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Full Length Research Paper

Genetic diversity and molecular discrimination of wild tea plants from Yunnan Province based on inter-simple sequence repeats (ISSR) markers

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To efficiently assess and discriminate wild tea germplasms, inter-simple sequence repeats (ISSR) were used to determine genetic relationships among 40 wild tea plants. A total of 275 bands were generated with 15 ISSR primers, of which 274 (99.6%) were polymorphic. The mean genetic similarity coefficient, the mean Nei's gene diversity (*h*) and the mean Shannon's information index (*I*) of tea cultivars were 0.4180, 0.3797 and 0.5586, respectively. This suggested that the genetic diversity of wild tea trees and the genetic base were very wide. Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis showed clear genetic relationships among these germplasms, and the major clusters were related to known pedigree relationships. Besides, from the bands amplified, there were three independent ways to identify the tea varieties, such as unique ISSR markers, unique band patterns and a combination of the band patterns provided by different primers. Finally, the ISSR fingerprints of 40 tea germplasms were constructed by the combination of the band patterns. This research indicated that ISSR markers were very effective in differentiating the wild tea varieties, too.

Key words: Wild tea plants, inter-simple sequence repeats (ISSR), molecular discrimination, genetic diversity.

INTRODUCTION

Wild tea germplasm resources are mainly distributed in the south and southwest of China, and are especially abundant in Yunnan province of China and northeast India and Cambodia (Sharma et al., 2010). At present, people have found wild, transitional and cultivated *Camellia sinensis* (L.) O. Kunzte in Yunnan province. The systematic-and-comparative study on *C. sinensis* (L.) O. Kunzte showed that the morphological characters and genetic base of tea germplasm resources showed continuous and gradual change from wild to cultivated, presenting relatively abundant diversity (Sharma et al.,2010). Because of its untouchable existing environment, not only that tea germplasms have been keeping aboriginal characters in evolution, also its gene constitutes are relatively pure, so they are living fossils for studying the origin and evolution of tea plants.

In the recent thirty years, more than thirty new tea varieties were bred in virtue of using "Yunnan tea cultivars" from tea germplasms as cross-bred parents, including twenty-three "Qianmei" cultivars and seven "Pingyun" cultivars, which had been authorized as countrywide and provincial well-bred (Ye and Ge, 1997). With the advances in tea breeding technology, the technology of sexual hybridization breeding, mutagenic

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Abbreviations: ISSR, Inter-simple sequence repeats; **SCAR,** sequence characterized amplified regions; **RAPD,** randomly amplified polymorphic DNA; **AFLP,** amplified fragment length polymorphisms; **RFLP,** restriction fragment length polymorphism; **CTAB,** cetyltrimethylammonium bromide.

breeding, polyploid breeding were developed, and a series of good tea varieties, which can adapt to different environmental conditions, cultivation technology, rearing technology and for other uses, were obtained. The objectives of tea variety breeding are to identify and create genetic variability, to assemble the productive genotypes, and to match the genotype to the appropriate environment. Therefore, the characterization and evaluation of genetic diversity of tea varieties are important for long-term improvement in tea yield, quality and resistance to diseases. Many approaches have been reported for assessing genetic diversity in tea germplasm resources (Sealy, 1958; Du et al., 1990; Chen et al., 2000).

Traditional approaches for measuring genetic diversity depend on the ability to resolve differences in morphological characters of tea germplasms. Because of its cross-pollinating nature, each cultivar is a high heterogeneous genotype with a long generation period. It must be evaluated in the course of several years, so morphological-agronomic characterization of tea germplasm is time consuming and labor intensive. In addition, morphological characters were controlled by minor polygenic traits and were affected easily by environmental effects and growth practices. These features make the identification of tea species very difficult. Currently, other methods for varietal identification of tea germplasms are based on isozymes and protein analysis by electrophoresis. However, the effectiveness of protein analysis for varietal identification is limited, since protein polymorphism is not so high (Lu et al., 1992). Thus, closely related varieties may be indistinguishable. In contrast to protein markers, DNA-based markers are unaffected by environment, detectable at all stages of development, and ubiquitous in number covering the entire genome. They also have the advantage of being abundant, highly polymorphic and analytically simple. Therefore, they play an increasingly important role in the identification and measurement of genetic diversity of plants (Zhou et al., 2004; Eshghi and Akhundova, 2010; Barakat et al., 2010; Abbasi et al., 2010). In the past ten years, genetic identities and relationships of tea plants have been determined using randomly amplified polymorphic DNA (RAPD) (Wachira et al., 1997; Kaundun et al., 2000; Chen and Yamaguchi, 2002, 2005), amplified fragment length polymorphisms (AFLP) (Mishra and S Sen-Mandi, 2004; Balasaravanan et al., 2003; Sharma et al., 2010) and restriction fragment length polymorphism (RFLP) (Matsumoto et al., 2002; Devarumath et al., 2002). Since 1994, a molecular marker technique called inter simple sequence repeat (ISSR) has been available (Zietkiewicz et al., 1994; Yu et al., 2012). ISSRs have been used to characterize genetic diversity in tea species (Lin et al., 2007; Yao et al., 2007; Liu et al., 2009). While these studies focused on identification and genetic variation among different species of clonal cultivated varieties (Devarumath et al., 2002), very little is known about the genetic variation in wild tea resources. The identification and estimation of genetic relationships of wild tea germplasm resources is important because it provides the basic information for breeding programs and can ultimately have a direct effect on tea production and quality.

So, application of DNA marker technology in molecular identification and diversity analysis of gene resources in wild tea germplasms has very important practical value and theoretical significance in germplasm identification, genetic improvement, germplasm protection, core germplasm construction, and importantly, agronomic gene orientation. In this work, our objectives were to assess the genetic diversity and construct the fingerprinting of wild tea germplasm resources in Yunnan of China using ISSR markers to resolve the problem of naming of tea germplasms, for instance, synonym and homonym, to understand the genetic base of wild tea trees, and to provide the theoretical base for genetic improvement, selection of cross-bred parents and property rights protection of tea varieties.

MATERIALS AND METHODS

Forty wild tea germplasm lines, described in Table 1, were used. Plant materials from the China National Germplasm Tea Repositories (CNGTR) at the Tea Research Institute of Yunnan Academy of Agricultural Sciences (TRIYAAS), were sampled (Table 1) using ISSR analysis during 2011. These materials were taken at minimum intervals of 5 m from each other to avoid confusion of each species.

DNA isolation

Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Liu et al., 2006). Obtained DNA pellet was washed three times with 70% ethanol, vacuum dried and dissolved in 100 µl ddH_2O after treating with 5 µl of RNaseA (10 mg/ml), the quantity and quality of the DNA was checked with spectrophotometer (Genesys 10UV, USA) and agarose gel (0.8%) electrophoresis after digestion with RNase. Absorbance ratio between 260 and 280 nm was computed and the quality of the genomic DNA was confirmed. Final sample was stored at 4°C for downstream applications.

ISSR amplification, separation and visualization

ISSR primers (synthesized by Shanghai Bioasia Technology Co. Ltd., China) were synthesized based on di- and tri-nucleotide repeats (GA, GT, CT and GTC) as a core sequence with a *T*m value range of 40.0 to 58.0. Screening was carried out with 40 primers. Out of which, 15 primers which gave clear banding pattern were used for confirmatory studies (Table 2). Polymerase chain reaction (PCR) reactions (Liu et al., 2006) were carried out in a volume of 10 µl including 40 ng of total DNA, 10 × PCR buffer (200 mmol/L Tris-HCl pH 8.4, 500 mmol/L KCl), 2.0 mmol/L MgC_{l2}, 0.20 mmol/L of each dNTP, 4 pmol/L of each primer and 0.5 U of *Taq* DNA polymerase. The optimum annealing temperature was determined for each primer. Amplification was carried out in a programmable peltier thermo cycler PTC 200 (MJ Research, USA). Amplification protocol includes initial denaturation for 5 min at 94°C followed by 39 successive cycles of 60 s denaturation at 94°C,

Table 1. Tea varieties analyzed in this study.

S/N	Variety	Origins	Scientific name	Type
1	Heitiaozicha	Changning, Yunnan	*C. sinensis* var assamica	Wild
2	Niuzhaidachashu	Yanjin, Yunnan	*C. sinensis* arborescens	Wild
3	Huangniheyecha	Fuyuan, Yunnan	*C. irrawadiensis*	Wild
4	Danuocha	Chuxiong, Yunnan	*C. atrothea*	Wild
5	Gejiedaye	Jinggu, Yunnan	*C. sinensis* (L.) O. kunzte	Wild
6	Xinhuakucha	Tengchong, Yunnan	*C .sinensis* var assamica	Wild
7	Maandacha 1	Weixin, Yunnan	*C. sinensis* arborescens	Wild
8	Yangchajieyecha 1	Yuanjiang, Yunnan	*C. taliensis*	Wild
9	Shuixiedashucha	Yongping, Yunnan	*C. taliensis*	Wild
10	Longdaoyecha-1	Ruili, Yunnan	*C. taliensis*	Wild
11	Mangbingdashancha 2	Zhenkang, Yunnan	*C. sinensis*	Wild
12	Denggadaheicha 2	Ruili, Yunnan	C. sinensis var assamica	Wild
13	Tujiecha	Nanhua, Yunnan	*C. atrothea*	Wild
14	Youdianbaohongcha	Changning, Yunnan	*C. irrawadiensis*	Wild
15	Longshandashancha	Longling, Yunnan	*C. irrawadiensis*	Wild
16	Shangyunbaohongcha	Tengchong, Yunnan	*C. sinensis* var assamica	Wild
17	Mangshuibaohongcha	Changning, Yunnan	*C. taliensis*	Wild
18	Badadachashu	Menghai, Yunnan	*C. taliensis*	Wild
19	Dapingdayecha	Yuanyang, Yunnan	*C.sinensis* var assamica	Wild
20	Bazhaisecha	Maguan, Yunnan	*C. sinensis* var dehungensis	Wild
21	Hehuacunshancha	Lianghe, Yunnan	*C. taliensis*	Wild
22	Mengsongdachashu	Menghai, Yunnan	*C. taliensis*	Wild
23	Goujieqingcha	Changning, Yunnan	*C. gymnogyna*	Wild
24	Yuanjiangyecha	Yuanjiang, Yunnan	*C. atrothea*	Wild
25	Tongchangkucha	Jinping, Yunnan	*C.sinensis* var kucha	Wild
26	Chahedacha	Fuyuan, Yunnan	*C. tachangensis*	Wild
27	Gaoliangyecha	Shizong, Yunnan	*C.kwangnanica*	Wild
28	Dachangdashancha	Lianghe, Yunnan	*C. taliensis*	Wild
29	Xiangzhuqingyecha	Fengqing, Yunnan	C.*taliensis*	Wild
30	Nuoliangqunti	Changyuan, Yunnan	*C. taliensis*	Wild
31	Mengwenyecha	Luxi, Yunnan	*C. taliensis*	Wild
32	Jiangdonghuangyecha	Luxi, Yunnan	*C. taliensis*	Wild
33	Yongbaomenggaocha	Yunxian, Yunnan	*C. taliensis*	Wild
34	Fengshandashancha	Fengqing, Yunnan	*C. taliensis*	Wild
35	Longchuanyecha	Longchuan, Yunnan	*C. taliensis*	Wild
36	Qinglongdashucha	Daguan, Yunnan	*C. gymnogynoides*	Wild
37	Nanwangcha	Mengla, Yunnan	*C. sinensis* (L.) O.kunzte	Wild
38	Jingguquntі	Jinggu, Yunnan	*C. sinensis* (L.)O.kunzte	Wild
39	Shilicha	Jinping, Yunnan	*C. sinensis* (L.)O.kunzte	Wild
40	Mangbingdashancha 1	Zhenkang, Yunnan	*C. taliensis* (L.)O.kunzte	Wild

temperature of 150°C with 1 × TBE buffer (100 mmol/L Trisborate, annealing for 30 s at respective *T*m values of the selected primers and 2 min elongation at 72°C. Final elongation was performed for 7 min at 72°C. Amplifications were checked by separating on 6% polymeric acrylamide gel electrophoresis for 4 h at a constant pH 8.0, 2 mmol/L EDTA), in 2000 ml of distilled water) running buffer. Finally, the gel was silver-stained, visualized under ultraviolet light, photographed and documented. The analysis was performed for all the samples at least three times with each selected primers. Molecular sizes of the amplified fragments were roughly estimated using a 3000 bp ladder (Shanghai Bioasia Technology Co. Ltd., China).

Data analysis

Scanned gel image was analyzed using DNA marker ladder (Shanghai Bioasia Technology Co. Ltd., China) for fragment length calibration. Only distinct, reproducible, well-resolved amplified fragments were scored manually for the band presence (1) and

Table 2. The name and sequence of primers and amplified results.

Primer code	Sequence of primer	Tm (°C)	Tn (°C)	Number of bands scored	Number of polymorphic bands	Percentage of polymorphic bands
S807	$(AG)_8T$	52.18	56	13	13	100
S808	$(AG)_8C$	54.59	56	12	12	100
S810	$(GA)_8T$	52.18	53	22	22	100
S811	$(GA)_8C$	54.59	54	12	12	100
S835	$(AG)_8YC$	56.16	57	17	16	94.12
S836	$(AG)_8YA$	53.88	54	14	14	100
S840	$(GA)_8YT$	53.88	54	12	12	100
S841	$(GA)_8YC$	56.16	55	22	22	100
S856	$(AC)_8YA$	53.88	56	18	18	100
S890	$(CT)_8A$	53.80	55	15	15	100
ISSR2	$(AG)_8(CT)A$	55.41	56	28	28	100
ISSR3	$(GA)_8(CT)T$	55.41	58	29	29	100
ISSR4	$(TC)_8(AG)T$	55.41	58	23	23	100
ISSR5	$(TC)_8(AG)G$	57.56	56	25	25	100
ISSR8	$(AC)_8T$	52.18	52	13	13	100
Average		54.53	55.3	18.3	18.2	99.6

Tm, Melting temperature; Tn, annealing temperature; Y = (C, G).

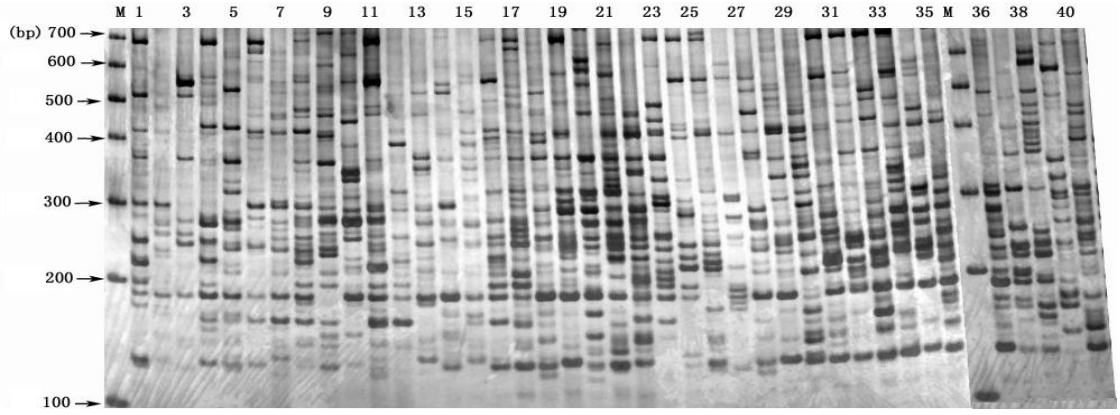

Figure 1. Amplification electrophoresis parameter with primer S835.

absence (0) for each of the ISSR markers, and different bands of different primers were used for constructing the fingerprinting of tea germplasms. Nei's gene diversity (Nei, 1973), Shannon's information index (Lewontin, 1972), genetic similarity and genetic distance estimated by Nei's coefficient between pairs and dendrograms based on the unweighed pair group method with arithmetical mean (UPGMA) were analyzed using Popgene software, version 3.5 (Rohlf, 2000).

RESULTS

Genetic variation and cluster analysis of tea varieties

Size of the polymorphic amplified fragments ranged from 100 to 3000 bp (Figure 1). The banding pattern of these primers on an average showed about 99.6% polymorphism (Table 2). Amplified fragments from 0.1 to 1.2 kb were present in almost all accessions (Figure 1). Using the data from all PCR amplification bands shown by 15 ISSR markers, the genetic similarity matrix among all sources used in the present work was obtained by multivariate analysis using Nei's coefficient. Similarity coefficients ranged from 0.2823 to 0.7094 with an average of 0.4180. The highest genetic similarity coefficient (0.7094) was found between "Jiangdonghuangyecha" and "Gejiedayecha", which indicates that they are closely related. The lowest genetic similarity coefficient (0.2823) was found between

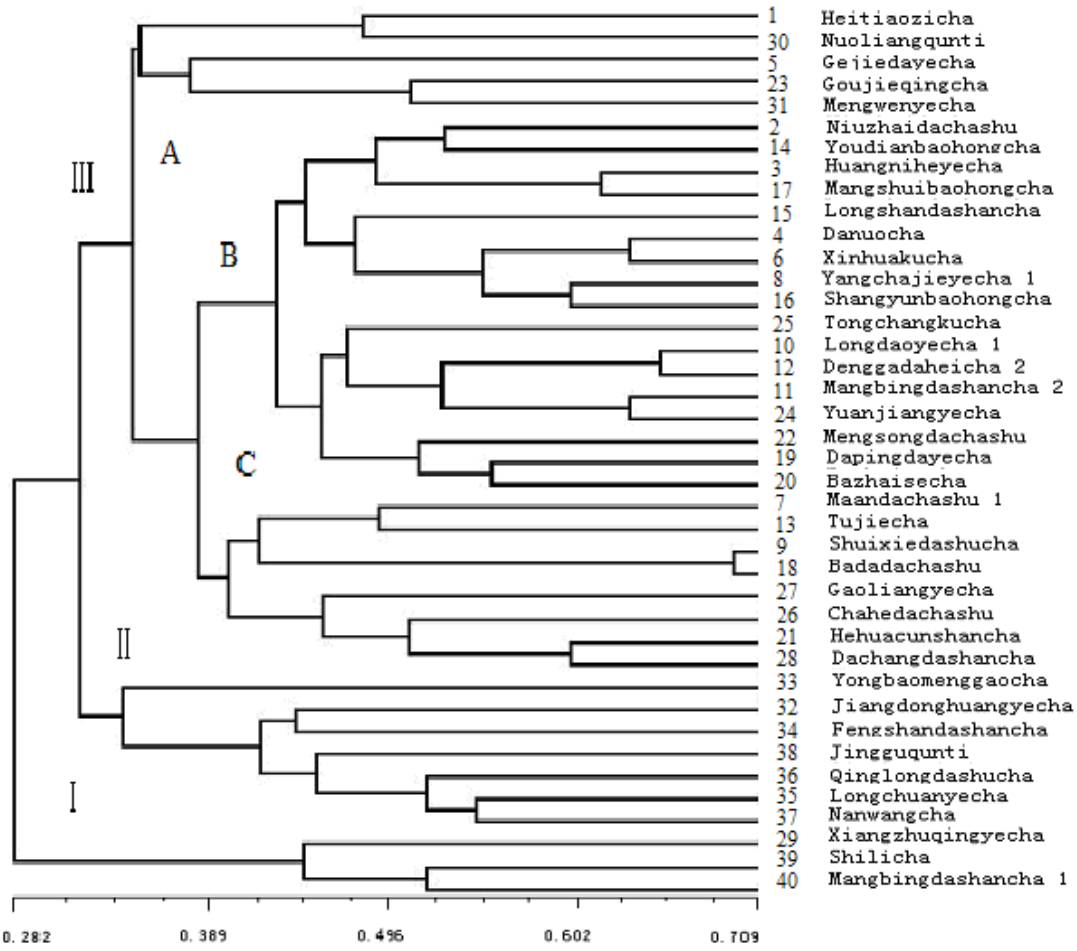

Figure 2. Dendrogram illustrating the phylogenetic relationship among 40 tea germplasms based on ISSR markers.

"Badadachashu" and "Shuixiedashucha", which indicated that they were relatively distant in relationship. The mean Nei's genetic diversity and Shannon's information index among tea varieties were 0.3797 and 0.5586, respectively, indicating that genetic distances among wild tea accessions were bigger, and their genetic base is wider.

A dendrogram was obtained by UPGMA method using the total number of amplified ISSR fragments. The dendrogram consisted of three main clusters (Figure 2), forming three distinct tea varieties groups (similarity coefficient value is equal to 0.3350). UPGMA cluster analysis showed clear genetic relationships among the 40 tea varieties and the major clusters were related to known pedigree relationships. "Xiangzhuqingyecha", formed the A subgroup, showing that it had a remote relationship with the B and C subgroup. The B subgroup contained seventeen varieties, which could be divided into two subgroups again. The C subgroup consisted of eight tea germplasms, containing "Maandachashu 1", "Tujiecha", "Shuixiedashucha", "Badadachashu", "Gaoliangyecha", "Chahedacha", "Hehuacunshancha" and "Dachangdashancha" in their pedigree. The dendrogram also indicated that mostly wild tea germplasms from the same area could not be grouped together except for "Longdaoyecha 1" and "Denggadaheicha 2" from Ruili county of Yunnan Province. To sum up, in this study, all wild tea varieties had rather high genetic diversity. The main reason is that tea plant itself not only has rather high heterozygosity, but is the cross-pollinating plant. In addition, long-time transfer growth in new environment also has some effect on genetic information of tea trees. Consequently, abundant genetic diversity of wild tea trees results from comprehensive result of all kinds of factors. Further research needs to be carried out on this.

Levels of polymorphism and molecular identification of tea germplasms revealed by ISSR-PCR markers

A total of 275 reliable fragments were obtained. The number of fragments per primer ranged from 12 to 29 with the average number of bands per primer being 18.3; among them, 274 bands were polymorphic with a ratio

Table 3. Specific markers that can be used for discrimination of cultivars.

Discriminable variety	Specific marker	Criteria
Denggadaheicha-2	S841-900 bp	Presence
Tujiecha	S841-1000 bp	Presence
Heitiaozicha	S841-300 bp	Absence
Yangchajieyecha 1	ISSR5-300 bp	Absence
Gejiedaye	S856-300 bp, S841-500 bp	Presence
	ISSR4-500 bp, S810-300 bp	Absence
Dachangdashancha	S811-400 bp, S841-300 bp	Absence
Xiangzhuqingyecha	S841-500 bp ,S836-300 bp	Presence
	ISSR5-400 bp	Absence
Mangbingdashancha 1	S841-500 bp, S836-400 bp	Presence
Hehuacunshancha	S841-200 bp, S836-300 bp	Absence
Jingguqunti	ISSR5-900 bp	Absence
Maandachashu	S810-400 bp	Absence
Longshandashancha	ISSR4-700 bp, ISSR3-500 bp	Absence
Danuocha	ISSR4-600 bp	Absence
Shangyunbaohongcha	ISSR3-300 bp	Absence
Shuixiedashucha	S811-400 bp	Absence

of 99.6%. The number of polymorphic bands per primer was 18.2. The results of PCR amplification are given in Tables 2 and 3 and Figure 1.

Some polymorphic bands produced by ISSR primers were unique and could be used to discriminate the tea varieties. In this study, fifteen tea varieties could be distinguished by using twenty-four specific ISSR markers, which showed either presence or absence of bands generated by nine primers (Table 3). By amplification of ISSR markers, different bands of the primer were generated because of the difference in tea varieties, for example, amplified bands from the primer S811 can be used for distinguishing twelve tea germplasms (Table 4), and the combination of the different bands can be used to distinguish tea varieties. With this, all tea varieties analyzed could be effectively distinguished by a combination of the band patterns provided by different primers (Table 4). The fingerprinting of 40 wild tea varieties could be constructed with twenty-two bands from four different primers, and each tea variety has an exclusive fingerprinting, respectively (Figure 3). Furthermore, each tea variety could be easily distinguished with one another.

DISCUSSION

Previously, morphological characters (Sealy, 1958; Chang, 1984; Chen et al., 2000), esterase isozymes (Lu et al., 1992) and biochemical components (Du et al., 1990) were used to discriminate tea varieties. However, they were found not to be reproducible because of different developing stages, growing environments, cultivation conditions, seasons and even experimental conditions. RAPD also had been proven to be quite useful in woody plant DNA diversity, genetic relationships and identification studies (Chen and Yamaguchi, 2002, 2005). However, the genetic diversity, relationship and molecular identification of tea genetic resources of Yunnan Province, China using ISSR markers has hardly been reported yet. ISSR markers provide a convenient and rapid tool to differentiate closely related individuals at the inter-specific level because of high stability, good repeatability, speed and ease in handling (Wolfe and Liston, 1998).

In this paper, fifteen ISSR primers were used for constructing fingerprinting and estimating genetic diversity of tea germplasms. Using these primers, 275 discernible DNA fragments were generated and 274 of them were polymorphic. Twenty-four of the polymorphic bands could be considered tea germplasm variety-specific markers and fifteen tea varieties could be distinguished (Yao et al., 2007; Lin et al., 2007). These putative variety-specific ISSR markers could be transformed to sequence characterized amplification regions (SCARs) after sequencing and designing primer pairs to develop markers specific for varieties. In addition, the tea varieties selected could be unambiguously distinguished by three independent ways: Unique ISSR markers, unique band patterns and a combination of the band patterns provided by different primers, indicating that ISSRs were a very effective tool and robust method in the differentiation of tea varieties. The present study revealed relatively higher polymorphism (99.6%) in wild

Table 4. The name of tea varieties, ISSR band patterns and their identification capacity.

S/N	Variety	ISSR3	S811	ISSR2	S835	S811	ISSR3	ISSR3,	SSR3,
1	Heitiaozicha	[1]A	A	A	A	[2]+	+	+	+
2	Niuzhaidachashu	B	B	B	B	[3]-	+	+	+
3	Huangniheyecha	C	C	C	C	-	-	-	+
4	Danuocha	D	C	D	D	-	-	-	+
5	Gejiedaye	E	D	E	E	-	+	+	+
6	Xinhuakucha	F	E	F	F	-	+	+	+
7	Maandacha 1	G	F	G	E	-	+	+	+
8	Yangchajieyecha 1	H	D	H	G	-	+	+	+
9	Shuixiedashucha	I	G	I	E	+	+	+	+
10	Longdaoyecha-1	J	C	J	H	-	-	+	+
11	Mangbingdashancha 2	J	H	K	E	+	+	+	+
12	Denggadaheicha 2	K	F	B	I	-	-	-	+
13	Tujiecha	K	C	D	J	-	-	-	+
14	Youdianbaohongcha	K	B	L	B	-	-	+	+
15	Longshandashancha	K	I	M	C	+	+	+	+
16	Shangyunbaohongcha	L	F	N	B	-	+	+	+
17	Mangshuibaohongcha	M	B	O	K	-	+	+	+
18	Badadachashu	N	J	D	L	+	+	+	+
19	Dapingdayecha	N	K	C	M	-	-	-	+
20	Bazhaisecha	O	D	P	E	-	+	+	+
21	Hehuacunshancha	N	B	Q	N	-	-	+	+
22	Mengsongdachashu	P	L	C	O	+	+	+	+
23	Goujieqingcha	K	E	R	P	-	-	+	+
24	Yuanjiangyecha	Q	B	S	Q	-	+	+	+
25	Tongchangkucha	K	K	T	R	-	-	-	+
26	Chahedacha	N	E	U	I	-	-	+	+
27	Gaoliangyecha	R	B	M	S	-	+	+	+
28	Dachangdashancha	C	M	V	B	+	+	+	+
29	Xiangzhuqingyecha	S	N	W	T	+	+	+	+
30	Nuoliangqunti	K	O	X	U	+	+	+	+
31	Mengwenyecha	K	I	Y	I	-	-	+	+
32	Jiangdonghuangyecha	N	P	Z	I	+	+	+	+
33	Yongbaomenggaocha	K	E	Y	V	-	-	-	+
34	Fengshandashancha	K	D	AB	K	-	-	+	+
35	Longchuanyecha	K	B	BC	W	-	-	+	+
36	Qinglongdashucha	N	B	CD	X	-	-	+	+
37	Nanwangcha	J	Q	DE	Y	+	+	+	+
38	Jingguqunti	T	B	EF	Z	-	+	+	+
39	Shilicha	C	B	FG	AB	-	-	+	+
40	Mangbingdashancha 1	U	B	GH	BC	-	+	+	+
Total		[4]21	17	33	28	12	24	33	40

[1] Letters represent the band patterns; [2] "+" shows that the germplasm could be discriminated by the primer(s); [3] "-"shows that the germplasm could not be discriminated by the primer(s); [4] number shows that the number of the germplasm could be discriminated by the primer(s).

tea germplasms based on the statistical data. The mean genetic similarity coefficient was 0.4180, indicating that genetic diversity of tea varieties was very high, pedigree relationships was very remote, and the genetic base was very wide. The dendrogram obtained by the UPGMA method consisted of three major clusters (Figure 2): Cluster I, including three tea varieties; cluster II, including seven tea varieties; cluster III, including thirty tea

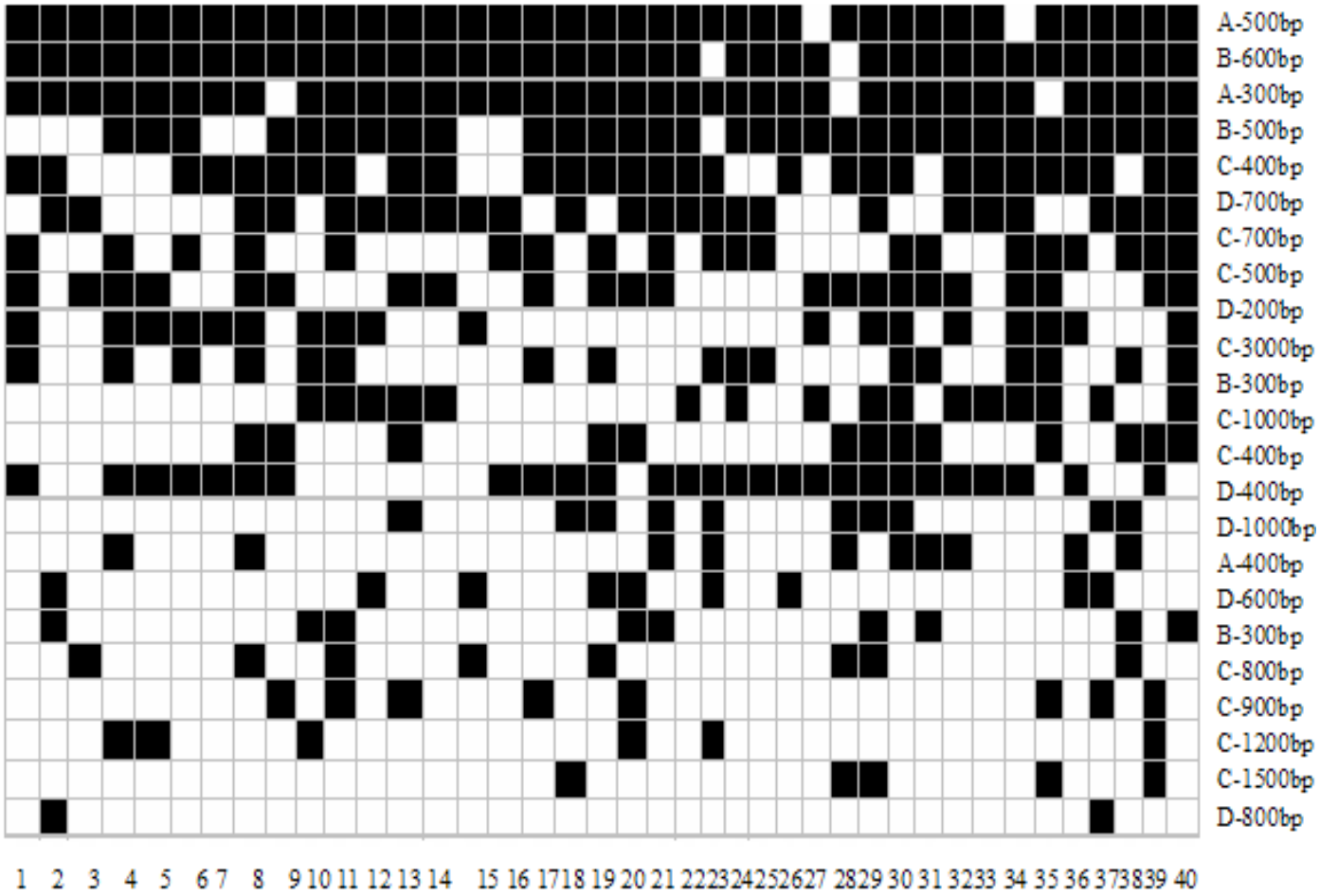

Figure 3. DNA Fingerprinting among 40 wild tea plants based on ISSR markers. For germplasms see Table 1 for details. Shaded blocks represent the presence of bands. Letters (A, B, C and D) represent different primers (S811, ISSR3, S835 and ISSR2), respectively.

varieties. The clustering result indicated that the genetic difference among tea germplasms was very large. A plausible explanation for this result is that they have rather complex genetic background. Their geographical isolation may cause the distinctness in their clustering behavior, that is to say, gene exchange with external tea varieties did not occur and hence the remote relationships was maintained between them (Yao et al., 2007; Liu et al., 2009). From cluster analysis among forty tea varieties, we also found that most tea varieties did not cluster together according to their regions of origin, it was possibly caused by discontinuous variation of long-term hybridization, cross-pollination and effect of natural environment (Yao et al., 2007; Lin et al., 2007).

In conclusion, ISSRs can be successfully employed to assess the level of polymorphism and diversity in wild tea varieties. The above results obtained by ISSR analysis of tea varieties may provide useful information for molecular identification, pedigree analysis, genetic improvement, germplasm conservation and construction of core collections in *C. sinensis (L.)* O. Kunzte.

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REFERENCES

Abbasi FM, Ahmad H, Perveen F, Inamullah, Sajid M, Brar DS (2010). Assesment of genomic relationship between Oryza sativa and Oryza australiensis. Afr. J. Biotechnol., 9(12): 1312-1316.

Balasaravanan T, Pius PK, Kumar RR, Muralecdharan N, Shusany AK (2003). Genetic diversity among south Indian tea germplasm (*Camellia Sinensis, C.assamica and C.assamica spp.lasiocalyx*) using AFLP markers. Plant Sci., I65: 365-372.

Barakat MN, Abdel Fattah RS, Badr M, El-Torky MG (2010). In vitro mutagenesis and identification of new variants via RAPD markers for improving Chrysanthemum morifolium. Afr. J. Agric. Res., 5(8):748-757.

Chang H T (1984). A revision of the tea resource plants. Acta Sci. Nat. Univ. Sunyatseni, (S), 23(1): 1-12.

Chen L, Yamaguchi S (2002). Genetic diversity and phylogeny of tea plant (*Camellia sinensis*) and its related species and varieties in the section *Thea* genus *Camellia* determined by randomly amplified polymorphic DNA analysis. J. Horticult. Sci. Biotechnol., 77(6): 729-732.

Chen L, Yamaguchi S (2005). RAPD markers for discriminating tea germplasms on the inter-specific level in China. Plant Breeding, 124(4): 404-409.

Chen L, Yu F L, Tong Q Q (2000). Discussions on phylogenetic classification and evolution of sect Thea. Tea Sci., 20(2): 89-94.

Du Q Z, Li M J, Liu W H, Wang H S(1990). Chemical and numerical taxonomies of section Thea plants. Tea Sci., 10: 1-12.

Eshghi R, Akhundova E (2010). Genetic diversity in hulless barley based on agromorphological traits and RAPD markers and comparison with storage protein analysis. Afr. J. Agri. Res., 5 (1): 97-107.

Kaundun SS, Zhyvoloup A, Park YG (2000). Evaluation of the genetic diversity among elite tea (*Camellia sinensis var.sinensis*) accessions using RAPD markers. Euphytica, 115: 7-16.

Lewontin RC (1972). Apportionment of human diversity. J. Evol. Biol., 6: 381-398.

Liu BY, Wang LY, Li YY, He W, Zhou J, Wang PS, Cheng H (2009). Genetic diversity in tea germplasms as revealed by ISSR markers[J]. Indian J. Agric. Sci., 79(9): 715-721.

Liu BY, Wang PS, Zhong HJ, Ji PZ, XuM, Cheng ZQ (2006). The ISSR-PCR reaction system's establishment about Yunnan section Thea plant. J. Yunnan Agric. Univ (sup.)., (3): 21-25.

Lin ZH, Chen RH, Chen CS (2007). Preliminary application of ISSR markers in the genetic relationship analysis of tea plants. J Tea Sci., 27(1): 45-50.

Lu C Y, Liu W H, Li M J(1992). Relationship between the evolutionary relatives and the variation of esterase isozymes in tea plant. J Tea Sci., 12: 15-20.

Mishra RK, Sen-Mandi S (2004). Genetic diversity estimates for Darjeeling tea clones based on amplified fragment length polymorphism markers. J Tea Sci., 24(2): 86-92.

Matsumoto S, Kiriiwa Y, Yakeda Y (2002). Diferentiation of Japanese green tea cultivars as revealed by RFLP analysis of phenylalanine ammonialyase DNA. Theor. Appl. Genet., 104: 998-1002.

Nei M (1973). Analysis of gene diversity in subdivided populations. Proc Natl. Acad. Sci., USA, 70: 3321-3323.

Rohlf FJ (2000). NTSYSpc: Numerical taxonomy and nadtivariate analysis system.Version 2.1. Exeter software Applied Biestatistics Inc., New York. USA.

Sharma RK, Negi MS, Sharma S, Bhardwaj P, Kumar R, Bhattachrya E, Tripathi SB, Vijayan D, Baruah A R, Das SC, Bera B, Rajkumar R, Thomas J, Sud RK, Muraleedharan N, Hazarika M, Lakshmikumaran M, Raina SN, Ahuja PS(2010). AFLP-Based Genetic Diversity Assessment of Commercially Important Tea Germplasm in India. Biochem. Genet., 48: 549-564.

Sealy JR (1958). A revision of the genus *Camellia*. Royal Horticultural Society, London. pp. 1-239

Wachira FN, Powell W, Waugh R (l997). An assessment of genetic diversity among *Camellia sinensis* L.(cultivated tea) and its wild relatives based on randomly amplified polymorphic DNA and organelle-specific STS. Heredity, 78: 603-611.

Wolfe A D, Liston A (1998). Contributions of the polymerase chain reaction to plant systematics. In: Soltis D E, Soltis P S, Doyle J J, eds. Molecular Systematics of Plants II: DNA Sequencing. Kluwer, New York, 43-86.

Yao MZ, Chen L, Wang XC (2007). Genetic diversity and relationship of conal tea cultivars in china revealed by ISSRmarkers. Acta Agron. Sin, 33(4): 598-604.

Ye NX, Ge JC (1997). Actuality and prospect of the research of Fujian tea cultivar germplasm. J. Fujian Tea, (1): 42-45.

Yu J, Jing ZB, Cheng JM (2012). Genetic diversity of Stipa bungeana populations in the Loess Plateau of China using inter-simple sequence repeat (ISSR) markers. Afr. J. Biotechnol., 11(34): 8414-8416.

Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176-183.

Zhou YQ, Jing JZ, Li ZY, Zhang BH, Jia JF (2004). Assessment of genetic diversity of *Rehmannia glutinosa* Libosh.f. *hueichingensis* (Chao et Schih) Hsiao germplasm detected by RAPDs and ISSRs. Heriditas, 26(6): 922-928.