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ANALYSIS OF GENETIC DIVERSITY IN DIFFERENT GEOGRAPHICAL POPULATIONS OF *BAUHINIA VARIEGATA* L. WITH SEQUENCE-RELATED POLYMORPHISM (SRAP) MARKERS

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Abstract

Bauhinia variegata L. (Orchid tree) is a popular ornamental plant in south China and has medicinal, environmental remediation, and environmental biomonitoring values. To examine the genetic diversity and differentiation of *B. variegata* in south China, the leaf samples were collected from seven geographic populations. The sequence-related amplified polymorphism (SRAP) markers were screened and 13 primer pairs were selected. Based on the 13 primer pairs, 168 bands were generated. It was found that out of 168 bands, 112 were polymorphic was 66.67%. The genetic Nei's distance among different *B. varigata* populations ranged from 0.2548 to 0.0470. The general Nei's genetic diversity index was 0.2265. The general Shannon's information index was 0.3405. Genetic differentiation (Gst) among populations was 0.4580. The population of *B. varigata* from Hong Kong was clustered into one separate group with other populations from mainland China. The SRAP data indicated that the *B. varigata* had certain genetic diversity and the gene flow occurred mainly intra-populations. The prepotency selection should be processed intra-population, and the germplasm introduction should focus on different regions for a wider collection range, which is in accordance with the expansive distribution of *B. variegata*.

Key words: South China, *Bauhinia variegata*, Populations, Genetic diversity, SRAP.

Introduction

Bauhinia variegata L. (Orchid tree), belonging to the family Fabaceae, is a deciduous tree with peculiar, hoof-like leaves, raceme. Flowers larger, scented, white or pink that bloom in late winter to early summer and are often grown for its ornamental value (Wu & Raven, 2010; Singh *et al.*, 2019). This plant is commonly applied in urban greening in South China and has an excellent capacity of tolerance and adaptation to city environments with considerable level of air pollution (Zhang *et al.*, 2022; Singh *et al.*, 2021). The species is also hardy against drought, wind and frost, and applied for wasteland recovery and afforestation (Yadav *et al.*, 2023). Moreover, this plant has great potential in medicine, environmental remediation, and environmental biomonitoring (More-Adate *et al.*, 2022; Jayashree *et al.*, 2021; Sharma *et al.*, 2019; de Lima *et al.*, 2017).

Many methods have been used to assess genetic diversity of plants. Molecular marker systems have been demonstrated to be useful tools for genetic diversity and phylogenetic analysis (Liu *et al.*, 2014; Xie *et al.*, 2015; Shinwari *et al.*, 2018; Jan *et al.*, 2019; Khan *et al.*, 2020; Sardar *et al.*, 2021). Sequencing-related amplification polymorphisms (SRAP) is a universal molecular marker developed for selective amplification of open reading frames (Li & Quiros, 2001). Polymorphisms are primarily caused by different promoters, introns, and spacers among different species and individuals. SRAP has been applied in molecular identification, genetic linkage map construction, gene tagging, genomic and cDNA fingerprinting, genetic diversity analysis and comparative genetics of different species (Qiao *et al.*, 2022; Xuan *et al.*, 2022; Liu *et al.*, 2016). Due to its simplicity and reliability throughout, SRAP is considered to have higher power in revealing genetic diversity than the other PCR-based technic, such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), and amplified fragment length polymorphism (AFLP) (Li & Quiros, 2001; Gulsen *et al.*, 2005).

Although some studies focused on the *B. variegata* have been done, the genetic diversity and differentiation among different *B. variegata* populations in south China has not been reported. Therefore, using the SRAP markers, this study was undertaken to analyze the genetic differentiation and gene flow levels of *B. variegata* populations in different regions and the outcome will also provide a reference for the conservation of germplasm resources, species introduction, and genetic improvement.

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Materials and Methods

Plant materials and genomic DNA extraction: Fresh leaves (3 to 5) were collected from different places in south China, including Guangdong province, Yunnan province, Fujian province, and Hong Kong SAR, where the *Bauhinia variegata* was used in landscaping (Table 1). Leaves were placed in hermetic bags and taken back to the laboratory. The leaves were frozen by liquid nitrogen and stored in a refrigerator at -80°C for further use.

Total genomic DNA was isolated from leaves using the modified cetyl-trimethylammonium bromide (CTAB) method (Porebski *et al.*, 1997). The DNA samples were verified using 1% agarose gel electrophoresis. The quality and quantity of DNA samples were measured by a NanoDrop 2000 ultra-micro spectrophotometer to ensure the A260/A280 and A260/A230 ratios were between 1.8 and 2.0, respectively.

SRAP analysis: PCR-based SRAP analysis was performed as described by Li & Quiro (2001). All reagents were purchased from Takara Bio (Otsu, Japan). The total volume of PCR reaction was 25 µL containing 50 ng genomic DNA, 200 µM dNTPs, 2.75 mM $MgCl_2$, 0.4 µM of each primer, 2.5 µL PCR buffer, 0.75 U *Taq* DNA polymerase, and sterile double-distill water. All PCR amplifications were carried out using a thermal cycler (Eppendorf, Germany) with the following program: initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 50°C for the set of primer combinations, then 72°C for 2 min, and ending with a final elongation at 72°C for 5 min. Thirteen pairs of primers screened from 594, including 10 forward and 9 reverse primers that identified consistently reproducible polymorphisms with clearly defined bands were used (Table 2). PCR products were resolved in a 6% polyacrylamide gel at 12.5 V cm^{-1} for 1.5 h and stained with silver nitrate ($AgNO_3$). Reliable and clearly distinguishable amplified bands of 100-1500 bp were scored as either 1 (present) or 0 (absent), and a SRAP data matrix was constructed.

Data analysis: POPGENE version 1.31 software was used to analyze genetic diversity and other related indicators, including the percentage of polymorphic bands (PPB), percentage of polymorphic loci (P), number of different alleles (Na), number of effective alleles (Ne), Nei's genetic diversity (H), Shannon's information index (I), total genetic diversity (Ht), mean genetic diversity (Hs), coefficient of gene differentiation intra populations(Gst), and gene flow (Nm) (Shen *et al.*, 2010; Yeh *et al.*, 1999; Yu *et al.*, 2014). Based on genetic distance, GenAIEx 6.501 was used to perform Molecular Variance Analysis (AMOVA). Based on the genetic consistency, NTSYS2.0 software was used to perform unweighted pair group arithmetic average (UPGMA) clustering.

Results

Screening SRAP primer: Genomic DNA extraction was detected by agarose electrophoresis. Clear bands with little residues were observed (partial results were shown in Fig. S1). Thirteen pairs of primers were selected for PCR of DNA samples from 7 geographical populations. The details of different primer combinations are given in Table 3.

The selected primers were used to amplify *B. variegata* materials and a total of 168 bands were obtained (Table 3). Among them, 112 bands were polymorphic, and the average percentage of polymorphic bands (PPB) reached 66.43%. The number of total bands using different primer combinations varied from 8 to 20, with an average of 12.92. The number of polymorphic bands using different primer combinations varied from 3 to 15, with an average of 8.62. The primer combination E5M1 obtained the highest PPB of 88.89% and the primer combination E12M9 had the lowest PPB of 30%.

Table 1. Locations where *B. variegata* samples were collected.

No.	Abbreviation	Provenance	Latitude, Longitude
1.	GZ	Guangzhou, Guangdong province	23.16° N, 113.27° E
2.	SG	Shaoguan, Guangdong province	24.82° N, 113.60° E
3.	DG	Dongguan, Guangdong province	23.05° N, 113.76° E
4.	MZ	Meizhou, Guangdong province	24.30° N, 116.12° E
5.	HK	Hong Kong SAR	22.29° N, 114.18° E
6.	YN	Sanbaozhuang, Yunnan province	25.40° N, 102.60° E
7.	SM	Sanming, Fujian province	26.27° N, 117.64° E

Table 2. Sequence of SRAP primers used in current study.

Primer name	Forward primer	Primer name	Reverse primer
E1	TGAGTCCAAACCGGATA	M1	GACTGCGTACGAATTAAT
E3	TGAGTCCAAACCGGAAT	M2	GACTGCGTACGAATTTGC
E5	TGAGTCCAAACCGGAAG	M9	GACTGCGTACGAATTTCA
E8	TGAGTCCAAACCGGTGC	M13	GACTGCGTACGAATTCTA
E12	TGAGTCCAAACCGGAGG	M16	GACTGCGTACGAATTGAT
E14	TGAGTCCAAACCGGAAC	M17	GACTGCGTACGAATTATG
E17	TGAGTCCAAACCGGTAG	M19	GACTGCGTACGAATTACG
E21	TGAGTCCAAACCGGTCA	M20	GACTGCGTACGAATTTAG
E24	TGAGTCCAAACCGGGAT	M21	GACTGCGTACGAATTTCG
E26	TTCAGGGTGGCCGGATG		

Note: The primers were in according to Li & Quiros (2001)

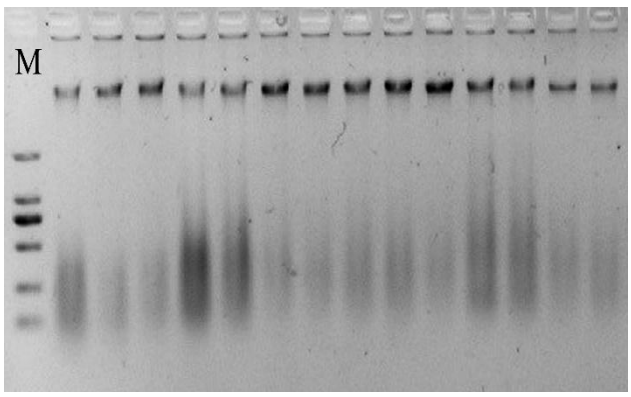

Fig. S1.

Table 3. Polymorphism data based on genetic analyses performed using 13 SRAP primer combinations.

Primer combination	Number of total bands	Number of polymorphic bands	PPB (%)
E17M13	13	7	53.85
E8M13	12	7	58.33
E8M21	10	3	30.00
E12M9	8	7	87.50
E3M16	14	9	64.29
E14M21	12	10	83.33
E21M19	12	7	58.33
E5M1	9	8	88.89
E26M2	9	6	66.67
E12M13	20	14	70.00
E3M17	20	15	75.00
E24M20	16	13	81.25
E1M17	13	6	46.15
Total	168	112	——
Average	12.92	8.62	66.43

Note: PPB, percentage of polymorphic bands

Table 4. Genetic diversities of *B. variegata* from 7 different geographical populations.

Geographical population	P (%)	Na	Ne	H	I
GZ	35.71	1.3571	1.2491	0.1404	0.2052
SG	33.93	1.3393	1.2270	0.1287	0.1887
DG	29.17	1.2917	1.1960	0.1101	0.1619
MZ	25.60	1.2560	1.1812	0.1017	0.1486
HK	12.50	1.1250	1.0986	0.0536	0.0771
YN	32.14	1.3214	1.1980	0.1138	0.1690
SM	33.93	1.3393	1.2195	0.1221	0.1791
average	29.00	1.3000	1.1956	0.1100	0.1614

Note: P, percentage of polymorphic loci; Na, number of alleles; Ne, number of effective alleles; H, Nei's genetic diversity; I, Shannon's information index

Table 6. Genetic differentiation of 7 geographical populations.

	Ht	Hs	Gst	Nm
Average	0.2031	0.1101	0.4580	0.5917
Standard deviation	0.0363	0.0155		

Note: Ht, total genetic diversity; Hs, mean genetic diversity; Gst, coefficient of gene differentiation; Nm, gene flow

Genetic diversity analysis: The statistical analysis of *B. variegata* from 7 different regions was performed with POPGEN 32 (Table 4). The percentage of polymorphic loci (P) varied from 35.71% (GZ) to 12.50% (HK), with an overall mean of 29%. In addition, the average values of the number of alleles (Na) and number of effective alleles (Ne) were 1.6667 and 1.3850 respectively. The Nei's genetic diversity (H) was between 0.1404 and 0.0536, with an average value of 0.1100. The Shannon's information index (I) was between 0.2052 and 0.0771, with an average value of 0.1614. Based on the parameters above, the genetic diversity of *B. variegata* in the provenance GZ was the highest and the lowest was in HK.

Genetic relationship and differentiation analysis: Nei's genetic diversity of different populations ranged from 0.2548 to 0.0470, supporting the close relationship among the *B. variegata* populations (Table 5). Long genetic distance clearly appeared between the populations from HK and DG, whereas the genetic distance between SM and SG was the closest, relationships indicating relatively distant with the former. The variation tendency of genetic identity was in accordance with the genetic distance, with a range from 0.9541 to 0.7900. The top number appeared between SM and SG and the genetic identity between HK and DG was the lowest.

In species scale, the total genetic diversity (Ht) was estimated to be 0.2031, with a 0.1101 in mean genetic diversity (Hs) calculation (Table 6). The coefficient of gene differentiation (Gst) intra populations was estimated to be 0.4580, indicating the genetic variation from inter populations was 45.80% of the total while the genetic variation from the intra populations was 54.20%. The reasonable genetic variation had occurred among different populations, in which the genetic variation of intra-population was slightly higher than inter-population. When gene flow (Nm) > 1, the gene flow could be detected inter populations. The larger the value, the stronger the gene flow that has occurred. In this study, Nm was 0.5917, supporting the lower gene flow among *B. variegata* populations. The results of AMOVA also showed that 39% of the genetic variation occurred among different populations, 61% of the variation existed within populations (Table 7).

Table 5. Analysis of Nei's genetic distance and genetic identity of *B. variegata* from 7 different regions.

	YN	SG	DG	SM	MZ	GZ	HK
YN	****	0.9111	0.8821	0.9038	0.9072	0.8750	0.8001
SG	0.0930	****	0.9496	0.9541	0.9401	0.9015	0.8052
DG	0.1254	0.0517	****	0.9259	0.8833	0.8706	0.7751
SM	0.1011	0.0470	0.0770	****	0.9504	0.9037	0.8046
MZ	0.0974	0.0617	0.1241	0.0509	****	0.8910	0.7900
GZ	0.1335	0.1037	0.1385	0.1012	0.1154	****	0.8369
HK	0.2230	0.2167	0.2548	0.2174	0.2357	0.1781	****

Note: **** stand for 1.0000. The genetic identity was above the asterisks and the genetic distance was below the asterisks

Table 7. AMOVA analysis of genetic variation in 7 geographic populations.

Variation source	Degree of freedom	Total variance	Mean variance	Variance composition	Percentage of total variance (%)
Inter population	6	471.47	78.58	5.69	39
Intra population	83	747.68	9.01	9.01	61
Total	89	1219.14		14.70	100

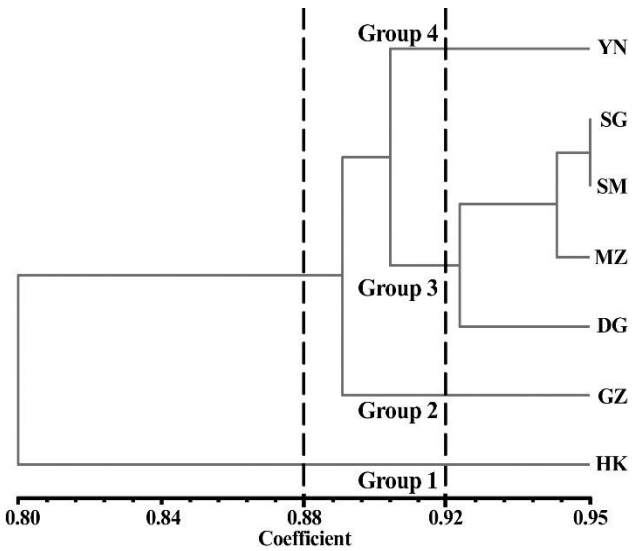

Fig. 1. The cluster map of seven *B. variegata* populations based on the genetic identity.

Clustering analysis: Based on the genetic identity, the UPGMA method was used to perform the clustering analysis among different *B. variegata* populations (Fig. 1). The seven populations were grouped into two categories when the threshold was 0.88. The population from Hong Kong was clustered into one group, and the other populations were clustered into the other big group. At 0.92, the populations could be divided into four categories. The populations from HK, GZ, and YN were clustered into separate groups, and the other populations were clustered into the other big group.

Discussion

Genetic diversity is an indispensable part of biodiversity. Evaluation of genetic diversity is a basis for identifying and conserving germplasm resources, studying the evolutionary origin of species, and choosing parents for hybrid offspring (Wang *et al.*, 2012; Jan *et al.*, 2018, 2024; Malook *et al.*, 2019). Molecular marker technology is a reliable method for examining the genetic diversity (Liao *et al.*, 2012; Liu *et al.*, 2014). SRAP is the molecular marker system developed for selective amplification of open reading frames with several advantages, such as simple experimental procedures, reasonable throughput rates, independence of environmental effects, low cost, and highly reproducible results (Li & Quiros, 2001).

In this study, SRAP molecular marker technology was used to study the genetic diversity from 7 provenances in *B. variegata*, and 168 bands were obtained. Among them, 112 were polymorphic, and each pair of primers amplified 12.92 bands and 8.62 polymorphic on average. The average polymorphism percentage was 66.43%, which was lower than that of *Pinus* taxa in China (94.8%) (Xie *et al.*, 2015), *Magnolia wufengensis* (88%) (Chen *et al.*, 2014), and *Simmondsia chinensis* (82.3%) (Kumar *et al.*, 2019); and higher than *Polygonum viviparum* (39.3%) (Lu *et al.*, 2008). The 13 pairs of SRAP selected primer in this study provided nice *B. variegata* genomic information, which could be used to study the genetic diversity of *Bauhinia* plants. The Ne obtained by coamplification of 13 pairs of primers was between 1.2491 and 1.0986, with an average value of 1.1956. Nei's genetic diversity ranged from 0.1404 to 0.0536, with an average value of 0.1100. These results showed that *B. variegata* exhibited a certain level of genetic variation at the species level.

The Nei's genetic distance between population from Hong Kong and the other 6 populations was greater than 0.20, with the 6 populations the genetic distance reached 0.0470-0.20 among each other, indicating the populations in this study belonged to a single species. According to the proposition that the genetic distance of a single species should range from 0.20 to 0.03 (Thorpe, 1982), the population from Hong Kong might have a distinctive origin compared with other 6 populations. Liao *et al.*, (2016) suggested that the SARP is a useful technic for the investigation of the population genetic diversity of broad-leaved tree species. The low genetic distance in this study among the 6 populations in mainland China showed a monophyletic relationship (Fig. 1). The restricted communication between population from Hong Kong and populations from mainland China may be the main reason for the lack of genetic exchange. Moreover, the narrow acreage of Hong Kong may influence the interaction between two varieties possibly play a negative role in the genetic distance. Based on the clustering, the distribution of populations from mainland China was correlated with the distinctive geographical conditions and the variety adapting to the extreme environment could be developed, providing fundamental knowledge on application and breeding of *B. variegata*.

Genetic difference among distinct populations can be represented by the coefficient of gene differentiation and genetic distance (Nei, 1987), and the gene flow that is caused by individual migration will increase the genetic change in intra population (Slatkin, 1987). The gene flow index (Nm) is a critical factor that affects the genetic structure and genetic differentiation among populations (Fan *et al.*, 2015). Wright (1949) proposed that gene flow possesses a deterrent function in the genetic differentiation in different regions when the gene flow index Nm > 1, conversely, the population tends to choose the other way. In this study, the Gst was 0.4580, indicating 45.80% of genetic variation occurred inter-population, with the remainder intra-population. The populations of *B. variegata* obtained a comparatively high level of genetic differentiation, possibly resulting from the low gene flow (Nm = 0.5917). The factors that affect the genetic structure of plant populations include the evolutionary history,

mutation, genetic drift, mating system, gene flow, natural selection, and life form (Wang *et al.*, 2014). Self-pollination a characteristic of *B. variegata* may hinder gene flow among populations. These results support the collection of germplasm resources, and the prepotency selection should be processed intra-population, and the germplasm introduction should focus on different regions for a wider collection range, which is in accordance with the extensive distribution of *B. variegata*.

Conclusion

The results of this study showed that the *Bauhinia variegata* grown in south China exhibited substantial population differentiation and had a low level of gene flow among populations. This study also demonstrated that SRAP molecular markers were effective for characterizing population genetic diversity and the genetic relationships of *Bauhinia* plants and suggested that they could be useful for investigating the population genetic diversity of other broad-leaves tree species. *In-situ* conservation may promote intra-population gene flow, while transfer of seedlings from different regions, especially Hong Kong, could increase gene flow among populations.

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Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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