Biochemical Genetics (2019) 57:555–570
https://doi.org/10.1007/s10528-019-09909-1

ORIGINAL ARTICLE

Analysis of Genetic Diversity and Development of a SCAR Marker for Green Tea (*Camellia sinensis*) Cultivars in Zhejiang Province: The Most Famous Green Tea-Producing Area in China

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Received: 9 July 2018 / Accepted: 28 January 2019 / Published online: 8 February 2019

Abstract

Camellia sinensis (L.) O. Kuntze is one of the most important non-alcoholic beverage crops in Asian and African countries. In recent years, many green tea cultivars have been released and played an important role in improving the production and quality of tea trees. The objectives of this study were to assess the genetic diversity of the eighteen main green tea cultivars in Zhejiang Province—the most famous green tea-producing area of China—using start codon-targeted (SCoT) markers and to develop a specific sequence-characterized amplified region (SCAR) marker for application in cultivar diagnosis. Thirty-one SCoT primers produced 264 loci, 226 of which were polymorphic. The genetic similarity coefficients among these green tea cultivars ranged from 0.587 to 0.814, indicating that a high level of genetic diversity was present. Both a UPGMA dendrogram and a PCoA plot grouped the tea cultivars into three groups. The partitioning of groups in the UPGMA and PCoA was similar, and much of the clustering was highly consistent with the classification of tea cultivars according to their genetic backgrounds. A unique SCoT band, SCoT4-1649, specific to the tea cultivar 'Yingshuang,' was transformed into a SCAR marker. This SCAR marker is highly useful for the identification and germplasm conservation of green tea cultivars.

Keywords Tea plant · Varieties · SCoT marker · Genetic diversity · SCAR marker

Yan-Xia Xu and Si-Yan Shen are contributed equally to this work.

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Introduction

The tea plant, *Camellia sinensis* (L.) O. Kuntze, is a perennial shrub plant in the genus *Camellia* of the family Theaceae. It has been one of the most widely consumed and oldest nonalcoholic beverage crops in Asian and African countries for more than 4000 years (Chen et al. 2007; Li et al. 2016; Yang and Liang 2014). Because it is rich in flavonoids, minerals, and trace elements, tea has numerous significant pharmacological effects on human health, including preventing low-density lipoprotein oxidation, enhancing immunity, and reducing the risk of cardiovascular disease, and cancer (Huang et al. 2016; Li et al. 2015; Xu et al. 2017; Zheng et al. 2014). As the world's largest tea-cultivating country, China has the largest number of tea plant accessions, most of which are cultivated in the southern and eastern provinces of China (Ahmed et al. 2014; Wang et al. 2016; Zhao et al. 2008). According to their different processing procedures, the main tea types of China are green tea, white tea, black tea, dark tea, yellow tea, and oolong tea (Yao et al. 2008, 2012). Zhejiang Province is the most famous green tea-producing area in China and worldwide (Lou and Sun 2013). According to the statistics of Zhejiang Province's Agriculture Department in 2016, the cultivated area of tea plants in Zhejiang was 1967 km^2, the agricultural output of tea was 15.5 billion Yuan, and the volume of green tea exports was 141,900 tons, which accounted for 52.38% of the total tea produced in China (Zhejiang Tea Industry Association 2017).

For a long time, the breeding of tea plants depended mostly on individual selection from natural populations and the hybrid progeny of uncontrolled pollination (Ma et al. 2010; Raina et al. 2012; Yao et al. 2008). However, understandings of genetic diversity and identification of varieties are important premises for modern tea plant-breeding programs. DNA molecular markers have the potential to reveal genetic diversity and to identify cultivars of tea germplasm. Recently, several different DNA markers have been used for genetic diversity, evolutionary relationship, linkage map, and cultivar identification in tea plants, such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR), and simple sequence repeat (SSR), etc (Mukhopadhyay et al. 2016).

Start codon-targeted (SCoT) polymorphism, a reliable gene-targeted marker technique, was developed based on the translation start codon (Collard and Mackill 2009). SCoT marker is correlated with functional genes and corresponding traits and requires no sequence information. Primers for the SCoT technique are designed based on the conserved region surrounding the translation initiation codon, ATG. Compared with other types of DNA molecular markers, such as RAPD, ISSR, and SSR, the SCoT technique is correlated with functional genes and corresponding traits, is more stable, and finds more polymorphisms. Over the past few years, SCoT markers have been popularly used in plant genetic diversity assessment and phylogenetic studies (Collard and Mackill 2009; Feng et al. 2015, 2016b; Guo et al. 2012; Luo et al. 2012, 2010; Xiong et al. 2011). In addition, sequence-characterized amplified region (SCAR) markers represent a specific,

defined genomic DNA fragment detected by PCR amplification using a pair of specific primers (Paran and Michelmore 1993). SCAR can be derived from RAPD (Correa et al. 2014; Cunha et al. 2016; Tigano et al. 2010), AFLP (Choi et al. 2008), ISSR (Kumar et al. 2018), and SCoT markers (Mulpuri et al. 2013; Rajesh et al. 2016). Compared with RAPDs, ISSRs, AFLPs, and similar markers, SCAR markers have been proven to be simpler and more reliable and have been widely used in plant identification at inter- and/or intraspecific levels (Lee et al. 2011; Marieschi et al. 2016).

In this study, SCoT markers were applied to examine the genetic diversity of the green tea cultivars in Zhejiang Province of China. Furthermore, a SCAR marker that could be used to detect tea cultivar, 'Yingshuang,' was developed based on a specific SCoT fragment.

Materials and Method

Plant Materials and DNA Extraction

A total of 18 green tea cultivars were collected, including the 14 main cultivars of Zhejiang Province ('Longjing 43' 'Longjing Changye,' 'Zhongcha 102,' 'Zhongcha 108,' 'Jiaming 1,' 'Fuding Dabaicha,' 'Maolv,' 'Yingshuang,' 'Chunyu 1,' 'Chunyu 2,' 'Zhenong 113,' 'Zhenong 117,' 'Jinfeng,' and 'Cuifeng') and four cultivars selected by our research institute ('Zhongcha 125,' 'Zhongcha 126,' 'Zhongcha 127' and 'Zhongcha 128'). Relevant information about sampled cultivars is shown in Table 1; morphological features of one bud and two tender leaves of samples are shown in Fig. 1. Further, 10 different individuals of 'Yingshuang' were used to validate the developed SCAR marker. All cultivars tested in this study were grown in the fields of the Tea Research Institute of the Chinese Academy of Agricultural Sciences in Hangzhou, China.

Total genomic DNA was isolated from fresh, young leaves of each sample using the Plant Genomic DNA Extraction kit (Shanghai Sangon Biological Engineering Technology and Service Co. Ltd., China) as described in previous studies (Yao et al. 2012). The integrity and quality of the genomic DNA were assessed using 0.8% agarose gel electrophoresis, and the DNA concentration was evaluated using a UV spectrometer.

SCoT-PCR

A total of 36 SCoT primers (synthesized by the Suzhou Hongxun Biotechnology Co. Ltd., China) following the previous study were used in this study (Collard and Mackill 2009). PCR was performed in 20 µl of PCR mixture solution that contained 1 µl of each primer (10 µM), 10 µl of 2×*EasyTaq* PCR SuperMix (Beijing TransGen Biotech Co., Ltd., China), 1 µl of genomic DNA template, and 8 µl of ddH_2O. PCR was performed with the following conditions: 94 °C for 5 min; 32 cycles of 94 °C for 50 s, 50–60 °C for 50 s (depending on the annealing temperature of each primer),

Table 1 List of tea cultivars included in the present study

Number	Cultivar name	Code	Voucher number	Location
1	*C. sinensis* 'Longjing 43'	Longjing 43	CS000755	Hangzhou, Zhejiang, China
2	*C. sinensis* 'Longjing Changye'	Longjing Changye	CS000467	Hangzhou, Zhejiang, China
3	*C. sinensis* 'Zhongcha 102'	Zhongcha 102	CS001756	Hangzhou, Zhejiang, China
4	*C. sinensis* 'Zhongcha 108'	Zhongcha 108	CS001881	Hangzhou, Zhejiang, China
5	*C. sinensis* 'Jiaming 1'	Jiaming 1	CS000474	Hangzhou, Zhejiang, China
6	*C. sinensis* 'Fuding Dabaicha'	Fuding Dabaicha	CS000273	Hangzhou, Zhejiang, China
7	*C. sinensis* 'Maolv'	Maolv	CS001739	Hangzhou, Zhejiang, China
8	*C. sinensis* 'Yingshuang'	Yingshuang	CS000505	Hangzhou, Zhejiang, China
9	*C. sinensis* 'Chunyu 1'	Chunyu 1	–	Hangzhou, Zhejiang, China
10	*C. sinensis* 'Chunyu 2'	Chunyu 2	–	Hangzhou, Zhejiang, China
11	*C. sinensis* 'Zhenong 113'	Zhenong 113	CS000512	Hangzhou, Zhejiang, China
12	*C. sinensis* 'Zhenong 117'	Zhenong 117	CS000514	Hangzhou, Zhejiang, China
13	*C. sinensis* 'Jinfeng'	Jinfeng	CS000511	Hangzhou, Zhejiang, China
14	*C. sinensis* 'Cuifeng'	Cuifeng	CS000517	Hangzhou, Zhejiang, China
15	*C. sinensis* 'Zhongcha 125'	Zhongcha 125	–	Hangzhou, Zhejiang, China
16	*C. sinensis* 'Zhongcha 126'	Zhongcha 126	–	Hangzhou, Zhejiang, China
17	*C. sinensis* 'Zhongcha 127'	Zhongcha 127	–	Hangzhou, Zhejiang, China
18	*C. sinensis* 'Zhongcha 128'	Zhongcha 128	–	Hangzhou, Zhejiang, China

and 72 °C for 1.5 min; and a final extension at 72 °C for 10 min. For SCoT marker profiling, PCR products were separated on 1.5% (W/V) agarose gels, followed by staining with GelStain (Beijing TransGen Biotech Co., Ltd., China) and photography using a Molecular Imager® Gel Doc™ XR+ System with in-built Image Lab™ Software system (Bio-Rad, Philadelphia, PA, USA). To verity the reproducibility of results, each reaction was repeated two times.

Data Analysis

The amplified bands were calculated by means of Bandscan 5.0 gel image analysis software assisted by manual correction. Only reproducible and unambiguous DNA bands were scored as present (1) or absent (0). A cluster analysis was performed using NTSYS-pc version 2.10e software (Rohlf 2000). A UPGMA (unweighted pair group method with an arithmetic mean) dendrogram was constructed based on

Fig. 1 The morphological features of one bud and two tender leaves of different green tea cultivars. Numbers 1–18: samples from 18 tea cultivars, the details of which are given in Table 1

similarity matrices calculated using the simple matching (SM) coefficient (Nei and Li 1979). Principal coordinate analysis (PCoA) was further used to demonstrate the multidimensional distribution of the tea cultivars in a scatter plot (Gower 1966).

Specific SCoT Fragment Selection, Cloning, and Sequencing

Any SCoT fragment present in a particular cultivar while being absent in all the other cultivars was considered a cultivar-specific marker. The selected cultivar-specific band was extracted and purified from the SCoT gel using a SanPrep Column DNA Gel Extraction Kit (Shanghai Sangon Biological Engineering Technology and Service Co. Ltd., China) and cloned into the pMD^{TM}19-T vector (Takara, China) according to the manufacturer's protocol. Recombinant clones were selected by red/white clone screening and sequenced at Shanghai Sunny Biotechnology Co. Ltd. (Shanghai, China).

SCAR Primer Design and Validation

The obtained sequence was BLAST searched using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and deposited in GenBank (Clark et al. 2016). Specific SCAR primers were designed based on the obtained sequence using Primer Premier 5 software (Lalitha 2000). SCAR amplification was performed in 20 µl of PCR mixture solution which contained 1 µl of forward primer (10µM), 1 µl of reverse primer (10 µM), 10 µl of 2×*EasyTaq* PCR SuperMix (Beijing TransGen Biotech Co., Ltd., China), 1 µl of genomic DNA template, and 7 µl of ddH_2O.

SCAR-PCR amplifications was performed under the following conditions: 94 °C for 5 min; 32 cycles of 94 °C for 50 s, 57 °C for 50 s, 72 °C for 1.5 min; and a final extension at 72 °C for 10 min. Furthermore, 10 selected individuals of 'Yingshuang' were used to validate the developed SCAR marker. The amplification products were separated and photographed as described in the section "SCoT-PCR."

Results

SCoT Polymorphisms

In total, 31 SCoT primers were selected for further study after the initial primer screening, and these selected primers generated a total of 264 reliable loci ranging from 4 (SCoT1) to 17 (SCoT33) (Table 2), with an average of 8.5 loci per primer. Among the generated loci, 226 were polymorphic. The number of polymorphic loci per primer ranged from 2 (SCoT1 and 24) to 14 (SCoT33), with an average of 7.3 loci per primer. The percentage of polymorphic loci ranged from 40.0% to 100.0%, with an average of 83.7% polymorphism. Two representative profiles (SCoT4 and 17) are shown in Fig. 2.

Genetic Diversity Among Tea Cultivars

Genetic diversity among the 18 tea cultivars was analyzed using a total of 264 SCoT loci based on the SM coefficient of the DNA banding pattern similarity. The genetic similarities among the 18 tea cultivars ranged from 0.587 ('Zhongcha 108'/'Jinfeng') to 0.814 ('Zhongcha 127'/'Zhongcha 128') (Table 3). A UPGMA dendrogram was constructed to infer the genetic diversity of these tea cultivars. In this study, all tea cultivars could be grouped into three main groups with a similarity index of 0.670 (Fig. 3). Group I comprised six cultivars, including 'Longjing 43,' 'Zhongcha 108,' 'Longjing Changye,' 'Zhongcha 102,' 'Jiaming 1,' and 'Fuding Dabaicha.' Six cultivars containing 'Chunyu 1,' 'Chunyu 2,' 'Zhongcha 125,' 'Zhongcha 126,' 'Zhongcha 127,' and 'Zhongcha 128' were grouped into Group II, while 'Maolv,' 'Yingshuang,' 'Zhenong 113,' 'Zhenong 117,' 'Jinfeng,' and 'Cuifeng' constituted Group III. To further understand the genetic relationships among the tested tea cultivars, a two-dimensional PCoA based on genetic similarity was performed (Fig. 4). In general, all green tea cultivars were clustered into three main groups, which were similar to the results shown by the UPGMA dendrogram (Figs. 3, 4). The first two principal axes explained 16.34% and 12.25% of the total molecular variation observed, respectively. Comparable results were obtained by both multivariate approaches (PCoA and UPGMA) in this study (Figs. 3, 4).

Development of the SCAR Marker

Among the 31 SCoT primers, the primer SCoT4 revealed an amplified DNA fragment of 1649 bp that was unique to green tea cultivar 'Yingshuang,' which could

Table 2 Sequences and polymorphism information of the 31 SCoT primers selected for this study

Primer code	Primer sequence (5′-3′)	Annealing temperature (°C)	No. of amplified loci	No. of polymorphic loci	Polymorphic loci (%)
SCoT1	CAACAATGGCTACCACCA	49.86	4	2	50.0
SCoT2	CAACAATGGCTACCACCC	50.73	6	5	83.3
SCoT3	CAACAATGGCTACCACCG	51.27	7	6	85.7
SCoT4	CAACAATGGCTACCACCT	49.5	11	8	72.7
SCoT5	CAACAATGGCTACCACGA	50.1	10	10	100.0
SCoT6	CAACAATGGCTACCACGC	52.05	7	6	85.7
SCoT7	CAACAATGGCTACCACGG	51.27	6	5	83.3
SCoT8	CAACAATGGCTACCACGT	50.41	9	8	88.9
SCoT9	CAACAATGGCTACCAGCA	50.32	7	5	71.4
SCoT10	CAACAATGGCTACCAGCC	51.19	7	6	85.7
SCoT11	AAGCAATGGCTACCACCA	51.37	7	5	71.4
SCoT12	ACGACATGGCGACCAACG	55.93	11	10	90.9
SCoT13	ACGACATGGCGACCATCG	55.39	8	8	100.0
SCoT14	ACGACATGGCGACCACGC	58.58	7	6	85.7
SCoT15	ACGACATGGCGACCGCGA	59.85	8	7	87.5
SCoT16	ACCATGGCTACCACCGAC	54.05	10	8	80.0
SCoT17	ACCATGGCTACCACCGAG	53.71	12	11	91.7
SCoT18	ACCATGGCTACCACCGCC	57.09	10	9	90.0
SCoT19	ACCATGGCTACCACCGGC	57.09	6	5	83.3
SCoT20	ACCATGGCTACCACCGCG	57.53	11	8	72.7
SCoT23	CACCATGGCTACCACCAG	52.43	5	4	80.0
SCoT24	CACCATGGCTACCACCAT	51.58	5	2	40.0
SCoT25	ACCATGGCTACCACCGGG	56.35	8	8	100.0
SCoT26	ACCATGGCTACCACCGTC	54.05	9	8	88.9
SCoT28	CCATGGCTACCACCGCCA	57.10	9	9	100.0
SCoT30	CCATGGCTACCACCGGCG	58.32	11	11	100.0
SCoT31	CCATGGCTACCACCGCCT	56.77	7	7	100.0
SCoT33	CCATGGCTACCACCGCAG	55.62	17	14	82.4
SCoT34	ACCATGGCTACCACCGCA	56.27	14	13	92.9
SCoT35	CATGGCTACCACCGGCCC	57.9	10	9	90.0
SCoT36	GCAACAATGGCTACCACC	51.53	5	3	60.0
Average	–	–	8.5	7.3	83.7
Total	–	–	264	226	–

provide a molecular tool for 'Yingshuang' identification. This DNA fragment (named SCoT4-1649) was cloned, sequenced, and deposited in GenBank (GenBank accession number: MG702647). The nucleotide sequence of SCoT4-1649 contained 58.94% A + T and 41.06% G + C, as shown in Fig. 5. The blast results showed that the fragment SCoT4-1649 had a high similarity (80.0%) with the partial coding sequence of gag-pol mRNA from *C. sinensis* (GenBank accession number:

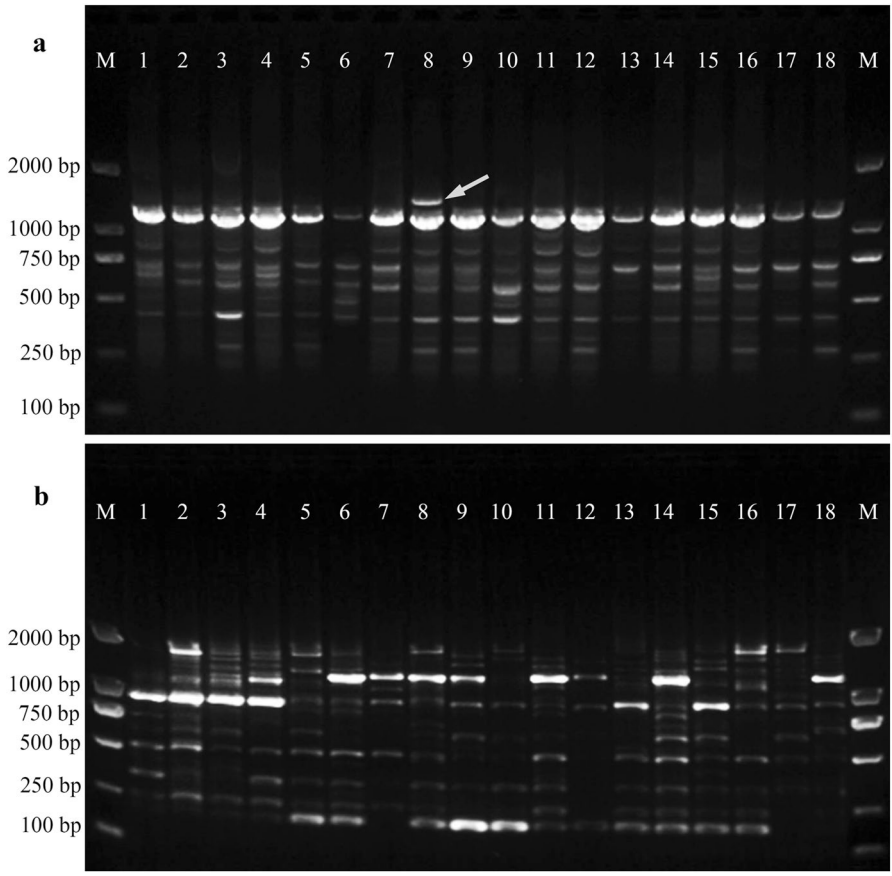

Fig. 2 Amplification profiles of primers SCoT4 (**a**) and SCoT17 (**b**). *Lane M*: *Trans2K* DNA Marker; *Lanes 1–18*: genomic DNA samples from 18 green tea cultivars (1–18) as listed in Table 1. Arrowhead represents the specifically amplified band from the targeted green tea cultivar, 'Yingshuang'

KJ946251). A SCAR primer pair, named S4YS, was developed based on the fragment SCoT4-1649 (Fig. 5; Table 4).

Amplification of the Designed SCAR Primers

SCAR-PCR with the primer pair S4YSF/R was used to amplify fragments from 18 tea cultivars at the optimum working annealing temperature of 57 °C. A clear single amplicon of 1346 bp was produced for 'Yingshuang,' but no amplicons were produced for the other 17 tea cultivars (Fig. 6a). To further verify the specificity of this SCAR marker, PCR analysis of ten different samples of 'Yingshuang' using the primer pair S4YSF/R was performed, and all samples of 'Yingshuang' produced the specific amplicon at 1346 bp (Fig. 6b).

Table 3 Pairwise similarity coefficients calculated by SCoT markers. Lanes 1–18: genotypes of the 18 green tea samples (1–18) shown in Table 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.000																	
2	0.754	1.000																
3	0.697	0.761	1.000															
4	0.795	0.761	0.735	1.000														
5	0.780	0.723	0.727	0.742	1.000													
6	0.731	0.720	0.693	0.678	0.769	1.000												
7	0.670	0.652	0.686	0.633	0.678	0.652	1.000											
8	0.727	0.670	0.697	0.705	0.674	0.708	0.754	1.000										
9	0.640	0.598	0.663	0.640	0.678	0.644	0.682	0.723	1.000									
10	0.629	0.610	0.598	0.629	0.659	0.625	0.633	0.667	0.716	1.000								
11	0.663	0.652	0.655	0.617	0.633	0.727	0.727	0.761	0.712	0.663	1.000							
12	0.659	0.617	0.636	0.652	0.682	0.693	0.648	0.712	0.655	0.682	0.799	1.000						
13	0.617	0.659	0.602	0.587	0.617	0.682	0.652	0.655	0.606	0.655	0.773	0.746	1.000					
14	0.678	0.659	0.655	0.640	0.655	0.682	0.667	0.686	0.644	0.678	0.780	0.739	0.803	1.000				
15	0.659	0.633	0.629	0.644	0.674	0.633	0.617	0.682	0.678	0.712	0.670	0.652	0.678	0.777	1.000			
16	0.606	0.617	0.674	0.636	0.636	0.617	0.640	0.621	0.693	0.629	0.670	0.636	0.617	0.693	0.788	1.000		
17	0.652	0.595	0.644	0.621	0.652	0.640	0.625	0.659	0.663	0.705	0.701	0.674	0.670	0.761	0.765	0.780	1.000	
18	0.655	0.652	0.663	0.648	0.648	0.644	0.621	0.678	0.674	0.678	0.689	0.655	0.659	0.720	0.754	0.769	0.814	1.000

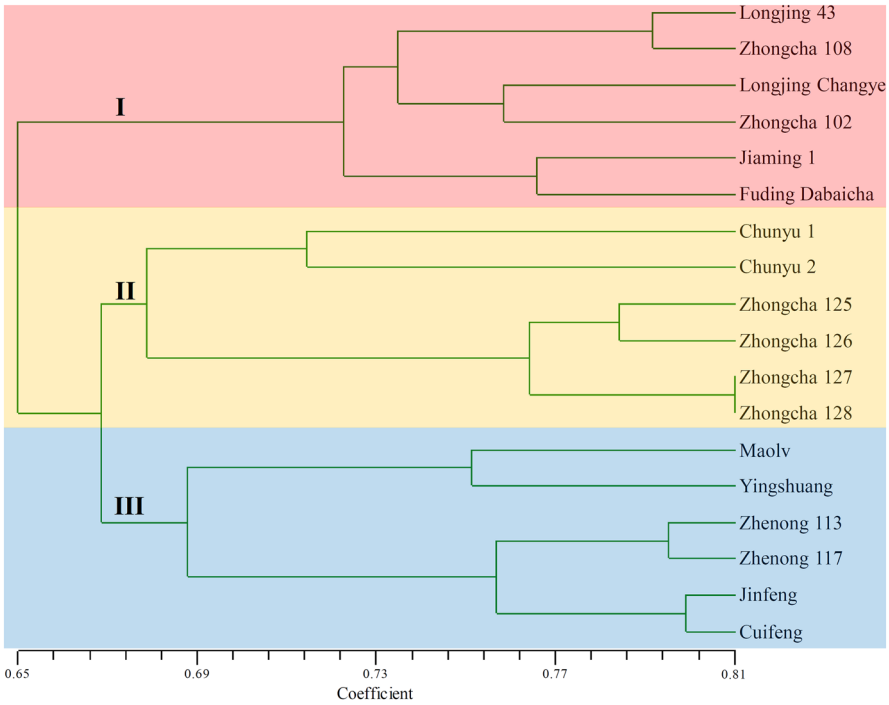

Fig. 3 UPGMA dendrogram of the tea cultivars in this study based on genetic similarities of DNA fingerprinting patterns from the 31 SCoT primers. Numbers (I–III) indicate that the tested tea cultivars were grouped into three groups with a similarity of 0.670

Discussion

Genetic diversity is an important basis for plant breeding and genetic conservation of tea cultivars, and DNA molecular marker techniques provide a simple and an effective approach for studying the genetic diversity of tea cultivars based on nucleic acid polymorphisms (Yu et al. 2017). Different from RAPD, ISSR, AFLP, and similar marker techniques, the SCoT marker method is correlated to functional genes and their corresponding traits, and thus it is more suitable for marker-assisted breeding (Collard and Mackill 2009; Feng et al. 2016b; Mulpuri et al. 2013). In recent years, SCoT markers have popularly been used to reveal genetic diversity in many plants, including *Jatropha curcas* (Mulpuri et al. 2013), *Quercus brantii* (Alikhani et al. 2014), *Diospyros* germplasm (Deng et al. 2015), *Chrysanthemum morifolium* (Feng et al. 2016b), and *Vigna unguiculata* (Igwe et al. 2017). To the best of our knowledge, this is the first time that SCoT markers have been used to infer the extent of genetic diversity and develop a SCAR marker for green tea cultivars in Zhejiang Province of China in such a large sample size, and thus, this study has expanded the application of SCoT markers to the plant field.

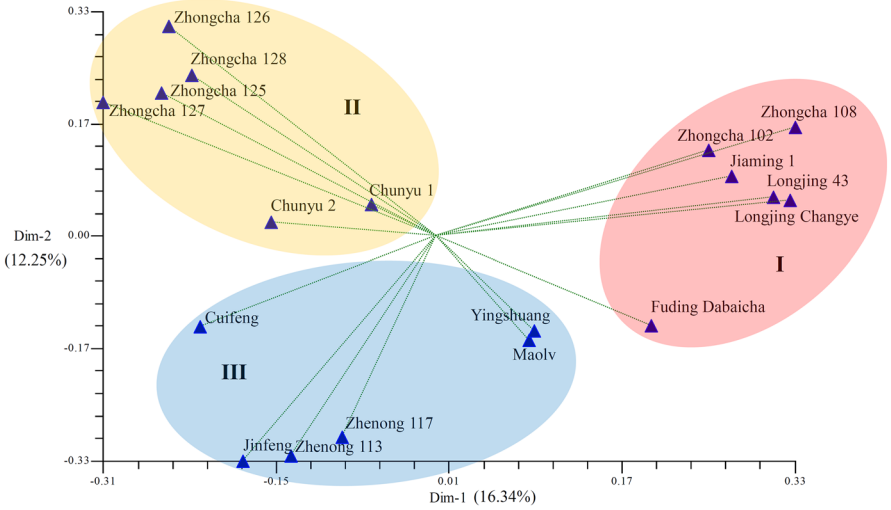

Fig. 4 Two-dimensional projection of a PCoA of 18 green tea samples based on the SCoT markers along the first two principal axes. The first two principal axes explained 16.34% and 12.25% of the total molecular variations observed, respectively. Numbers (I–III) indicate the grouping of the tested tea cultivars into three groups

TCTAGAGATTCAACAATGGCTACCACCTCACCATGAGATTGACTTCTCAATTGATTTAGTGCCTAGCAC
GACACCGATCTCTATGGCACCGTATAGATTTGCACTCGC**TGAGCTGAGTGAGTTGAA**GATTTA
ACTCCAAGAATTGTTAGACAAGAGTTTCATCCACCCTAGTAAGTCGCCTTAGGGAGCATCGACTCTCT
TTGTTAAGAAACATGATGGTGCCTTACAACTTTGTATTGATTACCAGAAGTTAAATCAAGTAACTGTT
AAGAACAAGTACCCAATGCCTCGTATAGATGATTTGTTTGATCAATCGAAGGAGTTTTGTTGTTTCTCT
GAGATAGACTTGAGATCAGGTTATCACCAGTTGAGGGTAAGGGAGGAAGATATCCAAAAGACAGTAT
TCCATATGCGATATGGCCATTATGAGTTTCTAGTGATGCCTTTTGGGCTAACCAATGCACCCGCCGTAT
TTATGGATTTGATAAATCGGATCTTTTGCGAGTTCCTAGACCGTTTGGTAGTGGTCTTTGTTAATGATA
TTTTGATTTACTCACCCTCCGAGGAGGAACACGAGGAACACTTATGTGTTGTGCTTGAGTTGCTTAGGG
CCCATAAACTATATGCTATGTTTGGAAAATGTGAATTCTGGTTGAGTGGAGTAAAGTTCTTAGGCCAC
GTGGTATCCGGGGAAGGAGTGACTGTAGATTCATCCAAGATAGAAGCCACACAAGATTGGAAACAAT
CCAAGAATGTCGTTGAGATCTGTAGTTTCTTGGGGTTGGCTGGGTATTATCATCGGTTTGTGAAAGATT
TCTCTAGATTAACTTCACCATTGACTAGATGTCACGACCCTAACCTGCCCTTGTAGGTTTAGTGCCATA
ACCGGCCAGTAATCCTGGGATTACCGAAGGCCTCTAAACAGATTAAACAAAATACAGGTGCTAGCGG
AAGCAAATACTAACATATACGTCCATAGAGTTGTGTAGACATACATAGGACAGAAAATTTAAAACTT
AATTTCCATAATTAAGATGTCATGCCCACGTCACCATTCATACATACAGACCAACAAAAGACTTGTTC
AAACTTAATAAAAGAAACACAAAAGATCATTAACATGATTCATAAAAGCTTGACCGCTACAAAAGGG
AACCTCCTCGGGAGACACTGTGGCTGACTGACCAAAACAACCCTCCAGGTAACAACTTAGGCCTGGTC
ACACATCTGCTCACCTACATACATAAATAATACATTGAGCTGGAGCCCAGTGAATAATAATAGAATGA
AGGATTTATGAATGATGTCCAAACAGTTTCAAGAATCAGAGTTCATATCAGAAACAGATTCGGATTCA
CATATTTAACCAGAAGTCATAAACAATTATATGTGGTACATCCATTAGCTATCATATCATCTTAGCCCT
AGGACCCATA**TTCATAGTCAGTACCGAC**GACCCCAAAGGTCCATATCTTGTGCACAAGTAACCA
TGGTATTGTTTTCCCCAGGCAAAGGCCATGTATAAACCACGGTATTGTTTCCCCTAGGTAAGGGCCAT
ATGATCATAAGCCACGGTATTGTTTCCCCCGGGCGAGGGCCATATAAACATATGCCACGATATTGTTT
CCCCCAGGTGGTAGCCATTGTTG

Fig. 5 Nucleotide sequence of the SCoT amplicon (SCoT4–1649) specific to the tea cultivar 'Yingshuang.' The underlined sequences represent the forward primer and reverse primer of the SCAR marker S4YS

Table 4 Characteristics of the SCAR marker developed from primer SCoT4 for 'Yingshuang'

Species-specific locus	Original specific sequence length (bp)	SCAR primer pair	SCAR primer sequence (5′-3′)	Tm (°C)	Working annealing temperature (°C)	Aplicon length (bp)
SCoT4–1649	1649	S4YSF	TGAGCTGAGTGAGTTGAA	58	57	1346
		S4YSR	GTCGGTACTGACTATGAA	55		

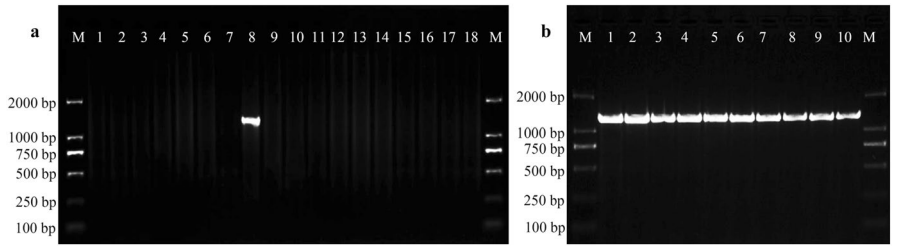

Fig. 6 Amplification profiles of the primer pair S4YSF/S4YSR. **a** Amplification profile of the primer pair S4YSF/S4YSR in the 18 green tea samples. *Lane M*: *Trans2K* DNA Marker; *Lanes 1–18*: genomic DNA samples from 18 green tea cultivars (1–18) as listed in Table 1. **b** Amplification profiles of the primer pair S4YSF/S4YSR in ten different individuals of 'Yingshuang.' *Lane M*: *Trans2K* DNA Marker

In this study, 83.7% of the amplified SCoT loci were polymorphic, which was relatively high compared to the polymorphism rates detected by SCoT analysis of 76.19% in mango cultivars (Luo et al. 2010), 36.76% in peanut cultivars (Xiong et al. 2011), and 73.82% in *Mangifera indica* (Luo et al. 2012). The high-polymorphism rates detected in this study indicate the presence of considerable genetic diversity among the tested green tea cultivars. In this study, the UPGMA dendrogram constructed using genetic similarity derived from SCoT-genotyping patterns revealed three groups among the 18 green tea cultivars at a genetic similarity value of 0.670 (Fig. 3), which is very close to the similarity level of 0.624 found for cultivar groupings in medicinal *Chrysanthemum morifolium* using SCoT markers (Feng et al. 2016b). The UPGMA dendrogram and the PCoA plot indicated that most tea cultivars with similar genetic backgrounds were grouped together (Figs. 3, 4). For example, 'Longjing 43,' 'Zhongcha 108,' 'Longjing Changye,' and 'Zhongcha 102,' all of which were selected from the 'Longjing' population in Hangzhou of Zhejiang, China (Yang and Liang 2014), showed close relationships within Group I. 'Maolv' and 'Yingshuang,' which were both selected from the same parents 'Fuding Dabaicha' (Yang and Liang 2014), were grouped closely within Group III. Compared with 'Maolv' and 'Yingshuang,' 'Zhenong 113,' 'Zhenong 117,' 'Jinfeng,' and 'Cuifeng,' all selected from the natural hybrid offspring of 'Fuding Dabaicha' and 'Yunnan Dayecha' (Yang and Liang 2014), showed closer relationships within Group III.

Some studies have shown that SCoT markers can be utilized not only for genetic diversity research but also for the identification of plants, by converting SCoT markers into SCAR markers (Hao et al. 2018; Mulpuri et al. 2013; Rajesh et al. 2016). Morphologically, green tea cultivars are difficult to identify because of their extremely similar shapes (Fig. 1). The S4YS marker developed in the present study produced a 1346 bp amplicon in 'Yingshuang,' while no amplification was observed in other green tea cultivars. Therefore, this SCAR marker might be used in the identification and authentication of 'Yingshuang' from morphologically similar green tea cultivars mainly grown in Zhejiang Province of China. Notably, the length of the PCR product detected by the S4YS marker is 1346 bp, which is 303 bp shorter than the length of the original selected DNA fragment (SCoT4–1649) (Figs. 5, 6). The advantage of such a SCAR marker design method is that it can increase

the specificity and stability of the primers, especially when the DNA is partially degraded (Choi et al. 2008; Lee et al. 2011; Marieschi et al. 2016; Richero et al. 2013). An increasing number of studies have reported that SCAR markers could be used for authentication of plant species, such as *Casuarina equisetifolia* (Ghosh et al. 2011), *Jatropha curcas* (Mulpuri et al. 2013), *Punica granatum* (Marieschi et al. 2016), and *Ocimum tenuiflorum* (Kumar et al. 2018). SCAR markers have also been proven useful in the breeding programs of crops such as soybean (Gavioli et al. 2007), persimmon (Kanzaki et al. 2010), mung bean (Dhole and Reddy 2013), wheat (Kim et al. 2016), and sugar cane (Khan et al. 2017).

In conclusion, our study has provided important information on the genetic diversity and identification methods of green tea cultivars in Zhejiang Province of China. The results of this study demonstrate that SCoT markers are a highly polymorphic, efficient, and powerful tool to examine the genetic diversity of tea cultivars. Both a UPGMA dendrogram and PCoA analysis clustered the tea cultivars tested in this study into three groups mainly in accordance with their known genetic backgrounds. The SCAR marker developed in our study provides a rapid method for the genotypic identification of green tea cultivars and will thus support molecular-assisted breeding programs.

Acknowledgements This work was supported by the National Natural Science Foundation of China (No. 31600562), the Central Public-interest Scientific Institution Basal Research Fund (No.1610212018013), the Chinese Academy of Agricultural Sciences through the Agricultural Science and Technology Innovation Program (CAAS-ASTIP-2017-TRICAAS), and the Earmarked Fund for China Agriculture Research System (CARS-019).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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