



Genetic diversity and differentiation in *Camellia reticulata* (Theaceae) polyploid complex revealed by ISSR and ploidy

B.-Y. Wang and Z.-Y. Ruan

Research Institute of Resource Insects, Chinese Academy of Forestry,
Kunming, P.R. China

Corresponding author: B.-Y. Wang
E-mail: wbykm@yahoo.com.cn

Genet. Mol. Res. 11 (1): 503-511 (2012)
Received August 3, 2011
Accepted January 9, 2012
Published March 6, 2012
DOI <http://dx.doi.org/10.4238/2012.March.6.3>

ABSTRACT. *Camellia reticulata* is a well-known ornamental and oil plant that is endemic to southwest China. This species shows three cell ploidies, i.e., diploidy, tetraploidy and hexaploidy. We made the first investigation of genetic diversity and differentiation of natural populations of *C. reticulata*, and 114 individuals from 6 populations were sampled. Cytogeography results showed that ploidy is invariable within populations and evenly distributed. A relatively high level of genetic diversity was found in *C. reticulata*, both at the species level (PPB = 88.89%; H = 0.2809; I = 0.4278) and at the population level (mean PPB = 42.13%; mean H = 0.14; mean I = 0.21). We found a relatively low degree of differentiation among ploidies ($G_{ST} = 0.2384$; AMOVA = 10.26%) and a relatively high degree of differentiation among populations ($G_{CS} = 0.3807$; AMOVA = 48.75%). The high genetic diversity can be explained by its biological character, wide distribution and ploidies, and the special genetic structure can be ascribed to polyploid origin from hybridization with different *Camellia* spp. This information will be useful for the introduction, conservation and further studies of *C. reticulata* and related species.

Key words: *Camellia reticulata*; Genetic diversity; ISSR; Ploidies

INTRODUCTION

Camellia reticulata Lindl is an evergreen shrub or arbor with a natural distribution in Yunnan and the region southwest of Sichuan, China (Ming, 2000). It was first identified in 1827 based on a cultivar from Tengchong, Yunnan, and its relatives in the field were found in the 1940s; the specimens were hexaploid (Ming, 1998). The diploidy and tetraploidy of *C. reticulata* were discovered in 1994 and 1997, respectively (Xia et al., 1994; Gu, 1997). These 3 cell ploidies are the currently established ploidies in *C. reticulata*. Although some cytology studies have been carried out on *C. reticulata* (Kondo et al., 1986; Xiao et al., 1993; Xia et al., 1994), cytogeography in *C. reticulata* has not been examined, and whether different ploidies occur within a population is still unknown.

C. reticulata is not only valued as a flowering ornamental but also as an oil plant. It has been cultivated in China for more than 1300 years. It is famous worldwide for its large flowers, bright and charming colors, multiple cultivars, and long flowering season. Its seeds, widely cultivated in the southwest of China, have high oil content, and its oil is rich in unsaturated fatty acids, vitamin E, and other physiologically active substances. Despite its great value, the numbers and areas of natural populations have decreased rapidly because of overharvesting and habitat destruction. Genetic diversity and a natural distribution pattern are significant for the use and conservation of *C. reticulata*, but studies on *C. reticulata* genetic diversity and its pattern of natural populations have not yet been performed.

Polyploid complexes are prevalent in herbage, but wood polyploid complexes are seldom seen. *C. reticulata* is one of them. Origin, differentiation, and distribution are basic aspects in the understanding of a species, but these characteristics are complicated in *C. reticulata*, not only because it is a polyploid complex but also because it is sympatric with some related species. Studies have provided evidence of the origin of its polyploid populations in Panzhihua, Sichuan, China (Gu and Xiao, 2003; Liu and Gu, 2011), but no information is available about other polyploid populations. Studying genetic differentiation among ploidies and populations provides an overall understanding of *C. reticulata* and information from which to speculate about the course of its origin, differentiation, and distribution.

Intersimple sequence repeat (ISSR) is a polymerase chain reaction (PCR)-based technique that amplifies sequences between adjacent, inversely oriented microsatellites using an SSR-containing primer (Zietkiewicz et al., 1994). The strategy is especially attractive because it avoids the need to carry out the costly cloning and sequencing inherent in the original microsatellite-based approach. Moreover, locus-specific probes and microsatellite-containing sequences of interest can be developed by isolating and cloning or reamplifying individual bands (Wu et al., 1994; Zietkiewicz et al., 1994). ISSR has been profitably used for studies of genetic diversity in various plants at the species and population levels (Kumar, 2002; Nybom, 2004; Luo et al., 2010; Wang, 2010). Because ISSR primers are longer, they may show greater repeatability and stability of map position in the genome in comparisons of closely related individuals (Zietkiewicz et al., 1994).

In this study, we investigate the chromosome number and the genetic diversity and pattern of *C. reticulata* in its natural range. The aims of this study were to clarify the chromosome number (ploidies) of *C. reticulata* populations, estimate genetic diversity at both the species and the population level, analyze the genetic differentiation among ploidies and populations of *C. reticulata*, and discuss the implications of our study on the conservation, use, and management of this valuable resource.

MATERIAL AND METHODS

Six populations were sampled from within their natural distributions in southwest China (Table 1; Figure 1). Fresh leaves were collected and dried with silica in a resealable plastic bag for approximately 2 weeks, and genomic DNA was extracted and isolated from the dry leaves according to a modified cetyltrimethylammonium bromide procedure (Wang, 2003).

Table 1. Information on the *Camellia reticulata* populations studied.

Population name	Location	Latitude (N)	Longitude (E)	Height (m)
Tengchong	Yunnan, Tengchong	25.11	98.28	2005
Yanbian	Sichuan, Yanbian	27.12	101.28	2443
Huidong	Sichuan, Huidong	26.77	102.69	2250
Xichang	Sichuan, Xichang	27.73	102.35	2197
Fuming	Yunnan, Fuming	25.53	102.62	1627
Yunxian	Yunnan, Yunxian	24.30	100.14	2087



Figure 1. Locations of the *Camellia reticulata* populations studied.

bands ranging in size from 200 to 2000 bp. Five to nine loci were scored per primer. At the species level, the PPB, Nei's genetic diversity, and the Shannon diversity index were 88.89%, 0.2809, and 0.4278, respectively. Within the populations, the PPB ranged from 25.00 to 52.78%, Nei's genetic diversity between the populations ranged from 0.0981 to 0.1611, and the Shannon diversity index between the populations ranged from 0.1429 to 0.2426 (see Table 3).

Table 3. Genetic diversity and ploidy of *Camellia reticulata*.

Population	N	H	I	PPB (%)	Chromosome number
Tengchong	20	0.1518 (0.2003)	0.2247 (0.2873)	41.67	90
Yanbian	19	0.1438 (0.1998)	0.2126 (0.2852)	39.81	30
Huidong	20	0.1358 (0.1795)	0.2112 (0.2584)	52.78	60
Xichang	16	0.1336 (0.1892)	0.2009 (0.2725)	40.74	60
Fuming	20	0.1611 (0.1997)	0.2426 (0.2828)	52.78	90
Yunxian	19	0.0981 (0.1823)	0.1429 (0.2603)	25.00	90
Total	114	0.2809 (0.1703)	0.4278 (0.2280)	88.89	

H = Nei's genetic diversity; I = Shannon diversity index; PPB = percentage of polymorphic bands.

Differentiation

The coefficient of differentiation among ploidies (G_{ST}) was 0.2384, and that among populations within ploidies (G_{CS}) was 0.3807 (Table 4). Analysis of molecular variance showed a genetic differentiation among the ploidies and among the populations within ploidies in *C. reticulata*. Of the total genetic diversity, 10.26% resided among ploidies, 48.75% resided among the populations within ploidies, and the rest (40.99%) resided among individuals within populations (Table 5). Indirect estimates of the level of gene flow (N_m) between ploidies and populations were calculated to be 0.8134 and 1.5977 individuals per generation, respectively.

Table 4. Coefficient of differentiation for *Camellia reticulata*.

Parameter	H_T	H_C	H_S	G_{ST}	G_{CS}	$N_m (G_{ST})$	$N_m (G_{CS})$
Mean	0.2912	0.1374	0.2218	0.2384	0.3807	0.8134	1.5977
SD	0.0292	0.0112	0.0208				

Table 5. Analysis of molecular variance (AMOVA) for six populations of *Camellia reticulata*.

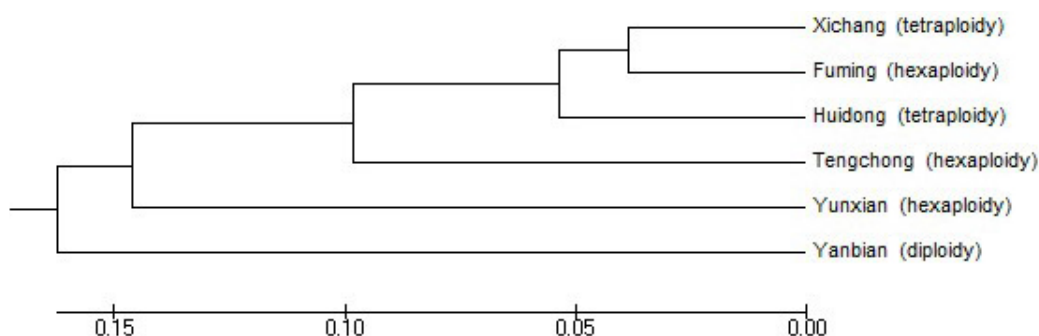
Source of variation	d.f.	SS	MS	VC	% of total variance	P
Among ploidies	2	493.4	246.7	1.83	10.26%	0.15
Among populations within ploidies	3	481.9	160.6	8.69	48.75%	<0.001
Within populations	108	788.9	7.3	7.3	40.99%	<0.001

d.f. = degrees of freedom; SS = sum of square; MS = mean square; VC = variance component.

Genetic distance between populations ranged from 0.0769 to 0.4042, and geographic distance between populations ranged from 111.81 to 498.39 km (Table 6). The Mantel test, which shows the coefficient of correlation between genetic and geographic distances was neither high ($R = 0.20735$) nor significant ($P = 0.2443$). The relationships among the 6 populations are shown in a dendrogram based on Nei's genetic distance, and the populations with different ploidies were not divided into different clusters (Figure 2).

Table 6. Genetic distance (below diagonal) and geographic distance (above diagonal) between populations of *Camellia reticulata*.

	Tengchong	Yanbian	Huidong	Xichang	Fuming	Yunxian
Tengchong		372.71	477.42	498.39	440.41	209.55
Yanbian	0.3153		146.19	125.49	223.58	330.12
Huidong	0.2239	0.2830		111.81	135.66	374.43
Xichang	0.1953	0.3547	0.1232		247.83	440.92
Fuming	0.1691	0.2650	0.0913	0.0769		222.69
Yunxian	0.3803	0.4042	0.2445	0.2815	0.2623	

**Figure 2.** UPGMA dendrogram based on Nei's genetic distance.

DISCUSSION

Although morphologic character varied within populations, the chromosome number was invariable within populations. Our results, together with those of earlier studies (Kondo et al., 1986; Gu, 1997; Ming, 2000), have clarified the distribution of ploidies in *C. reticulata*. The diploidy live in only one location in Yanbian, Sichuan, and the tetraploidy live in a narrow zone adjacent to the diploidy. The hexaploidy occupy the rest of the distribution. The regular distribution of ploidies may be related to the course of polyploidization and spread; when a species spreads, it has a greater chance of having higher ploidy, and higher ploidy can spread further because it may have greater adaptive capability.

With polymorphic and monomorphic loci used for calculation, genetic diversity in *C. reticulata* was not only higher than the mean level in plants but also higher than that of relative species in *Camellia*. The mean PPB of plant species determined by RAPD markers is 62% (Zou et al., 2001). At the species level, the mean PPBs in gymnosperms, dicotyledons, and monocotyledons are 70.9, 44.8, and 59.2%, respectively, and at the population level, the mean PPBs in gymnosperms, dicotyledons, and monocotyledons are 57.7, 29.0, and 40.3%, respectively (Hamrick and Godt, 1989). In *Camellia japonica*, allozyme analysis revealed a PPB of 81% and an H of 0.322 at the species level; mean PPB was 62-69%, and mean H was 0.245-0.265 at the population level (Wendel and Parks, 1985; Chung and Kang, 1996; Chung and Chung, 2000). In *Camellia nitidissima*, RAPD and amplified fragment length polymorphism analysis revealed that PPB was 78.1-72.5% and H was 0.2698-0.2444 at the species level; mean PPB was 32.3-37.3% and mean H was 0.1069-0.1288 at the population level (Tang et al., 2006). In *Camellia sinensis* ISSR, RAPD, and allozyme analysis showed a PPB of 57.4-84%

at the species level; mean PPB was 21.4-50%, and mean I was 0.229 at the population level (Kaundun et al., 2000; Kumar, 2002; Chen et al., 2005; Thomas et al., 2006). In the present study, PPB was 88.89%, H was 0.2809, and I was 0.4278 at the species level; mean PPB was 42.12%, mean H was 0.1373, and I was 0.2058 at population level.

A high level of genetic diversity may be the result of the common character of *Camellia* species as well as the individuated character of *C. reticulata*, because the genetic diversity of species in *Camellia* is higher than the mean level of genetic diversity in other plants, and the genetic diversity of *C. reticulata* is higher than that of other *Camellia* species. A high level of genetic diversity may also be related to life form and mating system. Long-lived woody and outcrossing species have levels of genetic diversity that are higher than those of other life forms and species with different mating systems (Hamrick et al., 1992; Hamrick and Godt, 1996). Species in *Camellia* are all long-lived woody plants with outcrossing mating systems (Ming, 2000). *Camellia reticulata* has more genetic diversity than other *Camellia* species, which can be explained by its large geographic range and complex ploidies. *Camellia reticulata* is distributed from Yunnan to Sichuan Provinces in the southwest of China, and its distribution is wider than that of most *Camellia* species (Ming, 1998). Species with wider distribution tended to be more genetically diverse (Casiva et al., 2002). There are 3 cell ploidies in *C. reticulata*, and some polyploidy populations have been shown to originate through hybridization (Hamrick and Godt, 1996). Introgression of other *Camellia* species has also promoted genetic diversity in *C. reticulata*.

Most of the variation in *C. reticulata* occurs among populations ($G_{CS} = 0.3807$; 48.75% variation lies among populations within ploidies), and very little variation occurs among ploidies ($G_{ST} = 0.2384$; 10.26% variation lies among ploidies). This result can be explained by the origin of polyploidy. The distribution area of *C. reticulata* overlaps with other *Camellia* species such as *C. saluenensis*, *C. pitardii* var. *potardii*, *C. pitardii* var. *variabilis*, *C. mairei* var. *mairei*, *C. mairei* var. *lapidea*, *C. mairei* var. *velutina*, and *C. yunnanensis* (Ming, 1998). Some polyploidy populations of *C. reticulata* have been shown to originate through hybridization, and the local *Camellia* species participated in the origin of the polyploidy (Gu and Xiao, 2003; Liu and Gu, 2011). *Camellia reticulata* has overlapped different *Camellia* species in different locations, so different ploidy populations and populations with the same ploidy may have different genetic composition. Therefore, the main genetic differentiation is among populations but not among ploidies, and the populations do not cluster according to ploidies. Genetic distance is also not decided by geographic distance.

Although the genetic diversity is rich at the species level in *C. reticulata*, the genetic differentiation existed not only among ploidies but also among populations with the same ploidy, and some populations lacked members and genetic diversity. For example, the Yanbian population, the only recorded diploidy population in *C. reticulata*, was considered to be the ancestor of *C. reticulata*, and no more than 50 individuals remain. The Yunxian population, one of the hexaploidy populations, differs from the other hexaploidy populations in its large plant body, flowers, and fruits; only 19 individuals remain, and their genetic diversity is much lower than that of other populations. These 2 populations should be protected first. In conclusion, this study provides data on the cytogeography, genetic diversity and its distribution, differentiation among ploidies, and populations of *C. reticulata* and contributes to an overall understanding of the living status of the species. We expect this study to be helpful in resource conservation, introduction, and further studies of *C. reticulata* and related species.

ACKNOWLEDGMENTS

The research was funded by the Science and Technology Ministry and State Forestry Administration, China (#2009BADB1B01), and the Yunnan Provincial Science and Technology Department (#2009CD149).

REFERENCES

- Casiva PV, Saidman BO, Vilardi JC and Cialdella AM (2002). First comparative phenetic studies of Argentinean species of *Acacia* (Fabaceae), using morphometric, isozymal, and RAPD approaches. *Am. J. Bot.* 89: 843-853.
- Chen J, Wang P, Xia Y, Xu M, et al. (2005). Genetic diversity and differentiation of *Camellia sinensis* L. (cultivated tea) and its wild relatives in Yunnan province of China, revealed by morphology, biochemistry and allozyme studies. *Genet. Resour. Crop Evol.* 52: 41-52.
- Chung MG and Kang SS (1996). Genetic variation within and among populations of *Camellia japonica* (Theaceae) in Korea. *Can. J. Forest. Res.* 26: 537-542.
- Chung MG and Chung MY (2000). Levels and partitioning of genetic diversity of *Camellia japonica* (Theaceae) in Korea and Japan. *Silvae Genet.* 49: 119-124.
- Excoffier L, Smouse PE and Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.
- Gu ZJ (1997). The discovery of tetraploid *Camellia reticulata* and its implication in studies on the origin of this species. *Acta Phytotaxon. Sin.* 35: 107-116.
- Gu ZJ and Sun XF (1997). A karyomorphological study of seventeen species of Chinese camellis. *Acta Bot. Yunanica* 19: 159-170.
- Gu ZJ and Xiao H (2003). Physical mapping of the 18S-26S rDNA by fluorescent *in situ* hybridization (FISH) in *Camellia reticulata* polyploid complex (Theaceae). *Plant Sci.* 164: 279-285.
- Hamrick JL and Godt MJW (1989). Allozyme Diversity in Plant Species. In: Plant Population Genetics, Breeding and Genetic Resources (Brown AHD, Clegg MT, Kahler AL and Weir BS, eds.). Sinauer, Sunderland, 43-63.
- Hamrick JL and Godt MJW (1996). Effects of life history traits on genetic diversity in plant species. *Phil. Trans. R. Soc. Lond. B* 351: 1291-1298.
- Hamrick JL, Godt MJW and Sherman-Broyles SL (1992). Factors influencing levels of genetic diversity in woody plant species. *New Forest.* 6: 95-124.
- Kaundun SS, Zhyvoloup A and Park YG (2000). Evaluation of the genetic diversity among elite tea (*Camellia sinensis* var. *sinensis*) accessions using RAPD markers. *Euphytica* 115: 7-16.
- Kondo K, Gu ZJ, Na HY and Xia L (1986). A cytological study of *Camellia reticulata* and its closely related species in Yunnan, China. *Kromosomo II* 43-44: 1405-1419.
- Kumar MT (2002). Assessment of genetic diversity of tea (*Camellia sinensis* (L.) O. Kuntze) by inter-simple sequence repeat polymerase chain reaction. *Euphytica* 128: 307-315.
- Liu LQ and Gu ZJ (2011). Genomic *in situ* hybridization identifies genome donors of *Camellia reticulata* (Theaceae). *Plant Sci.* 180: 554-559.
- Luo ZB, Zhuang GQ, Ma DW and Chen F (2010). Genetic diversity of *Pogonatherum paniceum* (Lam.) Hack in Southwest China revealed by ISSR. *Afr. J. Biotechnol.* 9: 1416-1422.
- Ming TL (1998). The Classification, Differentiation and Distribution of the Genus *Camellia* Sect. *Camellia*. *Acta Bot. Yunanica* 20: 127-148.
- Ming TL (2000). Monograph of the Genus *Camellia*. Yunnan Science and Technology Press, Kunming.
- Nei M (1973). Analysis of gene diversity in subdivided populations. *P. Natl. Acad. Sci. U. S. A.* 70: 3321-3323.
- Nybom H (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Mol. Ecol.* 13: 1143-1155.
- Tang S, Bin X, Wang L and Zhong Y (2006). Genetic diversity and population structure of yellow camellia (*Camellia nitidissima*) in China as revealed by RAPD and AFLP markers. *Biochem. Genet.* 44: 449-461.
- Thomas J, Vijayan D, Joshi SD, Joseph LS, et al. (2006). Genetic integrity of somaclonal variants in tea (*Camellia sinensis* (L.) O. Kuntze) as revealed by inter simple sequence repeats. *J. Biotechnol.* 123: 149-154.
- Wang BY (2003). The Population Genetics in *Paeonia delevayi*. Master's thesis, Yunnan University, Kunming.
- Wang G (2010). Genetic diversity of ancient male ginkgo trees by ISSR analysis. *J. Beijing Forest. U.* 32: 39-45.
- Wendel JF and Parks CR (1985). Genetic diversity and population structure in *Camellia japonica* L. (Theaceae). *Am. J.*

- Bot.* 72: 52-65.
- Wu KS, Jones R, Danneberger L and Scolnik PA (1994). Detection of microsatellite polymorphisms without cloning. *Nucleic Acids Res.* 22: 3257-3258.
- Xia LF, Gu ZJ, Wang ZL, Xiao TJ, et al. (1994). Dawn on the origin of *Camellia reticulata* - the new discovery of its wild diploid in Jinshajiang valley. *Acta Bot. Yunnanica* 16: 255-262.
- Xiao TJ, Gu ZJ and Xia LF (1993). A study of meiosis of 9 species in genus *Camellia*. *Acta Bot. Sin.* 15: 167-172.
- Yeh FC, Yang R, Boyle TJ, Ye Z, et al (2000). PopGene32, Microsoft Windows-Based Freeware for Population Genetic Analysis. Version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton.
- Zietkiewicz E, Rafalski A and Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.
- Zou YQ, Ge S and Wang XD (2001). Molecular Marker in Plant Systematics and Evolution. Science Press, Beijing, 112.