Postprint: Genetic Relationship Analysis and Fingerprint Map Construction of Bougainvillea Based on ISSR Molecular Markers

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Abstract

This study optimized the ISSR-PCR reaction system and protocol for Bougainvillea by examining DNA template concentration, primer concentration, annealing temperature, and number of cycles. PCR amplification was performed on 131 Bougainvillea varieties using 11 ISSR primers, and the amplification products were detected via agarose gel electrophoresis to analyze genetic diversity and genetic distance among varieties, and to construct dendrograms and fingerprinting profiles. The results showed that in the optimized ISSR-PCR reaction system, the DNA template concentration was 0.5 ng · L ^{1}, the primer concentration was 0.5 M · L ^{1}, the optimal annealing temperatures for primers UBC813, UBC814, UBC815, UBC823, UBC824, UBC835, UBC840, UBC841, UBC843, UBC844, and UBC876 were 52.3, 55.9, 54.3, 54.3, 53.6, 56.2, 56.2, 51.9, 54.4, 54, and 50 °C, respectively, and the number of cycles was 32. The 11 ISSR primers amplified a total of 161 bands from the 131 Bougainvillea varieties, of which 156 were polymorphic bands, with a polymorphism ratio of 96.89%. For individual primers, the number of alleles, effective number of alleles, Nei' s gene diversity index, and Shannon' s information index ranged from 1.86–2.00, 1.33–1.68, 0.21–0.39, and 0.34–0.57, respectively, with average values of 1.969, 1.478, 0.294, and 0.447. Primer UBC841 exhibited the highest discrimination rate (80.92%), enabling effective identification of 106 varieties; combined with primer UBC876, all 131 Bougainvillea varieties could be completely distinguished, establishing fingerprinting profiles for each variety. The genetic distance among Bougainvillea varieties ranged from 0.00–0.60, with an average of 0.365, indicating relatively low genetic diversity. At a genetic distance of 0.58, the 131 varieties were divided into six major groups. Cluster analysis revealed that most varieties of the same species clustered together; however, some varieties within the same species did not cluster in the same group or subgroup, while varieties from multiple species sometimes clustered

within the same group. This study accurately revealed the genetic diversity of Bougainvillea germplasm resources, and the established fingerprinting profiles provide a reliable technique and effective tool for Bougainvillea variety registration, intellectual property protection, and variety identification.

Full Text

Abstract

This study optimized the ISSR-PCR reaction system and protocol for Bougainvillea by examining DNA template concentration, primer concentration, annealing temperature, and cycle number. Eleven ISSR primers were used to amplify DNA from 131 Bougainvillea cultivars, and the amplification products were detected via agarose gel electrophoresis to analyze genetic diversity and genetic distance, construct a cultivar dendrogram, and establish fingerprint profiles. The optimized ISSR-PCR system employed a DNA template concentration of 0.5 ng · L ^{1} and primer concentration of 0.5 M · L ^{1}. The optimal annealing temperatures for primers UBC813, UBC814, UBC815, UBC823, UBC824, UBC835, UBC840, UBC841, UBC843, UBC844, and UBC876 were 52.3, 55.9, 54.3, 54.3, 53.6, 56.2, 56.2, 51.9, 54.4, 54, and 50 °C, respectively, with 32 amplification cycles. The 11 ISSR primers amplified 161 bands across the 131 cultivars, of which 156 were polymorphic, yielding a polymorphism rate of 96.89%. Per primer, the number of alleles, effective number of alleles, Nei' s gene diversity index, and Shannon' s information index ranged from 1.86–2.00, 1.33–1.68, 0.21–0.39, and 0.34–0.57, respectively, with averages of 1.969, 1.478, 0.294, and 0.447. Primer UBC841 exhibited the highest discrimination rate (80.92%), enabling effective identification of 106 cultivars. Combined with primer UBC876, all 131 Bougainvillea cultivars could be completely distinguished, establishing a unique fingerprint for each cultivar. Genetic distances among cultivars ranged from 0.00 to 0.60, with a mean of 0.365, indicating relatively low genetic diversity. At a genetic distance of 0.58, the 131 cultivars clustered into six major groups. The cluster analysis revealed that most cultivars of the same species grouped together, though some cultivars within a species were distributed across different clusters or subclusters, while cultivars from multiple species occasionally clustered together. This study accurately revealed the genetic diversity of Bougainvillea germplasm resources, and the established fingerprints provide a reliable technique and effective tool for cultivar registration, intellectual property protection, and cultivar identification.

Keywords: Bougainvillea, ISSR marker, genetic diversity, genetic relationship, fingerprint

Introduction

Bougainvillea (*Bougainvillea spectabilis* Willd.) belongs to the family Nyctaginaceae, order Thymelaeceae, and is widely used in landscaping throughout tropi-

cal and subtropical regions. With over a century of cultivation history in China, Bougainvillea encompasses numerous cultivars and abundant bud sport variants, with approximately 200 introduced and selected varieties. However, their genetic relationships remain unclear, and issues of synonymy and homonymy are common. Current research on Bougainvillea has primarily focused on propagation and cultivation techniques (Zhou, 2008; Moneruzzaman et al., 2010; Sun et al., 2017) and physicochemical studies (Zhao et al., 2014; Figueroa et al., 2014; Marana et al., 2015; Chauhan et al., 2016), while genetic diversity research remains limited. Notably, only one study has employed Inter-Simple Sequence Repeats (ISSR) molecular markers to analyze genetic relationships among Bougainvillea germplasm resources, and that analysis included only 68 cultivars (Li et al., 2011). No reports have documented the use of ISSR markers for large-scale cultivar identification or fingerprint construction in Bougainvillea.

ISSR molecular marker technology, developed based on Simple Sequence Repeats (SSR), involves designing primers with 1–4 additional bases at one end of an SSR to detect DNA sequence polymorphisms between two closely positioned, oppositely oriented SSRs. Its principle is similar to that of SSR markers (Shao, 2017; Zhou, 2014). ISSR markers offer several advantages: simple primer design, higher polymorphism than Random Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP) markers, ease of operation, low DNA requirement, strong stability, good reproducibility, and cost-effectiveness with high safety (Tan, 2014). ISSR markers have been widely applied in genetic relationship analysis (Li, 2014), genetic diversity assessment (Wang, 2015; Liang, 2018), fingerprint construction (Wang, 2016), cultivar identification (Ali, 2015; Jedrzejczyk et al., 2018; Sun et al., 2017), purity testing (Guan et al., 2013), and genetic stability analysis (Reza et al., 2017). This study investigated 131 Bougainvillea cultivars using ISSR markers to analyze their genetic diversity, determine genetic relationships, and conduct cultivar identification and fingerprint construction, thereby providing reliable techniques and effective tools for germplasm conservation, intellectual property protection, and cultivar clarification.

Materials and Methods

Plant Materials

The 131 Bougainvillea cultivars examined in this study are listed in Table 1 . Eighteen cultivars (Nos. 1–18) were maintained at the Institute of Landscape Horticulture, Guangxi Forestry Research Institute, while 113 cultivars (Nos. 19–131) were collected from the Tropical Crops Germplasm Resources Institute, Chinese Academy of Tropical Agricultural Sciences. Young leaves were harvested from healthy, pest-free plants for immediate DNA extraction and stored at –80 °C until use.

Primer Synthesis and Screening

Primer sequences were selected from 100 ISSR primers published by the University of British Columbia and synthesized by Suzhou Genewiz Biotechnology Co., Ltd. Primers exhibiting high polymorphism, strong stability, and good reproducibility were selected for genetic relationship analysis and fingerprint construction.

DNA Extraction and Quality Assessment

Genomic DNA was extracted from young leaves using a Rapid Plant Genomic DNA Extraction Kit (Aidlab Biotechnologies Co., Ltd., Beijing). DNA concentration was assessed using 1.2% agarose gel electrophoresis and UV spectrophotometry.

Optimization of ISSR-PCR Reaction System

Based on relevant literature (Li et al., 2010), the initial amplification reaction system was set at 20 L, containing 10 L 2× DreamTaq mix, 1 L ISSR primer (10 M), 8 L RNase-free water, and 1 L template gDNA (20–30 ng). Key factors affecting DNA amplification were optimized, including template DNA concentration (using the 'Auratus' cultivar, *Bougainvillea spectabilis* 'Auratus') and primer concentration. Template DNA concentrations of 400, 200, 50, 20, 10, 5, 0.2 ng · L ^{1} (seven levels) and primer concentrations of 40, 20, 15, 10, 4, 2 pmol · L ^{1} (six levels) were tested using primer UBC815 to identify optimal conditions.

PCR amplification was performed on a HeMa9600 thermal cycler (Shenzhen Zhuhai Hema Co., Ltd.) with the following initial program: 94 °C for 5 min; 38 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 2 min; final hold at 4 °C. Subsequently, annealing temperature trials were conducted for 11 selected primers, and cycle numbers were tested at gradients of 20, 23, 26, 29, 32, 35, 38, and 42 cycles.

PCR products were detected using 1.5% agarose gel electrophoresis with GoldView staining at a voltage not exceeding 5 V/cm in 1× TAE buffer for 90 min, and imaged using a JS-1075 gel imaging system.

Validation of ISSR-PCR System

DNA from 18 randomly selected Bougainvillea cultivars was used as template to validate the optimized ISSR-PCR system using polymorphic primers, assessing system stability.

Data Processing and Analysis

Amplification products were manually scored. Using a 5,000 bp marker as standard, clear and reproducible bands were recorded: presence scored as "1" and

absence or ambiguous bands as "0". The band data were converted into a binary matrix. PopGene32 software was used to analyze genetic parameters including percentage of polymorphic loci (PPL), observed number of alleles (Na), effective number of alleles (Ne), Nei' s gene diversity index (H), Shannon' s information index (I), genetic distance (GD), and genetic similarity coefficient (GS). Based on genetic distance and similarity coefficients, cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in NTSYSpc 2.1 (Rohlf et al., 2000) to construct a DNA digital fingerprint for Bougainvillea cultivars.

Results

Genomic DNA Extraction and Quality Assessment

Genomic DNA extracted using the rapid extraction kit produced clear, bright bands without smearing or impurities in agarose gel electrophoresis (Figure 1 [Figure 1: see original paper] shows results for 20 samples). UV spectrophotometry revealed OD /OD ratios of 1.8–2.0 and concentrations of 200–400 ng · L ^{1}, indicating high-quality DNA suitable for subsequent experiments.

Optimization of ISSR-PCR Conditions

Effects of DNA Template Concentration DNA template concentration and quality are critical for ISSR-PCR amplification. Using primer UBC815, amplification products (Figure 2 [Figure 2: see original paper]) showed that bands could be obtained across 0.01–50 ng · L ^{1}, but amplification was poor at 0.01, 0.05, 10, and 50 ng · L ^{1}. No significant differences were observed between 0.1–2 ng · L ^{1}. After repeated trials, 0.5 ng · L ^{1} yielded the clearest and most stable bands.

Effects of Primer Concentration Amplification occurred across 0.1–2.0 M · L ^{1} primer concentrations, but bands were fewer and fainter at 0.1, 0.2, and 2.0 M · L ^{1}. Optimal amplification was achieved at 0.5–1.0 M · L ^{1}. Based on repeated experiments, 0.5 M · L ^{1} was selected for subsequent trials (Figure 3 [Figure 3: see original paper]).

Effects of Annealing Temperature Using the optimized reaction system, annealing temperature trials for 11 primers identified optimal temperatures: UBC813 (52.3 °C), UBC814 (55.9 °C), UBC815 (54.3 °C), UBC823 (54.3 °C), UBC824 (53.6 °C), UBC835 (56.2 °C), UBC840 (56.2 °C), UBC841 (51.9 °C), UBC843 (54.4 °C), UBC844 (54 °C), and UBC876 (50 °C). Representative annealing temperature optimization gels are shown in Figures 4 [Figure 4: see original paper], 5 [Figure 5: see original paper], and 6 [Figure 6: see original paper].

Effects of Cycle Number While increasing cycle number can enhance product yield, excessive reaction time increases nonspecific amplification and is limited by component availability (e.g., declining polymerase activity). Appropriate cycle number is therefore crucial. ISSR-PCR results at different cycle numbers (Figure 7 [Figure 7: see original paper]) showed no amplification at 20–26 cycles, but clear bands at 29–41 cycles. The brightest, clearest bands were obtained at 32 cycles, with minimal improvement beyond this. For time and cost efficiency, 32 cycles was selected.

Stability Validation of ISSR-PCR System

Eighteen samples representing *B. spectabilis*, *B. glabra*, *B.* × *buttiana*, *B. peruviana*, and *Bougainvillea* sp. were selected to validate the optimized system using primer UBC824 (Figure 8 [Figure 8: see original paper]). Results demonstrated excellent stability with clear, reproducible, highly polymorphic bands, confirming suitability for subsequent analyses.

ISSR Primer Screening

From 100 ISSR primers, 11 were selected through primary and secondary screening based on abundant, clear, reproducible amplification. Representative screening results are shown in Figures 9 [Figure 9: see original paper] and 10 [Figure 10: see original paper].

Genetic Analysis

Polymorphism of ISSR Primers The 11 selected primers, chosen for abundant, clear, polymorphic, and reproducible amplification, were used to analyze 131 Bougainvillea cultivars (Table 3). These primers amplified 161 total bands (average 14.6 per primer), with 156 polymorphic bands (96.89% polymorphism). Individual primer amplification ranged from 12–20 bands, with UBC841 producing the most (20 bands) and UBC814, UBC815, and UBC843 the fewest (12 bands each). All primers except UBC823, UBC835, and UBC840 achieved 100% polymorphism, indicating excellent polymorphic potential. Representative amplification with UBC815 is shown in Figure 11 [Figure 11: see original paper].

Genetic Diversity of Bougainvillea Germplasm Binary data matrices were analyzed using PopGene32 software (Table 4). Per primer, observed allele number ranged 1.86–2.00 (mean 1.969); effective allele number ranged 1.33 (UBC840)–1.68 (UBC876) (mean 1.478); Nei' s gene diversity index ranged 0.21 (UBC840)–0.39 (UBC841) (mean 0.294); Shannon' s information index ranged 0.34 (UBC823, UBC840)–0.57 (UBC841) (mean 0.447). UBC841 and UBC876 showed the highest values across all parameters, while UBC840 showed the lowest effective allele number, Nei' s index, and Shannon' s index. These results indicate relatively limited genetic diversity in Bougainvillea.

UPGMA Cluster Analysis Based on 11 ISSR primers, genetic distances among 131 cultivars ranged 0.00–0.60 (mean 0.365). UPGMA cluster analysis produced a dendrogram (Figure 12 [Figure 12: see original paper]) revealing six major groups at a genetic distance of 0.58.

Group 1 comprised 25 cultivars across four species: 13 *B. glabra*, 4 *B. spectabilis*, 1 *B. × buttiana*, 1 *B. peruviana*, and 6 *Bougainvillea* sp. At a genetic distance of 0.54, Group 1 subdivided into three subgroups (1-I, 1-II, 1-III). Subgroup 1-I included eight *B. glabra* cultivars (Nos. 1, 3, 7, 19, 101, 127). Subgroup 1-II included three *B. glabra* (37, 40, 41), two *B. spectabilis* (40, 84), one *B. × buttiana* (51), one *B. peruviana* (76), and three *Bougainvillea* sp. (92, 130, 131). Subgroup 1-III contained only two *B. glabra* cultivars (2, 4).

Group 2 included nine cultivars: five *B. glabra* (25, 26, 27, 29, 28), one *B. × buttiana* (45), and three *Bougainvillea* sp. (110, 112, 111).

Group 3 contained 86 cultivars: six *B. glabra*, 29 *B. × buttiana*, six *B. spectabilis*, nine *B. peruviana*, two *B. × spectoglabra* hybrids, and 33 *Bougainvillea* sp. At a genetic distance of 0.56, Group 3 subdivided into four subgroups (3-I, 3-II, 3-III, 3-IV). Subgroup 3-I included two *B. glabra* (5, 6), five *B. × buttiana* (18, 17, 14, 15, 16), two *B. spectabilis* (8, 10), and two *B. × spectoglabra* hybrids (13, 12). Subgroup 3-II contained only one *Bougainvillea* sp. (86). Subgroup 3-III further divided into seven mini-clusters (3-IIIA through 3-IIIG) at a genetic distance of 0.53. Subgroup 3-IV included one *B. glabra* (24), one *B. × buttiana* (52), one *B. peruviana* (68), and two *Bougainvillea* sp. (87, 105).

Group 4 comprised one *B. glabra* (30) and one *B. spectabilis* (79). **Group 5** included one *B. glabra* (35) and three *Bougainvillea* sp. (124, 100, 123). **Group 6** contained four *B. glabra* (22, 32, 33, 34) and one *Bougainvillea* sp. (103).

All 131 cultivars were successfully distinguished, demonstrating ISSR markers' effectiveness for Bougainvillea germplasm analysis and cultivar identification.

Fingerprint Construction The 11 ISSR primers completely distinguished all 131 cultivars. Electrophoretic bands were scored as "1"(present) or "0"(absent) to construct a binary fingerprint matrix (Table 5). Primer UBC841 showed the highest discrimination rate (80.92%), identifying 106 cultivars alone. Combined with UBC876, all 131 cultivars were uniquely identified. UBC841 amplified 20 polymorphic loci (2500, 2200, 2000, 1600, 1500, 1450, 1100, 1000, 950, 850, 750, 700, 650, 550, 450, 400, 350, 150, 250, and 2900 bp). UBC876 amplified 15 polymorphic loci (2800, 2500, 2000, 1800, 1500, 1350, 1150, 1050, 900, 850, 750, 650, 550, 3100, and 500 bp). UBC876 generated 15-digit fingerprints for cultivars 12, 14, 31, 39, 42, 43, 44, 48, 58, 64, 65, 66, 67, 70, 82, 85, 86, 88, 90, 97, 98, 99, 101, 107, and 126, while UBC841 generated 20-digit fingerprints for the remaining cultivars.

Discussion

ISSR System Optimization

DNA template quantity critically affects PCR amplification. In this study, amplification occurred across 0.01–50 ng· L ^{1}, but bands were unclear and diffuse at excessively high or low concentrations (Wang et al., 2010). Repeated trials identified 0.5 ng· L ^{1} as optimal, producing the clearest, most stable bands. Primer concentration directly influences PCR results: excessive concentrations increase mismatching and nonspecific amplification while promoting primer-dimer formation, whereas insufficient concentrations reduce product yield (Liu, 2014). PCR specificity is also affected by annealing temperature, which depends on base composition, concentration, primer length, and target sequence length (Zhang, 2014); thus, optimal temperatures vary by primer. This study determined optimal annealing temperatures through gradient trials. Cycle number is decisive for amplification: more cycles increase nonspecific products, and amplification capacity declines with polymerase activity. Typically, 30–40 cycles are recommended (Feng et al., 2004); this study found 32 cycles optimal.

Genetic Diversity and Relationship Analysis in Bougainvillea

Using the optimized ISSR-PCR system, 11 highly polymorphic, reproducible, and stable primers amplified 161 bands across 131 cultivars, with 156 polymorphic bands (96.7% polymorphism)—substantially higher than RAPD (67.4% polymorphism) (Richa et al., 2009) and isozyme (75.5% polymorphism) (Yin et al., 2001) markers. However, Nei' s gene diversity index (mean 0.29) and Shannon' s information index (mean 0.45) were low, with high genetic similarity coefficients (0.64–0.95), indicating limited genetic diversity among tested cultivars. This aligns with Huang (2010) (genetic similarity 0.50–0.97 among 68 Bougainvillea accessions) and Richa et al. (2009) (similarity 0.51–0.94 among 30 accessions). Limited genetic diversity may stem from Bougainvillea' s specialized floral structure and low pollen viability, which restrict hybrid breeding and favor vegetative propagation, narrowing the gene pool and increasing genetic similarity (Huang, 2010).

Genetic distances of 0.00–0.60 (mean 0.365) enabled effective identification of all 131 cultivars, demonstrating ISSR reliability for Bougainvillea cultivar discrimination. Clustering at a genetic distance of 0.58 revealed six groups, showing differentiation among cultivars with discernible patterns: most cultivars within a species clustered together (e.g., 60.0% of 30 *B. glabra* cultivars in Groups 1–2; 93.5% of 31 *B. × buttiana* in Group 3; 81.8% of 11 *B. peruviana* in Group 3). The two hybrids (Nos. 12, 13) clustered in Subgroup 3-I. However, some intraspecific cultivars were distributed across different clusters (e.g., *B. glabra* cultivars 'Mrs. Eva' and 'Snow Purple' in Groups 4 and 5, respectively; 'Alba' , 'Royal Purple' , 'Singapore White' , and 'Singapore Beauty' in Group 6). One *B. × buttiana* cultivar ('Los Banos Variegata') clustered in Group 1, likely reflecting its *B. glabra* parentage. Additionally, multiple species clustered

together in Group 3, possibly due to long-term adaptation to similar environments converging traits and underlying genes.

Compared with Li et al. (2011) ISSR analysis of 68 cultivars, 16 shared cultivars showed similar clustering relationships (e.g., 'Variegata' , 'Senjakala' , 'Golden Lady' , and 'China Beauty' in Subgroup 1-I; 'Barbara Karst' , 'Imperial Delight' , 'Rosa' , 'Pink Pixie' , 'Thimma' , and 'Temple Fire' in Subgroup 3-I). However, ISSR-based clustering did not strictly correspond to traditional morphological classification. For example, *B. glabra* 'Mrs. Eva' (No. 30) and *B. spectabilis* 'Splendens' (No. 79), morphologically distinct and belonging to different species, clustered together, indicating discrepancies between molecular and phenotypic classifications.

This study elucidated genetic relationships among Bougainvillea cultivars and successfully established molecular fingerprints, providing valuable information for future germplasm conservation and cultivar identification.

References

JEDRZEJCZYK I, REWERS M, 2018. Genome size and ISSR markers for *Mentha* L. (Lamiaceae) genetic diversity assessment and species identification[J]. *Ind Crop Prod*, 120: 171-179.

MONERUZZAMAN K M, HOSSAIN ABMS, NORMANIZA O, et al., 2010. Effects of removal of young leaves and kinetin on inflorescence development and bract enlargement of *Bougainvillea glabra* var. "Elizabeth Angus" [J]. *Aust J Crop Sci*, 4(7): 467-473.

REZA RAJI M R, LOTFI M, TOHIDFAR M, et al., 2018. Somatic embryogenesis of muskmelon (*Cucumis melo* L.) and genetic stability assessment of regenerants using flow cytometry and ISSR markers[J]. *Protoplasma*, 255(3): 873-883.

RICHA S, SUDHIR S, ARVIND S, et al., 2009. RAPD-based genetic relationships in different Bougainvillea cultivars. *Crop Breed Appl Biot*, 9: 154-163.

ALI QS, 2015. Identification and diversity analysis of Wintersweet (*Chimonanthus Praecox*) crossing progenies using SSR molecular markers[D]. Wuhan: Huazhong Agricultural University.

FENG FJ, WANG FY, LIU T, 2004. The influence factors of the ISSR-PCR experiment system on *Pinus koraiensis* Sieb. et Zucc[J]. *Chin Bull Bot*, 21(3): 326-331.

GUAN J, JIAO XH, WU JX, et al., 2013. Identification of Asian lily hybrid F1 by using ISSR[J]. *Mol Plant Breed*, 11(3): 415-420.

HUANG YJ, 2010. ISSR analysis of germplasm resources of *Bougainvillea Brasiliensis* Raeusch[D]. Fuzhou: Fujian Agriculture & Forestry University.

LI FY, HUANG YJ, WU SH, et al., 2011. ISSR analysis of germplasm resources of *Bougainvillea spectabilis* Willd[J]. *Chin J Trop Crops*, 32(9): 1692-1696.

LI GS, 2014. The research of ISSR on relationship of 5 species of wild *Camellia* of genus *Camellia* and fingerprint construction[D]. Changsha: Central South University of Forestry & Technology.

LIANG Y, 2018. Genetic diversity analysis of *Caragana* Fabr. in inner Mongolia desert area[D]. Huhehaote: Inner Mongolia University.

SHAO ZT, 2017. Studies on genetic relationship of culinary Rhubarb and official Rheum based on ISSR and SRAP markers[D]. Xinxiang: Henan Institute of Science and Technology.

SUN LN, LIN M, LI JH, et al., 2017. Callus induction of *Bougainvillea spectabilis* cultivars[J]. *J W Chin For Sci*, 46(2): 157-160.

SUN LN, TANG Q, WANG HX, et al., 2017. Identification of *Bougainvillea spectabilis* radiation mutant based on ISSR markers[J]. *Guangxi For Sci*, 46(3): 276-279.

SUN LN, WANG HX, GONG JY, et al., 2013. Cutting propagation of *Bougainvillea glabra* and its effecting factors[J]. *Guangxi For Sci*, 42(2): 183-185.

TAN HQ, 2014. Genetic diversity analysis and varietal identification among 68 Chinese asparagus bean (*Vigna unguiculata* ssp. *sesquipedialis*) cultivars based on RAPD, ISSR and morphological markers[D]. Yaan: Sichuan Agricultural University.

WANG L, 2015. Analysis on genetic diversity of *Forsythia suspensa* by ISSR marker[D]. Taiyuan: Shanxi Agricultural University.

WANG LJ, 2016. A study on the genetic diversity and the identification of DNA barcoding of medicinal plants *Rosa* L. [D]. Zhengzhou: Henan Agricultural University.

WANG WX, LAI FX, HONG LY, et al., 2010. Effects of DNA concentrations and storage conditions on PCR detection of transgenic rice [J]. *J Agric Biotechnol*, 18(5): 846-852.

YIN JM, OU WJ, ZHANG X, 2001. Protein analysis of genetic relationship of Bougainvillea cultivars[J]. *Chin J Trop Crops*, 22(4): 61-66.

ZHANG R, 2014. Analysis on genetic diversity of medicinal *Astragalus* by ISSR marker [D]. Taiyuan: Shanxi Agricultural University.

ZHOU LY, 2014. ISSR analysis on the diversity of oil-tea *Camellia* germplasm[D]. Changsha: Hunan Normal University.

ZHOU Q, 2008. Research on germplasm resource and propagational technique of *Bougainvillea* L. in China [J]. *Chin Agric Sci Bull*, 24(12): 321-324.