

## 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.

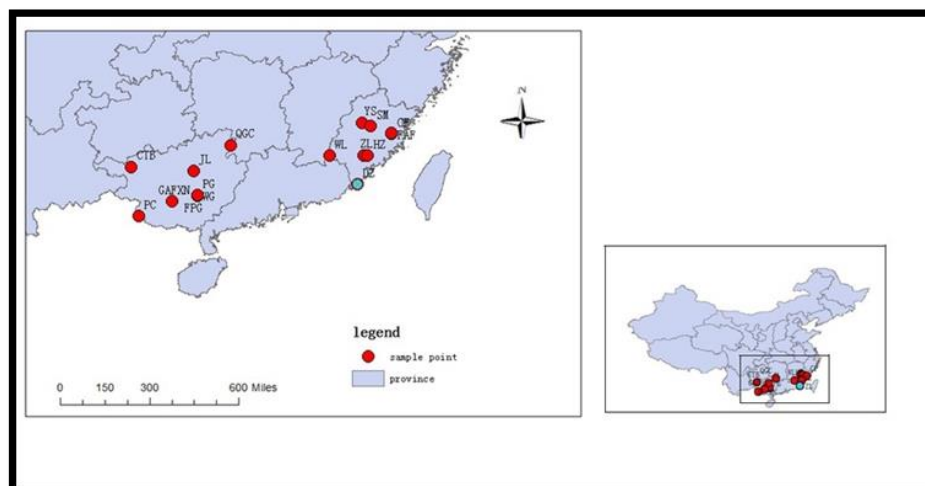
## Materials and methods

### *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/ $\mu$ 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  $\mu$ L containing 2.5 mM MgCl<sub>2</sub>, 0.25 mM dNTPs, 1.25 U of Taq DNA polymerase (TaKaRa Biotechnology, Dalian, China), 0.6  $\mu$ M primer, 10 ng genomic DNA, and 2  $\mu$ 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  $\mu$ 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**

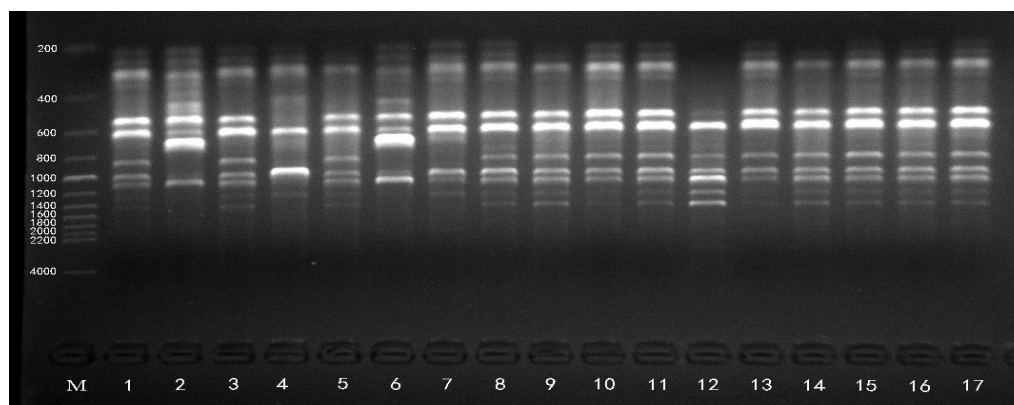
### **ISSR polymorphism**

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) <sub>8</sub> C  | 52.5    | 10                     | 9                           |
| UBC818  | (CA) <sub>8</sub> G  | 54.6    | 11                     | 11                          |
| UBC826  | (AC) <sub>8</sub> C  | 53.2    | 15                     | 15                          |
| UBC827  | (AC) <sub>8</sub> G  | 54.6    | 11                     | 10                          |
| UBC835  | (AG) <sub>8</sub> YC | 56.2    | 10                     | 9                           |
| UBC836  | (AG) <sub>8</sub> YA | 53.9    | 14                     | 11                          |
| UBC840  | (GA) <sub>8</sub> YT | 51.0    | 10                     | 9                           |
| UBC841  | (GA) <sub>8</sub> YC | 53.2    | 9                      | 8                           |
| UBC842  | (GA) <sub>8</sub> YG | 53.2    | 9                      | 9                           |
| UBC850  | (GT) <sub>8</sub> YC | 56.2    | 12                     | 8                           |
| UBC856  | (AC) <sub>8</sub> YA | 53.9    | 13                     | 12                          |
| UBC857  | (AC) <sub>8</sub> YG | 56.2    | 10                     | 9                           |
| UBC859  | (TG) <sub>8</sub> RC | 56.2    | 12                     | 10                          |
| UBC886  | VDV(CT) <sub>7</sub> | 53.0    | 12                     | 10                          |
| UBC888  | BDB(CA) <sub>7</sub> | 53.8    | 12                     | 12                          |
| UBC889  | DBD(AC) <sub>7</sub> | 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.

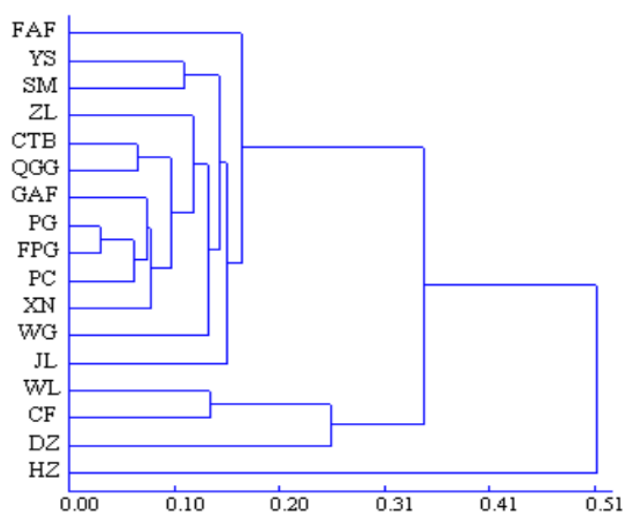
The UPGMA tree that was constructed based on genetic distance (*D*) demonstrated that the 17 accessions clustered into six groups (Fig. 3a). Group A, C, E and F comprised one accession each, FAF, JL, DZ and HZ respectively. However, Group B consisted of eleven accessions, namely, YS, SM, ZL, CTB, QGG, 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).

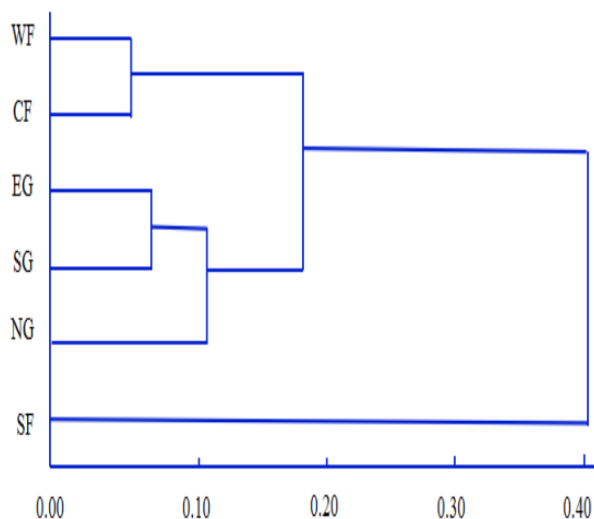
**Table 5.** Nei's (1978) genetic similarity coefficient (GSC) (above diagonal) and genetic distance (D) (below diagonal) between populations

| 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   |