833	31
TXS-265	13.06	−1.388	1.613	5.912	30
TXS-267	14.314	−1.195	1.943	6.575	20
TXS-268	16.472	−3.745	2.226	7.082	13
TXS-270	13.361	−1.325	1.788	6.091	26
TXS-276	12.877	−1.063	1.86	5.936	27
TXS-349	16.23	−2.921	2.282	7.149	11
TXS-363	12.779	−0.921	1.844	5.918	29
TXS-365	14.714	−1.365	1.844	6.706	18
TXS-366	14.65	−1.933	1.859	6.563	21
TXS-370	16.1	−3.405	2.052	6.953	15
TXS-400	20.197	−4.234	2.879	8.781	2
TXS-578	16.309	−3.182	2.141	7.109	12
TXS-666	15.245	−2.475	2.172	6.775	17

Table 6.
Comprehensive score and ranking of principal components of 33 black-heart wood Chinese fir.

3.2.1.2 Statistics of graft survival rate

The construction of iron-heart *Cunninghamia lanceolate* germplasm resource nursery was uniformly carried out by splitting, and from the results (**Table 8**), the average survival rate of grafting was 83%, among which the minimum survival rate of grafting with number TXS-35 was 50%, and the number TXS-30, the highest

Code	Breast diameter/cm	Altitude/m	GPS(E,N)
TXS-1	72.0	895	110.246958, 28.835133
TXS-2	48.2	899	110.247068, 28.835290
TXS-3	41.1	845	110.247144, 28.833239
TXS-4	43.2	505	110.260574, 28.814858
TXS-5	29.5	560	110.260804, 28.814984
TXS-6	44.1	563	110.260804, 28.814984
TXS-7	63.0	813	110.246442, 28.822674
TXS-8	25.3	804	110.246186, 28.822969
TXS-9	51.5	800	110.247032, 28.823265
TXS-10	47.0	807	110.247682, 28.823303
TXS-11	43.0	894	110.245943, 28.834792
TXS-12	35.5	1000	110.242849, 28.834510
TXS-13	37.4	1010	110.243119, 28.835489
TXS-14	47.0	904	110.245980, 28.835239
TXS-15	46.0	894	110.246676, 28.834436
TXS-16	43.2	648	110.268425, 28.798110
TXS-17	26.9	632	110.268312, 28.797980
TXS-18	27.7	648	110.268386, 28.798233
TXS-19	40.6	632	110.267831, 28.797993
TXS-20	34.4	629	110.267767, 28.796707
TXS-21	54.7	902	110.247119, 28.835550
TXS-22	54.2	901	110.246885, 28.835296
TXS-23	73.0	895	110.246958, 28.835133
TXS-24	48.2	899	110.247968, 28.835290
TXS-25	41.1	845	110.247444, 28.833239
TXS-26	43.2	505	110.260574, 28.814858
TXS-27	29.5	560	110.260804, 28.814984
TXS-28	52.3	490	110.261149, 28.814843
TXS-29	38.7	514	110.261320, 28.814457
TXS-30	27.3	511	110.261126, 28.814625
TXS-31	29.4	524	110.260469, 28.814065
TXS-32	29.0	502	110.259730, 28.813559
TXS-33	37.7	494	110.269097, 28.814165
TXS-34	28.0	525	110.259179, 28.814850
TXS-35	31.8	523	110.250914, 28.814820

Table 7.
The information of the 35 Iron-heart Cunninghamia lanceolate.

survival rate of grafting is 96%. It shows that TXS-30 has a high degree of adherence to the test forest fir, and it is suitable as a material for remote preservation of iron-heart *Cunninghamia lanceolate* germplasm. Experiments have proved that it is feasible and suitable to use the test forest of Chinese fir in Majiang Town as a place

Code	Graft survival rate (%)	Average number of branches	Rootstock trail (cm)	Rootstock height (cm)	Average total growth (cm)
TXS-1	88	7	3.50	32.10	53.40
TXS-2	67	6	2.18	19.00	38.68
TXS-3	70	7	2.76	40.11	41.59
TXS-4	69	8	2.47	19.45	19.83
TXS-5	69	5	1.50	34.00	17.00
TXS-6	85	6	2.77	28.25	24.93
TXS-7	80	7	1.87	16.67	27.67
TXS-8	80	8	1.90	12.30	24.50
TXS-9	90	9	2.35	19.35	38.50
TXS-10	70	5	2.09	20.36	28.00
TXS-11	95	6	2.59	36.88	23.90
TXS-12	85	5	3.23	35.63	33.75
TXS-13	86	6	2.74	33.99	43.91
TXS-14	88	7	2.85	34.61	28.35
TXS-15	89	8	3.51	31.66	35.55
TXS-16	95	7	2.40	32.00	25.30
TXS-17	79	6	2.83	35.00	49.13
TXS-18	89	7	2.40	34.33	34.67
TXS-19	93	8	2.10	28.00	16.00
TXS-20	88	9	2.15	31.00	30.03
TXS-21	87	9	3.00	26.00	49.75
TXS-22	87	7	2.00	20.87	28.82
TXS-23	92	7	2.59	35.59	36.88
TXS-24	91	6	2.77	41.04	42.34
TXS-25	95	7	2.46	21.15	25.35
TXS-26	89	8	3.46	23.66	26.50
TXS-27	89	9	3.03	27.00	35.57
TXS-28	93	7	1.90	20.50	51.30
TXS-29	92	6	2.65	32.35	22.34
TXS-30	96	7	3.10	34.50	31.55
TXS-31	94	8	2.13	20.85	27.15
TXS-32	76	9	2.74	21.70	18.58
TXS-33	77	10	2.56	30.38	24.16
TXS-34	65	8	3.45	41.90	28.55
TXS-35	50	9	1.86	21.00	28.92

Table 8.
Statistics of grafting survival rate of iron-heart Cunninghamia lanceolate.

where the iron-heart *Cunninghamia lanceolate* is preserved in a different place, and the method of splitting can realize the clonal reproduction of iron-heart *Cunninghamia lanceolate* and has a higher survival rate.

3.3 Analysis of sub-populations genetic structure

3.3.1 Genetic diversity

The evolutionary potential and adaptation of a species are reflected by its genetic diversity, the more genetic variation a species has, the more adaptive it is. The study of the genetic diversity of iron-heart *Cunninghamia lanceolate* is necessary to understand its biological characteristics. In total, 133 alleles were observed among all samples for 15 polymorphic loci, which is higher than the amount previously reported. This difference may have been caused by the sample size, reproductive properties, and molecular marker characteristics of the species. The microsatellites used in the study yielded moderately to highly variable allele numbers per locus, in which 15 SSR primer pairs generated a total of 133 alleles, with a mean of 8.87 alleles at each locus, ranging from 5 for the contig5410_1886A locus to 18 for the contig406_1209 locus, except the two loci CLSSR6 and CLSSR8. Both the CLSSR6 locus and CLSSR8 locus had only 2 alleles, producing the lowest Ne (0.641, 0.691). The expected and observed heterozygosity of all the loci ranged from 0.442 to 0.870 and from 0.270 to 0.700, with averages of 0.654 and 0.474, respectively (**Table 9**). As an important index for measuring the genetic diversity of a population, the He of the SSRs was 0.654, which indicated that a higher genetic diversity existed in the population, suggesting that these accessions varied with high genetic diversity. The high genetic diversity may be due to being a predominantly outcrossing species. Meanwhile, the Ne was significantly smaller than the Na for each loci, which may be because the natural ecological conditions became severe suddenly during the process of alternation generation because of the high altitude of the site, and collapse of the large population occurred, leading to the loss of rare alleles in the population and the bottleneck effect. The results also revealed a range of PIC values from 0.348 (CLEER6) to 0.858 (contig406_1209C), and among these, the values of three loci (contig476_526D, 0.421; CLSSR6, 0.348; and CLSSR8, 0.374) were less than 0.5, indicating that the other 12 primers were accessible for identifying the genetic diversity of Chinese fir in Xioxi, Hunan Province. The average Shannon's Information Index (I) value was 1.350, with a minimum of 0.285 (contig 406_1209C) and a maximum of 0.641 (CLSSR8). However, the effective number of alleles (Ne) ranged from 1.792 to 7.677 per locus for all accessions, and the mean value was 3.325. Overall, the mean values of Ne, He, Ho, PIC, Fst, and Gst were 1.933, 0.654, 0.474, 0.566, 0.090, and 0.076, respectively.

There were high levels of differentiation and genetic diversity at these loci. The 15 polymorphic loci showed that the G'stN value was between 0.259 (contig6319_250C) and −0.001 (CLSSR6), with an average value of 0.083. This finding shows that the genetic difference among populations was 8.3%, and 91.7% of the genetic difference existed among individuals in the population. The average Nm of 15 SSR loci in nine populations was 9.163, indicating that gene exchange was frequent.

Na: Number of alleles; Ne: effective number of alleles; I: shannon's Information Index; Ho: Observed heterozygosity; He: Expected heterozygosity with populations; G'stN: Nei's standardized Gst; PIC: The polymorphism information content; Nm = [(1/Fst)-1]/4; Fis (Inbreeding coefficient within individuals) = (Hs-Ho)/Hs; Fst (Inbreeding coefficient within subpopulations) = (Ht-Hs)/Ht; Gis (Analog of Fst, adjusted for bias) = (cHs-Ho)/cHs; Gst (Analog of Fst, adjusted for bias) = (cHt-cHs)/cHt.***

The highest number of alleles was observed in population JZW-3 (Na = 8), and three populations (LYP-2, LYP-3, and LYP-4) had the lowest number of alleles, which was only 4. The observed heterozygosity within a population ranged from

Locus	Na	Ne	I	Ho	He	G'stN	PIC	Nm	Fis	Fit	Fst	Gst
contig476_526D	6	1.792	0.948	0.429	0.442	0.009	0.421	11.812	0.037	0.057	0.021	0.008
contig7616_683B	16	4.088	1.680	0.700	0.755	0.015	0.724	9.161	0.031	0.057	0.027	0.013
contig4728_384B	14	2.304	1.340	0.548	0.566	0.009	0.548	12.039	−0.002	0.018	0.020	0.008
contig5410_1886A	5	3.879	1.389	0.592	0.742	0.042	0.695	4.600	0.193	0.234	0.052	0.038
contig16181_1285C	7	2.811	1.220	0.598	0.644	0.002	0.577	16.098	0.037	0.051	0.015	0.002
contig406_1209C	18	7.677	2.285	0.657	0.870	0.019	0.858	7.211	0.199	0.225	0.034	0.017
Unigene633	7	3.878	1.488	0.391	0.742	0.206	0.701	0.997	0.304	0.444	0.200	0.187
Unigene754	5	3.148	1.282	0.422	0.682	0.232	0.626	0.866	0.201	0.380	0.224	0.212
Unigene840	7	3.081	1.340	0.644	0.675	0.075	0.630	2.948	−0.027	0.053	0.078	0.067
Unigene1061	10	4.504	1.691	0.369	0.778	0.106	0.746	1.961	0.514	0.569	0.113	0.095
Unigene491	12	3.208	1.591	0.270	0.688	0.160	0.652	1.298	0.527	0.603	0.162	0.145
contig6319_250C	7	2.335	1.105	0.448	0.572	0.259	0.524	0.751	−0.082	0.189	0.250	0.237
contig1560_1789D	15	3.368	1.551	0.423	0.703	0.131	0.661	1.614	0.397	0.478	0.134	0.119
CLSSR6	2	1.815	0.641	0.332	0.449	−0.001	0.348	17.026	0.238	0.249	0.014	−0.001
CLSSR8	2	1.993	0.691	0.290	0.498	−0.014	0.374	49.057	0.413	0.416	0.005	−0.012
Mean	8.87	3.325	1.350	0.474	0.654	0.083	0.566	9.163	0.199	0.268	0.090	0.076

Table 9.
Characterization of 15 simple sequence repeat loci in iron-heart Cunninghamia lanceolate *based on 548 accessions representing 9 sampling sites.*

0.416 to 0.506, varying little. The mean of the expected heterozygosity within populations was significantly higher than the observed heterozygosity (Ho) within populations, while the highest value was found for population LYP-1 (He = 0.637), and the lowest value of 0.524 was found in LYP-4 (**Table S2**). LYP-4 was the least diverse population (I = 0.997 and He = 0.524) of all the sites sampled. The highest genetic diversity was recorded for sites located in JWZ-2, JWZ-3, and LYP-1 (I = 1.244, 1.294, and 1.241 and He = 0.622, 0.636, and 0.637, respectively). In **Figure 3**, the geographic distribution of the population diversity based on Fp and He is presented, which indicated that JZW- (1,2,3) was likely the center of genetic diversity of this Chinese fir variety.

Molecular variance analysis was used to assess the population differentiation among 9 subgroups, which demonstrated that approximately 11% of the total variance was explained among the groups and 89% of the total variance was explained within accessions (**Table 10**). The population differentiation study that included red-heartwood Chinese fir and clones from six different provinces produced similar results to our study and identified a slightly higher genetic variance in subgroups. However, a moderate degree of variability was present among some populations. Previous studies [38] have shown that severe genetic drift, which might be intensified by long-term habitat isolation, is widespread in small populations. This effect will result in a low level of genetic diversity within a population and genetic differentiation among populations. Meanwhile, the results were almost consistent with G'stN = 0.083, indicating that variation mainly existed between individuals, so it was unreasonable to divide the groups according to geographical locations and administrative boundaries.

3.3.2 Genetic structure and divergence

The study of population structure is important for the formulation of strategies utilizing special germplasms for breeding objectives and conserving species effectively. Meanwhile, the genetic structure largely determines the evolutionary potential of a species or population. To verify the results of the neighbor-joining cluster analysis and PCA principal component analysis, the results of 15 pairs of SSR primer polymorphisms of 548 wild germplasm resources in Xiaoxi, Hunan Province, were further analyzed by STRUCTURE v2.3.4. The results showed that L(K) increased with the increase of K. A clear peak appeared at the value of ΔK at K = 2 (**Figure 4A** and **B**). When k = 2, ΔK reached the peak value, which indicated that the 548 accessions were clearly differentiated into two clusters according to STRUCTURE analysis (**Figure 4**). All the accessions from JZW-2, JZW-3, and LYP-1 were present

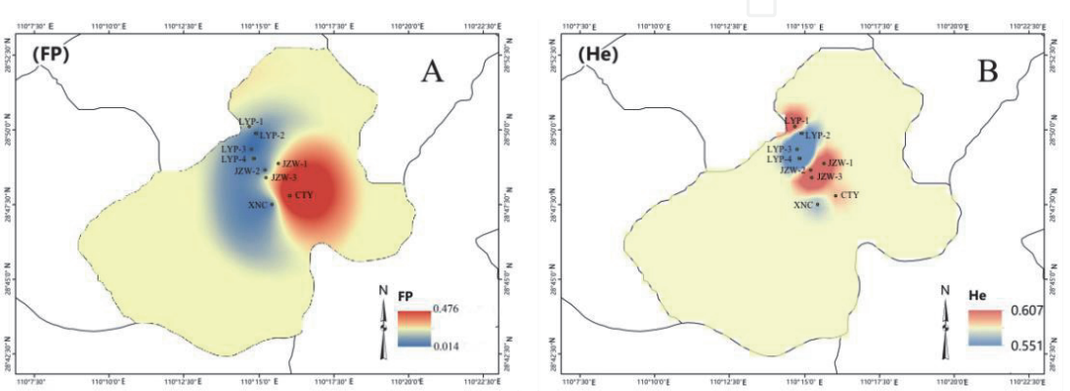


Figure 3.
Distribution of population diversity based on the expected heterozygosity and private allele frequency. (A) The private allele frequency (Fp) in all populations. (B) The expected heterozygosity (He) in all populations.

Source of variation	Degree of freedom	Sum of squares	Variance component estimates	Percentage of variation (%)
Among populations	9	804.900	1.541	11
Between samples within populations	539	6443.894	11.955	89
Total	548	7248.794	13.496	100

Table 10.
Analysis of molecular variance (AMOVA) among populations of iron-heart Cunninghamia lanceolate.

in two clusters, with approximately one-half of each population in each cluster, which can be considered admixed. Materials from different sources were distributed in the populations, there was no obvious regional differentiation, and the results of the population structure analysis were consistent with the results of SSR genetic diversity clustering. According to previous research, in the genetic structure analysis of a structured population, when the genetic component (Q value) of material is ≥0.6, the genetic background of the material is relatively simple, and when the Q value is <0.6, the genetic background of the material is relatively complex. With the increase of the K value (k = 3, k = 4), a new gene classification appeared in the wild Chinese fir population, but the high variance was inconsistent (**Figure 4D**). The clustering of CTY, JZW-1, JZW-2, JZW-3, and XNC showed some evidence that these populations can be broken down into further clusters, while LYP-1, LYP-2, LYP-3, and LYP-4 were relatively stable for higher K values. Excluding the CTY and JZW-1 populations, a new gene classification appeared in the other seven populations, which showed that there were significant differences among other populations. This finding suggested that the heterozygosity and genetic background of the wild Chinese fir are higher. When K = 4, the population was divided into four groups. The accessions that originated from the same population, including JZW-1, JZW-3, and XNC, were divided into different clusters. This result indicated that the four clusters are not geographically independent. Several populations (i.e., the LYP-3 and LYP-4 populations) that consisted of a single genetic component might have experienced founder effects or significant bottlenecking. The results also show low levels of mixing, which account for the hybridization or outcrossing of individuals between populations. Classifying accessions according to administrative boundaries and geographical distributions is very subjective, and it is very difficult to grade traits accurately in the provenance of this specific Chinese fir. In some cases, the population structure may not be predicted via administrative boundaries and geographical distributions. Therefore, the relationship between the population structure and phylogenetic clustering is not obvious, which is consistent with previous research results [22] for the Chinese fir. Wind pollination and a high natural outcrossing frequency among the species may lead to inconsistencies in population classifications and geographical locations. As a result, the geographical origin and genetic structure of a population should be simultaneously considered for the screening of this special germplasm breeding material. That is, geographical features are not obvious among distribution regions. From the principal component analysis results, we were able to identify two main populations with some sub-populations in each group. Obviously, the distributions of accessions from the same location in the two groups were not concentrated and scattered in each group. Additionally, one group contained all the individuals from JZW-1 and approximately 60% of the accessions from the other three locations (ZJW-2, JZW-3, and LYP-1), which occupied approximately 40% in the other group.

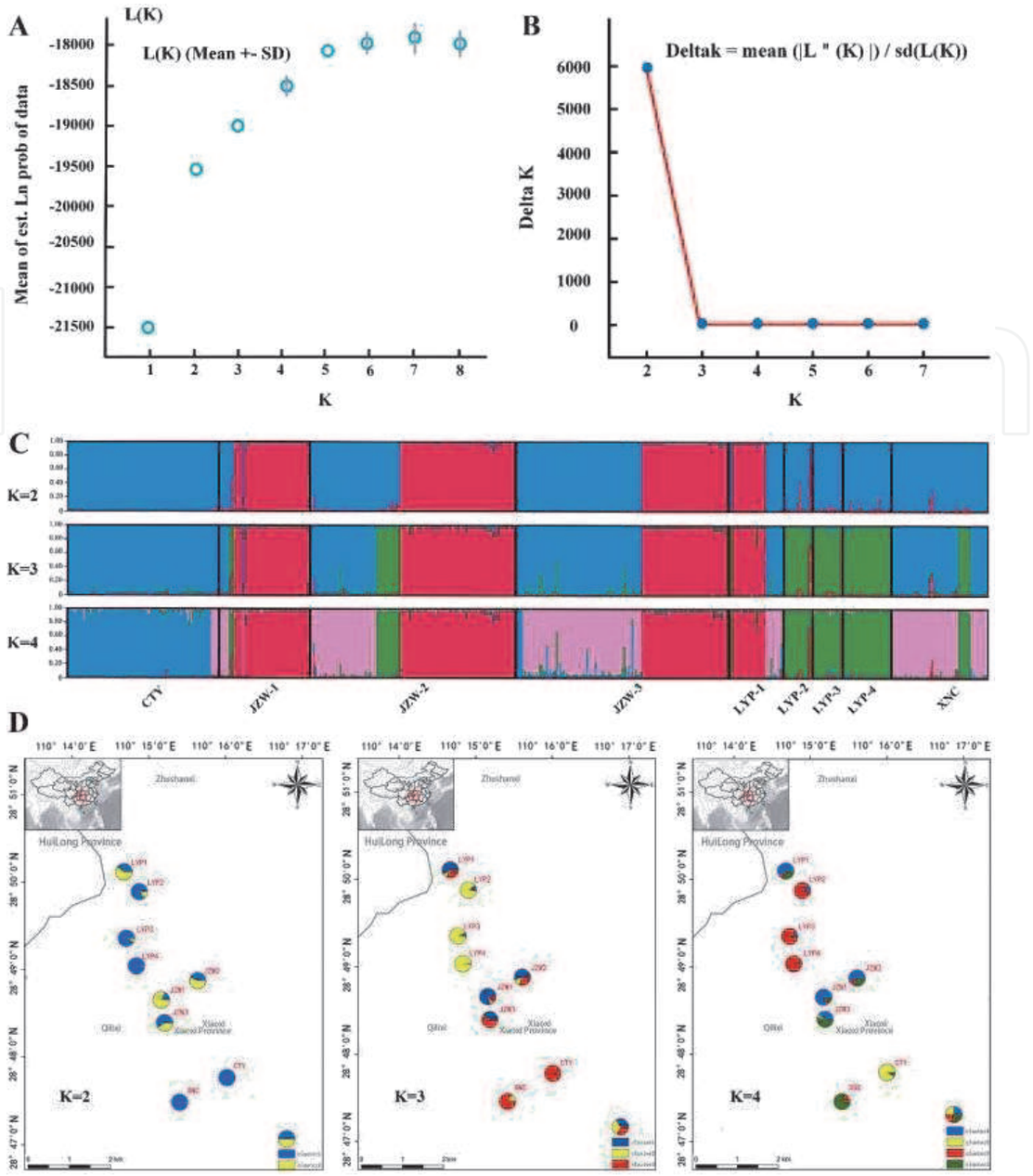


Figure 4.
Population classification based on the consensus of STRUCTURE analysis across 10 replications for per K clusters. (A) Circles with standard deviations represent the average log-likelihoods across per K runs independently. (B) Solid circles indicate the values of Evanno's ΔK based on the rate of change of the log-likelihood. (C) Bar plots express the population structure. The number of clusters is shown from K = 2 to K = 4. Vertical bars represent each genotype, and the length of each colored bar represents the proportion of membership for each cluster. (D) the distribution of 2 to 4 clusters of 9 populations is visualized as a pie chart, with each population divided into colored segments based on the proportion of its members in a given cluster.

The lowest Gst and Fst values between populations JWZ-2 and LYP1 were 0.004 and 0.010, respectively (**Table S3**). The highest values, which were 0.104 for Fst and 0.093 for Gst, were observed between populations CTY and LYP-2. Most of the values for both parameters were within the limits of moderate genetic differentiation between populations (**Table S3**).

4. Discussion

Seed yield and quality are the basis for the collection and preservation of improved seeds and the construction of seed orchards, which has a great impact on

the efficiency of plantation and industrial development in the later stage [39, 40]. It is found that the variation range of seed and cone traits is 12.18–51.34%, among which the variation of cone length, cone width, cone length-width ratio, seed length, seed width, and seed length-width ratio is relatively small, indicating that these seed traits of iron-heart *Cunninghamia lanceolate* are relatively stable [41, 42]. The order of coefficient of variation from large to small is: seed germination rate > seed quality > seed length to width ratio > seed length > cone seed yield > seed width > total cone mass > seed goodness > cone length > seed quality (1000) > cone length to width ratio > cone width. The results of the analysis of variance showed that among families, the differences of total cone quality, seed quality, seed germination rate, seed goodness, seed quality (1000), seed width, and cone length-width ratio were very significant ($P < 0.001$), the differences of cone length were significant ($P < 0.01$), and the differences of the other four traits were not significant. The results showed that the phenotypic characters of different Iron-heart *Cunninghamia lanceolate* families had high diversity and rich variation.

Genetic diversity of a species reflects its evolutionary potential and allows for evolution and adaptation. The more abundant the genetic variation of a species is, the more adaptable it is. Thus, it is necessary to study the genetic diversity of a species to understand its biological properties [43]. All previous studies on this species revealed a relatively high level of genetic diversity [22]. In the current study, 15 SSR markers were used to evaluate the population genetics of a large number of specific Chinese fir individuals across its distribution range in Xiaoxi Hunan. Amplification results of the 548 germplasms only existed Hunan Xiaoxi gave a total of 133 alleles with a mean of 8.87 at each locus, a value higher than those in previous reports [1, 22]. The difference may relate to the reproductive attributes of this species, the sample size, and/or the characteristics of the molecular markers. Understanding population structure is useful for developing strategies for the conservation of new species and effectively utilizing genotypes for breeding purposes. Genetic distance is commonly used to describe the genetic structure of a population and the differences among populations [44]. The evolutionary potential of a species or population depends to a large extent on the genetic structure of the population [45]. The results of the STRUCTURE analysis performed for this study indicate that the most likely genetic structure of the 548 studied accessions is two clusters.

5. Conclusions

Through this study, we constructed a germplasm resource nursery of Iron-heart China fir, and the grafting survival rate was as high as 83%. 27 families of iron-heart *Cunninghamia lanceolate* seeds were collected, and the highest germination rate was 68%; 15 highly polymorphic and stable SSR markers were selected to analyze the genetic structure of the natural population of iron-heart *Cunninghamia lanceolate*. In total, the study got 133 alleles, and the GestN's = 0.083. AMOVA analysis showed that the variation among populations was only 11%, and 89% of the variation came from individuals. In addition, STRUCTURE analysis showed that the whole samples could be divided into two groups, and there was no correlation between population division and geographical location. This study will lay a foundation for the protection of the new species of Iron-heart *Cunninghamia lanceolate*. In this study, only the genetic structure of its natural population was analyzed, but the heartwood variation was not deeply discussed. In addition, we only used the single method of STRUCTURE to analyze its genetic structure and did not use PCA, neighbor-joining (NJ) cluster analysis, and other methods to analyze its genetic structure. This will be what we will study in the next step.

Acknowledgements

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A. Summary materials

Gst values above the diagonal; Fst values below the diagonal.

Code	DBH (cm)	Altitude (m)	GPS(E,N)
TXS-1	72.00	895	110.246958, 28.835133
TXS-2	48.20	899	110.247068, 28.835290
TXS-3	41.10	845	110.247144, 28.833239
TXS-4	43.20	505	110.260574, 28.814858
TXS-5	29.50	560	110.260804, 28.814984
TXS-6	44.10	563	110.260804, 28.814984
TXS-7	63.00	813	110.246442, 28.822674
TXS-8	25.30	804	110.246186, 28.822969
TXS-9	51.50	800	110.247032, 28.823265
TXS-10	47.00	807	110.247682, 28.823303
TXS-11	43.00	894	110.245943, 28.834792
TXS-12	35.50	1000	110.242849, 28.834510
TXS-13	37.40	1010	110.243119, 28.835489
TXS-14	47.00	904	110.245980, 28.835239
TXS-15	46.00	894	110.246676, 28.834436
TXS-16	43.20	648	110.268425, 28.798110
TXS-17	26.90	632	110.268312, 28.797980
TXS-18	27.70	648	110.268386, 28.798233
TXS-19	40.60	632	110.267831, 28.797993
TXS-20	34.40	629	110.267767, 28.796707
TXS-21	54.70	902	110.247119, 28.835550
TXS-22	54.20	901	110.246885, 28.835296
TXS-23	73.00	895	110.246958, 28.835133
TXS-24	48.20	899	110.247968, 28.835290
TXS-25	41.10	845	110.247444, 28.833239
TXS-26	43.20	505	110.260574, 28.814858
TXS-27	29.50	560	110.260804, 28.814984
TXS-28	52.30	490	110.261149, 28.814843
TXS-29	38.70	514	110.261320, 28.814457
TXS-30	27.30	511	110.261126, 28.814625

Code	DBH (cm)	Altitude (m)	GPS(E,N)
TXS-31	29.40	524	110.260469, 28.814065
TXS-32	29.00	502	110.259730, 28.813559
TXS-33	37.70	494	110.269097, 28.814165
TXS-34	28.00	525	110.259179, 28.814850
TXS-35	31.80	523	110.250914, 28.814820

Table S1.
The information of the 35 Iron-heart Cunninghamia lanceolate.

Code	Plot	Position	longitude	latitude	Altitude(m)
1	1	CTY	110.266812	28.793035	534
2	1	CTY	110.266115	28.794041	541
3	1	CTY	110.266582	28.795033	544
4	1	CTY	110.267011	28.794845	551
5	1	CTY	110.267154	28.795096	514
6	1	CTY	110.267154	28.795096	561
7	1	CTY	110.2683	28.794092	605
8	1	CTY	110.2683	28.794092	596
9	1	CTY	110.269446	28.794093	602
10	1	CTY	110.269446	28.794093	616
14	1	CTY	110.271739	28.793089	654
15	4	JZW3	110.263718	28.803132	658
16	4	JZW3	110.264863	28.804133	655
17	1	CTY	110.269446	28.792084	660
18	4	JZW3	110.264863	28.804133	653
19	1	CTY	110.274031	28.792087	652
20	1	CTY	110.274031	28.792087	654
21	1	CTY	110.274031	28.792087	656
22	1	CTY	110.274031	28.792087	661
23	4	JZW3	110.262572	28.804132	660
24	1	CTY	110.271739	28.794094	659
25	1	CTY	110.271739	28.794094	644
26	1	CTY	110.272311	28.793592	646
27	1	CTY	110.272549	28.79341	664
28	1	CTY	110.272455	28.793361	650
29	1	CTY	110.272322	28.793322	651
30	1	CTY	110.272446	28.793236	645
31	1	CTY	110.272293	28.793381	642
32	1	CTY	110.272159	28.793616	623
33	1	CTY	110.272141	28.793757	653

Code	Plot	Position	longitude	latitude	Altitude(m)
34	1	CTY	110.27215	28.793773	654
35	1	CTY	110.272276	28.793624	663
36	1	CTY	110.272132	28.79378	658
37	1	CTY	110.272061	28.793863	668
38	1	CTY	110.272061	28.793863	663
39	1	CTY	110.272178	28.793881	655
40	1	CTY	110.272162	28.793761	661
41	1	CTY	110.272151	28.796853	666
42	1	CTY	110.272276	28.794812	657
43	1	CTY	110.269445	28.788067	650
44	1	CTY	110.272123	28.794133	657
45	1	CTY	110.272101	28.793965	645
46	1	CTY	110.27215	28.794034	646
47	1	CTY	110.272509	28.794644	630
48	1	CTY	110.272473	28.794675	627
49	1	CTY	110.272419	28.794675	625
50	1	CTY	110.272401	28.794754	630
51	1	CTY	110.272491	28.794675	632
52	1	CTY	110.272347	28.794769	635
53	1	CTY	110.272419	28.794691	645
54	1	CTY	110.272437	28.794738	645
55	1	CTY	110.272419	28.794691	650
56	1	CTY	110.272456	28.794738	651
57	1	CTY	110.272456	28.794973	650
58	1	CTY	110.272599	28.79491	653
59	1	CTY	110.272813	28.794754	653
60	1	CTY	110.272706	28.794722	638
61	1	CTY	110.272675	28.794859	675
62	1	CTY	110.272804	28.794785	655
63	1	CTY	110.272666	28.794701	673
64	1	CTY	110.27274	28.794741	675
65	1	CTY	110.273003	28.794652	676
66	1	CTY	110.272473	28.794785	677
67	1	CTY	110.273064	28.794565	711
68	1	CTY	110.273064	28.794565	680
69	1	CTY	110.273064	28.794565	688
70	1	CTY	110.273261	28.794511	681
71	1	CTY	110.273097	28.794544	674
72	1	CTY	110.273178	28.794603	668
73	1	CTY	110.273231	28.794833	655

Code	Plot	Position	longitude	latitude	Altitude(m)
74	1	CTY	110.273231	28.948323	671
75	1	CTY	110.273214	28.794826	668
76	1	CTY	110.273208	28.794858	658
77	1	CTY	110.27334	28.794583	667
78	1	CTY	110.27344	28.794662	663
79	1	CTY	110.273415	28.794634	662
80	1	CTY	110.273119	28.794773	660
81	1	CTY	110.273501	28.794945	679
82	1	CTY	110.273369	28.794766	675
83	1	CTY	110.273626	28.795024	613
84	1	CTY	110.27365	28.795094	630
85	1	CTY	110.273573	28.795127	657
86	1	CTY	110.273598	28.795052	665
87	1	CTY	110.273357	28.79507	653
88	1	CTY	110.273261	28.795105	789
89	1	CTY	110.27327	28.794935	870
90	1	CTY	110.273327	28.794876	759
91	1	CTY	110.273147	28.795255	667
92	1	CTY	110.272935	28.795291	682
93	1	CTY	110.273253	28.795221	660
94	1	CTY	110.27328	28.795224	674
95	1	CTY	110.273167	28.795279	655
97	1	CTY	110.27311	28.795333	666
98	1	CTY	110.273139	28.79538	691
99	1	CTY	110.273337	28.795049	653
100	1	CTY	110.273321	28.794943	635
101	2	JZW-1	110.258688	28.811154	486
102	2	JZW-1	110.25895	28.811245	482
103	3	JZW-2	110.261468	28.812119	496
104	3	JZW-2	110.261475	28.812175	504
107	3	JZW-2	110.261663	28.81301	549
108	3	JZW-2	110.261508	28.813028	526
109	3	JZW-2	110.26122	28.813561	527
110	3	JZW-2	110.261226	28.813602	520
112	3	JZW-2	110.260904	28.81422	509
113	3	JZW-2	110.260998	28.814105	506
114	3	JZW-2	110.260803	28.814323	497
115	3	JZW-2	110.260723	28.814317	482
117	3	JZW-2	110.26169	28.815117	507
118	3	JZW-2	110.262119	28.815117	500

Code	Plot	Position	longitude	latitude	Altitude(m)
119	3	JZW-2	110.26169	28.815493	510
120	3	JZW-2	110.26169	28.815493	509
121	3	JZW-2	110.26169	28.815493	515
122	3	JZW-2	110.26169	28.81474	526
123	3	JZW-2	110.262549	28.81474	539
124	3	JZW-2	110.262549	28.81474	513
125	3	JZW-2	110.262549	28.81474	519
127	3	JZW-2	110.263409	28.81474	531
128	3	JZW-2	110.26169	28.81474	534
129	3	JZW-2	110.26169	28.81474	535
130	3	JZW-2	110.263409	28.81474	537
131	3	JZW-2	110.263409	28.81474	531
132	3	JZW-2	110.263409	28.81474	552
134	3	JZW-2	110.26169	28.81474	538
136	3	JZW-2	110.26169	28.81474	525
137	3	JZW-2	110.26169	28.81474	521
138	3	JZW-2	110.26169	28.81474	537
139	3	JZW-2	110.26169	28.81474	535
140	3	JZW2	110.26169	28.81474	528
141	3	JZW-2	110.26169	28.81474	519
142	3	JZW-2	110.26169	28.81474	524
143	3	JZW-2	110.26169	28.81474	523
144	2	JZW-1	110.256532	28.813234	522
145	3	JZW-2	110.26169	28.81474	514
146	3	JZW-2	110.26169	28.81474	525
147	3	JZW2	110.26169	28.81474	513
148	3	JZW-2	110.26169	28.81474	510
149	3	JZW-2	110.26169	28.81474	511
150	3	JZW-2	110.26169	28.81474	512
151	9	XNC	110.25808	28.794866	426
152	9	XNC	110.258057	28.794869	425
153	9	XNC	110.25805	28.794869	427
154	9	XNC	110.257734	28.792924	352
155	2	JZW-1	110.255928	28.81311	500
156	2	JZW-1	110.255961	28.812925	513
157	9	XNC	110.257942	28.792232	496
158	9	XNC	110.257963	28.792241	499
159	9	XNC	110.258036	28.792211	495
160	9	XNC	110.257983	28.792156	492
161	9	XNC	110.256324	28.792974	556

Code	Plot	Position	longitude	latitude	Altitude(m)
162	9	XNC	110.25626	28.793065	554
163	9	XNC	110.256173	28.793191	519
164	9	XNC	110.256173	28.793197	547
165	9	XNC	110.25619	28.793335	548
166	9	XNC	110.256159	28.7934	545
167	9	XNC	110.256149	28.793409	548
168	9	XNC	110.256129	28.793385	548
169	9	XNC	110.256116	28.793521	531
170	9	XNC	110.256096	28.793444	543
171	9	XNC	110.256096	28.793556	542
172	9	XNC	110.256069	28.793668	533
173	9	XNC	110.256089	28.793644	534
174	9	XNC	110.256109	28.793574	530
175	9	XNC	110.256123	28.793633	527
176	9	XNC	110.256129	28.793627	523
177	9	XNC	110.256096	28.793739	520
178	9	XNC	110.256082	28.793733	515
179	9	XNC	110.255975	28.793344	500
180	9	XNC	110.256076	28.79355	526
181	9	XNC	110.25668	28.791991	534
182	9	XNC	110.256928	28.792044	542
183	9	XNC	110.256814	28.791926	535
184	9	XNC	110.256754	28.791779	527
185	9	XNC	110.2567	28.791744	524
186	9	XNC	110.256673	28.791732	538
187	9	XNC	110.256512	28.791849	526
188	9	XNC	110.256458	28.791808	526
189	9	XNC	110.256458	28.79182	532
190	9	XNC	110.256519	28.791908	530
191	9	XNC	110.256465	28.791902	540
192	9	XNC	110.256378	28.791745	536
194	9	XNC	110.256438	28.791685	542
195	9	XNC	110.256371	28.791633	556
196	9	XNC	110.256358	28.791508	553
197	9	XNC	110.256445	28.791402	552
198	9	XNC	110.256438	28.79139	552
199	9	XNC	110.255995	28.791137	544
200	9	XNC	110.25621	28.790978	545
201	5	LYP-1	110.251672	28.831943	928
202	5	LYP-1	110.251716	28.83204	920

Code	Plot	Position	longitude	latitude	Altitude(m)
203	6	LYP-2	110.251766	28.830129	862
204	6	LYP-2	110.251838	28.830039	882
205	6	LYP-2	110.251952	28.829865	845
206	6	LYP-2	110.251776	28.829784	872
207	6	LYP-2	110.251688	28.829844	871
208	6	LYP-2	110.252861	28.829196	796
210	6	LYP-2	110.253689	28.828283	755
211	6	LYP-2	110.253985	28.827786	749
212	6	LYP-2	110.253501	28.827892	730
213	6	LYP-2	110.254058	28.827841	738
214	6	LYP-2	110.253728	28.827494	733
215	6	LYP-2	110.254178	28.827329	733
216	6	LYP-2	110.254169	28.827263	734
217	6	LYP-2	110.254318	28.827022	728
218	6	LYP-2	110.254701	28.826678	710
220	7	LYP-3	110.251102	28.819344	879
221	7	LYP-3	110.250925	28.819311	889
222	7	LYP-3	110.251247	28.819485	860
223	7	LYP-3	110.25086	28.819912	875
224	7	LYP-3	110.250989	28.820227	878
225	7	LYP-3	110.250962	28.819546	870
226	7	LYP-3	110.251204	28.819504	840
228	7	LYP-3	110.25115	28.819278	835
229	7	LYP-3	110.251005	28.81924	837
230	7	LYP-3	110.251354	28.819156	830
232	7	LYP-3	110.251451	28.819231	830
234	7	LYP-3	110.251268	28.818977	814
236	7	LYP-3	110.2513	28.818902	733
237	7	LYP-3	110.251434	28.818902	678
238	7	LYP-3	110.251366	28.818901	810
239	7	LYP-3	110.251472	28.818635	807
240	7	LYP-3	110.251367	28.818623	806
241	8	LYP-4	110.253403	28.814614	785
242	8	LYP-4	110.253464	28.814627	780
243	8	LYP-4	110.253504	28.814843	780
244	8	LYP-4	110.253303	28.814612	790
245	8	LYP-4	110.252381	28.814914	787
246	8	LYP4	110.252431	28.814457	787
247	8	LYP-4	110.252565	28.814573	785
248	8	LYP-4	110.252531	28.814643	770

Code	Plot	Position	longitude	latitude	Altitude(m)
249	8	LYP-4	110.252415	28.814638	780
250	8	LYP-4	110.25249	28.81451	790
251	8	LYP-4	110.252389	28.814524	780
252	8	LYP-4	110.252617	28.814707	780
253	8	LYP-4	110.252464	28.814759	822
254	8	LYP-4	110.252586	28.814907	823
255	8	LYP-4	110.252485	28.814505	815
256	8	LYP-4	110.252474	28.81493	825
257	8	LYP-4	110.252442	28.81495	826
258	8	LYP-4	110.252474	28.81493	825
259	8	LYP-4	110.252432	28.81475	836
260	2	JZW-1	110.259991	28.808601	830
261	2	JZW-1	110.259993	28.808731	819
263	8	LYP-4	110.251947	28.814613	824
264	8	LYP-4	110.251909	28.814514	837
265	8	LYP-4	110.251896	28.814477	840
266	8	LYP-4	110.251815	28.814618	834
267	8	LYP-4	110.25207	28.81447	825
268	8	LYP-4	110.252282	28.814319	819
269	8	LYP-4	110.252118	28.814293	821
270	8	LYP-4	110.252175	28.814322	821
271	8	LYP-4	110.252244	28.814284	815
272	8	LYP-4	110.251362	28.814845	799
273	8	LYP-4	110.251322	28.814858	808
274	8	LYP-4	110.251597	28.813805	808
275	8	LYP-4	110.2515	28.813742	823
276	3	JZW-2	110.264737	28.810652	525
277	3	JZW-2	110.260078	28.814223	485
278	3	JZW-2	110.260078	28.814175	488
279	3	JZW-2	110.260024	28.814317	491
280	3	JZW-2	110.25997	28.814364	509
281	3	JZW-2	110.259863	28.814599	504
282	3	JZW-2	110.259863	28.814599	504
283	3	JZW-2	110.259809	28.814646	510
284	3	JZW-2	110.259809	28.814693	514
285	3	JZW-2	110.259702	28.814787	513
286	3	JZW-2	110.259756	28.814787	513
287	3	JZW-2	110.259702	28.814787	518
288	3	JZW-2	110.259648	28.814787	520
289	3	JZW-2	110.259648	28.814882	525

Code	Plot	Position	longitude	latitude	Altitude(m)
291	3	JZW-2	110.259326	28.814976	535
293	3	JZW-2	110.259218	28.814882	525
295	3	JZW-2	110.259218	28.814699	523
296	3	JZW-2	110.259218	28.814599	526
297	3	JZW-2	110.259326	28.814552	516
298	3	JZW-2	110.259433	28.814458	510
299	3	JZW-2	110.259863	28.813611	464
300	2	JZW-1	110.263011	28.80788	498
301	2	JZW-1	110.263	28.808087	503
302	2	JZW-1	110.263183	28.808167	506
303	2	JZW-1	110.263571	28.808077	510
304	3	JZW-2	110.265989	28.811247	545
305	3	JZW-2	110.266004	28.811278	576
306	3	JZW-2	110.266103	28.811241	523
307	3	JZW-2	110.266153	28.811309	535
308	3	JZW-2	110.266199	28.811258	537
309	3	JZW-2	110.266199	28.811259	537
310	3	JZW-2	110.266079	28.811436	520
311	3	JZW-2	110.266025	28.811335	520
312	3	JZW-2	110.265996	28.811373	532
313	3	JZW-2	110.265996	28.811372	520
314	3	JZW-2	110.265858	28.811433	528
315	3	JZW-2	110.265956	28.81142	531
316	3	JZW-2	110.265863	28.811323	529
317	3	JZW-2	110.265504	28.811433	525
318	3	JZW-2	110.265889	28.81137	529
319	3	JZW-2	110.265938	28.811365	530
320	3	JZW-2	110.265968	28.81137	531
321	3	JZW-2	110.265889	28.811301	529
323	3	JZW-2	110.265745	28.81161	526
324	3	JZW-2	110.265873	28.811646	525
325	3	JZW-2	110.265485	28.811865	536
326	3	JZW-2	110.265524	28.811862	537
327	3	JZW-2	110.265556	28.811825	537
328	3	JZW-2	110.265618	28.811889	526
330	3	JZW-2	110.265554	28.811823	530
331	3	JZW-2	110.265658	28.811802	530
332	3	JZW-2	110.265503	28.811767	535
333	3	JZW-2	110.265296	28.811861	557
334	3	JZW-2	110.265234	28.811896	539

Code	Plot	Position	longitude	latitude	Altitude(m)
335	7	LYP-3	110.265138	28.811964	538
335	3	JZW-2	110.265138	28.811964	538
336	3	JZW-2	110.264905	28.811888	542
337	3	JZW-2	110.264886	28.811812	535
338	3	JZW-2	110.264902	28.811727	537
339	3	JZW-2	110.264918	28.811686	543
340	3	JZW-2	110.265194	28.811692	538
341	3	JZW-2	110.265055	28.811593	540
342	3	JZW-2	110.265127	28.811549	532
343	3	JZW-2	110.265103	28.811499	532
344	3	JZW-2	110.265036	28.811453	535
345	3	JZW-2	110.265021	28.811431	540
346	3	JZW-2	110.265059	28.811403	538
347	3	JZW-2	110.264996	28.811403	535
348	3	JZW-2	110.265917	28.811525	542
349	3	JZW-2	110.265937	28.811543	525
350	3	JZW-2	110.265909	28.811615	529
351	3	JZW-2	110.266003	28.811554	530
352	3	JZW-2	110.266267	28.811835	540
353	3	JZW-2	110.266255	28.811816	533
354	3	JZW-2	110.266272	28.811721	541
355	3	JZW-2	110.266164	28.811771	538
356	3	JZW-2	110.265991	28.811558	532
357	3	JZW-2	110.266228	28.811586	540
358	3	JZW-2	110.266193	28.811927	535
359	3	JZW-2	110.265963	28.811565	545
360	3	JZW-2	110.26604	28.811553	533
362	3	JZW-2	110.266012	28.811557	537
363	6	LYP-2	110.252271	28.829358	818
364	6	LYP-2	110.252219	28.829951	854
365	5	LYP-1	110.251349	28.831102	899
366	5	LYP-1	110.251304	28.831128	899
367	5	LYP-1	110.250948	28.831558	922
368	5	LYP-1	110.250874	28.831577	930
369	5	LYP-1	110.250689	28.831557	932
370	5	LYP-1	110.250634	28.832051	900
371	5	LYP-1	110.250478	28.832025	940
372	5	LYP-1	110.250434	28.832028	940
373	2	JZW-1	110.263912	28.808815	945
374	5	LYP-1	110.250163	28.832358	945

Code	Plot	Position	longitude	latitude	Altitude(m)
375	5	LYP-1	110.249569	28.832626	998
376	5	LYP-1	110.249466	28.832862	1015
377	5	LYP-1	110.249088	28.832724	1030
378	5	LYP-1	110.249297	28.832514	1011
379	5	LYP-1	110.249066	28.832626	1033
380	5	LYP-1	110.248575	28.83256	1035
381	5	LYP-1	110.248598	28.832476	1040
382	5	LYP-1	110.24879	28.832071	1050
383	5	LYP-1	110.248354	28.832044	1028
385	5	LYP-1	110.248287	28.83145	1022
386	5	LYP-1	110.248318	28.831274	1005
389	5	LYP-1	110.248173	28.831164	990
390	5	LYP-1	110.248311	28.831247	991
392	5	LYP-1	110.248629	28.830985	980
393	5	LYP-1	110.248575	28.831086	967
394	5	LYP-1	110.248873	28.831389	950
395	5	LYP-1	110.249581	28.830962	934
396	5	LYP-1	110.249954	28.831276	938
397	5	LYP-1	110.250058	28.831746	937
398	5	LYP-1	110.251929	28.832356	921
399	5	LYP-1	110.251779	28.832887	921
400	5	LYP-1	110.251693	28.832149	921
401	9	XNC	110.256284	28.79092	542
402	9	XNC	110.256519	28.79092	542
403	9	XNC	110.256485	28.790973	540
404	9	XNC	110.256586	28.791055	539
405	9	XNC	110.256586	28.791043	539
406	9	XNC	110.256693	28.790949	538
407	9	XNC	110.256687	28.790949	546
408	9	XNC	110.256942	28.790949	540
409	9	XNC	110.25721	28.790172	513
410	9	XNC	110.257351	28.790431	492
411	4	JZW-3	110.257822	28.804113	467
412	4	JZW-3	110.257472	28.804066	477
413	4	JZW-3	110.257472	28.804066	477
414	4	JZW-3	110.257284	28.803972	491
415	4	JZW-3	110.257123	28.803996	496
416	4	JZW-3	110.257043	28.804031	498
417	4	JZW-3	110.256922	28.804561	524
418	4	JZW-3	110.256855	28.804572	508

Code	Plot	Position	longitude	latitude	Altitude(m)
419	4	JZW-3	110.256761	28.804631	517
420	4	JZW-3	110.25668	28.804678	494
421	4	JZW-3	110.256667	28.804702	515
422	4	JZW-3	110.256613	28.804725	519
423	4	JZW-3	110.256519	28.804737	520
424	4	JZW-3	110.256411	28.804808	512
425	4	JZW-3	110.256304	28.804878	526
426	4	JZW-3	110.25625	28.804867	520
427	4	JZW-3	110.256264	28.80486	526
428	4	JZW-3	110.256129	28.80489	528
429	4	JZW-3	110.256035	28.80489	527
430	4	JZW-3	110.255982	28.804937	530
431	4	JZW-3	110.255901	28.805067	541
432	4	JZW-3	110.25582	28.805055	536
433	4	JZW-3	110.25578	28.805043	537
434	4	JZW-3	110.255753	28.805079	542
435	4	JZW-3	110.255673	28.805149	537
436	4	JZW-3	110.255646	28.805161	538
437	4	JZW-3	110.255485	28.805196	543
438	4	JZW-3	110.255471	28.805208	550
439	4	JZW-3	110.255216	28.805302	558
440	4	JZW-3	110.254894	28.805396	559
441	4	JZW-3	110.254692	28.80542	553
442	4	JZW-3	110.254692	28.805408	565
443	4	JZW-3	110.254571	28.805396	564
444	4	JZW-3	110.25445	28.805396	568
445	4	JZW-3	110.254316	28.805361	566
446	4	JZW-3	110.254236	28.805349	567
447	4	JZW-3	110.254155	28.805337	569
448	4	JZW-3	110.254061	28.80533	570
449	4	JZW-3	110.253954	28.805349	572
450	4	JZW-3	110.253859	28.805361	576
452	4	JZW-3	110.253094	28.805585	579
453	4	JZW-3	110.253121	28.805561	599
454	4	JZW-3	110.253013	28.805632	595
455	4	JZW-3	110.252664	28.805679	611
456	4	JZW-3	110.252557	28.805655	612
457	4	JZW-3	110.252369	28.805702	618
458	4	JZW-3	110.252315	28.805702	622
459	4	JZW-3	110.252208	28.805749	626

Code	Plot	Position	longitude	latitude	Altitude(m)
460	4	JZW-3	110.251885	28.805843	637
461	4	JZW-3	110.251858	28.805867	642
462	4	JZW-3	110.251724	28.805867	649
463	4	JZW-3	110.25167	28.805867	652
464	4	JZW-3	110.251563	28.805941	659
465	4	JZW-3	110.251536	28.805914	654
466	4	JZW-3	110.251429	28.805891	651
467	4	JZW-3	110.251429	28.805914	650
468	4	JZW-3	110.251402	28.805914	648
469	4	JZW-3	110.251348	28.805961	644
470	4	JZW-3	110.251187	28.806008	648
471	4	JZW-3	110.25116	28.806126	652
472	4	JZW-3	110.25116	28.806126	648
473	4	JZW-3	110.25116	28.806102	644
474	4	JZW-3	110.251026	28.807115	641
475	4	JZW-3	110.250918	28.807044	660
476	4	JZW-3	110.250864	28.807185	662
477	4	JZW-3	110.250891	28.807303	658
478	4	JZW-3	110.250891	28.807303	657
479	4	JZW-3	110.250918	28.807303	656
480	4	JZW-3	110.250891	28.80735	654
481	4	JZW-3	110.250811	28.80742	658
482	4	JZW-3	110.250838	28.807444	659
483	2	JZW-1	110.250945	28.81041	671
485	2	JZW-1	110.251052	28.810457	663
486	2	JZW-1	110.251106	28.81048	662
487	2	JZW-1	110.25116	28.810504	663
488	2	JZW-1	110.251267	28.810551	659
489	2	JZW-1	110.251321	28.810551	661
490	2	JZW-1	110.252557	28.810716	629
491	2	JZW-1	110.252637	28.810833	624
492	2	JZW-1	110.252745	28.810904	624
493	2	JZW-1	110.253013	28.810951	607
494	2	JZW-1	110.252986	28.81097	601
495	2	JZW-1	110.253121	28.810998	613
496	2	JZW-1	110.253121	28.81099	612
497	2	JZW-1	110.253175	28.811092	609
498	2	JZW-1	110.253443	28.811116	613
499	2	JZW-1	110.253604	28.811116	605
500	2	JZW-1	110.253631	28.811163	603

Code	Plot	Position	longitude	latitude	Altitude(m)
501	2	JZW-1	110.25398	28.811092	597
502	2	JZW-1	110.255135	28.810716	574
503	2	JZW-1	110.255243	28.810669	568
504	2	JZW-1	110.255323	28.810598	550
505	2	JZW-1	110.256049	28.81041	533
506	4	JZW-3	110.256908	28.80808	519
507	4	JZW-3	110.256908	28.80808	516
508	4	JZW-3	110.256425	28.80808	526
509	4	JZW-3	110.255699	28.807938	543
510	4	JZW-3	110.255565	28.807915	549
511	4	JZW-3	110.255565	28.807915	546
512	4	JZW-3	110.25535	28.807915	555
513	4	JZW-3	110.255377	28.807915	553
514	4	JZW-3	110.25527	28.807985	553
515	4	JZW-3	110.254921	28.807891	577
516	4	JZW-3	110.254894	28.807868	579
517	4	JZW-3	110.254894	28.807868	579
518	4	JZW-3	110.254706	28.807868	572
519	4	JZW-3	110.254706	28.807821	569
520	4	JZW-3	110.254652	28.807844	563
521	4	JZW-3	110.254652	28.807844	572
522	4	JZW-3	110.254437	28.807868	573
523	4	JZW-3	110.254437	28.807821	567
524	4	JZW-3	110.254168	28.807844	588
525	4	JZW-3	110.254115	28.80775	588
527	4	JZW-3	110.253363	28.807585	609
528	4	JZW-3	110.253148	28.807562	608
529	4	JZW-3	110.253443	28.807632	604
530	4	JZW-3	110.253094	28.807632	608
531	4	JZW3	110.25296	28.807656	609
532	4	JZW-3	110.25296	28.807656	608
533	4	JZW-3	110.252933	28.807679	612
534	4	JZW-3	110.25296	28.807656	612
535	4	JZW-3	110.252879	28.807656	615
536	4	JZW-3	110.252718	28.807632	620
537	4	JZW-3	110.252691	28.80775	621
538	4	JZW-3	110.252745	28.807726	611
539	4	JZW-3	110.25261	28.80775	613
540	4	JZW-3	110.252208	28.807774	612
541	4	JZW-3	110.252208	28.80775	622

Code	Plot	Position	longitude	latitude	Altitude(m)
542	4	JZW-3	110.252154	28.807726	619
543	4	JZW-3	110.252019	28.807797	630
544	4	JZW-3	110.251885	28.807868	634
545	4	JZW-3	110.251778	28.807821	642
546	4	JZW-3	110.251697	28.807726	650
547	4	JZW-3	110.25167	28.807891	644
548	4	JZW-3	110.251697	28.807938	651
549	4	JZW-3	110.251563	28.807962	655
550	4	JZW-3	110.25124	28.807915	659
551	4	JZW-3	110.25124	28.807915	653
552	4	JZW-3	110.251267	28.807962	656
553	4	JZW-3	110.25124	28.807962	652
554	4	JZW-3	110.250999	28.80815	662
555	4	JZW-3	110.250999	28.808197	659
556	4	JZW-3	110.250838	28.808174	664
557	4	JZW-3	110.250784	28.808197	665
558	4	JZW-3	110.250596	28.808221	606
559	2	JZW-1	110.248554	28.809421	717
560	2	JZW-1	110.247829	28.810645	756
561	2	JZW-1	110.247775	28.810692	751
562	2	JZW-1	110.247829	28.810645	750
563	2	JZW-1	110.247775	28.810692	750
564	2	JZW-1	110.247829	28.810645	749
565	2	JZW-1	110.247775	28.810692	749
566	2	JZW-1	110.24756	28.811116	754
567	2	JZW-1	110.247829	28.811728	760
568	2	JZW-1	110.247883	28.811822	762
569	2	JZW-1	110.24799	28.81187	764
570	2	JZW-1	110.248044	28.811916	759
571	2	JZW-1	110.248151	28.811916	765
572	2	JZW-1	110.248474	28.81201	752
573	2	JZW-1	110.250085	28.810127	727
574	2	JZW-1	110.2503	28.810221	698
575	2	JZW-1	110.250408	28.810221	684
576	3	JZW-2	110.259756	28.813516	477
577	3	JZW-2	110.259756	28.813516	497
579	3	JZW-2	110.259541	28.813611	505
580	3	JZW-2	110.259541	28.813611	501

Table S2.
Location and number of trees sampled for 9 populations in provenance.

Pop	Na	Ne	I	Ho	Fp	He	uHe	F
CTY	7	2.870	1.174	0.497	0.533	0.594	0.598	0.164
JZW-1	6	2.755	1.179	0.452	0.000	0.599	0.604	0.240
JZW-2	7	3.070	1.244	0.455	0.200	0.622	0.625	0.244
JZW-3	8	3.183	1.294	0.497	0.200	0.636	0.639	0.205
LYP-1	5	3.272	1.241	0.452	0.133	0.637	0.647	0.257
LYP-2	4	2.463	1.015	0.442	0.000	0.536	0.554	0.173
LYP-3	4	2.452	1.013	0.416	0.000	0.538	0.554	0.220
LYP-4	4	2.453	0.997	0.506	0.067	0.524	0.534	0.035
XNC	5	2.792	1.108	0.465	0.000	0.574	0.579	0.181
Mean		2.812	1.141	0.465	0.126	0.584	0.593	0.191

Na: number of different alleles; Ne: number of effective alleles; I: Shannon's Information Index; Ho: observed heterozygosity; He: expected heterozygosity with populations; uHe: unbiased expected heterozygosity with populations; F: fixation Index; Fp: no. private alleles (no. of alleles unique to a single population).

Table S3.
Genetic diversity parameters of 9 populations of Chinese fir. All values were multilocus estimates based on 15 microsatellite loci.

	CTY	JZW-1	JZW-2	JZW-3	LYP-1	LYP-2	LYP-3	LYP-4	XNC
CTY		0.088	0.069	0.038	0.057	0.093	0.081	0.080	0.052
JZW-1	0.092		0.014	0.025	0.013	0.067	0.064	0.069	0.071
JZW-2	0.072	0.018		0.018	0.004	0.051	0.039	0.049	0.037
JZW-3	0.041	0.029	0.020		0.010	0.051	0.038	0.044	0.024
LYP-1	0.063	0.021	0.010	0.016		0.045	0.034	0.041	0.043
LYP-2	0.104	0.080	0.062	0.062	0.060		0.006	0.026	0.063
LYP-3	0.091	0.077	0.050	0.048	0.049	0.025		0.016	0.049
LYP-4	0.087	0.077	0.055	0.051	0.051	0.040	0.030		0.059
XNC	0.057	0.076	0.041	0.027	0.051	0.076	0.061	0.066	

Table S4.
Pair-wise estimates of genetic differentiation between Chinese fir populations using Fst and Gst coefficients based on 15 SSR markers.

	CTY	JZW-1	JZW-2	JZW-3	LYP-1	LYP-2	LYP-3	LYP-4
JZW-1	0.357							
JZW-2	0.275	0.059						
JZW-3	0.144	0.101	0.073					
LYP-1	0.242	0.071	0.035	0.059				
LYP-2	0.356	0.255	0.197	0.201	0.192			
LYP-3	0.302	0.244	0.152	0.148	0.151	0.062		
LYP-4	0.273	0.236	0.164	0.150	0.153	0.099	0.073	
XNC	0.184	0.266	0.136	0.088	0.177	0.228	0.176	0.188

Table S5.
Genetic distance between the different population.

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References

[1] Lin E, Zhuang H, Yu J, Liu X, et al. Genome survey of Chinese fir (*Cunninghamia lanceolata*): Identification of genomic SSRs and demonstration of their utility in genetic diversity analysis. Scientific Reports. 2020;**10**(1):4698

[2] Wang Z, Chen J, Liu W, et al. Transcriptome characteristics and six alternative expressed genes positively correlated with the phase transition of annual cambial activities in Chinese Fir (*Cunninghamia lanceolata* (Lamb.) Hook). PLoS One. 2013;**8**(8):e71562

[3] Huang L, Zhu Y, Yang J, et al. Shoot blight on Chinese fir (*Cunninghamia lanceolata*) is caused by bipolaris oryzae. Plant Disease. 2018;**102**(3):500-506

[4] Chen M, Wang C, Fei B, et al. Biological degradation of Chinese fir with trametes versicolor (L.) lloyd. Materials (Basel). 2017;**10**(7):834

[5] Bao W, Qu Y, Shan X, Wan Y. Screening and validation of housekeeping genes of the root and cotyledon of *Cunninghamia lanceolata* under abiotic stresses by using quantitative real-time PCR. International Journal of Molecular Sciences. 2016;**17**(8):1198

[6] Zhang X, Peng JY, Shi JT, et al. Wood anatomical characteristics and physical-mechanical properties of dark-brown heart Cunninham lanceolate from Hunan. Journal of Southwest Forestry University (Natural Sciences). 2021; **41**(1):155-160

[7] You R, Zhu N, Deng X, et al. Variation in wood physical properties and effects of climate for different geographic sources of Chinese fir in subtropical area of China. Scientific Reports. 2021;**11**(1):1-11

[8] McDonald MB. Seed quality assessment. Seed Science Research. 1998;**8**(2):265-276

[9] Jiang SB, Zhang Y, Wei XL, et al. Growth and photosynthetic physiology for two groups of Phoebe bournei plus tree seedlings. Journal of Zhejiang A & F University. 2016;**33**(1):51-59

[10] Bai TD, Yu CL, Gan ZC, et al. Association of cone and seed traits of Pinus yunnanensis var. tenuifolia with geo-meteorological factors. Chinese Journal of Plant Ecology. 2020;**44**(12): 1224-1235

[11] Kramer AT. Hidden variation: cultivars and wild plants differ in trait variation with surprising root trait outcomes. Restoration Ecology. 2021; **29**(2):e13336

[12] Wang Y, Zheng RH, Chen XC, et al. Conservation and utilization status and development strategy of Chinese fir Germplasm Resources. Fujian Forestry Science and Technology. 2007;**1**:128-132

[13] Cun C, Ding C, Huang Q, et al. Construction of phenotypic core collection of populus deltoides. Forest Research. 2021;**34**(2):1-11

[14] Huzar-Novakowiski J, Dorrance A. Genetic diversity and population structure of pythium irregulare from soybean and corn production fields in Ohio. Plant Disease. 2018;**102**(10): 1989-2000

[15] Soorni A, Fatahi R, Haak D, et al. Assessment of genetic diversity and population structure in iranian cannabis germplasm. Scientific Reports. 2017; **7**(1):15668

[16] King B. Which morphological characters are influential in a Bayesian phylogenetic analysis? Examples from the earliest osteichthyans. Biology Letters. 2019;**15**(7):20190288

[17] Sundar I, Sakthivel N. Advances in selectable marker genes for plant

transformation. Journal of Plant Physiology. 2008;**165**(16):1698-1716

[18] Darbani B, Eimanifar A, Stewart C, et al. Methods to produce marker-free transgenic plants. Biotechnology Journal. 2007;**2**(1):83-90

[19] Jayabalan S, Pulipati S, Ramasamy K, et al. Analysis of genetic diversity and population structure using SSR markers and validation of a Cleavage Amplified Polymorphic Sequences (CAPS) marker involving the sodium transporter OsHKT1;5 in saline tolerant rice (*Oryza sativa* L.) landraces. Gene. 2019;**713**:143976

[20] Singh A, Chaurasia S, Kumar S, et al. Identification, analysis and development of salt responsive candidate gene based SSR markers in wheat. BMC Plant Biology. 2018;**18**(1):249

[21] Duan H, Cao S, Zheng H, et al. Genetic characterization of Chinese fir from six provinces in southern china and construction of a core collection. Scientific Reports. 2017;**7**(1):13814

[22] Duan H, Hu R, Wu B, et al. Genetic characterization of red-colored heartwood genotypes of Chinese fir using simple sequence repeat (SSR) markers. Genetics and Molecular Research. 2015;**14**(4):18552-18561

[23] Feng Y, Yang Z, Wang J, et al. Development and characterization of SSR markers from Pinus massoniana and their transferability to P. elliottii, P. caribaea and P. yunnanensis. Genetics and Molecular Research. 2014;**13**(1): 1508-1513

[24] Zeng J, Chen J, Kou Y, et al. Application of EST-SSR markers developed from the transcriptome of Torreya grandis (*Taxaceae*), a threatened nut-yielding conifer tree. PeerJ. 2018;**6**:e5606

[25] Song Y, Li M, Xu J, et al. Polymorphic microsatellite markers in the traditional Chinese medicinal plant Paris polyphylla var. yunnanensis. Genetics and Molecular Research. 2015; **14**(3):9939-9942

[26] Chutimanitsakun Y, Nipper R, Cuesta-Marcos A, et al. Construction and application for QTL analysis of a Restriction Site Associated DNA (RAD) linkage map in barley. BMC Genomics. 2011;**12**(1):4

[27] Taramino G, Tarchini R, Ferrario S, et al. Characterization and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor*. Theoretical and Applied Genetics. 1997;**95**(1–2):66-72

[28] Cao WB, Yin F, Zhang CX. Ecological and economic development of China's National Nature Reserve—Taking the investigation of the Creek National Nature Reserve of Yongshun County of Hunan Province as an example. Journal of Southwest Minzu University (Natural Science Edition). 2017;**43**(2):198-203

[29] Wen Y, Ueno S, Han W. Development and characterization of 28 polymorphic EST-SSR markers for *Cunninghamia lanceolata* (Taxodiaceae) based on transcriptome sequences. Silvae Genetica. 2013;**62**(1–6):137-141

[30] Zhang S. Variation regularity analyzation of material properties and association SSR locus mining for *Cunninghamia lanceolata* [master thesis]. Hangzhou, Zhejiang, China: Zhejiang A & F University; 2015

[31] Hang Y, Yu J, Zhou S, et al. Development and application of SSR loci from functional genes involved in wood formation in cunninghamia lanceolata. Journal of Agricultural Biotechnology. 2019;**27**(01):34-42

[32] Harrell Jr FE, Dupont C. Hmisc: Harrell miscellaneous. package version 3.17 -1. 2015. Accessed on 12 December 2015 [OL] https://CAN.-project.org/package=Hmisc

[33] Wei T, Simko V. R package "corrplot": Visualization of a Correlation Matrix (Version 0.84). [OL]. https://github.com/taiyun/corrplot. Accessed 12 Dec 2017

[34] Rod P, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics. 2012;**28**(19):2537-2539

[35] Meirmans PG. AMOVA-based clustering of population genetic data. The Journal of Heredity. 2012;**103**(5): 744-750

[36] Rosenberg NA. Distruct: a program for the graphical display of population structure. Molecular Ecology Notes. 2004;**4**(1):137-138

[37] Blanco-Guillot F, Castañeda-Cediel ML, Cruz-Hervert P, et al. Genotyping and spatial analysis of pulmonary tuberculosis and diabetes cases in the state of Veracruz Mexico. PLoS One. 2018;**13**(3):e0193911

[38] Ellstrand NC, Elam DR. Population genetic consequences of small population size: Implications for plant conservation. Annual Review of Ecology and Systematics. 1993;**24**(1):217-242

[39] Gough RE. Seed quality: basic mechanisms and agricultural implications. Florida: CRC Press; 2020

[40] Chen L, Chen Q, Chen DX, et al. Study on cone and seed qualities among different cunninghamia lanceolata clones. Guangxi Forestry Science. 2021; **50**(1):1-7

[41] Zhang Z, Zhang HG, Zhou Y, et al. Variation of seed characters in Korean pine (Pinus koraiensis) multi-clonal populations. Journal of Beijing Forestry University. 2015;**37**(2):67-78

[42] Mo C, Zhang HG, Zhang HX, et al. Variations in nutrition compositions and morphology characteristics in different hybrid combination of Korean pine (Pinus koraiensis). Bulletin of Botanical Research. 2017;**37**(5):700-708

[43] Zhang X, Su H, Yang J, Feng L, Li Z, Zhao G. Population genetic structure, migration, and polyploidy origin of a medicinal species Gynostemma pentaphyllum (Cucurbitaceae). Ecology and Evolution. 2019;**9**(19):11145-11170

[44] Nei M, Takezaki N. The root of the phylogenetic tree of human populations. Molecular Biology and Evolution. 1996; **13**(1):170-177

[45] Loveless MD, Hamrick JL. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics. 1984;**15**(1):65-95