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GENETIC DIVERSITY AND RELATIONSHIP OF DONGTING BILUOCHUN TEA GERMPLASM IN SUZHOU REVEALED BY SSR MARKERS

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Abstract

Dongting Biluochun (DTBLC) tea is one of the top famous tea in China, which is originated from Suzhou city, Jiangsu Province, China. Generally, DTBLC tea is processed using 'Dongting population', which have a great genetic diversity. However, little information has been found so far on its genetic diversity. In order to unveil the relationship among the DTBLC germplasm, 68 tea accessions collected from Suzhou geographical origin growing area were analyzed by using 36 pairs of polymorphic simple sequence repeat (SSR) primers. Finally, a total of 68 tea accessions generated 158 alleles with a mean of 4.39 alleles per locus and 314 genotypes with a mean of 8.72. The polymorphism information content (PIC) was between 0.09 and 0.76, and the mean value was 0.53; the gene diversity index (*H*) ranged from 0.10 to 0.79, with an average of 0.56, while the average observed heterozygosity (*H*o) was 0.50 varied from 0.04 to 0.71. The coefficient of the genetic similarity of SSR makers among the tea accessions ranged from 0.49 to 0.89. These results indicated that a considerable genetic diversity existed among these tea accessions. According to the clustering results, 68 tea accessions were clustered into 5 categories with no obvious geographical distribution characteristics based on the unweighted pair group method of arithmetic mean (UPGMA), which hinted that the territorial division of protection for geographic indication-DTBLC tea was appropriate. However, dendrogram based on Nei's genetic distance revealed complex genetic relationships among DTBLC tea germplasm resources, and these collections possessed impressive level of genetic diversity which could be important for subsequent selective breeding, utilization, and conservation of tea germplasm resources in Jiangsu, China.

Key words: Dongting Biluochun tea, Genetic diversity, SSR markers.

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is an important commercial crop, which is mostly cultivated in the tropical and subtropical countries of Asia, Africa namely China, India, Bangladesh, Sri Lanka, Vietnam, Kenya and some areas of Latin America such as Argentina etc. It has become a daily drink for billions of people across the world. Tea plant is originated from China, with abundant and diverse germplasm resources. Currently, there are more than 3,000 tea accessions collected and ex-situ conserved in the China National Germplasm Tea Repository (Yao *et al.*, 2012). Excellent germplasm resources are the important substantial basis of tea breeding and variety innovation. Nevertheless, due to self-incompatibility and cross-fertilization, tea plant is highly heterogeneous, resulting in broad genetic variation. Therefore, it is of great scientific values to clarify the genetic relationships among cultivars and to explore heterotic groups on the basis of cultivar classification using molecular markers (Fang *et al.*, 2011). The knowledge of understanding the genetic background, diversity and relationship will also greatly help to select parents for the current and long-term success of tea breeding programs (Pandolfi *et al.*, 2009).

Molecular marker have proven to be a powerful approach for the study of genetic variation, relationships, molecular identification, and phylogeny of different cultivars, as well as for cultivar discrimination and fingerprinting of plant species (Boczkowska *et al.*, 2012; Frascaroli *et al.*, 2013; El-Esawi *et al.*, 2016; Lassois *et al.*, 2016). The different types of molecular markers such as random amplification of polymorphic DNA (RAPD) (Arakawa *et al.*, 2016), restriction fragment length polymorphism (RFLP), inter simple sequence repeats (ISSR) (Devarumath *et al.*, 2002; Liu *et al.*, 2015), amplified fragment length polymorphism (AFLP) (Sharma *et al.*, 2010; Ji *et al.*, 2012), single nucleotide polymorphism (SNP) (Ma *et al.*, 2015), and microsatellites or simple sequence repeats (SSR) (Taniguchi *et al.*, 2014; Liu *et al.*, 2017) have already been used for genetic and genomic analysis in tea plants. Of these approaches, SSR has become an important technique used in tea plant genetics and breeding for its multi-allelic nature, co-dominant inheritance, relative abundance, extensive genome coverage and simple detection (Zhao *et al.*, 2008). The SSR markers have been used successfully in genetic diversity and relationship (Fang *et al.*, 2011), the origin of tea gemplasm (Meegahakumbura *et al.*, 2016), the selection core collection (Taniguchi *et al.*, 2014), and the construction of genetic linkage maps in tea plants (Tan *et al.*, 2016).

Dongting Biluochun (DTBLC) green tea is a very famous tea named by Kangxi Emperor of Qing dynasty, produced in Suzhou area, Jiangsu Province, China. It is well known for its unique floral aroma, fruity flavor,

delicate appearance and silvery green with pekoe as well as its glorious history (Wang *et al.*, 2016), which was honoured as "Tea fairy" and "The first tea of China". This kind of green tea contains bioactive components such as tea polyphenols, caffeine, catechins, free amino acids, vitamins and other beneficial substances, and also have plenty of health benefits like anticancer, anti-inflammatory effects and oxidant activity, etc (McKay & Blumberg, 2002). The tea varieties suited to process DTBLC tea requires early sprouting, many hairy, short internode and abundant biochemical components, which could be the breeding objectives of DTBLC tea. The quality and quantity of DTBLC tea is tightly linked with the tea germplasm grown in Suzhou Dongting Mountain. The kind of tea is processed using a special DTBLC tea germplasm named 'Dongting population' with a great genetic diversity. Nevertheless, the genetic diversity of the DTBLC tea germplasm has not been investigated until now. The objective of this research is to assess the genotypic variability and relationship among DTBLC genetic resources distributed in Suzhou. In addition, this study will provide a scientific basis for future discovery and selective breeding of DTBLC tea.

Materials and Methods

Plant materials and DNA isolation: A total of 68 tea accessions in Table 1 were collected from Dongting Biluochun Plantation (Suzhou, Jiangsu Province, China). The phenotype of partial tea accessions of 'Dongting population' is shown in Fig. 1. One-bud-and-two-leaf-shoots were harvested from all accessions and immediately frozen in liquid nitrogen, and then stored at -80°C until further use. Total genomic DNA was extracted from young leaves according to the CTAB method described by Chen & Yamaguchi (2005).

SSR analysis: Thirty-six pairs of SSR marker were used for individual genotyping (Ma *et al.*, 2010) (Table 2). PCR amplifications were performed in 10 µl reaction mixtures, consisting of 50 ng template DNA, 10 × PCR buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl], 2.5 mM $MgCl_2$, 10mM dNTPs, 10 µM of each primer and 0.5 U *Taq* DNA polymerase (Takara, Dalian, China). PCR reactions were carried out at an initial denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 30 s, 46-60°C for 30 s, and 72°C for 50 s with a final extension step at 72°C for 10 min. Amplification products were separated on a 8% polyacrylamide gel for 90 min at a constant voltage of 150 V with 1 × TBE running buffer. Finally, the gels were stained with silver nitrate solution, visualized under ultraviolet light and scored manually.

Data analysis: The polymorphic bands were scored qualitatively as either 1 for presence or 0 for absence across all the genotypes. To calculate the genetic distance among tea accessions and construct the genetic similarity matrix, the population genetic parameters, including the number of alleles (N_A), genotype (N_o), observed heterozygosity (H_o), gene diversity (H), and polymorphism information content (PIC), were estimated using PowerMarker V3.25 software (Liu & Muse, 2005). Jaccard similarity coefficients were calculated to determine similarity indices between the genotypes. Cluster analysis was performed and dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed according to Jaccard's similarity coefficient using the NTSYS-PC ver.2.10 software (Rohlf, 2000). Genetic relationships among the genotypes were also analyzed by principal coordinate analysis (PCA) using the NTSYS-PC ver. 2.10 software (Rohlf, 2000).

Results

Genetic diversity analysis: The 36 SSR primer pairs yielded amenable and reproducible amplicons in samples of 68 tea accessions, and a total of 158 alleles and 314 genotypes were detected (Fig. 2, Table 2). The number of alleles per locus varied from 2 (CSR553, CSR557) to 7 (CSR564, CSR570) with an average of 4.39, and the amplified genotypes with a variation range of 3 (CSR553, CSR557) - 19 (CSR564), averagely 8.72 genotypes were amplified with each primer pair. The polymorphic information content (PIC) value of 36 SSR loci had greater amplitude, ranging from 0.09 (CSR557) to 0.76 (CSR564), and the mean value was 0.51, which was higher than 0.50, indicating great genetic differences and rich genetic diversity among tea germplasm resources in Suzhou. The results of gene diversity assay showed that Nei's gene diversity (H) varied from 0.10 (CSR557) to 0.79 (CSR564) with an average of 0.56; the observed heterozygosity (H_o) was between 0.04 (CSR557) and 0.71 (CSR564, CSR570, CSR581, CSR593), with an average of 0.50. These results indicated that the tea germplasm in Suzhou had relatively high genetic diversity.

Cluster analysis: The genetic similarity coefficient among tea germplasm tested ranged from 0.49 to 0.89, with larger amplitude. To be specific, the genetic similarity coefficient between Xinawu 5 and Shangjin 4 was the minimum (0.49), the value between Xinawu 3 and Chawan 4, Xinawu 4 and Chawan 3 was 0.50, indicating that these varieties exhibited a distant relationship among 68 tea accessions; while the genetic similarity coefficient between Ganshanling 1 and Yuhuatai 4 was large (0.89), indicating that these two cultivars had a close relationship (Table 2).

To demonstrate the relationships among populations, a dendrogram was constructed using UPGMA based on the similarity coefficient (Fig. 3). The clustering result showed that except for Chawan 3, Xinawu 7 and Xinawu 8 which were clustered into a solitary group, the other 65 accessions were clustered into 5 groups at the similarity coefficient of 0.65. Group I contained 47 lines in 4 subgroups, group II contained 8 lines, group III and IV both consisted of 4 lines, while group V consisted of only 2 lines, respectively.

Table 1. Detailed information for the 68 tea plant accessions used in this study.

No.	Cultivars name	Type	No.	Cultivars name	Type
1.	Shangjin 1	Landrace	35	Ganshanling 5	Landrace
2.	Shangjin 2	Landrace	36	Ganshanling 6	Landrace
3.	Shangjin 3	Landrace	37	Ganshanling 7	Landrace
4.	Shangjin 4	Landrace	38	Ganshanling 8	Landrace
5.	Luhua	Landrace	39	Ganshanling 9	Landrace
6.	Chawan 1	Landrace	40	Ganshanling 10	Landrace
7.	Chawan 2	Landrace	41	Ganshanling 11	Landrace
8.	Chawan 3	Landrace	42	Ganshanling 12	Landrace
9.	Chawan 4	Landrace	43	Ganshanling 13	Landrace
10.	Chawan 5	Landrace	44	Ganshanling 14	Landrace
11.	Chawan 6	Landrace	45	Ganshanling 15	Landrace
12.	Chawan 7	Landrace	46	Ganshanling 16	Landrace
13.	Chawan 8	Landrace	47	Ganshanling 17	Landrace
14.	Chawan 9	Landrace	48	Chawan 10	Landrace
15.	Baisha	Landrace	49	Chawan 11	Landrace
16.	Beiwang 1	Landrace	50	Chawan 12	Landrace
17.	Beiwang 2	Landrace	51	Chawan 13	Landrace
18.	Beiwang 3	Landrace	52	Chawan 14	Landrace
19.	Beiwang 4	Landrace	53	Chawan 15	Landrace
20.	Beiwang 5	Landrace	54	Chawan 16	Landrace
21.	Beiwang 6	Landrace	55	Chawan 17	Landrace
22.	Beiwang 7	Landrace	56	Chawan 18	Landrace
23.	Beiwang 8	Landrace	57	Chawan 19	Landrace
24.	Lingxia 1	Landrace	58	Xinawu 1	Landrace
25.	Lingxia 2	Landrace	59	Xinawu 2	Landrace
26.	Lingxia 3	Landrace	60	Xinawu 3	Landrace
27.	Yuhuatai 1	Landrace	61	Xinawu 4	Landrace
28.	Yuhuatai 2	Landrace	62	Xinawu 5	Landrace
29.	Yuhuatai 3	Landrace	63	Xinawu 6	Landrace
30.	Yuhuatai 4	Landrace	64	Xinawu 7	Landrace
31.	Ganshanling 1	Landrace	65	Xinawu 8	Landrace
32.	Ganshanling 2	Landrace	66	Xinawu 9	Landrace
33.	Ganshanling 3	Landrace	67	Lingyunbaihao	Landrace
34.	Ganshanling 4	Landrace	68	Fudingdabaicha	National seed

Principal component analysis: A multivariate approach was used to supplement the cluster analysis. Associations among the 68 genotypes were examined with principal component analysis (PCA) (Fig. 4). Principal coordinates 1, 2, and 3 accounted for 5.91, 4.75 and 4.03 % of the total variation, respectively. The three dimensional (3D) scatter plot based on the first, second and third principal components of the 68 accessions indicated that the relationship among all the tea accessions could be discerned from different levels and directions. Comparing the clustering of genotypes in the dendrogram obtained through UPGMA analysis with the clustering pattern of genotypes obtained on the basis of PCA (Figs. 3 and 4), the results showed that there was a diversity of relationship in the 68 tea germplasms.

Discussions

Evaluation and conservation of germplasm are the basic parts of plant breeding programs. SSR markers have been used to evaluate genetic diversity and relationship in tea plants. These studies include previous reports on 185 tea cultivars from 14 provinces in China using 48 SSR markers (Fang *et al.*, 2011), 80 genotype using 36 newly developed SSR markers (Liu *et al.*, 2017), 128 elite clonal tea cultivars using 30 SSR markers (Tan *et al.*, 2015) and 183 individuals using 406 SSR markers to construct a genetic map (Ma *et al.*, 2014). However, there is no report on the diversity of DTBLC teas which are collected from Suzhou. This is the first study to assess the genotypic variability and relationship at molecular level among DTBLC genetic resources using the powerful microsatellite technique.

Fig. 1. The phenotype of partial tea accessions of 'Dongting population'.

Table 2. Amplification information of 36 SSR primer pairs in 68 tea germplasms.

Primer code	Repeat motif	Expected product size (bp)	T_a (℃)	Allele No (a)	Genotype (No)	Gene diversity (*H*)	Obs. Heterozygosity (*Ho*)	PIC
CSR550	(AG)9	110-130	46	4.00	9.00	0.56	0.51	0.51
CSR551	(TTTC)5	135-145	56	4.00	7.00	0.62	0.50	0.55
CSR552	(TC)10	130-140	56	4.00	6.00	0.50	0.44	0.44
CSR553	(TC)13	320-340	56	2.00	3.00	0.30	0.22	0.26
CSR554	(AG)12	95-120	56	4.00	7.00	0.52	0.47	0.46
CSR555	(GA)9	105-120	48	4.00	6.00	0.61	0.56	0.54
CSR556	(AG)9	145-165	58	3.00	5.00	0.52	0.49	0.46
CSR557	(AG)14	115-130	60	2.00	3.00	0.10	0.04	0.09
CSR558	(CT)10	125-140	58	3.00	4.00	0.50	0.46	0.41
CSR559	(GGT)6	170-180	58	5.00	10.00	0.48	0.41	0.45
CSR560	(AG)9	135-150	58	4.00	8.00	0.48	0.38	0.43
CSR561	(GTG)6	260-280	58	3.00	5.00	0.51	0.59	0.42
CSR562	(CGC)7	130-140	60	5.00	8.00	0.50	0.54	0.45
CSR563	(CAC)6	325-350	56	5.00	10.00	0.55	0.59	0.49
CSR564	(CACCAT)4	260-275	60	7.00	19.00	0.79	0.71	0.76
CSR566	(CTT)9	280-300	58	6.00	14.00	0.74	0.59	0.70
CSR567	(TCA)6	250-270	58	4.00	10.00	0.57	0.44	0.51
CSR568	(CT)12	180-195	58	5.00	8.00	0.52	0.56	0.46
CSR570	(CT)8	165-180	58	7.00	13.00	0.73	0.71	0.70
CSR571	(TGC)8	145-155	58	4.00	9.00	0.50	0.29	0.44
CSR573	(CT)15	110-125	58	6.00	12.00	0.68	0.54	0.64
CSR575	(CA)14	170-180	52	5.00	10.00	0.62	0.49	0.57
CSR576	(TTGTT)5	260-275	54	5.00	12.00	0.56	0.32	0.53
CSR578	(AG)11	220-270	52	4.00	6.00	0.50	0.40	0.41
CSR581	(TGTTTTT)2	270-295	56	5.00	11.00	0.69	0.71	0.65
CSR582	(AG)12	350-370	60	5.00	12.00	0.56	0.47	0.52
CSR584	(CTCAAT)5	195-215	60	6.00	15.00	0.75	0.69	0.71
CSR585	(CT)9	150-170	54	4.00	10.00	0.69	0.56	0.64
CSR588	(CT)11	160-175	58	5.00	11.00	0.68	0.63	0.63
CSR589	(GA)9	350-370	48	3.00	5.00	0.48	0.44	0.41
CSR590	(TC)10	275-290	60	4.00	8.00	0.52	0.44	0.47
CSR591	(TTA)6	225-240	52	5.00	9.00	0.53	0.54	0.48
CSR593	(TAG)5	320-340	58	5.00	8.00	0.63	0.71	0.57
CSR594	(CT)16	135-155	58	4.00	9.00	0.59	0.56	0.55
CSR597	(CT)9	220-240	58	4.00	7.00	0.60	0.59	0.52
CSR598	(CT)16	120-145	52	3.00	5.00	0.50	0.49	0.42
Mean	-	-	-	4.39	8.72	0.56	0.50	0.51

* *Note:* PIC: Pholymorphism information content; Ta: Annealing temperature

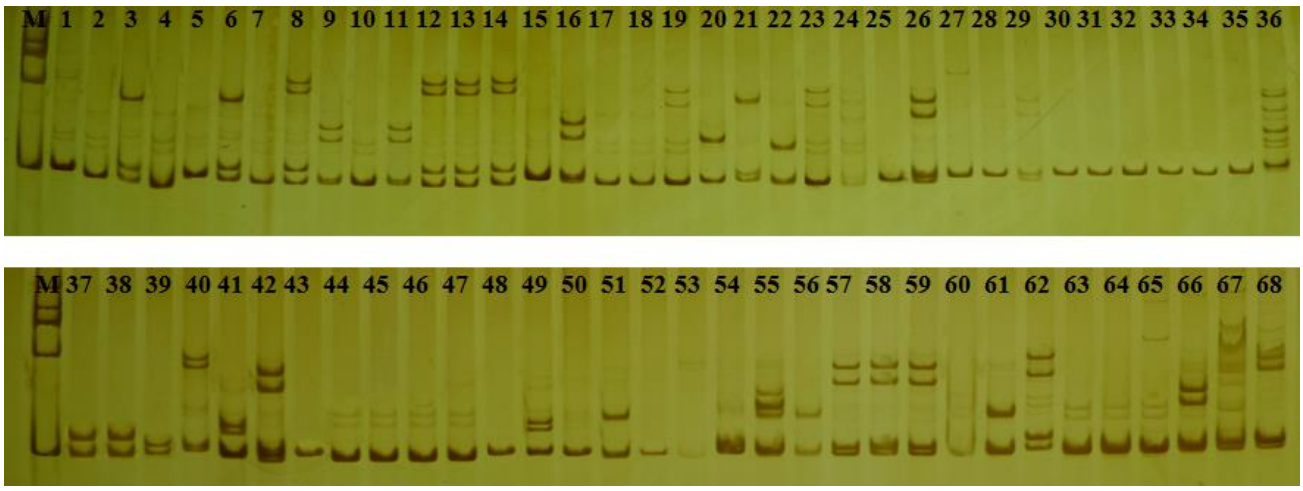

Fig. 2. The amplification result of primer pair CSR564 for 68 tea germplasm accessions.
Note: The lane numbers identify serial number of tea accessions as designated on Table 1; M stands for DNA ladder.

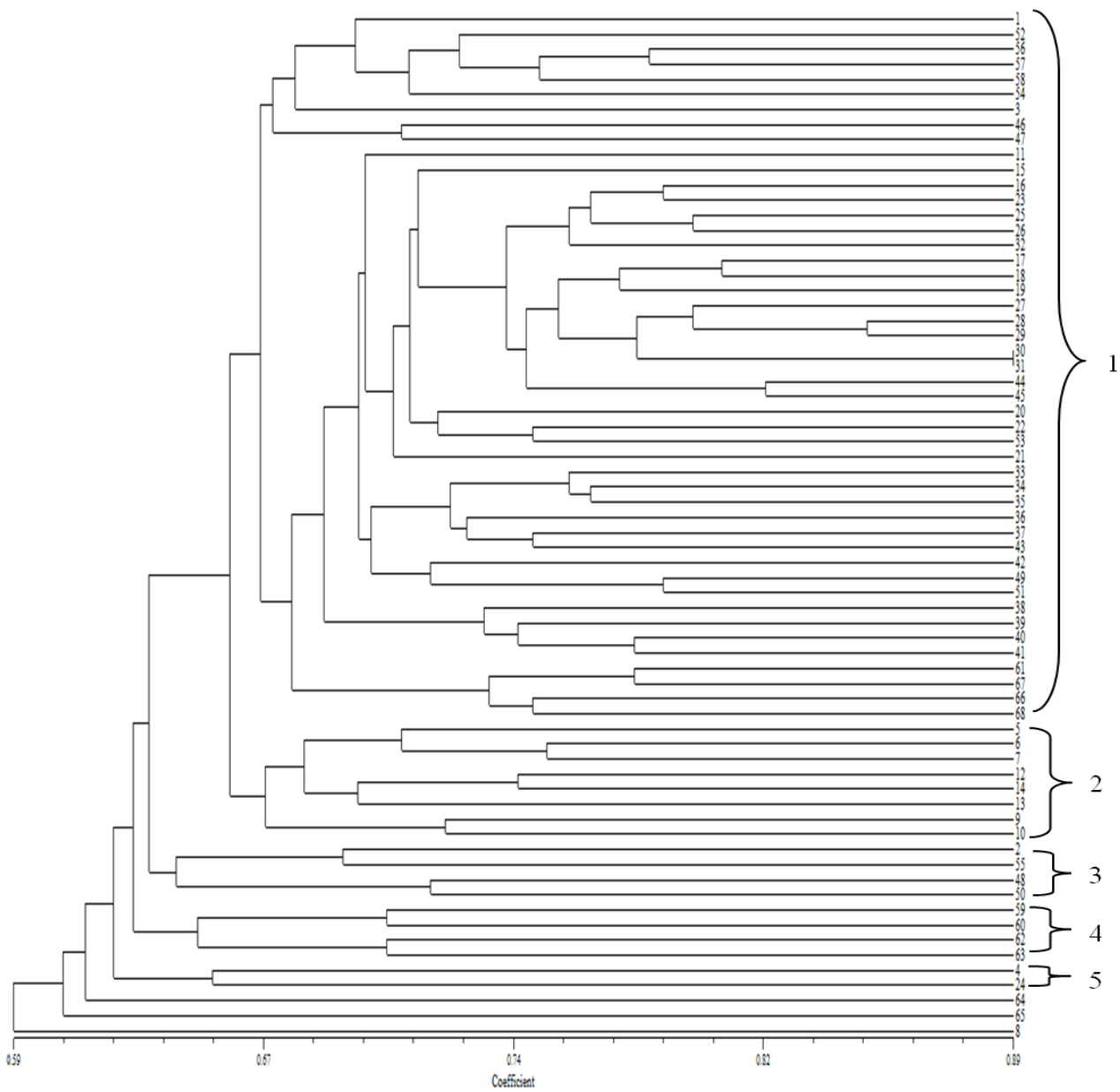

Fig. 3. The phylogenetic dendrogram of 68 tea germplasm accessions constructed from SSR data using UPGMA cluster analysis. The coefficient of similarity were calculated through the NTSYS 2.10 sofware.

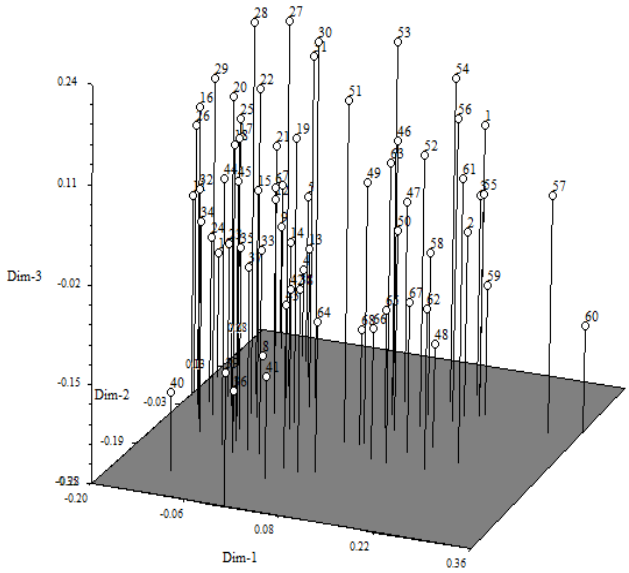

Fig. 4. Three-dimensional plots of 68 tea accessions by PCA analysis based on SSR data.

In the present study, we investigated DNA polymorphisms within and/or among DTBLC tea plant varieties based on SSR markers and attempted varietal classification based on allele frequencies at each locus. Among the 68 tea germplasm resources, the number of alleles per locus at each SSR loci ranged from 2 to 7 with an average of 4.39. Compared with the polymorphisms at SSR loci reported in maize (alleles per locus ranged from 2 to 13 with an average of 6.5 (Labate *et al.*, 2003), or cucumber accessions and inbred lines (alleles per locus ranged from 2 to 8 with an average of 3.44 (Mu *et al.*, 2008), our results showed that there was a high degree of genetic diversity in DTBLC.

Polymorphic information content (PIC) is considered as one of the important features of the molecular markers and could be used to assess to evaluate the genetic diversity (Ni *et al.*, 2002). PIC values ranged from 0.09 to 0.76, and classified 18 SSR loci as highly informative (PIC>0.5), 17 are reasonably informative (0.25< PIC<0.5), and 1 are only slightly informative (PIC < 0.25). The average PIC value of all SSR markers was 0.53, suggesting that high levels of genetic diversity among tea germplasm resources, which was in agreement with the previous reports (Bali *et al.*, 2013; Tan *et al.*, 2015). However, this average value was lower than that reported by Liu *et al*., (2017) of tea plant (0.862), but higher than that reported by Fang *et al*., (2011) for another 185 tea plant accessions (0.495). The difference of these studies may be attributed to the differences in the tea accessions used or the SSR loci used in this study.

Cluster analysis can reflect both the pedigree relationships and the geographic distances among plant species and varieties. Most accessions were clustered according to geographical origins and genetic backgrounds. It showed that Chawan 3, Xinawu 7 and Xinawu 8 were separated from other accessions in the cluster dendrogram, and the reason for this behavior calls for further research as it may indicate unique tea accessions with special breeding and production value. Compared with previous reports, the 68 tea germplasm resources with genetic similarities ranging from 0.49 to 0.89 had larger diversity than other tea germplasm populations, such as the Hunan cultivar population (Yang *et al.*, 2009), and the Chinese clonal cultivar population (Yao *et al.*, 2007). This could be explained by the fact that Suzhou has been recognised as the center of origin of DTBLC tea in the China.

Compared the principal component analysis and cluster analysis of the SSR data, our results ravealed a complexity of the relationship among accessions. Consequently, studies on the genetic relationships among species, should be integrated the use of these two complementary methods to give mutual authentication and subsequently more accurate and reliable results.

In conclusions, according to the present and previous results, there was a high degree of genetic diversity in DTBLC tea accessions. Large genetic variations can provide the opportunity to select and breed new cultivars with high yield and quality. Results indicate a considerable level of genetic variation in most of the tea accessions, which is very useful for tea breeding programs in Jiangsu, China.

Conclusions

Genomic SSR markers are more informative for estimation of genetic diversity among the tea plants due to the occurrence of more alleles in microsatellite regions. In this work, we have characterized for the first time the genetic diversity and relationship of DTBLC tea germplasm as revealed by SSR markers. Using 36 SSR primer pairs, 68 accessions of tea germplasm from Suzhou, China were evaluated and a total of 158 alleles and 314 genotypes were detected. The average gene diversity and PIC were 0.56 and 0.51, respectively. These tea resources showed relatively high diversity and wide genetic background. Five similar groups were obtained using UPGMA cluster and PCA methods, with the first 3 components accounting for 14.69% of the total variation. Only Chawan 3, Xinawu 7 and Xinawu 8 were uniquely classified while the rest of the accessions were grouped together. Therefore, the cluster dendrogram indicates no clear differences in geographic distribution characteristics among the 68 accessions tested in this study.

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References

Arakawa, O., S. Kikuya, P. Pungpomin, S. Zhang and N. Tanaka. 2016. Accumulation of anthocyanin in apples in response to blue light at 450 nm: Recommendations for producing quality fruit color under global warming. *Eur. J. Hort.. Sci*., 81(6): 297-302.

Bali, S., S.N. Raina, V. Bhat, R.K. Aggarwal and S. Goel. 2013. Development of a set of genomic microsatellite markers in tea (*Camellia* L.) (camelliaceae). *Mol. Breed.*, 32(3): 735-741.

Boczkowska, M., Z. Bulińska-Radomska and J. Nowosielski. 2012. AFLP analysis of genetic diversity in five accessions of polish runner bean (*Phaseolus coccineus* L.). *Genet. Resour. Crop Ev*., 59(4): 473-478.

Chen, L. and S. Yamaguchi. 2005. Rapd markers for discriminating tea germplasms at the inter-specific level in china. *Plant Breed.*, 124(4): 404-409.

Devarumath, R., S. Nandy, V. Rani, S. Marimuthu, N. Muraleedharan and S. Raina. 2002. RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica* ssp. *Assamica* (assam-india type). *Plant Cell Rep*., 21(2): 166-173.

El-Esawi, M.A., K. Germaine, P. Bourke and R. Malone. 2016. Genetic diversity and population structure of brassica oleracea germplasm in ireland using SSR markers. *Cr. Biol*., 339(3-4): 133-140.

Fang, W., H. Cheng, Y. Duan, X. Jiang and X. Li. 2011. Genetic diversity and relationship of clonal tea (*Camellia sinensis*) cultivars in China as revealed by SSR markers. *Plant Syst. Evol*., 298(2): 469-483.

Frascaroli, E., T.A. Schrag and A.E. Melchinger. 2013. Genetic diversity analysis of elite european maize (*Zea mays* L.) inbred lines using AFLP, SSR, and SNP markers reveals ascertainment bias for a subset of SNPs. *Theor. Appl. Genet*., 126(1): 133-141.

Ji, P.Z., J. Gao, M.Z. Liang and X.Q. Huang. 2012. AFLP analysis of genetic variation among cloned, seed produced and wild Camellia sinensis var. assamica tea plant in Yunnan, China. *Pak. J. Bot.*,44(6): 1989-1992.

Labate, J.A., K.R. Lamkey, S.E. Mitchell, S. Kresovich, H. Sullivan and J.S. Smith. 2003. Molecular and historical aspects of corn belt dent diversity. *Crop Sci*., 43(1): 80-91.

Lassois, L., C. Denancé, E. Ravon, A. Guyader, R. Guisnel, L. Hibrand-Saint-Oyant, C. Poncet, P. Lasserre-Zuber, L. Feugey and C.E. Durel. 2016. Genetic diversity, population structure, parentage analysis, and construction of core collections in the french apple germplasm based on ssr markers. *Plant Mol. Biol. Rep*., 34(4): 827-844.

Liu, B.Y., C.M. Zhao, X.M. Sun, Y.Y. Li, H.B. Jiang, Y.G. Wang, L. Ma and Z.F. Duan. 2015. Establishment of DNA fingerprinting in clonal tea improved cultivars from yunnan of china using ISSR markers. *Pak. J. Bot.*, 47(4): 1333-1340.

Liu, K. and S.V. Muse. 2005. Powermarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics*, 21(9): 2128-2129.

Liu, S., H. Liu, A. Wu, Y. Hou, Y. An and C. Wei. 2017. Construction of fingerprinting for tea plant (*Camellia sinensis*) accessions using new genomic SSR markers. *Mol. Breed.*, 37(8).

Ma, J.Q., L. Huang, C.L. Ma, J.Q. Jin, C.F. Li, R.K. Wang, H.K. Zheng, M.Z. Yao and L. Chen. 2015. Large-scale SNP discovery and genotyping for constructing a high-density genetic map of tea plant using specific-locus amplified fragment sequencing (SLAFseq). *PloS One*, 10(6): e0128798.

Ma, J.Q., M.Z. Yao, C.L. Ma, X.C. Wang, J.Q. Jin, X.M. Wang and L. Chen. 2014. Construction of a SSR-based genetic map and identification of QTLs for catechins content in tea plant (*Camellia sinensis*). *PLoS One*, 9(3): e93131.

Ma, J.Q., Y.H. Zhou, C.L. Ma, M.Z. Yao, J.Q. Jin, X.C. Wang and L. Chen. 2010. Identification and characterization of 74 novel polymorphic est-ssr markers in the tea plant, *Camellia sinensis* (Theaceae). *Amer. J. Bot*., 97(12): e153-e156.

Mckay, D.L. and J.B. Blumberg. 2002. The role of tea in human health: an update. *J. Am. Coll. Nutr*., 21(1):1-13.

Meegahakumbura, M., M.C. Wambulwa, K. Thapa, M. Li, M. Möller, J. Xu, J. Yang, B. Liu, S. Ranjitkar and J. Liu. 2016. Indications for three independent domestication events for the tea plant (*Camellia sinensis* (L.) O. Kuntze) and new insights into the origin of tea germplasm in china and india revealed by nuclear microsatellites. *PloS One*, 11(5): e0155369.

Mu, S.Q., X.F. Gu, S.P. Zhang, X.W. Wang and Y. Wang. 2008. Genetic diversity of cucumber (*Cucumis sativus* L.) germplasm by SSR. *Acta Hortic. Sinica*, 9: 1, 323-321, 330.

Ni, J., P.M. Colowit and D.J. Mackill, 2002. Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Sci*., 42(2): 601-607.

Pandolfi, C., S. Mugnai, E. Azzarello, S. Bergamasco, E. Masi and S. Mancuso. 2009. Artificial neural networks as a tool for plant identification: A case study on vietnamese tea accessions. *Euphytica*, 166(3): 411-421.

Rohlf, F.J. 2000. Statistical power comparisons among alternative morphometric methods. *Amer. J. Phys. Anth.*., 111(4): 463.

Sharma, R.K., M.S. Negi, S. Sharma, P. Bhardwaj, R. Kumar, E. Bhattachrya, S.B. Tripathi, D. Vijayan, A.R. Baruah, S.C. Das, B. Bera, R. Rajkumar, J. Thomas, R.K. Sud, N. Muraleedharan, M. Hazarika, M. Lakshmikumaran, S.N. Raina and P.S. Ahuja. 2010. AFLP-based genetic diversity assessment of commercially important tea germplasm in india. *Biochem. Genet*., 48(7-8): 549-564.

Tan, L.Q., L.Y. Wang, L.Y. Xu, L.Y. Wu, M. Peng, C.C. Zhang, K. Wei, P.X. Bai, H.L. Li and H. Cheng. 2016. SSR-based genetic mapping and qtl analysis for timing of spring bud flush, young shoot color, and mature leaf size in tea plant (*Camellia sinensis*). *Tree Genet. Genome*, 12(3): 52.

Tan, L.Q., M. Peng, L.Y. Xu, L.Y. Wang, S.X. Chen, Y. Zou, G.N. Qi and H. Cheng. 2015. Fingerprinting 128 chinese clonal tea cultivars using ssr markers provides new insights into their pedigree relationships. *Tree Genet. Genome*., 11(5).

Taniguchi, F., K. Kimura, T. Saba, A. Ogino, S. Yamaguchi and J. Tanaka. 2014. Worldwide core collections of tea (*Camellia sinensis*) based on ssr markers. *Tree Genet. Genome*, 10(6): 1555-1565.

Wang, C., S. Lv, Y. Wu, M. Lian, X. Gao and Q. Meng. 2016. Study of aroma formation and transformation during the manufacturing process of biluochun green tea in yunnan province by HS-SPME and GC-MS. *J. Sci. Food Agr*., 96(13): 4492-4498.

Yang, Y., Z. Liu, Y. Zhao, G. Liang and X. Zhao. 2009. Genetic diversity and relationship of huangjincha cultivar based on est-ssr markers. *J. Tea Sci*., 29(3): 236-242.

Yao, M., L. Chen, X. Wang, L. Zhao and Y. Yang. 2007. Genetic diversity and relationship of clonal tea cultivars in china revealed by ISSR markers. *Acta Agron. Sin.*,33(4): 598-604.

Yao, M.Z., C.L. Ma, T.T. Qiao, J.Q. Jin and L. Chen. 2012. Diversity distribution and population structure of tea germplasms in china revealed by EST-SSR markers. *Tree Genet. Genome*., 8(1): 205-220.

Zhao, L.P., Z. Liu, L. Chen, M.Z. Yao and X.C. Wang. 2008. Generation and characterization of 24 novel est derived microsatellites from tea plant (*Camellia sinensis*) and cross-species amplification in its closely related species and varieties. *Conserv. Genet*., 9(5): 1327-1331.

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