Systematic Evaluation of Landrace Tea Populations in Northern Sichuan, China, Based on Morphology, DNA Markers, and Biochemistry Analyses

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Abstract. Landrace tea populations are important recourses for germplasm conservation and selection of elite tea clone cultivars. To understand their genetic diversity and use them effectively for breeding, two traditional landrace tea populations, Beichuan Taizicha (BCTZ) and Nanjiang Dayecha (NJDY), localized to northern Sichuan, were evaluated for morphological characters, simple sequence repeat (SSR)-based DNA markers and the contents of biochemical components. A wide range of morphological variation and a moderately high level of DNA polymorphism were observed from both BCTZ and NJDY. NJDY had on average, bigger leaves, larger flowers, higher total catechins (TCs), and greater gene diversity (GD) than BCTZ. Interestingly, samples from BCTZ had a wide range in the ratio of galloylated catechins to nongalloylated catechins (G/NG) (1.83–8.12, cv = 48.8%), whereas samples from NJDY were more variable in total amino acid (TAA) content (25.3–50.8 mg·g⁻¹ dry weight) than those from BCTZ. We concluded that the two *Camellia sinensis* landrace populations are of great interest for both individual selection breeding and scientific studies.

As one of the most popular and oldest beverages in the world, tea is habitually and socially consumed by billions of people (Xia et al., 2017). The tea plant (*C. sinensis*), which is commercially cultivated on more than 3.80 million hectares of land, is of increasing economic and cultural importance (<http://faostat.fao.org>). There are two types of tea plant plantations according to the propagation method: one is clonally planted using cuttings from a few elite cultivars; the other is landrace population that developed from selected seeds (also called as Jats, Chen et al., 2007). Currently, clonal tea plantations are more prevalent because of the higher yield and uniform quality, and nearly, all newly developed tea plantations are clonal. However, some landrace populations also have good performance in yield and quality. These landraces are selected by tea farmers and passed from generation to generation, using seeds for hundreds of years. Unlike clonal plants, the landrace populations are

genetically polymorphic; therefore, they represent valuable genetic resources for breeding and diversity conservation. Actually, many elite clones that are widely used currently are selected from landraces (Chen et al., 2007).

Located in southwest China, Sichuan is one of the proposed regions of origin for *C. sinensis* (Zhong, 1980). It is also regarded as the first place where humans started to cultivate tea before the Han Dynasty (200 BC, Chen and Pei, 2003). Thousands of years of cultivation and conventional selective breeding efforts have resulted in a large number of *C. sinensis* landraces that have adapted to diverse habitats around the Sichuan basin. Among them, Nanjiang Dayecha (NJDY), Guling Niupicha (GLNP), Chongqing Pipacha (CQPP), and BCTZ are the most famous, and they were approved as Provincial Tea Landrace Populations by the *Sichuan Crop Variety Approval Committee* in 1985 and 1989 (China Tea Varieties Compilation Committee, 2001). The core distribution areas of the four landrace populations in Sichuan are shown in Fig. 1.

However, those landraces are being driven out of crop areas as a result of the wide use of clonal cultivars. In Nanjiang County, the area of origin of NJDY, the percentage of landrace populations has decreased from more than 90% in 1980 to 35% in 2013 (Nanjiang Agriculture Bureau). Another issue threatening the diversity of landrace tea is the abandonment of numerous tea

fields with landrace populations (Fig. 2). The situation is even worse for BCTZ as many old landrace tea plantations were destroyed in the devastating Wenchuan earthquake in May 2008 and more were abandoned because many tea farmers moved out after that disaster. Therefore, there is an urgent need to systematically evaluate those valuable landrace populations for better use and proper conservation before they disappear.

Morphological and biochemical analyses (Chen and Zhou, 2005; Chen et al., 2005; Jin et al., 2014) and SSR markers (Fang et al., 2012; Tan et al., 2015; Yao et al., 2012a) have been frequently used to investigate the genetic diversity of tea germplasm. Previously, we evaluated the CQPP and GLNP landrace populations using these analyses, and we identified a high level of genetic diversity and many individuals with potential breeding value (such as high amino acid or caffeine content) (Liu et al., 2014; Wang et al., 2012; Xie et al., 2015). However, partially because of the far distance and inconvenient traffic conditions, the systematic assessment of tea landrace populations from northern Sichuan (i.e., BCTZ and NJDY) is still lacking. In this study, by combining morphological analyses, SSR markers, and biochemistry techniques, we aim to reveal the diversity and differentiation of the other two famous landrace populations of *C. sinensis* in Sichuan. The purposes of this study are to 1) understand the genetic diversity of BCTZ and NJDY; 2) select individuals with potential for breeding; and 3) provide guidance for germplasm conservation efforts.

Materials and Methods

Plant materials. We investigated the distribution areas of BCTZ and NJDY in 2012 and 2013. With the government's and local tea farmers' help, we found several plantations of BCTZ in Chengjia and Guanlin in Beichuan County. NJDY plantations were found in Xialiang, Yuandingzi, Huitang, and Guimin in Nanjiang County. The distribution areas were mapped in Fig. 1. The tea plants identified (Fig. 2) are ≈60 to hundreds of years old according to the local famers and our rough estimations. The trees were grown from seeds, as the morphological traits were apparently different from each other. To include the most possible diversity for each population, we randomly chose samples from multiple sites. For BCTZ, we chose seven sites (numbered one to seven; lat. 31°50'N–32°05'N, long. 104°32'E–104°37'E; altitude 870–1160 m; Fig. 1B) and 5–14 plants were randomly selected from each site (66 in total) for observation and sampling. For NJDY, eight sites (A–H; lat. 32°00'N–32°28'N, long. 107°06'E–107°46'E; altitude 880–1300 m; Fig. 1C) were located and 52 plants (NJ01–NJ52) were selected (4–10 from each site). Because many of the selected sites were abandoned or had been under poor management for years, we lightly trimmed the selected plants and fertilized them with

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0.2 kg of compound fertilizer (N:P:K = 0.2:0.12:0.14) for each plant in the February and October of 2015. Pests and diseases were also under strict control.

Morphological analyses. Morphological observations were performed on each plant according to the Guidelines for the Conduct of Tests for Distinctness, Uniformity and

Stability—Tea [*C. sinensis* (L.) O. Kuntze]—UPOV (TG238/1). Eight characteristics of the mature leaf (length, width, texture of the upper surface, vein number,

Fig. 1. The core distribution areas of four provincial landrace populations in Sichuan (A) and the sampling sites of BCTZ (B) and NJDY (C) in this study. BCTZ = Beichuan Taizicha; NJDY = Nanjiang Dayecha; CQPP = Chongqing Pipacha; GLNP = Guling Niupicha.

Fig. 2. Representative pictures of plants from Beichuan Taizicha (A–D) and Nanjiang Dayecha (E–G) investigated in this study.

leaf tooth number, undulation of margin, shape of apex, and shape of base) and five characteristics of the flower (diameter, petal number, position of style splitting, style length, and petal color) were recorded for each plant in Sept. and Oct. 2015. The leaf area (leaf length $\times$ width $\times$ 0.7), leaf shape index (leaf length/width), and density of leaf teeth (leaf teeth number/leaf length) were also calculated.

DNA extraction and SSR marker genotyping. Young leaves were sampled from each plant and then stored in liquid nitrogen until DNA extraction. Genomic DNA of each sample was extracted using a Tiangen DNA Kit (DP305, Beijing, China). The DNA concentration was measured with a NanoDrop 2000c spectrophotometer (Thermal, Waltham, MA) and then adjusted to 20 ng· μ L⁻¹ for polymerase chain reaction (PCR) amplifications. Initially, we screened 24 SSR markers that had been selected from references (Fang et al., 2012; Jin et al., 2007; Ma et al., 2014; Tan et al., 2015) with 12 DNA templates. Ten markers with high polymorphisms and low null allele rates were used for the final analysis. The methods for marker genotyping followed previously described methods (Tan et al., 2013). Briefly, PCR amplifications were performed in 10- μ L reaction mixtures and the products were resolved on 8% polyacrylamide gels and visualized using silver staining.

Biochemical analysis. In the spring of 2016, we collected the first batch of “one bud and two leaves” samples from 11 BCTZ individuals and eight NJDY individuals. These individuals were selected because their yields were relatively high, so enough fresh leaves could be harvested for analyses. Dried samples for biochemical analyses were prepared according to Jin et al. (2014). The water extract and caffeine content were determined using the Chinese National Standard GB/T8305–2002 and GB/T8312–2013. Catechin content was extracted and analyzed using high-performance liquid chromatography methods according to GB/T8313–2008 with minor modifications. Briefly, 0.2000 g of powdered sample was extracted twice by intermittent shaking in 5 mL 70% methanol and then centrifuged at 3500 rpm for 10 min. The extract was diluted 10 times with pure water and filtered through a 0.22 μ m millipore filter into auto sampler vials. An Agilent ZORBAX SB-C18 column (Agilent, Santa Clara, CA) was used in the analysis. For free amino acid (FAA) quantification, a Waters AccQ-Tag kit (Waters, Milford, MA) was used following the manufacturer’s protocol. The derivatized sample solution (1 μ L) was subjected to chromatographic analysis using an AccQ-Tag column (4 μ m, 3.9 $\times$ 150 mm; Waters). The content of each chemical in the tea leaf extract was calculated from the calibration curve of the standard chemical integral area. All biochemical analyses were performed with three replications and the means were used for data analysis.

Data analysis. Excel 2010 was used for morphological and biochemical trait data

analyses, including the calculation of means, SD, and cv. Student’s *t* test was used to determine if there was a significant difference between means of the two populations. Significance was accepted at $P < 0.05$. PowerMarker (Liu and Muse, 2005) and PopGene32 (Yeh et al., 1997) were used to calculate the number of alleles (N_A), observed heterozygosity (H_O), GD , and Nei’s genetic differentiation coefficient (G_{ST}). A phylogenetic tree was constructed based on Nei’s genetic distances and the neighbor-joining method and viewed using MEGA 4.0 (Tamura et al., 2007). The TC content and total FAAs were calculated as the sum of all individual components. The formulas for catechin index (CI) and the ratio of G/NG are as follows:

$$CI = (EC + ECG)/(EGC + EGCG) \\ (Jin et al., 2014),$$

$$G/NG = (EC + C + EGC + GC)/ \\ (ECG + CG + EGCG + GCG),$$

where EC was epicatechin, ECG was epicatechin gallate, EGC was epigallocatechin, EGCG was epigallocatechin gallate, C was catechin, GC was galocatechin, CG was catechin gallate, and GCG was galocatechin gallate.

Results

Morphological traits. Results from the polymorphic leaf and flower trait analyses are shown in Fig. 3 and Table 1. The leaf areas of the 62 BCTZ plants varied from 9.00 to 42.56 cm² (with an average of 22.56 cm²), whereas data from 52 NJDY plants varied from 10.57 to 49.67 cm² (with an average of 29.84 cm²). According to the average values, both landraces belong to the middle-sized leaf group (20–40 cm²), but the diversity is quite high with variation coefficients more than 30%. Both BCTZ and NJDY had all three types of upper surface textures, leaf margin undulation, and apex shapes, and the percentages of each type in the two populations were similar. For leaf base shape, only two types (acute and obtuse) were observed.

Compared with the average values of BCTZ, NJDY had a larger flower size (3.69 vs. 3.41 cm), more petals (6.55 vs. 6.49) but the same flower style length (1.33 cm). Most plants of the two populations had white petals but 12.1% of BCTZ and 7.7% of NJDY had light green petals. Three types of style splitting positions (low, medium, and high) were observed in both populations. Among the quantitative morphological characters investigated, leaf length, leaf vine number, leaf shape index, and density of leaf teeth had a higher cv in NJDY than in BCTZ; other characters had the opposite trend (Table 1).

SSR analysis. Results from the SSR marker genotyping analysis are shown in Table 2. A total of 47 alleles were detected

with 10 SSR markers. Specifically, the allele number (N_A) of each marker varied from three to eight, with an average of 4.3 in BCTZ and 4.5 in NJDY per locus. Among the detected alleles, there are six specific alleles that were only detected in one of the two populations (Table 2): two in BCTZ and four in NJDY. The observed heterozygosity (H_O) of the tested markers varied from 0.172 to 0.800 and Shannon’s information index (SI) varied from 0.337 to 1.691. The average values of H_O and SI of BCTZ are lower than those of NJDY (Table 2). Both populations showed a moderately high level of overall diversity, with an average GD of 0.583 in BCTZ and 0.632 in NJDY. The overall inbreeding coefficient (F_{IS}) values were 0.015 in BCTZ and 0.018 in NJDY, suggesting very low inbreeding rates. Nei’s genetic differentiation coefficient (G_{ST}) between the two populations was 0.021, indicating the differentiation between the two populations is not considerable.

Based on the SSR marker data, the 118 tested samples could be combined into four groups as shown in the NJ tree (Fig. 4). Group I includes 28 samples and 25 of them are from NJDY. Group II is a mixture of BCTZ and NJDY; it contains 20 samples in total with 11 from NJDY and nine from BCTZ. Group III also has 22 samples and four are NJDY. Group IV is the largest group with 48 samples and most of them (36) are BCTZ. Six NJDY samples formed a subgroup while another six were mixed with BCTZ samples in group IV. A few samples had a very close relationship, such as NJ01 and NJ04, NJ42 and NJ43, but all samples have unique genotype combinations, confirming that the included samples grew from seeds (not clones). Overall, although individuals from the same landrace clustered more closely, BCTZ and NJDY could not be clearly separated based on the marker data, suggesting the genetic differentiation between them is not significant.

Biochemical analysis. The biochemical results of 19 individuals that had a relatively high yield are shown in Table 3 and Fig. 5. Extracted water content ranged from 38.61% to 46.15% and the means of two populations were almost the same. Caffeine content ranged from 3.01 to 4.08 mg·g⁻¹ in BCTZ and 2.75 to 3.47 mg·g⁻¹ in NJDY and on average, BCTZ had a higher caffeine content than NJDY ($P < 0.05$). The TCs in NJDY was significantly higher than BCTZ ($P < 0.05$). Among the eight types of catechins, EGCG was most abundant in all tested samples, accounting for 44.2% to 62.9% (average 54.0%) of TCs (Fig. 5). The next-most abundant catechins were either ECG or EGC. The CIs was at a relatively lower level in both populations, with a mean of 0.28 in BCTZ and 0.26 in NJDY. Interestingly, the ratio of G/NG varied significantly in BCTZ, from 1.83 to 8.12 with a cv of 48.82%, but this ratio only varied from 2.29 to 3.90 in NJDY.

The TAA content in NJDY ranged from 25.25 to 50.08 mg·g⁻¹, with a cv of 23.79% (Table 3). In comparison, BCTZ had much

Fig. 3. Histogram distributions of the polymorphic morphological traits of Beichuan Taizicha (BCTZ) and Nanjiang Dayecha (NJDY).

less variation in TAA ($cv = 7.36\%$). Among the 21 detected types of amino acids, theanine (The) was the most abundant, accounting for 46.53% to 65.77% of the TAA content. The average percentage of theanine of the TAA content (The/TAA) in NJDY reached 63.07%, which was significantly higher than in BCTZ (57.49%, $P < 0.01$).

Discussion

Sichuan is especially rich in tea plant germplasm, including numerous wild plants and many landrace populations (Hou et al., 1998; Yao et al., 2012b). This rich genetic diversity provides an excellent source of new elite cultivars to be incorporated into selective breeding. In this study, we systematically

investigated the genetic diversity of two well-known landraces that have been cultivated in northern mountainous areas of Sichuan Province for a long time. A moderately high level of diversity was observed in both populations in morphological, SSR marker analyses and biochemical investigations.

Although the average leaf areas of the two populations belonged to the middle-sized

Table 1. Variation of the quantitative characteristics of leaf and flower in BCTZ and NJDY.

		BCTZ (<i>n</i> = 66)			NJDY (<i>n</i> = 52)		
	Characters	Range	Mean ± SD	cv (%)	Range	Mean ± SD	cv (%)
Leaf	Length (cm)	5.06–11.21	8.43 ± 1.41**	16.77	4.75–12.95	9.97 ± 1.99	20.00
	Width (cm)	2.33–5.63	3.72 ± 0.74**	19.95	2.10–5.60	4.19 ± 0.68	16.18
	Leaf area (cm ²)	9.00–42.56	22.56 ± 7.85**	34.78	10.57–49.67	29.84 ± 9.44	31.64
	Leaf shape index	1.76–3.41	2.29 ± 0.28	12.37	1.49–4.19	2.40 ± 0.47	19.61
	Leaf vine number	5.60–11.80	7.77 ± 0.95*	12.28	7.00–14.00	8.23 ± 1.15	14.03
	Leaf tooth number	21.50–40.50	30.00 ± 4.42*	14.73	20.00–40.00	31 ± 2.99	9.40
	Leaf tooth density (per cm)	2.59–5.93	3.62 ± 0.61**	16.73	2.25–6.75	3.37 ± 0.94	27.91
Flower	Diameter (cm)	2.05–4.85	3.41 ± 0.50**	14.62	2.23–4.51	3.69 ± 0.47	12.61
	Petal number	5.20–8.00	6.55 ± 0.68	10.39	5.13–9.00	6.49 ± 0.64	9.85
	Length of style (cm)	0.74–2.02	1.33 ± 0.27	19.89	0.97–1.88	1.33 ± 0.15	11.35

Means of BCTZ that are followed by * ($P < 0.05$) or ** ($P < 0.01$) indicate significant differences between BCTZ and NJDY.
BCTZ = Beichuan Taizicha; NJDY = Nanjiang Dayecha.

Table 2. Genetic parameters based on SSR markers.

Marker	N_A ²		H_O		SI		GD		F_{IS}		G_{ST}
	BCTZ	NJDY	BCTZ	NJDY	BCTZ	NJDY	BCTZ	NJDY	BCTZ	NJDY	
A114	3	3	0.508	0.588	0.667	0.680	0.416	0.469	−0.025	−0.021	0.002
A18	5	7(2)	0.677	0.569	1.463	1.615	0.746	0.771	0.126	0.217	0.011
P05	4	4	0.621	0.615	1.124	1.123	0.616	0.625	−0.006	0.004	0.080
TM056	4	4	0.591	0.673	1.210	1.205	0.660	0.674	0.049	0.057	0.014
TM132	4	4	0.172	0.420	0.337	0.865	0.175	0.426	0.061	−0.013	0.044
TM169	3	3	0.569	0.481	0.961	0.977	0.554	0.600	−0.040	0.212	0.008
TM209	5(1)	5(1)	0.636	0.706	1.243	1.150	0.655	0.633	0.019	−0.115	0.036
TM237	3	4(1)	0.508	0.750	0.836	1.210	0.489	0.651	0.004	−0.181	0.006
CsFM1508	4	4	0.758	0.720	1.369	1.347	0.744	0.721	0.000	−0.015	0.006
CsFM1599	8(1)	7	0.800	0.720	1.691	1.546	0.773	0.752	−0.034	0.038	0.003
Mean	4.3	4.5	0.584	0.624	1.091	1.172	0.583	0.632	0.015	0.018	0.021

²Numbers in parentheses are private alleles only detected in that cultivar.

N_A = allele number; H_O = observed heterozygosity; SI = Shannon's information index; GD = gene diversity; F_{IS} = inbreeding coefficient; G_{ST} = Nei's genetic differentiation coefficient; SSR = simple sequence repeat; BCTZ = Bichuan Taizicha; NJDY = Nanjiang Dayecha.

Fig. 4. Neighbor-joining phylogenetic tree based on the genotypes of simple sequence repeat markers.

group, we also found small-leaf (leaf area < 20 cm²) and large-leaf individuals (40–60 cm²) in both populations. Similar observations were made in the GLNP landrace in Sichuan (Xie et al., 2015), whereas in the CQPP we observed middle, large and extra-large (>60 cm²) leaves (Wang and Tang, 2012). Therefore, all four leaf-size types of cultivated tea could be found in the traditional landraces in Sichuan. Similarly, all four leaf shapes (indicated by leaf shape index), three leaf upper surface texture types, and margin undulation types could be found in both BCTZ and NJDY. These results showed a high level of variation of the leaf characters within the Sichuan landrace tea populations. However, as the leaf traits might be significantly influenced by plant age and environment factors, which were not under a strict control in this study, the observed morphological diversity may be overestimated.

The genetic parameters based on SSR marker genotypes, including N_A , H_O , and GD , revealed moderately high polymorphisms in the two landrace populations. These landraces have been grown in a limited area for a long time; therefore, the high diversity is likely because of the outcrossing nature of *C. sinensis*, low selection pressure, and frequent gene exchanges resulting from human activities (Chen et al., 2007; Paul et al., 1997). Although the BCTZ sample size was larger, relatively higher N_A , H_O , and GD were detected in NJDY. Generally, the

Table 3. The range and variation of water extract, caffeine, catechins, amino acids and the ratios in BCTZ and NJDY.

Characters	BCTZ (n = 11)			NJDY (n = 8)		
	Range	Mean $\pm$ sd	cv (%)	Range	Mean $\pm$ sd	cv (%)
Water extract (%)	41.05–46.33	43.39 $\pm$ 1.73	3.99	38.61–46.15	43.16 $\pm$ 2.71	6.27
Caffeine (mg·g ⁻¹)	3.01–4.08	3.32 $\pm$ 0.29*	8.72	2.75–3.47	3.06 $\pm$ 0.25	8.19
TC (mg·g ⁻¹)	126.62–156.83	138.53 $\pm$ 0.76*	5.47	119.17–181.77	157.27 $\pm$ 08.89	12.01
EGCG (mg·g ⁻¹)	61.70–92.51	75.45 $\pm$ 11.17	14.81	63.37–104.58	84.18 $\pm$ 13.26	15.75
G/NG	1.83–8.12	4.09 $\pm$ 1.99*	48.82	2.29–3.90	2.92 $\pm$ 0.65	22.20
CI	0.23–0.35	0.28 $\pm$ 0.04	13.51	0.24–0.31	0.26 $\pm$ 0.02	9.09
TAA (mg·g ⁻¹)	35.29–43.71	39.28 $\pm$ 2.89	7.36	25.25–50.80	34.37 $\pm$ 8.18	23.79
Theanine (mg·g ⁻¹)	17.59–27.60	22.61 $\pm$ 3.01	13.32	16.05–32.05	21.66 $\pm$ 5.13	23.69
The/TAA (%)	46.53–65.77	57.49 $\pm$ 5.59**	9.72	58.89–65.77	63.07 $\pm$ 2.19	3.48

Means of BCTZ that are followed by * ($P < 0.05$) or ** ($P < 0.01$) indicate significant differences between BCTZ and NJDY.

BCTZ = Bichuan Taizicha; NJDY = Nanjiang Dayecha; TC = total catechins; EGCG = epigallocatechin gallate; G/NG = the ratio of galloylated catechins to nongalloylated catechins; CI = catechin index; TAA = total amino acids; The/TAA = the percentage of theanine in total amino acids.

Fig. 5. The total content and fractions of the main components of catechins (left) and amino acids (right) in the 19 samples. EGCG = epigallocatechin gallate; ECG = epicatechin gallate; EGC = epigallocatechin; The = theanine; Glu = glutamic acid; Gln = glutamine; Asp = aspartic acid; Arg = arginine. The “Others” were the sums of the less abundant catechins (left) or amino acids (right). Error bars represented the sd of three replications were shown for EGCG, total catechins, The and total amino acids.

samples from the same population grouped more closely, but the two populations could not be divided into two groups based on the SSR marker data (Fig. 4). The genetic differentiation between the two landraces is not significant ($G_{ST} = 0.021$), which is also supported by the morphological observations.

The most important components for tea quality are water extract, tea catechins, caffeine, and amino acid content (Chen and Zhou, 2005). The average water extract of the samples in this study (43.26%) was comparable with the average of 596 tea accessions collected in China (44.7% with a range from 24.4% to 57.0%; Chen and Zhou, 2005). The total content of catechins (TC) in the 19 samples was also within the range of 403 tea accessions (56.6–231.9 mg·g⁻¹; mean = 154.5 mg·g⁻¹) investigated by Jin et al. (2014). In that study, EGCG was the most abundant catechin, accounting for an average of 60.9% of the TC. In our study, EGCG was also the most abundant catechin but the average percentage was only 54.0%. Traditionally, because of the high level of EGCG and caffeine, green teas produced in Sichuan were thought to be bitterer than teas from other places (Xu et al., 2010). This does

not appear to be the case with BCTZ and NJDY, and the bitterness of the teas produced from them was indeed not as strong as others from Sichuan (data not shown). The unique genetic characters and the high altitudes (870–1300 m) where they grow may contribute to the lower bitterness (Chen et al., 2014; Han et al., 2017). The FAA content is especially critical for green tea quality. Among the 19 plants analyzed, one from NJDY (NJ51) had 50.8 mg of FAA per gram of dry sample. In addition, the TC of this plant was the lowest (119.17 mg·g⁻¹), indicating it is a very promising candidate for the selection of an elite green clone (Han et al., 2017).

The CI has been frequently used as a biochemical marker for studying the genetic diversity and tea quality of tea germplasm (Gulati et al., 2009; Jin et al., 2014; Magoma et al., 2000; Saravanan et al., 2005). A recent study showed that functional SNP allelic variants within *F3'5'H* governing catechin contents and CI in *C. sinensis* (Jin et al., 2017). In this study, the average CI was 0.28 in BCTZ and 0.26 in NJDY, which is lower than the average (0.36) of 403 tea accessions in China (Jin et al., 2014) but higher than the average (0.22) of 26 tea clones in India

(Saravanan et al., 2005). Another interesting character of catechins is the ratio of G/NG, which is also largely genetically determined (Cui et al., 2016; Liu et al., 2012). This ratio indicated a very high level of diversity in BCTZ with a cv of 48%. It is of great interest to find the genetic basis underlying this trait. Based on our biochemical analyses, four individuals (BC17, low G/NG; BC28 and BC37, high G/NG; NJ51, higher FAA, and low TC) have been clonally propagated by cutting. They will be subjected to further selection tests and genetic studies.

Preliminary protection measures of the landraces have been carried out in Beichuan County. The local government identified the oldest tea plants and set up a protection plate beside each plant (Fig. 2C). However, only hundreds of plants were targeted; thus, the diversity of the whole population has not been well protected. We suggest, besides the oldest plants, the government should protect the landrace plantations (at least a part of them) from being replaced by clonal tea cultivars. The local farmers should be trained to improve the management of the landrace tea plantations. Based on the biochemical results, BCTZ and NJDY are good for green tea production. Therefore, using them to

produce high-quality green tea that can yield profit for tea farmers is a long-term strategy to protect these landraces. Furthermore, collecting seeds from the landraces at different sites and growing them in the Sichuan Germplasm Tea Repositories (Mingshan, Sichuan) is also an effective approach for germplasm conservation.

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