

GENETIC DIVERSITY ANALYSIS OF *DENDROCALAMOPSIS BEECHEYANA* VAR. *PUBESCENS* BASED ON ISSR MARKERS

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Abstract. *Dendrocalamopsis beecheyana* var. *pubescens* is native to the southern provinces of China. In this study, the genetic diversity of 17 accessions from 6 populations of Fujian and Guangxi provinces were assessed. Genetic diversity was estimated using ISSR (inter-simple sequence repeat) markers. Sixteen selected primers produced 185 ISSR loci, of which 167 (90.27%) were found to be polymorphic. Dendrogram analyses revealed that the 17 accessions formed six major groups, while the six populations were clustered into three major groups. A high genetic diversity was identified at the species level, with the percentage of polymorphic band (PPB), Nei's gene diversity (*He*) and Shannon's information index (*I*) was found to be 90.27%, 0.2497, and 0.3949, respectively. In addition, Nei's differentiation coefficient (*Gst*) was relatively high (0.5399) and a low rate of gene flow (*Nm*) of 0.4261 was observed. The results clearly show that there is a high degree of genetic variation at the species level and a low degree of genetic variation at the population level. Moreover, ISSR molecular markers could be effectively utilized for the characterization of *D. beecheyana* var. *pubescens* accessions and identification of the relationships within populations.

Keywords: *D. beecheyana* var. *pubescens* primers, genetic variation, Fujian, species level

Introduction

Dendrocalamopsis beecheyana var. *pubescens* is considered to be an important ornamental bamboo with low cold tolerance, which is widely cultivated in the southern region of Guangdong Province, Hongkong, and Taiwan (Editorial Committee of Chinese Flora of Chinese Academy of Sciences, 1996). In China, this bamboo species is used for the manufacture of construction materials, while the bamboo shoots are used in the traditional Chinese cuisine of Fujian and Guangxi Provinces. In addition, it has been used for soil and water conservation and also to improve the environment in the coastal town of Zhangzhou in Fujian Province (Li et al., 2017). Thus, this bamboo has gained its value for ornamental, ecological, economic, and social purposes and is characterized by its wide adaptability, fast breeding, and highly developed root system (Desai et al., 2015).

To date, genetic diversity analysis of bamboo species has been done with molecular marker variations using PCR amplification (Barkley et al., 2005; Tian et al., 2012; Yang et al., 2012). However, studies on *D. beecheyana* var. *pubescens* have been limited to tissue culture, physiological biochemistry, and cultivation techniques with little information reported on genetic diversity. Considering the rich genetic diversity of bamboo species, its genetic diversity analyses can help to determine the genetic

resources of the species, and also the selection and conservation of improved cultivars.

In recent years, a number of molecular marker technologies have been developed and widely applied in genetic diversity and DNA fingerprinting studies including random amplified polymorphic DNA (RAPD) markers, restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, and inter-simple sequence repeat (ISSR) markers (Grover et al., 2016). RAPD has been regarded as an approach that exhibited high efficiency, sensitivity, and specificity, with rapid and simple method to analyze genomic DNA; however, it is incapable of discriminating between heterozygotes and homozygotes (Williams et al., 1990). The RFLP method offers large numbers of markers, but requires larger quantities of DNA from fresh samples (Yang et al., 1996). Similarly, AFLP markers offer high resolution, stability, and efficiency, but the requirement of high-purity DNA and restriction enzyme makes it expensive to use (Vos et al., 1995). ISSR markers provide a simple, rapid, and highly efficient method that is comparatively economical (Zietkiewicz et al., 1994). Moreover, this technique can detect abundant polymorphic fragments without previous knowledge of the position of the target DNA sequences. Furthermore, ISSR method exhibited high reproducibility since it used long primer lengths and high annealing temperatures (Archak et al., 2003). Due to these advantages, ISSR method was selected to study the analysis of genetic diversity among *D. beecheyana* var. *pubescens* accessions. In the present study, we applied this approach to estimate the genetic diversity within and among the populations of *D. beecheyana* var. *pubescens* collected from different regions in Fujian and Guangxi Provinces. Moreover, due to limited information based on ISSR marker few related report about the genetic diversity was compared with other species, such as mango (Luo et al., 2011), cucumber and apricot (Li et al., 2014), and *Platycodon grandiflorus* (Nie et al., 2014). These analyses provided insights into the germplasm resources and genetic relationships of natural bamboos populations that can help inbreeding improved varieties and for conservation.

Materials and methods

Plant materials

A total of 17 accessions of *D. beecheyana* var. *pubescens* from 6 natural populations of Fujian and Guangxi Province were used in this study. The geographical location of each population was determined using GPS and are listed in *Table 1*. The samples were cultivated at the Chishan state-owned forest farm, Dongshan, Fujian, China. Fresh young leaves were collected for the analyses (*Fig. 1*).

DNA extraction

Total genomic DNA from each accession was extracted using a Biospin Plant Genomic DNA Extraction kit following the manufacturer's instructions. The qualitative and quantitative analysis of DNA was performed on 1% agarose gels using an ultraviolet spectrophotometer (NANODROP 2000c Thermo Fisher, Beijing, China). The extracted DNA was diluted to 25 ng/ μ L and stored at -20 °C for future use.

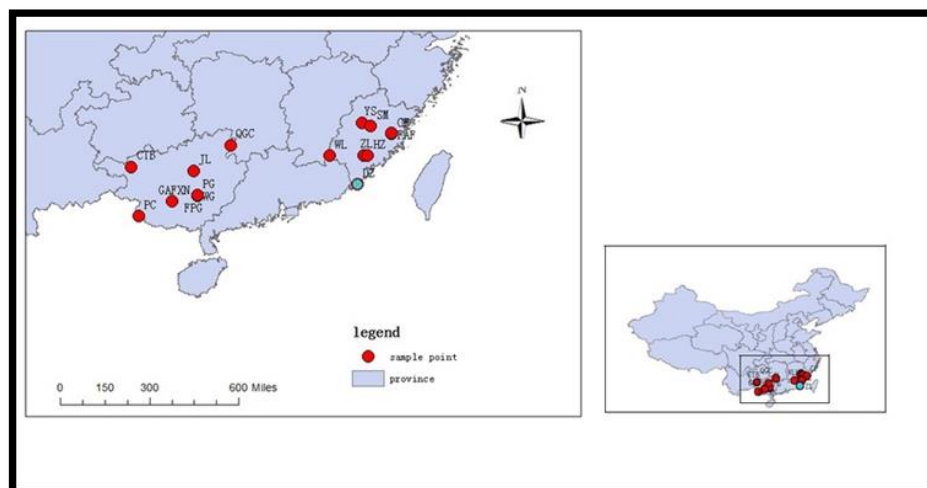


Figure 1. Map showing the origins of accessions

Table 1. Sampling locations of the 17 *D. beecheyana* var. *pubescens* accessions

No.	Accession	Population	Location	Latitude (N)	Longitude (E)
1	YS	Western Fujian Province	Yongan, Sanming	25°98'	117°37'
2	SM	Western Fujian Province	Sanming	26°27'	117°62'
3	WL	Western Fujian Province	Wuping, Longyan	25°01'	116°01'
4	ZL	Western Fujian Province	Zhangping, Longyan	25°03'	117°42'
5	FAF	Central Fujian Province	Fujian Academy of Forestry	26°08'	119°03'
6	CF	Central Fujian Province	Cangshan, Fuzhou	26°05'	119°03'
7	HZ	Southern Fujian Province	Huaan, Zhangzhou	25°02'	117°53'
8	DZ	Southern Fujian Province	Dongshan, Zhangzhou	23°07'	117°43'
9	WG	Eastern Guangxi Province	Watang Town, Guigang	23°04'	109°36'
10	PG	Eastern Guangxi Province	Pingtian Mountain, Guigang	23°06'	109°36'
11	FPG	Eastern Guangxi Province	Forest farm of Pingtian Mountain, Guigang	23°06'	109°36'
12	GAF	Southern Guangxi Province	Guangxi Academy of Forestry	22°49'	108°21'
13	XN	Southern Guangxi Province	Xingning, Nanning	22°48'	108°22'
14	PC	Southern Guangxi Province	Pingxiang, Chongzuo	22°05'	106°45'
15	CTB	Northern Guangxi Province	Cen Wang Laoshan Mountain, Tianlin, Baise	24°29'	106°23'
16	QGG	Northern Guangxi Province	Qianjia Dong, Guanyang, Guilin	25°32'	111°13'
17	JL	Northern Guangxi Province	Jinxiu Mountain, Liuzhou	24°18'	109°25'

Primer selection and ISSR-PCR amplification

In order to select appropriate primers for *D. beecheyana* var. *pubescens*, 100 ISSR primer sequences (*Supp. Tab 1*) provided by the University of British, Columbia (UBC) (<https://www.ubc.ca/>) in Canada were screened using an optimized ISSR-PCR system by the Shanghai Sangon Biological Engineering Technology Service Co. Ltd., China.

ISSR-PCR amplifications were performed in a total reaction volume of 20 μ L containing 2.5 mM MgCl₂, 0.25 mM dNTPs, 1.25 U of Taq DNA polymerase (TaKaRa Biotechnology, Dalian, China), 0.6 μ M primer, 10 ng genomic DNA, and 2 μ L 10 \times buffer. The reactions were performed in a TProfessional Thermocycler (Biometra, Gottingen, Germany) with the following conditions: initial denaturation at 94 °C for 5 min; followed by 40 cycles of 45 s at 94 °C, 30 s at annealing temperature (50-60 °C), 90 s at 72 °C; and final extension at 72 °C for 10 min. The PCR products were stored at 4 °C until further use. These PCR products (0.5 μ g/mL in 1 \times TBE buffer (445 mmol/L Tris, 445 mmol/L borate, 10 mmol/L EDTA)) were subjected to electrophoresis on a 1.5% agarose gel at 120 V for approximately 1 h and the gel was later analyzed using Gel Documentation System (Bio-Rad, Hercules, California, USA).

Data analysis

Polymorphic DNA fragments, obtained with reproducible bands, were scored as present (1) or absent (2). The binary data matrix was analyzed using POPGENE32 (Yeh et al., 2000) to obtain the following genetic diversity parameters within and among populations: percentage of polymorphic bands (*PPB*), number of alleles (*Na*), effective number of alleles (*Ne*), Nei's gene diversity (*He*), Shannon's information index (*I*), total genetic diversity (*Ht*), genetic diversity within populations (*Hs*), coefficient of gene differentiation (*Gst*), and gene flow (*Nm*). A cluster analysis was performed using the DATA processing system (DPS) program and a dendrogram was constructed using an unweighted pair-group method with arithmetic averaging (UPGMA).

Results

ISSR polymorphism

The ISSR primer screening yielded 16 primers exhibiting high polymorphism and stability (*Table 2*). These ISSR primers generated 185 amplified fragments with an average of 11.6 bands per primer and 167 bands (90.27%) were polymorphisms. The fragment sizes ranged from 200 to 2200 bp and the number of bands varied from 9 to 15. The highest proportion (100%) of the polymorphic bands was obtained with the primers UBC818, UBC826, UBC842, UBC888, and UBC889, while the lowest proportion (66.67%) was obtained with the primer UBC850. The results of the amplification using the primer UBC856 for all 17 accessions are illustrated in *Figure 2*. This example showed the high genetic diversity present in the 17 accessions.

Genetic diversity

At the species level, *Na*, *Ne*, *He*, *I* and *PPB* were found to be 1.9027, 1.3873, 0.2497, 0.3949 and 90.27%, respectively that were relatively higher than populations (*Table 3*). The accessions from the Western Fujian population exhibited highest values for the genetic diversity parameters, with *Na* = 1.5676, *Ne* = 1.4243, *He* = 0.2315, *I* = 0.3358,

and $PPB = 56.76\%$. However, genetic diversity in this population was observed to be lower than that at the species level. The lowest level of genetic diversity was found in the Eastern Guangxi population ($N_a = 1.1568$, $N_e = 1.1239$, $H_e = 0.0673$, $I = 0.0968$, $PPB = 15.68\%$). At the population level, the values for N_a , N_e , H_e , I and PPB were found to be in the range of 1.1568–1.5676, 1.1239–1.4243, 0.0673–0.2315, 0.0968–0.3358, and 15.68–56.76% respectively. Based on these results, it can be concluded that the highest genetic variations were observed in the Western Fujian population, while the lowest variations were observed in the Eastern Guangxi and Southern Guangxi populations.

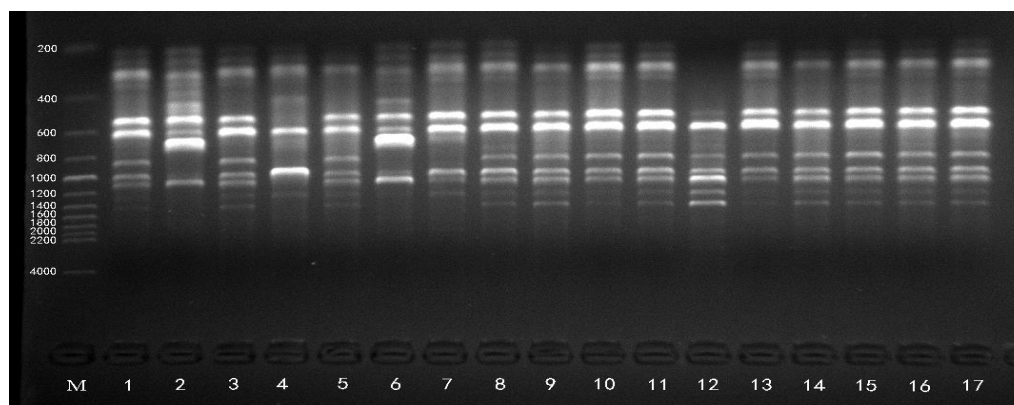


Figure 2. Amplification products from the 17 *D. beecheyana* var. *pubescens* accessions amplified using the primer UBC856

Table 2. ISSR primers used to analyze *D. beecheyana* var. *pubescens* accessions ($R = A, G$; $Y = C, T$)

Primers	Sequence (5'–3')	Tm (°C)	Number of bands scored	Number of polymorphic bands
UBC811	(GA) ₈ C	52.5	10	9
UBC818	(CA) ₈ G	54.6	11	11
UBC826	(AC) ₈ C	53.2	15	15
UBC827	(AC) ₈ G	54.6	11	10
UBC835	(AG) ₈ YC	56.2	10	9
UBC836	(AG) ₈ YA	53.9	14	11
UBC840	(GA) ₈ YT	51.0	10	9
UBC841	(GA) ₈ YC	53.2	9	8
UBC842	(GA) ₈ YG	53.2	9	9
UBC850	(GT) ₈ YC	56.2	12	8
UBC856	(AC) ₈ YA	53.9	13	12
UBC857	(AC) ₈ YG	56.2	10	9
UBC859	(TG) ₈ RC	56.2	12	10
UBC886	VDV(CT) ₇	53.0	12	10
UBC888	BDB(CA) ₇	53.8	12	12
UBC889	DBD(AC) ₇	55.0	15	15
Total			185	167
Mean			11.6	10.4

Table 3. Genetic diversity between populations of *D. beecheyana* var. *pubescens*

Population	Pop ID	<i>Na</i>	<i>Ne</i>	<i>He</i>	<i>I</i>	PPB (%)
Western Fujian Province	WF	1.5676	1.4243	0.2315	0.3358	56.76
Central Fujian Province	CF	1.4000	1.2828	0.1657	0.2419	40.00
Southern Fujian Province	SF	1.4378	1.3096	0.1814	0.2648	43.78
Eastern Guangxi Province	EG	1.1568	1.1239	0.0673	0.0968	15.68
Southern Guangxi Province	SG	1.1568	1.1267	0.0684	0.0979	15.68
Northern Guangxi Province	NG	1.2000	1.1679	0.0895	0.1274	20.00
Average		1.3198	1.2392	0.1340	0.1941	31.98
Average species level		1.9027	1.3873	0.2497	0.3949	90.27

Na: number of alleles; *Ne*: effective number of alleles; *I*: Shannon's information index; *He*: Nei's gene diversity; PPB: percentage of polymorphic bands

Genetic differentiation and relationships

From Nei's genetic diversity, genetic differentiation (*Gst*) was estimated as 0.5399, indicating that 53.99% of the genetic variation was distributed within populations. The level of the gene flow (*Nm*) was found to be 0.4261 individuals per generation within the six populations, thus, demonstrating that there was minimum gene flow between the populations (Table 4).

Table 4. Nei's analysis of genetic variability of the six populations

	<i>Ht</i>	<i>Hs</i>	<i>Gst</i>	<i>Nm</i>
Mean	0.2912	0.1340	0.5399	0.4261
SD	0.0253	0.0082		

Ht: total genetic diversity; *Hs*: genetic diversity within populations; *Gst*: coefficient of gene differentiation; *Nm*: gene flow

Genetic diversity was assessed using genetic distances (*D*) and genetic similarity coefficients (*GSC*). It was observed that *D* ranged from 0.0564 (between Western Fujian and Central Fujian populations) to 0.4590 (between Southern Fujian and Eastern Guangxi populations) and *GSC* ranged from 0.6319 (between Southern Fujian and Eastern Guangxi populations) to 0.9451 (Western Fujian and Central Fujian populations) (Table 5). This analysis showed similar trends in *D* and *GSC* levels. The highest *GSC* (0.9451) and the lowest *D* (0.0564) were found between the Western Fujian and Central Fujian populations, indicating the closest relationship between them. Moreover, the lowest *GSC* (0.6319) and the highest *D* (0.4590) were found between the Southern Fujian and Eastern Guangxi populations, indicating a distant relationship was between these populations.

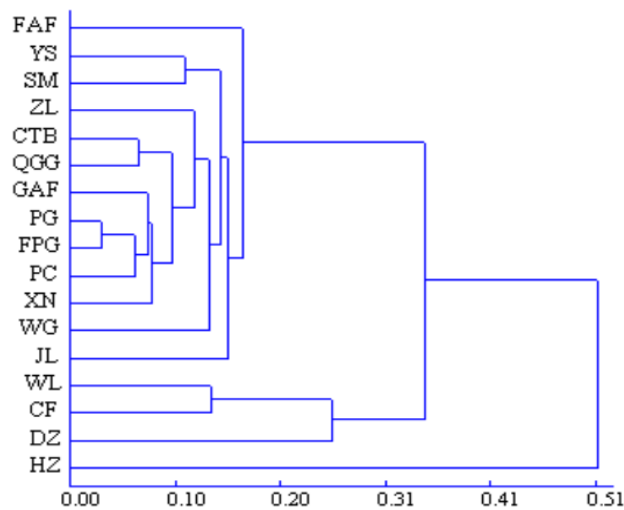
The UPGMA tree that was constructed based on genetic distance (*D*) demonstrated that the 17 accessions clustered into six groups (Fig. 3a). Group A, C, E and F comprised one accession each, FAF, JL, DZ and HZ respectively. However, Group B consisted of eleven accessions, namely, YS, SM, ZL, CTB, QGG, GAF, PG, FPG, PC, XN, and WG. Group D consisted two accessions, WL and CF. An UPGMA tree constructed from the population data generated three clusters (Fig. 3b). Cluster I

included two populations (WF and CF), cluster II contained three populations (EG, SG and NG), and cluster III consisted one population (SF).

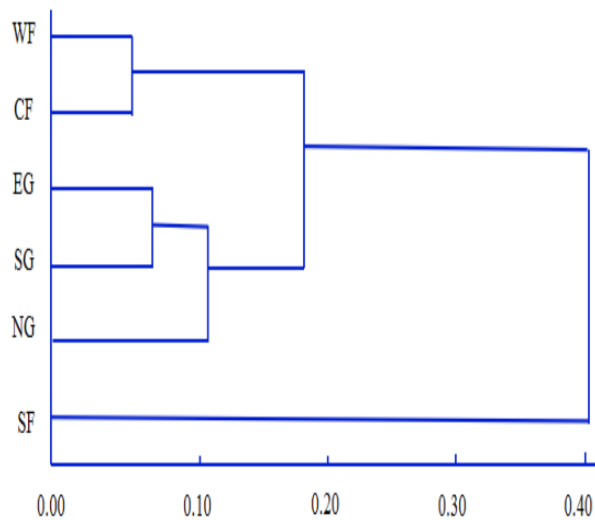
Table 5. Nei's (1978) genetic similarity coefficient (GSC) (above diagonal) and genetic distance (D) (below diagonal) between populations

Pop ID*	WF	CF	SF	EG	SG	NG
WF	****	0.9451	0.7410	0.8669	0.8697	0.8728
CF	0.0564	****	0.7911	0.8295	0.8073	0.8004
SF	0.2998	0.2343	****	0.6319	0.6368	0.6717
EG	0.1428	0.1869	0.4590	****	0.9339	0.8912
SG	0.1396	0.2141	0.4513	0.0684	****	0.9071
NG	0.1360	0.2226	0.3979	0.1151	0.0976	****

*The identities of the various populations are explained in Table 3



(a)



(b)

Figure 3. UPGMA dendrogram of *D. beecheyana* var. *pubescens* based on genetic distances indicating the clustering relationships of accessions (a) and populations (b)

Discussion

Polymorphism analysis

In this study, after a preliminary screening of 100 ISSR primers, those primers were selected that showed clear, reproducible, and polymorphic bands. The primers that generated fuzzy bands or failed to generate an amplification product were rejected, including those that showed monomorphic bands due to the formation of dimers in the primers. The 16 selected primers were used for the analysis of the 17 *D. beecheyana* var. *pubescens* accessions. The selected primers produced an average of 11.6 bands, which is lower than that observed in other bamboo species (Desai et al., 2015). The present study confirmed that the selection of competent primers is very important when using ISSR markers (Safaei et al., 2016).

Genetic diversity

Genetic diversity data is an essential component of a breeding program. In the present study, 17 accessions of *D. beecheyana* var. *pubescens* showed higher levels of genetic diversity at the species level compared to the population level. However, the accessions demonstrated relatively high levels of diversity within the six populations. These results are consistent with the data reported in *Jatropha* previously (Arolu et al., 2015). It was observed that the genetic diversity of *D. beecheyana* var. *pubescens* ($PPB_s = 90.27\%$, $He_s = 0.2497$) at the species level was higher than that of the short-lived perennial species *Blysmus sinocompressus* ($PPB_s = 74.76\%$, $He_s = 0.1668$) (Hu et al., 2016) and mating breeding system species *Platycodon grandiflorum* ($PPB_s = 76.29\%$, $He_s = 0.2326$) (Nie et al., 2014). At the population level, the average He_p in *D. beecheyana* var. *pubescens* was 0.1340 (Table 3) that was almost similar to that of *Taxus fuana* (0.1165) (Shah et al., 2008) but lower than that of apricot (0.1738) (Liu et al., 2016). Low He_p value is attributed to low genetic diversity. Similarly, the low average value of I_p (0.1941) also indicated a low level of genetic diversity (Table 3). High value have been reported in *Portulaca oleracea* ($I_p = 0.515$) (Alam et al., 2015). Further, the average value for PPB_s was found to be 31.98% that was lower than that of *Platycodon longiflorum* ($PPB_s = 55.67\%$) (Nie et al., 2014). Low genetic diversity within populations has been reported in other plant species, such as *Taxus fuana* (Shah et al., 2008) and *Monimopetalum chinense* (Xie et al., 2005).

Our results indicated that *D. beecheyana* var. *pubescens* showed considerable genetic variation between populations and high genetic diversity within populations. A mixed mating system has been recognized as an important factor to determine the level of genetic diversity in a plant species (Godt et al., 2001). However, other factors can also influence the genetic variation including, the geographic factor that can exhibits significant negative effect on genetic diversity and higher temperatures that can boost the seedling growth and increase genetic diversity. However, *D. beecheyana* var. *pubescens* flowers every 10–120 years in an unpredictable mode. The propagation is mainly dependent on selfing by underground rhizomes. Thus, in the present study, the breeding system was not likely to be a contributor towards genetic diversity.

Two possible explanations can be proposed for the high total genetic diversity within populations. Firstly, since *D. beecheyana* var. *pubescens* lives for many years before flowering, it is highly possible that habitat heterogeneity of the long-lived plants could have contributed to the pattern of genetic diversity in this species (Hu et al., 2016). Secondly, the geographical prevalence of this species might have supported high

genetic diversity compared to a species with restricted distribution (Godt and Hamrick, 1996). The populations of *D. beecheyana* var. *pubescens* examined in this study were narrowly distributed in Fujian and Guangxi Provinces. This restricted distribution might have resulted into low genetic diversity among populations (Ge et al., 2005).

Genetic differentiation and relationships

Various factors such as life history, geographic distribution, and gene flow within populations can influence the genetic structure of plant species (Hilfiker et al., 2004). In this study, we found that the genetic differentiation coefficient among populations of *D. beecheyana* var. *pubescens* ($Gst = 0.5399$) was higher than the average of other long-lived species ($Gst = 0.19$) (Nybom, 2004). This suggested that there was a high level of genetic differentiation in *D. beecheyana* var. *pubescens*. However, genetic variation in *D. beecheyana* var. *pubescens* in the Fujian and Guangxi Provinces was mainly observed at the intra-population level (53.99%) and inter-population level (46.01%). This can be attributed to the geographical location that might have had a significant influence on genetic differentiation leading to the higher genetic diversity at the intra-population level (Yuan et al., 2007). In addition, the complex topography of the region, including remote and isolated mountains, may have hindered the gene flow between populations, thus promoting population differentiation (Wheeler et al., 2011). In the present study, gene flow among *D. beecheyana* var. *pubescens* populations was found to be 0.4261 that indicated limited level of gene flow between populations and thus it could not prevent genetic differentiation at the intra-population level.

Genetic diversity was estimated using genetic distance (D) and genetic similarity coefficients (GSC), which ranged from 0.0564–0.4590 and 0.6319–0.9451, respectively. This range was consistent with a previous study that reported a range of 0.045455–0.454545 for D (Yuan et al., 2014), however, the GSC range (0.39–0.95) was lower compared to our study (Noroozisharaf et al., 2015). The GSC obtained from our ISSR analysis exhibited a high genetic diversity among *D. beecheyana* var. *pubescens* accessions. Moreover, the 17 accessions were found to form six major groups in the UPGMA dendrogram. The clustering in this dendrogram was not consistent with the geographical distribution, suggesting that there was no obvious correlation between genetic distance and geographic distance of accessions in Fujian and Guangxi Province. However, the 6 populations clustered into three major groups in the UPGMA dendrogram, and in particular, three populations, EG, SG and NG, formed a cluster with high genetic similarities due to the same place of origin. Therefore, based on the findings of this study, it can be concluded that the geographic distribution may have influenced the genetic distance or the genetic relationships of populations and the widespread accessions may have exhibited higher level of genetic diversity than the narrowly distributed ones. The population SF was found to be grouped away from other two groups, thus, supporting the hypothesis that these *D. beecheyana* var. *pubescens* accessions origin from SF were the independent species. Therefore, a high level of genetic variation can be estimated in widespread accessions.

Economical aspects and fiscal policy consideration

D. beecheyana var. *pubescens* as one of the protection forest was introduced to coastal regions of southern and eastern China for soil and water conservation and to improve the environment as well. Since, the southeast coast of China has been suffering

from typhoons, storm surges and other natural disasters throughout the year, it is crucial to strengthen the coastal shelterbelt system to minimize economic losses. Certainly, some capital investments and fiscal policies would be required to conserve the coastal shelterbelt. In this context, this study provided important genetic information for devising the conservation strategies of *D. beecheyana* var. *pubescens*.

One primary objective of conservation is to protect the genetic diversity. Considering the low genetic diversity among populations and high genetic variation within populations of *D. beecheyana* var. *pubescens* in China, the most effective strategy for maintaining genetic variation is to conserve and cultivate as many populations as possible. Moreover, transplanting individuals from other populations would enhance inter-population gene flow and genetic diversity. Thus, the foremost step for all populations in situ must be the prevention of the anthropogenic disturbance. Subsequently, we should make it a priority to cultivate the high genetic diversity of *D. beecheyana* var. *pubescens* in initial period. Since the WF population exhibited the highest level of genetic diversity, it should be preferred for cultivation. Further, the population with high genetic diversity may have the ability to adapt to the environment as compared to the population with low genetic diversity. For *ex situ* conservation, a germplasm bank is required to be established for this species.

Conclusion

In this study, the ISSR markers were found to be viable for detecting the high level of genetic diversity within populations and considerable genetic differentiation among populations. In addition, the polymorphism generated by ISSR markers was very high. Based on the findings of this study, it was observed that rich genetic diversity existed in *D. beecheyana* var. *pubescens*. Besides, the genetic differentiation and relationships obtained in this study may help in understanding and evaluating the conservation in China. The cluster results of 17 materials related to adaptability, resistance, water and soil conservation and growth period, which need to be further studies. It is necessary to strengthen the collection, identification and excavation of the germplasm resources of *D. beecheyana* var. *pubescens* to enrich the genetic diversity of breeding materials.

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APPENDIX

Table A1. Selected ISSR primers

Primer name sequence 5'-3'	Primer name sequence 5'-3'
801 ATA TAT ATA TAT ATA TT	851 GTG TGT GTG TGT GTG TYG
802 ATA TAT ATA TAT ATA TG	852 TCT CTC TCT CTC TCT CRA
803 ATA TAT ATA TAT ATA TC	853 TCT CTC TCT CTC TCT CRT
804 TAT ATA TAT ATA TAT AA	854 TCT CTC TCT CTC TCT CRG
805 TAT ATA TAT ATA TAT AC	855 ACA CAC ACA CAC ACA CYT
806 TAT ATA TAT ATA TAT AG	856 ACA CAC ACA CAC ACA CYA

807 AGA GAG AGA GAG AGA GT	857 ACA CAC ACA CAC ACA CYG
808 AGA GAG AGA GAG AGA GC	858 TGT GTG TGT GTG TGT GRT
809 AGA GAG AGA GAG AGA GG	859 TGT GTG TGT GTG TGT GRC
810 GAG AGA GAG AGA GAG AT	860 TGT GTG TGT GTG TGT GRA
811 GAG AGA GAG AGA GAG AC	861 ACC ACC ACC ACC ACC ACC
812 GAG AGA GAG AGA GAG AA	862 AGC AGC AGC AGC AGC AGC
813 CTC TCT CTC TCT CTC TT	863 AGT AGT AGT AGT AGT AGT
814 CTC TCT CTC TCT CTC TA	864 ATG ATG ATG ATG ATG ATG
815 CTC TCT CTC TCT CTC TG	865 CCG CCG CCG CCG CCG CCG
816 CAC ACA CAC ACA CAC AT	866 CTC CTC CTC CTC CTC CTC
817 CAC ACA CAC ACA CAC AA	867 GGC GGC GGC GGC GGC GGC
818 CAC ACA CAC ACA CAC AG	868 GAA GAA GAA GAA GAA GAA
819 GTG TGT GTG TGT GTG TA	869 GTT GTT GTT GTT GTT GTT
820 GTG TGT GTG TGT GTG TC	870 TGC TGC TGC TGC TGC TGC
821 GTG TGT GTG TGT GTG TT	871 TAT TAT TAT TAT TAT TAT
822 TCT CTC TCT CTC TCT CA	872 GAT AGA TAG ATA GAT A
823 TCT CTC TCT CTC TCT CC	873 GAC AGA CAG ACA GAC A
824 TCT CTC TCT CTC TCT CG	874 CCC TCC CTC CCT CCC T
825 ACA CAC ACA CAC ACA CT	875 CTA GCT AGC TAG CTA G
826 ACA CAC ACA CAC ACA CC	876 GAT AGA TAG ACA GAC A
827 ACA CAC ACA CAC ACA CG	877 TGC ATG CAT GCA TGC A
828 TGT GTG TGT GTG TGT GA	878 GGA TGG ATG GAT GGA T
829 TGT GTG TGT GTG TGT GC	879 CTT CAC TTC ACT TCA
830 TGT GTG TGT GTG TGT GG	880 GGA GAG GAG AGG AGA
831 ATA TAT ATA TAT ATA TYA	881 GGG TGG GGT GGG GTG
832 ATA TAT ATA TAT ATA TYC	882 VBV ATA TAT ATA TAT AT
833 ATA TAT ATA TAT ATA TYG	883 BVB TAT ATA TAT ATA TA
834 AGA GAG AGA GAG AGA GYT	884 HBH AGA GAG AGA GAG AG
835 AGA GAG AGA GAG AGA GYC	885 BHB GAG AGA GAG AGA GA
836 AGA GAG AGA GAG AGA GYA	886 VDV CTC TCT CTC TCT CT
837 TAT ATA TAT ATA TAT ART	888 BDB CAC ACA CAC ACA CA
838 TAT ATA TAT ATA TAT ARC	889 DBD ACA CAC ACA CAC AC
839 TAT ATA TAT ATA TAT ARG	890 VHV GTG TGT GTG TGT GT
840 GAG AGA GAG AGA GAG AYT	891 HVH TGT GTG TGT GTG TG
841 GAG AGA GAG AGA GAG AYC	892 TAG ATC TGA TAT CTG AAT TCC C
842 GAG AGA GAG AGA GAG AYG	893 NNN NNN NNN NNN NNN
844 CTC TCT CTC TCT CTC TRC	894 TGG TAG CTC TTG ATC ANN NNN
845 CTC TCT CTC TCT CTC TRG	895 AGA GTT GGT AGC TCT TGA TC
846 CAC ACA CAC ACA CAC ART	896 AGG TCG CGG CCG CNN NNN NAT G
847 CAC ACA CAC ACA CAC ARC	897 CCG ACT CGA GNN NNN NAT GTG G
848 CAC ACA CAC ACA CAC ARG	898 GAT CAA GCT TNN NNN NAT GTG G
849 GTG TGT GTG TGT GTG TYA	899 CAT GGT GTT GGT CAT TGT TCC A
850 GTG TGT GTG TGT GTG TYC	900 ACT TCC CCA CAG GTT AAC ACA