ARTICLE

Varietal identification of tea (Camellia sinensis) using nanofluidic array of single nucleotide polymorphism (SNP) markers

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Apart from water, tea is the world’s most widely consumed beverage. Tea is produced in more than 50 countries with an annual production of approximately 4.7 million tons. The market segment for specialty tea has been expanding rapidly owing to increased demand, resulting in higher revenues and profits for tea growers and the industry. Accurate varietal identification is critically important to ensure traceability and authentication of premium tea products, which in turn contribute to on-farm conservation of tea genetic diversity. Using a set of single nucleotide polymorphism (SNP) markers developed from the expressed sequence tag (EST) database of Camellia sinensis, we genotyped deoxyribonucleic acid (DNA) samples extracted from a diverse group of tea varieties, including both fresh and processed commercial loose-leaf teas. The validation led to the designation of 60 SNPs that unambiguously identified all 40 tested tea varieties with high statistical rigor (p<0.0001). Varietal authenticity and genetic relationships among the analyzed cultivars were further characterized by ordination and Bayesian clustering analysis. These SNP markers, in combination with a high-throughput genotyping protocol, effectively established and verified specific DNA fingerprints for all tested tea varieties. This method provides a powerful tool for variety authentication and quality control for the tea industry. It is also highly useful for the management of tea genetic resources and breeding, where accurate and efficient genotype identification is essential.

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INTRODUCTION

Tea is the most widely consumed beverage in the world next to water.1 With an annual global production of 4.72 million tons (http://faostat.fao.org/), tea represents a $40 billion-a-year industry, with significant expected growth in non-Asian regions (http://www.worldteaenews.com/; 2013). The tea plant, Camellia sinensis (L.) O. Kuntze, is a woody evergreen species in the family Theaceae, and in the subgenus section Thea.2–4 Its putative center of origin is in an area of South-East Asia that includes south and southwest China, Indo-China and northeastern India.2–5 Although tea is thought to have been domesticated in China, the exact region where tea came under cultivation is not clear and the ancestry of the cultigens has not been identified.3,6

Commercial tea products are classified into different categories based on processing techniques, i.e., manner of fermentation and oxidization. The common categories include green tea, black tea, oolong tea, white tea, yellow tea and dark tea. Within each category, a large number of varieties are used in tea production, often with greatly differing quality. It is estimated that in different regions of China, several thousand tea varieties are being cultivated. In addition, growing conditions, cultivation practices and harvesting time also significantly influence the quality and post-harvest attributes of tea.7

In spite of the significant effects of genotype on tea qualities, efficient methods for varietal authentication in the tea value chain have not yet been developed. Numerous instrumental methods to authenticate tea varieties have been investigated, among which near-infrared spectroscopy (1988) has been studied the most. This rapid and non-invasive method was employed by numerous investigators for authentication studies of tea.8–12 However, while near-infrared spectroscopy can effectively evaluate many quality attributes, accurate varietal identification remains an unsolved problem when large numbers of genotypes need to be examined. In addition to near-infrared spectroscopy, deoxyribonucleic acid (DNA)-based methods have been applied to identify plant species from a large array of commercial tea products.13 Microsatellite markers have been used in tea variety identification,14–22 In addition, sequence-tagged sites and cleaved amplified polymorphic sequences were applied to tea varietal identification.23,24 However, to date, the application of DNA fingerprinting has been used only for the differentiation of varieties, which precludes verification of large numbers of varieties through exact genotype matching. Moreover, resolving genotyping results from different labs, even with the use of microsatellite markers, has not been straightforward. It is difficult to standardize data generated from different genotyping platforms, and comparison of data is further complicated, because the same allele may be binned differently. Therefore, the use of simple sequence repeat (SSR)-based fingerprints for tea authentication can lead to false conclusions.

Recent progress in technology for plant genomics has led to the escalation in use of single nucleotide polymorphism (SNP) markers in DNA fingerprinting.25 The most abundant class of polymorphisms in plant genomes,26,27 SNPs have many advantages that are leading to their use as marker of choice. Unlike SSR markers, DNA separation by size is not required to analyze SNPs, and an assay array format or microchips can be used to accurately determine their identities. Because SNPs are biallelic and codominant markers,
the error rate in allele calling is much lower than with SSRs and quick, low-cost, multiplex genotyping techniques can be employed. These advantages have resulted in SNPs increasingly becoming the markers of choice for accurate genotype identification and in crop improvement. Using a nanofluidic system to analyze SNP markers, Fang et al. generated SNP fingerprint patterns for small quantities of DNA extracted from the seed coat of single cacao beans. Based on the SNP profiles, an assumed adulterant variety was unambiguously distinguished from the authentic beans by multilocus matching.

*Camellia sinensis* has a genome size of 4.0 Gb. Full genomic sequences of *Camellia sinensis* have not been developed; however, a substantial amount of transcriptome data and various expressed sequence tags (ESTs) have been developed from different tissues, including young roots, flower buds, immature seeds and roots. The publicly accessible EST databases offer a low-cost source for an effective first step in SNP discovery. The objectives of the present study were to develop SNP markers through the data mining of EST databases of tea plants and assess their potential application for tea varietal identification. The SNP resources reported herein represent the first study of EST-derived SNP validation in tea and demonstrate the utility of EST databases as an alternative approach for de novo SNP identification in species whose genome sequences are not yet available. These SNP markers, as well as the genotyping method, would be particularly useful for varietal authentication, germplasm management and tea breeding programs.

**MATERIALS AND METHODS**

Mining of putative SNPs from tea EST database

EST sequences of *Camellia sinensis* were obtained from the EST database at the NCBI GenBank (http://www.ncbi.nlm.nih.gov/). The FASTA-formatted files of EST sequences were downloaded and merged into a single dataset for data mining. The mRNA and cDNA sequences in the dataset were removed using the program EST-trimmer (http://pgrc.ipk-gatersleben.de/misa/download/est_trimmer.pl). Redundant entries were examined and excluded using the CD-HIT program with a 95% sequence similarity threshold. Only clusters that included at least six EST sequences, with a confidence score over 4, were accepted. In order to meet the requirements and constraints for primer design, all candidates for SNP markers with less than 60 nucleotides between two neighboring SNPs, and with flanking sequences less than 10 nucleotides long, were removed. A subset of the identified SNP sequences was then chosen for design and manufacture of primers to assay for SNPs in tea plant.

Validation of putative SNPs

To evaluate the putative SNP markers for suitability of varietal identification, we used a nanofluidic genotyping system and validated the SNPs for 40 diverse tea varieties (Table 1). Leaf samples of these varieties were obtained from the Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, China. Actively growing shoot and leaf samples from all varieties were harvested and dried in silica gel (Table 1).

DNA was extracted from dried tea leaves with the DNeasy® Plant Mini kit (Qiagen Inc., Valencia, CA, USA), which is based on the use of silica as an affinity matrix. The dry leaf tissue was placed in a 2-mL microcentrifuge tube with one ½-inch ceramic sphere and 0.15 g garnet matrix (Lysing Matrix A; QIAGEN, Hilden, Germany). The leaf samples were disrupted by high-speed shaking in a TissueLyser II (Qiagen Inc.) at 30 Hz for 1 min. Lysis solution (DNeasy® kit buffer AP1 containing 25 mg mL⁻¹ polyvinylpolypyrrolidone), along with ribonuclease I, was added to the powdered leaf samples and the mixture was incubated at 65 °C, as specified in the kit instructions. The remainder of the extraction method followed manufacturer’s suggestions. DNA was eluted from the silica column with two washes of 50 μL Buffer AE, which were pooled, resulting in 100 μL DNA solution. Using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA), DNA concentration was determined by absorbance at 260 nm. DNA purity was estimated by the 260 : 280 ratio and the 260 : 230 ratio. Ninety-six putative SNP sequences were submitted to the Assay Design Group at Fluidigm Corporation (South San Francisco, CA, USA) for design and manufacture of primers for a SNPtype™ genotyping panel. The assays were based on competitive allele-specific polymerase chain reaction (PCR) and enable bi-allelic scoring of SNPs at specific loci (KBioscience Ltd., Hoddesdon, UK). The Fluidigm SNPtype™ Genotyping Reagent Kit was used according to the manufacturer’s instructions. Using these primers, the isolated DNAs were subjected to Specific Target Amplification in order to enrich the SNP sequences of interest. Genotyping was performed on a nanofluidic 96.96 Dynamic Array™ IFC (Integrated Fluidic Circuit; Fluidigm Corp.). This chip automatically assembles PCR reactions, enabling simultaneous testing of up to 96 samples with 96 SNP markers. The use of a 96.96 Dynamic Array IFC for SNP genotyping of human samples was described by Wang et al. End-point fluorescent images of the 96.96 IFC were acquired on an EP1™ imager (Fluidigm Corp.). The data was analyzed with Fluidigm Genotyping Analysis Software.

**DATA ANALYSIS**

Informativeness of SNP markers

Key descriptive statistics for measuring the informativeness of these 96 SNP markers were calculated, including minor allele frequency, observed heterozygosity, expected heterozygosity, Shannon’s information index and inbreeding coefficient. The program

| Table 1. List of 40 Chinese tea varieties used in SNP genotyping |
|-------------------|-------------------|-------------------|-------------------|
| Number | Sample code | Name of variety | Source/origin |
| 1 | QH019 | QH019 | Qimen, Anhui |
| 2 | NNT001 | Qimen Qunti 1 | Anhui |
| 3 | NNT068 | Xicha 10 | Wuxi, Jiangsu |
| 4 | NNT069 | Biluochun | Wuxian, Suzhou, Jiangsu |
| 5 | NNT070 | Xicha 5 | Wuxi, Jiangsu |
| 6 | NNT092 | Tiantai Qunti | Tiantai, Zhejiang |
| 7 | NNT093 | Anjiabacha | Anji, Zhejiang |
| 8 | NNT094 | Jiukengzhong | Chun'an, Zhejiang |
| 9 | NNT095 | Longjing 43 | Hangzhou, Zhejiang |
| 10 | NNT096 | Zhongnong 12 | Hangzhou, Zhejiang |
| 11 | NNT097 | Byun | Hangzhou, Zhejiang |
| 12 | NNT098 | Hanlv | Hangzhou, Zhejiang |
| 13 | NNT100 | Zhongcha 102 | Hangzhou, Zhejiang |
| 14 | NNT101 | Juhaichun | Hangzhou, Zhejiang |
| 15 | NNT102 | Chulv | Hangzhou, Zhejiang |
| 16 | NNT103 | Zisun | Hangzhou, Zhejiang |
| 17 | NNT104 | Jiande Qunti | Jiande, Zhejiang |
| 18 | NNT105 | Juyan Qunti | Jinhua, Zhejiang |
| 19 | NNT106 | Leqing Qingcha 2 | Leqing, Zhejiang |
| 20 | NNT107 | Shuguchu | Linhai, Zhejiang |
| 21 | NNT021 | Liannan Dayecha | Liannan, Guangdong |
| 22 | NNT023 | Puning Xiaoyezhong | Puning, Guangdong |
| 23 | NNT024 | Qingsuizhong | Qingsui, Guangdong |
| 24 | NNT028 | Renhua Yuanye | Renhua, Guangdong |
| 25 | NNT029 | Longshan Kucha | Ruyuan, Guangdong |
| 26 | NNT032 | Beijue Danzhu | Longzhou, Chongzuo, Guangxi |
| 27 | NNT033 | Maoyu Danzhu | Longzhou, Chongzuo, Guangxi |
| 28 | NNT037 | Bengpo Dachashu | Longsheng, Guilin, Guangxi |
| 29 | NNT039 | Nuobingcha | Pingle, Guilin, Guangxi |
| 30 | NNT040 | Hexanzhong | Hexian, Hezhou, Guangxi |
| 31 | NNT041 | Xianggacha | Shaoping, Hezhou, Guangxi |
| 32 | NNT047 | Liubaocha | Guangzhou, Wuzhou, Guangxi |
| 33 | NNT012 | Chiyi | Anxi, Fujian |
| 34 | NNT013 | Qingxin Qian | Anxi, Fujian |
| 35 | NNT014 | Jianmian Qian | Anxi, Fujian |
| 36 | NNT015 | Anxi Baicha | Anxi, Fujian |
| 37 | NNT016 | Banqingming | Fuding, Fujian |
| 38 | NNT017 | Ajiawo Wulong | Jian’ou, Fujian |
| 39 | NNT018 | Tieluohan | Wuyi, Fujian |
| 40 | JX020 | Jinxuan | Taiwan, China |
GenAlEx 6.5 was used for computation. For genotype identification, pairwise multilocus matching was applied among individual samples using the same program. DNA samples that were fully matched at the genotyped SNP loci were declared the same genotype (or clones).

To assess the differentiation power of the SNP markers, we calculated the probability of identity (PID). We computed the PID among siblings, which was defined as the probability that two sibling individuals drawn at random from a population have the same multilocus genotype. This can be computed using the following equation:

\[ P_{ID_{sib}} = 0.25 \left( \frac{1}{2} \left( \sum p_i^2 \right)^2 \right) \]  

where \( p_i \) is the frequency of the \( i \)th allele.

Analysis of genetic relationship among varieties
Distance-based multivariate analysis was used to assess the relationship among the individual varieties. Pairwise genetic distances as defined by Peakall et al. were computed using the DISTANCE procedure implemented in GenAlEx 6.5. The same program was then used to perform Principal Coordinates Analysis (PCoA), based on the pairwise distance matrix. Both distance and covariance were standardized.

A model-based clustering algorithm implemented in the STRUCTURE software program was applied to the SNP data. This algorithm attempted to identify genetically distinct subpopulations based on allele frequencies. The admixture model was applied and the number of clusters (\( K \)-value), indicating the number of subpopulations the program attempted to find, was set from 1 to 10. The analyses were carried out without assuming any prior information about the genetic group or geographic origin of the samples. Ten independent runs were assessed for each fixed number of clusters (\( K \)), each consisting of \( 1 \times 10^6 \) iterations after a burn-in of \( 2 \times 10^6 \) iterations. The \( \Delta K \) value was used to detect the most probable number of clusters and the computation was performed using the online program STRUCTURE HARVESTER. Of the 10 independent runs, the one with the highest \( \ln Pr(\mathbf{X}|K) \) value (log probability or log likelihood) was chosen and represented as bar plots.

RESULTS

SNP discovery
A total of 202278 ESTs and mRNA nucleotide sequences were downloaded from NCBI (26 August 2013), after adapter removal, trimming and quality control, 124 647 higher quality sequences were selected. CAP3 program was used to assembly sequences into 20 934 contigs and 40 810 singletons with an average size of 4.005 sequences per contig under default parameter, among which putative SNPs were detected in 789 contigs using the QualitySNP program. All of these selected clusters included a minimum of six EST sequences. In total, we obtained 1786 putative EST-SNPs, of which, 1193 were transition types, including 672 C/T and 521 A/G. There were 593 transversion types, including 162 A/T, 142 A/C, 162 T/G and 127 C/G. To select high-quality SNPs for validation, candidate SNP sites with at least 60 bp before and after the site were filtered. We calculated the number of all sequences in a cluster and the number containing the SNP type in this cluster. We then selected 96 SNPs for validation by genotyping a test panel of tea varieties, including both clonal and seed populations. The flanking sequences and SNPs are listed as Supplementary Table 1.

Frequency of SNP markers and descriptive statistics
Out of the 96 genotyped SNP markers, 75 were successfully genotyped (Figure 1), while genotyping failed for 21 SNPs (21.9%), likely due to the sequence complexity or the presence of polymorphisms within flanking sequences. Among the successfully genotyped SNPs, 15 were monomorphic across the 40 tea varieties (i.e., only one SNP variant was identified in all individuals). These monomorphic markers likely resulted from errors in EST sequencing, which then led to incorrect identification of SNP. It is also possible...
that some of these SNPs may correspond to rare alleles that were not present in the test panel of tea varieties.

A total of 60 polymorphic SNPs were retained for further analysis. These 60 SNPs were reliably scored across the validation panel, and thus were considered true SNPs (Table 2). The minor allele frequency of these SNPs ranged from 0.03 to 0.485 with an average of 0.267. The mean observed heterozygosity was 0.324, ranging from 0.023 to 0.970, whereas the mean expected heterozygosity was 0.354 ranging from 0.045 to 0.579 (Table 2).

Variety authenticity
Repeated tests, using three independently extracted DNAs from the same tea variety, showed that the genotyping result was highly consistent. The seven repeatedly genotyped varieties (each with three replications) always generated the same SNP profiles. Individual genotype matching (pairwise comparisons) based on the 60 SNP markers showed that each of the 40 varieties has a unique SNP profile (Table 3). The probability that two tea varieties will have the same genotype at the 60 SNP loci is approximately 1 in 100 000 for the tested tea varieties, as computed by the mutliclouc matching procedure implemented in GenAlex 6.5.40

Genetic relationship among tea varieties
The genetic relationships among the 40 tea varieties are presented in the principal coordinates analysis plot (Figure 2). Each of the tested varieties was clearly separated from one another. Although the pattern of grouping was not apparent, it appeared that all the tested varieties could be grouped into three clusters. The first cluster was comprised mainly of the varieties from Southern China, including Guangxi and Guangdong. The second cluster included most of the varieties from Fujian Province, which is adjacent to Guangdong. The third cluster covered varieties from Zhejiang, Anhui and Jiangsu, including several of the well-known green tea varieties, such as 'Longjing' and 'Bi Yun'.

Population stratification of the 40 varieties, based on ΔK value computed by STRUCTURE HARVESTER, revealed two clusters as the most probable number of K (Figure 3) and the partition was largely compatible with the principle coordinate analysis (Figure 2). Almost all the varieties that fell into the cluster of Southern China (Guandong and Guangxi) in the PCoA were assigned to one Bayesian cluster. However, varieties which fell in the two clusters of Fujian and Eastern China in the PCoA were grouped in another single Bayesian cluster. Varieties ‘Shiguachu’ and ‘Yenhuiuyan’ were hybrids between the two clusters. This result appeared compatible with recent report based on SSR markers, where landraces from Southern China, and east coastal provinces (Zhejiang, Jiangsu and Anhui) were grouped in different clusters.

**DISCUSSION**

The market of specialty tea has been expanding rapidly at a global scale. So far, it is not possible to distinguish the genetic identity of a tea variety by other means, such as morphological and biochemical characteristics, especially for processed tea. Due to the insufficient throughput, accuracy and data standardization, the existing molecular marker-based technology, such as SSR marker fingerprinting, is of limited use. Tea leaf in commercial products usually contains a high level of polyphenolic and other PCR-inhibitory compounds and, in addition, there is residue from microorganisms that is a direct result of the fermentation and drying processes. Because of these problems, a robust analytical system is needed to genotype tea DNA.

In the present study, we demonstrated a DNA fingerprinting method that uses a small set of SNP markers to verify the genetic identity of a single bud or leaf. This method can handle a large amount of samples in a short period of time and the result is highly robust and repeatable. Our results showed that the nanofluidic array of SNP markers is particularly suitable for this purpose. The Specific Target Amplification protocol efficiently dealt with potential problems of the quality or quantity of DNA extracted from tea leaves. The Specific Target Amplification protocol, performed before genotyping, is a multiplex PCR reaction using primers for all loci of interest, but without targeting the specific alleles, thus proportionally increasing the copies of these loci. This procedure has been demonstrated to significantly improve call rates for SNPs in *Chenopodium quinoa*, *Amaranthus*, *Rosoideae* and cacao, and solved our problem of the low concentration of DNA obtained from processed commercial tea leaves. Results from the repeatedly genotyped leaves (three independent DNA extractions performed from the same tree) showed 100% concordance, suggesting that the nanofluidic system is a reliable platform for generating tea DNA fingerprints with high accuracy.

The effectiveness of individual identification via SNP fingerprints depends on the number of loci used for genotyping. An important statistical parameter for determining the number of loci required to identify all distinct individuals with a needed confidence level, is the PID. Multilocus PID values can be obtained by multiplying together single-locus PID values, assuming independence of loci. A stringent PID value is needed for domesticated crop species, because they often share similar ancestors. Therefore, PID calculated for sibs would provide a highly conservative boundary for domesticated crop species. The present result shows that using the 60 SNP loci, the chance of sampling identical genotypes from a random mating population would be 1 out 100 000. It thus predicts the high statistical power of using this set of SNPs for tea genotype verification.

Although the distance-based PCoA grouped the 40 varieties into three clusters, the Bayesian approach using STRUCTURE partitioned the tested varieties into two clusters (K = 2). The varieties from Fujian were assigned together with those from eastern China in the Bayesian clustering analysis. If Fujian was included as part of eastern China, the result generated by PCoA would be fully compatible with the Bayesian clustering analysis. Among the varieties from Eastern China, ‘Zhenong 12’ showed a membership of Southern China. This is because ‘Zhenong 12’ is a hybrid variety of ‘Fuding Dabaicha’ and ‘Yunan Daye’, and none of these parents were traditional varieties from eastern China. The result is also compatible with the groupings reported in Yao et al., where used microsatellite markers to assess genetic relationship among the Chinese tea varieties. In the report of Yao et al., the assigned clusters of Chinese tea varieties largely followed the geographical distribution, indicating that there was substantial regional differentiation among the Chinese tea landraces. Even so, less than 50% of the total variation can be explained by the PCoA, indicating the variation between clusters (or between regions) is relatively small. This observation supported the conclusion of Yao et al., which was based on SSR analysis, that most of the diversity was found within populations, whereas interpopulation variation accounted only for less than 5% of the total molecular variance. This result also supports previous reports that there might be a high level of inbreeding among the tested Chinese varieties, as indicated by a large inbreeding coefficient and reduced allelic diversity.

There remain a large number of EST sequences available for tea, offering an attractive alternative resource for *in silico* SNP identification. More SNP markers will be needed in order to assess genetic relationship among different varieties. In addition, validation of tea EST-derived SNPs in a large number of tea germplasm groups will help the identification of fully informative SNPs, but also will highlight the proportion of informative SNPs shared across different germplasm groups. More stringent SNP quality control will be needed to minimize PCR artifacts. Moreover, multiple leaf samples should be included in genotyping to examine genotyping repeatability. Sample of internal control needs to be applied to detect possible inconsistency between different plates.
Table 2. Minor allele frequency, heterozygosity and inbreeding coefficient of the 60 SNP loci scored on 40 Chinese tea varieties

| Marker name | Minor allele frequency | Expected heterozygosity | Observed heterozygosity | Inbreeding coefficient |
|-------------|------------------------|--------------------------|--------------------------|------------------------|
| Cs1         | 0.394                  | 0.478                    | 0.546                    | −0.127                 |
| Cs3         | 0.242                  | 0.367                    | 0.303                    | 0.190                  |
| Cs4         | 0.318                  | 0.451                    | 0.394                    | 0.142                  |
| Cs5         | 0.409                  | 0.484                    | 0.394                    | 0.200                  |
| Cs7         | 0.045                  | 0.087                    | 0.091                    | −0.032                 |
| Cs8         | 0.061                  | 0.114                    | 0.121                    | −0.049                 |
| Cs9         | 0.485                  | 0.500                    | 0.424                    | 0.166                  |
| Cs11        | 0.136                  | 0.236                    | 0.212                    | 0.115                  |
| Cs12        | 0.182                  | 0.298                    | 0.303                    | −0.003                 |
| Cs13        | 0.454                  | 0.562                    | 0.303                    | 0.473                  |
| Cs15        | 0.273                  | 0.397                    | 0.303                    | 0.251                  |
| Cs16        | 0.121                  | 0.219                    | 0.121                    | 0.458                  |
| Cs20        | 0.121                  | 0.213                    | 0.242                    | −0.123                 |
| Cs22        | 0.496                  | 0.424                    | 0.424                    | 0.160                  |
| Cs23        | 0.045                  | 0.087                    | 0.091                    | −0.032                 |
| Cs24        | 0.061                  | 0.114                    | 0.121                    | −0.049                 |
| Cs25        | 0.121                  | 0.213                    | 0.242                    | −0.123                 |
| Cs27        | 0.030                  | 0.059                    | 0.061                    | −0.016                 |
| Cs30        | 0.258                  | 0.383                    | 0.394                    | −0.015                 |
| Cs31        | 0.318                  | 0.434                    | 0.394                    | 0.107                  |
| Cs32        | 0.454                  | 0.522                    | 0.485                    | 0.086                  |
| Cs33        | 0.364                  | 0.463                    | 0.424                    | 0.099                  |
| Cs36        | 0.470                  | 0.498                    | 0.394                    | 0.224                  |
| Cs37        | 0.424                  | 0.556                    | 0.152                    | 0.735                  |
| Cs38        | 0.424                  | 0.579                    | 0.242                    | 0.591                  |
| Cs39        | 0.242                  | 0.367                    | 0.303                    | 0.190                  |
| Cs42        | 0.030                  | 0.059                    | 0.061                    | −0.016                 |
| Cs43        | 0.136                  | 0.236                    | 0.273                    | −0.143                 |
| Cs44        | 0.273                  | 0.397                    | 0.546                    | −0.362                 |
| Cs45        | 0.439                  | 0.493                    | 0.576                    | −0.154                 |
| Cs47        | 0.151                  | 0.257                    | 0.303                    | −0.164                 |
| Cs48        | 0.136                  | 0.236                    | 0.273                    | −0.143                 |
| Cs49        | 0.227                  | 0.351                    | 0.333                    | 0.066                  |
| Cs51        | 0.424                  | 0.489                    | 0.546                    | −0.101                 |
| Cs52        | 0.091                  | 0.165                    | 0.182                    | −0.085                 |
| Cs53        | 0.424                  | 0.577                    | 0.364                    | 0.383                  |
| Cs54        | 0.439                  | 0.517                    | 0.273                    | 0.485                  |
| Cs55        | 0.485                  | 0.555                    | 0.485                    | 0.141                  |
| Cs57        | 0.394                  | 0.478                    | 0.485                    | 0.000                  |
| Cs66        | 0.409                  | 0.484                    | 0.394                    | 0.200                  |
| Cs67        | 0.106                  | 0.190                    | 0.152                    | 0.216                  |
| Cs68        | 0.061                  | 0.114                    | 0.061                    | 0.480                  |
| Cs71        | 0.106                  | 0.190                    | 0.212                    | −0.103                 |
| Cs74        | 0.485                  | 0.500                    | 0.970                    | −0.939                 |
| Cs76        | 0.106                  | 0.190                    | 0.212                    | −0.103                 |
| Cs77        | 0.303                  | 0.439                    | 0.182                    | 0.596                  |
| Cs78        | 0.227                  | 0.351                    | 0.212                    | 0.409                  |
| Cs79        | 0.061                  | 0.114                    | 0.020                    | 0.984                  |
| Cs81        | 0.136                  | 0.242                    | 0.212                    | 0.139                  |
| Cs82        | 0.424                  | 0.549                    | 0.242                    | 0.569                  |
| Cs84        | 0.288                  | 0.410                    | 0.455                    | −0.093                 |
| Cs85        | 0.197                  | 0.316                    | 0.394                    | −0.231                 |
| Cs87        | 0.197                  | 0.316                    | 0.273                    | 0.153                  |
| Cs88        | 0.485                  | 0.500                    | 0.485                    | 0.045                  |
| Cs89        | 0.424                  | 0.489                    | 0.424                    | 0.147                  |
| Cs91        | 0.364                  | 0.463                    | 0.546                    | −0.164                 |
| Cs93        | 0.076                  | 0.140                    | 0.152                    | −0.067                 |
| Cs94        | 0.258                  | 0.396                    | 0.212                    | 0.477                  |
| Cs95        | 0.242                  | 0.367                    | 0.485                    | −0.306                 |
| Cs97        | 0.485                  | 0.499                    | 0.970                    | 0.939                  |
| Mean        | 0.267                  | 0.354                    | 0.324                    | 0.115                  |
This information is crucial for selecting an optimum core set of SNP markers for tea varietal identification, as well as for assisting tea germplasm management and breeding.

In conclusion, we conducted a pilot study on varietal authentication for tea. We developed a set of SNP markers and used them for varietal genotyping using a nanofluidic array. This technology...
enabled us to generate high quality SNP profiles based on DNA extracted from both fresh and processed tea products, including white, green, oolong and black tea. Together with forensic statistical tools, these SNP-based DNA fingerprints allowed unambiguous identification of all tested varieties. To our knowledge, this is the first authentication study in commercial tea products using molecular makers. This approach is robust for authentication verification of specialty tea varieties and thus, has a significant potential for practical application.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

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