Tea genetic resources in China

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ABSTRACT: Cultivation and consumption of tea in China has a history of about 5,000 years. Most scientists believe that tea plants originated from the Yunnan province in south-western China. China has abundant tea germplasms in the whole world. In the past two decades, significant progress has been made in collection, conservation, evaluation and utilization of tea genetic resources in China. Further collection of tea germplasm and association analysis of molecular markers on important agronomic traits of genetic resources are proposed.

Keywords: Tea genetic resources, collection, conservation, evaluation, utilization

Introduction

Tea was initially used as a medicinal drink in ancient China and later as a beverage for thousands of years. Its legend dates back to 2737 BC.[1] Tea plant originated from the Yunnan province in south-western China,[2,3] where there is an abundance of tea genetic resource in the entire world. The widely cultivated tea plant basically belongs to *Camellia sinensis* (L.) O. Kuntze and its varieties. In a larger sense, the tea genetic resources include all the species and varieties of *Camellia* L. Sect. *Thea* (L.) Dyer. The Sect. *Thea* is one of the 12 sections in Sealy's *Camellia* classification system,[4] one of the 18 sections in Chang's system[5] and one of the 14 sections in Ming's system,[6] respectively. With regard to the classification under section *Thea*, there are different systems citing 30 species and varieties,[5] 12 species and 6 varieties,[6] and 5 species and 2 varieties.[7] Tea genotypes are essential for breeding and biotechnology in offering valuable potential for the whole tea industry in future. In the past two decades, tea genetic resources have been widely collected, well conserved, broadly evaluated and utilized in China.

Collection and Conservation

The tea genetic resources have been investigated and collected from main tea-producing areas in China. They are largely preserved in the national permanent *ex situ* conservation facilities.

Collection

Investigation and collection of tea germplasm were initiated in early 1930s. During 1950–1960s, a wide collection was made with the foundation of new China. Since the 1980s, tea plant had been listed in the national crop exploration and collection plans. Initially, 410 accessions including some wild tea plants and new species were found and collected in Yunnan during 1981–1984. Later, 100 accessions were collected from the Shennongjia and Three Gorge area during 1985–1989. As the exploration in the Three Gorge Reservoir area was initiated and being carried out, an additional exploration was organized to save the genetic resources facing jeopardy. In this submerged region, totally 80 landraces were collected and preserved safely. During 1986–1990, 60 accessions of wild tea were collected from Hainan Island. During 1991–1994, 400 accessions were collected from Guizhou, Guangxi, Sichuan and Shanxi provinces. The majority of wild tea plants were classified into *C. crassicolumna* Chang, *C. tachangensis* F.C. Zhang and *C. taliensis* (W.W. Smith) Melchior. Meanwhile, 396 landraces and cultivars were also collected from Yunnan, Jiangxi, Fujian, Anhui, Zhejiang and other provinces during that period.

Conservation

In every tea research institute in China, there is a large or small tea germplasm garden. The permanent *ex situ* conservation facility is available at the China National Germplasm Tea Repository (CNGTR), including the Hangzhou Tea Repository (CNGHTR) in the Tea Research Institute Chinese Academy of Agricultural Sciences (TRICAAS) and the Menghai Tea Repository Branch (CNGMTRB) in the Tea Research Institute Yunnan Academy of Agricultural Sciences (TRIYAAS). These were established in 1990. Up to the end of 2010, totally 3,000 accessions including wild tea plants, landraces, improved cultivars, introduced cultivars

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*Author for correspondence. E-mail: tbtri@mail.hz.zj.cn and related species had been preserved in the CNGTR with the facilities of drainage and irrigation, fences and greenhouse to ensure safety. The number of collected and preserved tea genetic resources is increasing gradually. Total tea genetic resources preserved in China national and provincial tea germplasm repositories are estimated to be more than 10,000 accessions. Table 1 shows the geographical origin of tea genetic resources in the CNGTR in 2004. Table 2 shows the classification of tea genetic resources preserved in the CNGTR on the basis of latest taxonomic system proposed by Chen *et al.*[7]

Table 1. The geographical origin of tea genetic resources preserved in the CNGTR

Origin	No. of accessions	Origin	No. of accessions
Jiangsu	25	Sichuan	127
Zhejiang	270	Guizhou	43
Anhui	34	Yunnan	1,051
Fujian	296	Shanxi	32
Jiangxi	84	Gansu	1
Henan	5	Taiwan	11
Hubei	137	Chongqing	78
Hunan	55	Shandong	1
Guangdong	73	Others	128
Guangxi	149		
Hainan	65	Total	2,665

Table 2. Accessions of each species and varieties preserved in the CNGTR

Species and varieties*	No. of accessions
Camellia tachangensis F. C. Zhang	21
C. taliensis (W.W. Smith) Melchior	123
C. crassicolumna Chang	19
C. gymnogyna Chang	26
C. sinensis (L.) O. Kuntze	1427
C. sinensis var. *pubilimba* Chang	154
C. sinensis var. *assamica* (Masters) Chang	712
Camellia sp.	159
Related species in *Camellia*	24
Total	2,665

*Based on the latest classification system of section *Thea* proposed by Chen *et al.*[7]

Besides field conservation through planting, an *in vitro* conservation system was also developed using immature embryo and nodal segment tissue cultures. Whole plants could be developed from immature embryos, to be propagated successfully for more than 20 generations; no significant genetic shift was found after *in vitro* conservation.[8] Nodal segments (0.3 cm in length) were successfully induced to form callus which subsequently grew into plants.[9] The technology of cryopreservation of seed under extra-low temperature (–196°C) also was studied, but it could not be used for clonal varieties and infertile materials.[10] In addition, China is also conducting *in situ* conservation of tea germplasms in Yunnan and Fujian provinces.

Evaluation and Appraisal

On the basis of botanical characteristics as well as agronomic traits, some important factors such as tea quality, chemical components, tolerance and resistance to biotic and abiotic stresses of more than 1,500 accessions tea genetic resources are evaluated and appraised using multi-disciplinary approaches in the last 20 years.[11-14] Some accessions with special characteristics, such as extremely early and late sprouting in the spring, extremely large and small leaf blades, high and low tea polyphenol content, high and low caffeine content, high amino acids content, high tolerance to low temperature, were screened.[11-14] Two documents, namely 'Descriptors and Data Standard for Tea (*Camellia* spp.)'[15] and China Agricultural Standard 'Technical Code for Evaluating Crop Germplasm–Tea Plant (*Camellia sinensis*)'[16] have been published. They provide very important technical standards for evaluation and nationwide appraisal of tea genetic resources. Consequently, the results would be more reliable, reproducible and compatible for sharing.

Genetic resources of high quality made-tea and high levels of biochemical components

Thirty accessions suitable to make high quality green tea, 39 accessions for black tea and 16 accessions for oolong tea were screened. Some of the varieties are directly used in cultivation and production. Some accessions containing 38% or more of tea polyphenols were screened. A few containing more than 5.2% caffeine were found, which was higher by 30% over the common cultivars. Caffeine content in one was lower than 1.0%, it is a rare material that could to be employed in evolving a hybrid with low-caffeine content.

Variation of main quality components

There is significant variation in bio-chemical components of various genetic resources that originated from different regions in China.[14] Studies showed that average tea polyphenol content was 28.4%, ranging from 13.6% to 47.8%, which indicated a gradually increasing trend from northeastern to southern regions, the highest polyphenol being present in Yunnan. Catechin content varied from 81.9 to 262.7 g/kg, with an average of 144.6 g/kg; the accession with the highest catechins content was found in Hunan. The amino acids ranged 1.1–6.5%, with an average of 3.3%; amino acid content was much lower in the south compared to northern and eastern region. The average caffeine content was 4.2%, varying from 1.2% to 5.9%. Yunnan, then Fujian provinces, have plentiful high caffeine genetic resources. The average content of water extracts was 44.7%, varying from 24.4% to 57.0%. The variation pattern was similar to that of tea polyphenols. Tea genetic resources of transnormal components are valuable and they could be used directly or indirectly used for commercial functional components extract, breeding and production.

Study of Taxonomy and Evolution of Tea Plants

Based on the most important taxonomic systems of the genus *Camellia*,[4–6] and our long-term research on wild tea plants and other tea genetic resources all around the country, a new taxonomic system of section *Thea* was proposed.[7] The section *Thea* was revised into five species and two varieties, i.e. *C. tachangensis* F. C. Zhang, *C. taliensis* (W.W. Smith) Melchior, *C. crassicolumna* Chang, *C. gymnogyna* Chang, *C. sinensis* (L.) O. Kuntze, *C. sinensis* var. *assamica* (Masters) Kitamura and *C. sinensis* var. *pubilimba* Chang. *C. sinensis* (L.) O. Kuntze is widely distributed in China and other tea-growing countries. However, the other four species are concentrated in Yunnan, Guangxi and Guizhou provinces of China. Compared to Chang's (1998) and Ming's (2000) systems,[5,6] this proposal seems more concise and functional and was validated by molecular marker analysis.[17] The molecular phylogenetic dendrogram of section *Thea* was constructed based on the Nei and Li's similarity coefficient from pair-wise comparisons of RAPD (Random Amplified Polymorphic DNA) data.[18] The 24 species and varieties from Chang's system[5] were basically clustered into two groups, one with 5-loculate and the other with 3-loculate ovary. In these two groups, there are three and two subgroups, respectively. The molecular systematics including morphological classification is consistent with each other in general. The putative evolutionary tendency of tea plants was also proposed, probably with the existence of two parallel pathways: the absence and presence of pubescence on the ovary. The evolutionary tendency was from a 5-loculate to a 3-loculate ovary, arborescent to shrub by habit and large flowers and leaves to very small ones.

Descriptors and databases of the accessions of tea germplasm

Since 2003, a detailed database system of evaluated tea genetic resources has been established based on the descriptors and standardized data of tea germplasm.[15] Tea has 111 descriptors, including 26 for passport, 45 for morphological traits and biological characteristics, 29 for quality characteristics, 8 for abiotic tolerance and biotic resistance, the last 3 for chromosome ploidy, DNA markers and fingerprinting, and remarks (see Table 3). The information on the genomes has been digitally expressed for sharing of both information and genetic resources.

Core Collection of Tea Germplasm in China

After the establishment of an optimum sampling strategy for core collection of tea germplasm,[19] a set of 414 accessions of the primary collection was screened; 360 accessions were finally selected as the core collection of Chinese tea germplasm based on EST-SSR (Expressed Sequence Tag based Simple Sequence Repeat) analysis.

Application of DNA Markers Approaches on Tea Germplasm

A wide range of DNA markers such as RAPD, AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeat) and EST-SSR have been applied in the research of tea genetic resources. Presently, these markers have been proven to be robust and valuable in the research of genetic diversity and variation, introduction and spread, molecular identification and DNA fingerprinting, molecular phylogenetics, genetic stability and integrity and the establishment of the genetic linkage map for tea breeding. Ni *et al.*[20] published an excellent review on germplasm and breeding of tea plant based on DNA marker approach.

Utilization

The main purpose of conservation and evaluation of tea germplasms is for better utilization, either direct or indirect. Nevertheless, the currently effectively used genetic resources are <20% of the total, mainly owing to the following aspects:

Table 3. Descriptors of tea germplasm

No.	Code		Property*	Note or Unit
Passport				
1	101	Accession number	M	
2	102	GeneBank number	M	
3	103	Introduction number	C/Alien germplasm	
4	104	Collecting number	C/Wild and landrace	
5	105	Accession name	M	
6	106	Alien name	M	
7	107	Family	M	
8	108	Genus	M	
9	*109*	*Species*	*M*	
10	110	Country of origin	M	
11	111	Province of origin	M	
12	112	Origin	M	
13	113	Altitude	C/Wild and landrace	M
14	114	Longitude	C/Wild and landrace	
15	115	Latitude	C/Wild and landrace	
16	116	Sample source	M	
17	117	Donor institution	M	
18	118	Donor accession number	M	
19	119	Pedigree	C/Improved cultivar or Breeding line	
20	120	Breeding institution	C/Improved cultivar or Breeding line	
21	121	Releasing year	C/Improved cultivar	
22	122	Breeding methods	C/Improved cultivar or Breeding line	
23	123	Germplasm type	M	1: Wild; 2: Traditional cultivar/Landrace; 3: Advanced/Improved cultivar; 4: Breeding line; 5: Genetic stock; 6: Other
24	124	Propagating type	M	1: Sexual; 2: Asexual
25	125	Image filename	O	.jpg
26	126	Experiment location	M	
Morphological Traits and Biological Characteristics				
27	201	Plant type	M	1: Shrub; 2: Semi-arbor; 3: Arbor
28	202	Growth habit	M	1: Erect; 2: Semi-erect; 3: Horizontal spreading

No.	Code		Property*	Note or Unit
29	203	Sprouting density	O	1: Sparse; 2: Medium; 3: Dense
30	204	Date of 'one and a bud'	M	mm/dd
31	205	Date of 'two and a bud'	M	mm/dd
32	206	Young shoot colour	M	1: Whitish; 2: Yellow green; 3: Light green 4: Green; 5: Purple green
33	207	Young shoot pubescence	M	0: Absent; 1: Sparse; 2: Medium; 3: Dense; 4: Extremely dense
34	208	Length of 'three and a bud'	M	cm
35	209	Weight of 100 'three and a bud'	M	g
36	210	Leaf attitude	M	1: Erect; 2: Semi-erect; 3: Horizontal; 4: Drooping
37	211	Leaf length	M	cm
38	212	Leaf width	M	cm
39	213	Leaf size	M	1: Small; 2: Medium; 3: Large; 4: Extremely large
40	214	Leaf shape	M	1: Rounded; 2: Ovate; 3: Elliptic; 4: Oblong; 5: Lanceolate
41	215	Number of vein pairs	M	Pair
42	216	Leaf colour	M	1: Yellow green; 2: Light green; 3: Green; 4: Dark green
43	217	Leaf upper surface	O	1: Smooth; 2: Slightly rugose; 3: Rugose
44	218	Leaf cross section	O	1: Convex; 2: Flat; 3: Concave
45	219	Leaf texture	M	1: Soft; 2: Medium; 3: Hard
46	220	Sharpness of leaf sawtooth	O	1: Sharp; 2: Medium; 3: Obtuse
47	221	Density of leaf sawtooth	O	1: Sparse; 2: Medium; 3: Dense
48	222	Depth of leaf sawtooth	O	1: Flat; 2: Medium; 3: Deep
49	223	Leaf base shape	O	1: Acute; 2: Round
50	224	Leaf apex shape	M	1: Acute; 2: Attenuate; 3: Blunt; 4: Obtuse
51	225	Leaf margin undulation	M	1: Flat; 2: Slightly wavy; 3: Wavy
52	226	Stage of full blooming	O	
53	227	Number of calyxs	M	
54	228	Calyx color	M	1: Green; 2: Purple red
55	229	Calyx pubescence	M	1: Absent; 2: Present
56	230	Flower diameter	M	cm
57	231	Petal colour	M	1: White; 2: Greenish; 3: Pink
58	232	Petal texture	O	1: Thin; 2: Medium; 3: Thick
59	233	Number of petals	M	
60	234	Ovary pubescence	M	0: Absent; 1: Present
61	235	Style length	M	cm
62	236	Number of style splittings	M	
63	237	Position of style splitting	M	1: High; 2: Medium; 3: Low
64	238	Relative height between gynoecium and androecium	M	1: Gynoecium lower; 2: Gynoecium and androecium same height; 3: Gynoecium higher
65	239	Fruit shape	O	1: Global; 2: Kidney-shaped; 3: Triangular; 4: Quadrangle; 5: Cinquefoil-shaped

No.	Code		Property*	Note or Unit
66	240	Fruit diameter	O	cm
67	241	Thickness of carpodermis	O	cm
68	242	Seed shape	O	1: Round; 2: Semi-round; 3: Cone-shaped; 4: Like kidney-shaped; 5: Irregular
69	243	Seed diameter	O	cm
70	244	Seed colour	O	1: Brown; 2: Brown grey; 3: Grey
71	245	Weight of 100 seeds	O	g
Quality characteristics				
72	301	Processing suitability	M	1: Green tea; 2: Black tea; 3: Oolong tea; 4: Unsuitable
73	302	Other processing suitability	O	1: Green tea; 2: Black tea; 3: Oolong tea; 4: None
74	303	Total score of green tea	C/green tea	
75	304	Aroma score of green tea	C/green tea	
76	305	Characteristics of green tea aroma	C/green tea	
77	306	Taste score of green tea	C/green tea	
78	307	Characteristics of green tea taste	C/green tea	
79	308	Total score of black tea	C/black tea	
80	309	Aroma score of black tea	C/black tea	
81	310	Characteristics of black tea aroma	C/black tea	
82	311	Taste score of black tea	C/black tea	
83	312	Characteristics of black tea taste	C/black tea	
84	313	Total score of Oolong tea	C/Oolong tea	
85	314	Aroma score of Oolong tea	C/Oolong tea	
86	315	Characteristics of Oolong tea aroma	C/Oolong tea	
87	316	Taste score of Oolong tea	C/Oolong tea	
88	317	Characteristics of Oolong tea taste	C/Oolong tea	
89	318	Water extracts	M	%
90	319	Caffeine	M	%
91	320	Tea polyphenols	M	%
92	321	Amino acids	M	%
93	322	Ratio of polyphenols/ amino acids	O	
94	323	Theanine	O	%
95	324	Catechins	O	mg/g
96	325	Epigallocatechingallate	O	mg/g
97	326	Epigallocatechin	O	mg/g
98	327	Epicatechingallate	O	mg/g
99	328	Epicatechin	O	mg/g
100	329	Gallocatechin	O	mg/g

No.	Code		Property*	Note or Unit
Abiotic Tolerance				
101	401	Cold tolerance	O	3: Strong; 4: Relatively strong; 5: Medium; 7: Weak
102	402	Drought tolerance	O	3: Strong; 4: Relatively strong; 5: Medium; 7: Weak
Biotic Resistance				
103	501	Resistance to brown blight	O	3: Resistant; 5: Moderate resistant; 7: Susceptible; 9: High susceptible
104	502	Resistance to tea anthracnose	O	3: Resistant; 5: Moderate resistant; 7: Susceptible; 9: High susceptible
105	503	Resistance to blister blight	O	3: Resistant; 5: Moderate resistant; 7: Susceptible; 9: High susceptible
106	504	Resistance to tea leafhopper	O	3: Resistant; 5: Moderate resistant; 7: Susceptible; 9: High susceptible
107	505	Resistance to pink mite	O	3: Resistant; 5: Moderate resistant; 7: Susceptible; 9: High susceptible
108	506	Resistance to tea red spider mite	O	3: Resistant; 5: Moderate resistant; 7: Susceptible; 9: High susceptible
Others				
109	601	Chromosome ploidy	O	
110	602	Fingerprinting and molecular markers	O	
111	603	Remarks	O	

* M: Mandatory descriptors, O: Optional descriptors, C: Conditional descriptors for specific germplasms.

Direct use in tea production

About 40 of the excellent, high-quality genetic resources are widely dispersed in about 16,415 ha in several locations across China. The cultivated area has increased by 9,920 ha in the last 5 years. These genetic resources have made great contribution to the livelihood of local people in the mountainous and national minority areas.

Breeding

There are 123 national and more than 160 provincial registered tea cultivars in China. Among them, 91 national and 130 provincial clones have been bred by hybridization and systematic selection using the tea genetic resources. About one-third of these clones are the major cultivars and form the very basis of clonal tea cultivation in China. For example, Longjing 43 and Longjing Changye–bred by the TRICAAS – are the most popular clones in famous flat green tea areas such as Dragon Well tea. They are extended to more than 10 provinces. Yunkang 10, an excellent black tea clone bred by the TRIYAAS, selected from genetic resources in Mt. Nannushan, Yunnan province, is now becoming a predominant clone in Yunnan. Another excellent example is a temperature-sensitive mutant (albino tea cultivar);[21] the leaves of tender shoots become whitish in spring between 19°C and 22°C and turn green after the temperature is higher than 25°C, with very high amino acid content (6.2%) and low tea polyphenols (10.7%). It has been rapidly popularized in Zhejiang and nearby provinces, and the farmers get excellent income due to its high price owing to its wonderful quality. More and more similar white tea or yellow tea cultivars have been recently reported in the Zhejiang province.

Amongst the 109 new improved clones, 35 were obtained by cross-breeding, 70 through systematic selection from landraces and 4 by single plant selection. The breeders paid much attention to the combination of Yunnan Dayecha, which is from the original area of tea plants, and Fuding Dabaicha, a famous high aroma, high quality green tea clone. About 60 clones, accounting for 55% of the total area under clones, were derived from this combination, either through natural pollination or controlled hybridization.

Research Use

The preserved tea genetic resources offer plentiful research material for screening of high functional components, such as beta-carotene,[22] epigallocatechin gallate (EGCG), γ-GABA content and low fluoride content, high tolerance to the lead toxicity in soil, high nitrogen use efficiency for molecular systematics research of tea plants[17] and for functional genome analysis.[23]

Prospects

Collection of Tea Genetic Resources

Many tea genetic resources from their original habitats and other tea-growing areas were collected and conserved though a series of tea genetic resource investigation programmes. However, there are still many wild tea plants which have not been collected yet in the mountain regions, in particular in Yunnan province. These wild tea plants face extinction through destruction and exploitation by human activities. And some unique genes might be lost in wild tea or their relatives, whose collection should gain priority. In addition, many tea genetic resources held by breeders should also be collected and preserved in the CNGTR to prevent accidental loss.

Association Analysis of Molecular Markers to Important Agronomic Traits

The phenotypes of 1,500 accessions in the CNGTR had been evaluated so far. Additionally, hundreds of EST-derived SSR primers have been generated and characterized by the authors' using tea plant EST sequences. It is time to analyse the relationship between molecular markers and important agronomic traits. It will be very useful for marker-assisted selection in the planned improvement programme.

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Morphological variation in tea germplasm

Plate 1: Straight branch

Plate 2: Zigzag branch

Plate 3: Green-coloured young shoots

Plate 4: Whitish young shoots

Plate 5: Purple young shoots

Plate 6: Yellow young shoots