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Genetic diversity of *Camellia japonica* (Theaceae), a species endangered to East Asia, detected by inter-simple sequence repeat (ISSR)

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ABSTRACT

Inter-simple sequence repeat (ISSR) markers were used to investigate the genetic diversity within and among thirteen natural populations of *Camellia japonica* (L.). Twenty ISSR primers gave rise to 211 discernible DNA bands of which 190 (90.1%) were polymorphic. On average each primer gave rise to 10.55 bands including 9.50 bands with polymorphic profile. At the species level, high genetic diversity was detected (*PPB*: 90.1%; H_E: 0.3414; *H*: 0.5013). However, relatively low genetic diversity existed within populations. Shiko-2 exhibits the greatest level of variability (*PPB*: 76.8%; H_E: 0.2966; *H*: 0.4319), whereas XS presents its own variability at the lowest level (*PPB*: 67.3%; H_E: 0.2344; *H*: 0.3478). A relatively high level of genetic differentiation among populations was revealed by Nei's gene diversity statistics (21.3%), Shannon's information measure (21.4%) and analysis of molecular variance (AMOVA) (22.5%). There was significant correlation between genetic distance and geographic distance ($r = 0.8154$, $P < 0.05$).

1. Introduction

Oceanic islands are natural laboratories for studies of plant evolution (Crawford et al., 1987; Adsersen, 1995; Crawford and Stuessy, 1997). One feature of the floras of oceanic islands is the high number of endemics occurring in small areas. For example, there are 570 endemics species in Canary Islands (Fransisco-Ortega et al., 2000). Adaptive radiation into diverse habitats and genetic drift are often considered to be important factors producing such extensive speciation (Crawford et al., 1987; Fransisco-Ortega et al., 2000). However, island populations have a much higher risk of extinction than mainland populations (Diamond, 1984; Flesness, 1989; Case et al., 1992). Recorded extinctions since 1600 showed that substantial proportions of extinctions in vascular plants were of island forms (Olson, 1989). Major factors responsible for the high extinction rates of insular species include limited distribution area, habitat frangibility and small population size (Olson, 1989; Stone and Stone, 1989; Adsersen, 1991; Rieseberg and Swensen, 1996; Frankham, 1997).

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Genetic diversity is the raw material for evolutionary change (Frankel and Soulé, 1981). The analysis of genetic diversity is a key element for the study of biodiversity, ecosystem functioning, and the consequences of man-made impact on natural systems. Many studies suggest that island populations have lower genetic diversity than comparable mainland populations (Olson, 1989; Reid and Miller, 1989; Frankham, 1997). There are many factors that can contribute to lower genetic diversity of island compared with mainland populations, namely human activities, inbreeding depression, loss of genetic variation, accumulation of mildly deleterious mutations, and genetic adaptations to island environments (flightlessness, limited ability to avoid predators and diseases) (Olson, 1989; Myers, 1979; Vitousek, 1988; Atkinson, 1989; World Conservation Monitoring Centre, 1992).

Camellia japonica (L.), a member of Theaceae, is an evergreen broad-leaved woody species, which is widely distributed in China (in Zhejiang and Shandong provinces), Japan (on Honshu, Shikoku, and Kyushu Islands) and along the southern and western coast of the Korean Peninsula (Ueno et al., 1999; Gao et al., 2005). The plants are shrubs or small trees up to 3–10 m tall. Its flowers are bisexual and disposed in racemes. Their leaves alternate with serrate margin, and the seeds are small (length under 1 cm) (Zhang and Ren, 1998; Gao et al., 2005).

Inter-simple sequence repeats (ISSR) have been extensively used to characterize genetic diversity in plants (Tsumura et al., 1996; Camacho and Liston, 2001; Barth et al., 2002). The technique provides the following advantages: (1) no prior information or lengthy mapping studies are required; (2) development costs are low; and (3) laboratory protocols can easily be transferred between plants (Barth et al., 2002). Compared with RAPD, a series of studies have indicated that ISSR could be able to produce more reliable and reproducible bands because of the higher annealing temperature and longer sequence of ISSR primers (Nagaoka and Ogihara, 1997; Tsumura et al., 1996; Cao et al., 2006). Therefore, ISSR has proved to be useful in population genetic studies (Zietkiewicz et al., 1994; Barth et al., 2002; Esselman et al., 1999).

In this study, the main objectives were to reveal the level and partitioning of genetic diversity in *C. japonica* among thirteen populations using ISSR markers. It will provide the basic information for effective conservation.

2. Materials and methods

2.1. Sampling

Young leaf tissues of 390 individuals of *C. japonica* were collected from thirteen populations in China and Japan. The distribution of the populations studied is shown in Table 1. Fig. 1 shows collection information along with an interactive map, two voucher pictures and collector information of ZJJ population. The young leaf tissues were stored with silica gel in zip-lock bags until DNA extraction.

2.2. DNA extraction and ISSR-PCR amplification

Genomic DNA was extracted using the modified CTAB method (Doyle, 1991). DNA was determined qualitatively and quantitatively in 1% agarose gel buffered with 0.5× TBE. Eighty primers (synthesized by Shanghai Sangon Bioengineering Technology Service Co. Ltd., Shanghai, China) from the Biotechnology Laboratory, University of British Columbia (UBC set no. 9) were initially screened for PCR amplification and 20 primers (Table 2) that produced clear and reproducible banding patterns were chosen for our final analysis. ISSR amplification was performed in a volume of 20 μL containing 40 ng genomic DNA, 2.0 μL 10× Buffer, 1.5 mmol L^{-1} Mg^{2+}, 0.2 mmol L^{-1} dNTP, 0.6 μmol L^{-1} primer, and l U of *Taq* DNA polymerase. PCR amplifications were carried out in a GeneAmp 9700 DNA Thermal Cycler (PerkineElmer, USA), with initial denaturation for 5 min at 94 °C, followed by 40 cycles of denaturation for 40 s at 94 °C, annealing for 45 s at respective T_m values (Table 2) of the selected primers, and 1.5 min elongation at 72 °C. Final elongation was performed for 10 min at 72 °C. Amplification products were electrophoresed on a 1.5% agarose gel at 120 V for 1.5 h, stained with ethidium bromide and photographed under UV light.

Table 1
Locality of populations sampled of *Camellia japonica*.

Population	Number	Locality	Geographical location	Altitude/m
TH	30	Taohua Island, Zhejiang, China	29°48′N, 122°18′E	12
ZJJ	30	Zhujiajian Island, Zhejiang, China	29°25′N, 121°42′E	372
SCD	30	Putuo Island, Zhejiang, China	30°00′N, 122°24′E	288
HJ	30	Putuo Island, Zhejiang, China	30°00′N, 122°23′E	291
XS	30	Xiangshan, Zhejiang, China	29°36′N, 121°74′E	203
CMY	30	Changmenyan Island, Shandong, China	36°10′N, 120°56′E	36
BG*	30	Botanical Garden, Shandong, China	36°06′N, 120°34′E	10
WS*	30	Wusi Quare, Shandong, China	36°11′N, 120°53′E	3
Kago	30	Kagoshima, Japan	31°25′N, 130°35′E	144
Shiko-1	30	Shikoku Island, Japan	33°03′N, 132°58′E	120
Shiko-2	30	Shikoku Island, Japan	32°43′N, 133°00′E	12
Goto-1	30	Goto Island, Japan	32°40′N, 128°48′E	26
Goto-2	30	Goto Island, Japan	32°38′N, 128°51′E	36

Note: The individuals of BG* and WS* were immigrants from Changmenyan Island.