ISSN 1008-9209
CODEN ZXSKFJ

JOURNAL OF ZHEJIANG UNIVERSITY

AGRICULTURE AND LIFE SCIENCES

浙江大学学报

农业与生命科学版

ISSN 1008-9209
0 7>
9 771008 920140

4/2014
Vol.40 No.4 July 2014
中国·杭州 HANGZHOU, CHINA
Zhejiang Daxue Xuebao (Nongye yu Shengming Kexue Ban)

《浙江大学学报(农业与生命科学版)》编委会

Editorial Board of Journal of Zhejiang University (Agriculture & Life Sciences)

浙江大学学报
(农业与生命科学版)
Zhejiang Daxue Xuebao
(Nongye yu Shengming Kexue Ban)
第 40 卷 第 4 期(总第 185 期) 2014 年 7 月 20 日

ISSN 1008-9209 CN 33-1247/S
JOURNAL OF ZHEJIANG UNIVERSITY
(Agriculture & Life Sciences)
Vol.40 No.4 (General serial No.185) July 20, 2014
http://www.journals.zju.edu.cn/agr

目 次 Contents

CN33-1247/S * 1956 * b * A4 * 120 * zh+en * P * ¥20.00 * 1000 * 13 * 2014-07

责任编辑 胡清华
英文校对 I. H. Shamsi

浙江大学学报(农业与生命科学版) 40(4):404～412,2014
Journal of Zhejiang University (Agric. & Life Sci.)
http://www.journals.zju.edu.cn/agr
E-mail:zdxbnsb@zju.edu.cn **DOI**:10.3785/j.issn.1008-9209.2014.05.051

Entire chloroplast genome sequence of tea (*Camellia sinensis* cv. Longjing 43): a molecular phylogenetic analysis

Ye Xiaoqian[1], Zhao Zhonghui[1], Zhu Quanwu[1], Wang Yingying[2], Lin Zhangxiang[2], Ye Chuyu[2], Fan Longjiang[2*], Xu Hairong[1*] (*1. Institute of Tea Science, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China; 2. Zhejiang Key Laboratory of Crop Germplasm/Department of Agronomy, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China*)

Summary *Camellia sinensis* cv. Longjing 43 is a domestic variety of tea species and an important economic crop in China. In this study, we developed a rapid method to get the chloroplast (cp) genome and sequenced the entire cp genome sequence of *C. sinensis* cv. Longjing 43. The *C. sinensis* cv. Longjing 43 cp genome was 157 085 bp in length, which contained a large single-copy (LSC, 86 642 bp) region, a small single-copy (SSC, 18 283 bp) region, and two inverted repeat (IR, each with a size of 26 080 bp) regions. With the cp genome of Korean *C. sinensis* cultivar as a reference, 134 chloroplast genes were successfully annotated. There were 15 genes with non-synonymous mutations in the coding region and more than 100 polymorphic sites in the non-coding region, which could be the DNA markers for the determination of different *C. sinensis* varieties. We also investigated the relationship of 12 *C. sinensis* varieties in China based on several cp genomic regions, which contain many variant sites. The result showed that these varieties were divided into two groups with Lingyunbaimaocha in one group and the other 11 in another group. Among the other 11 varieties, the Longjingchangye, Longjingyuanye, Longjingguazi, and Zhongcha 102 had a closer relationship and were formed into one cluster with 100% support rate, demonstrating the reliability of the method that used the cp genome sequences to investigate the genetic relationships.

Key words *Camellia sinensis* cv. Longjing 43; chloroplast genome; high-throughput sequencing; genetic relationship; phylogenetic tree
CLC number S 571.1; Q 7 **Document code** A

茶树"龙井43"叶绿体基因组测序及其系统进化(英文).浙江大学学报(农业与生命科学版),2014,40(4):404-412

叶晓倩[1], 赵忠辉[1], 朱全武[1], 王营营[2], 林张翔[2], 叶楚玉[2], 樊龙江[2*], 须海荣[1*](1.浙江大学农业与生物技术学院茶叶研究所,杭州 310058;2.浙江大学农业与生物技术学院农学系/浙江省作物种质资源重点实验室,杭州 310058)

摘要 利用高通量测序技术对中国茶树品种"龙井43"叶绿体基因组的全序列进行测定,拼接补洞后再利用Sanger测序法对序列进行验证,最终得到"龙井43"叶绿体基因组全序列(GenBank登录号:KF562708.1).该基因

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* **Corresponding author:** Fan Longjiang, Tel: +86-571-88982730, E-mail: fanlj@zju.edu.cn; Xu Hairong, Tel: +86-571-88982215, E-mail: xuhr@zju.edu.cn
Biography: Ye Xiaoqian, E-mail: 21216079@zju.edu.cn
Received: 2014-05-05; **Accepted:** 2014-05-24; **Published online:** 2014-07-24
URL:http://www.cnki.net/kcms/doi/10.3785/j.issn.1008-9209.2014.05.051.html

组大小为 157 085 bp,其中大单拷贝区的长度为 86 642 bp,小单拷贝区的长度为 18 283 bp,反向重复区的长度为 26 080 bp,共注释叶绿体基因 134 个.与韩国茶树品种叶绿体基因组序列比对,在编码区发现 15 个基因发生了非同义突变,在非编码区有 100 多个多态性位点,这些突变可作为茶树品种鉴定的 DNA 标记.同时,选取 12 个中国茶树品种,对其叶绿体基因组上的特异性片段(ycf1,psbA-trnH,psbK-psbI-psbI)进行测序比对并构建系统进化树.结果表明:12 个茶树品种在进化树上分为 2 个亚群,其中“凌云白毛茶”单独形成一支,其他 11 个品种形成一支;而 11 个品种中,“龙井瓜子”“龙井长叶”“龙井圆叶”“中茶 102”形成一支,自展支持率为 100%.表明利用叶绿体基因片段可有效区分茶树品种间的亲缘关系.

关键词　龙井 43; 叶绿体基因组; 高通量测序; 亲缘关系; 系统发育树

The chloroplast is an organelle that plays an important role in photosynthesis[1]. It also carries out a number of other functions, including the synthesis of starch, fatty acids, pigments, and amino acids[2]. It is a semi-autonomous organelle that has a complete set of genetic system and an independent genome[3].

In 1909, C. E. Correns discovered a phenomenon that does not abide by the Mendelian laws of inheritance in *Mirabilis jalapa*. It was the first report to indicate that chloroplast may contain genetic information. The complete chloroplast (cp) genome sequences of *Nicotiana tabacum*[4] and *Marchantia polymorpha*[5] were first obtained in 1986. In recent years, the cp genomics has developed rapidly with the application of molecular biotechnologies and DNA sequencing techniques. Using the high-throughput sequencing technologies, several cp genome sequences were obtained, such as bamboo[6] and duckweed[7]. By the end of December 10, 2013, there were 420 complete cp genome sequences deposited in GenBank. The cp genome sequences are useful for the study of plant phylogenetics[8-10] and photosynthesis at the molecular level[11-12].

Camellia sinensis is an important crop used to make tea[13]. In recent years, many *C. sinensis* varieties with special characteristics including albino or etiolated buds have been found in China, such as Zhejianganjibaiyecha, Ningboqiannianxue, and Hunanbaojinhuangjinya. The resources of *C. sinensis* are thus much more abundant, and these varieties stimulate the enthusiasm of the farmers to conduct seed selection on *C. sinensis*. Due to the high heterozygosity, the research on the genome of *C. sinensis* is still in the initial stage. The relatively small cp genome will be helpful in investigating the albinism or etiolation mechanisms in *C. sinensis*. In this study, we obtained the complete cp genome sequences of Longjing 43, which is an important *C. sinensis* variety, based on high-throughput sequencing data. Furthermore, we investigated the relationship among different varieties of *C. sinensis* using cp genomes.

1　Materials and Methods

1.1　Materials

The fresh leaves of Longjing 43, Shuigu tea, Fudingdabai tea, Fujianshuixian, Maoxie, Pingyun, Linyunbaimao tea, Zhenong 25 and Wuniuzao were collected from Huajiachi campus of Zhejiang University. The fresh leaves of Longjingchangye, Longjingguazi, Longjingyuanye and Zhongcha 102 were collected from the Chinese Tea Research Institute.

1.2　Methods

1.2.1　DNA sequencing and chloroplast genome assembly of Longjing 43　The DNA of Longjing 43 was extracted using the plant genome DNA extraction kit (Transgen Company). Raw sequence reads were generated using the Illumina Hiseq 2000 platform and Bowtie 2[14] (http://bowtie-bio.sourceforge.net/index.shtml) was used to map clean reads with the cp genome of Korean *Camellia* (GenBank number: NC_020019.1) as a reference. Chloroplast genome-related reads were then collected. Assembly of the mapped reads was then performed using CLC software (http://www.clcbio.com). The gaps were filled up by GapCloser[15] (http://soap.genomics.org.cn/soapdenovo.html) (Fig. 1). Primers used in the PCR amplification were designed by Primer Premier 5.0 (Table 1). PCR products were sequenced using the Sanger method.

1.2.2　Genome annotation　The cp genome of

Longjing 43 was annotated using DOGMA[16] (http://dogma.ccbb.utexas.edu/). The final result was submitted to GenBank with the number KF562708.1. The annotated files were used to draw gene maps using the GenomeVx tool[17] (http://wolfe.gen.tcd.ie/GenomeVx/).

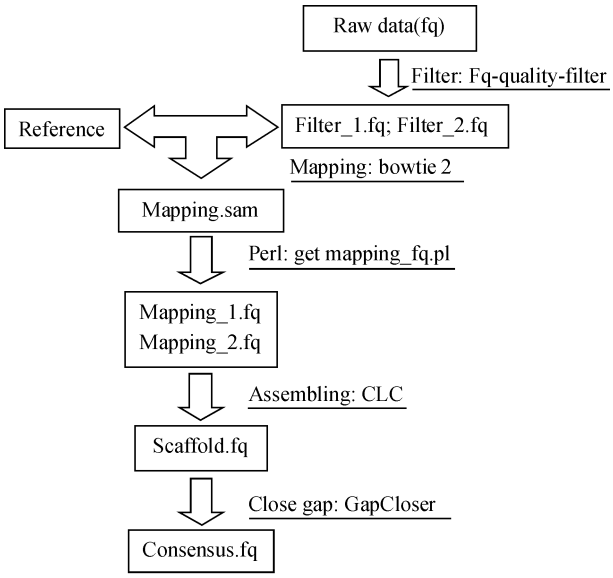


Fig.1 Assembly of the Longjing 43 chloroplast genome based on the high-throughput sequencing data

1.2.3 Phylogenetic relationships among 12 varieties of *C. sinensis* Total DNA was extracted from fresh leaves of the 12 varieties of *C. sinensis* by the CTAB method[18]. PCRs for three regions (ycf1, psbA-trnH, psbK-psbI-psbI) with condensed variant sites were carried out in 50 μL reaction mixture containing 25 μL 2× *Taq* PCR Master Mix, 2 μL of each primer (10 μmol/L) (Table 1), 19 μL ddH_2O, and 2 μL DNA samples. Amplification was carried out under the following condition: 94 ℃ for 4 min, 50 - 55 ℃ (the temperature dependents on primers) for 30 s, 72 ℃ for 30 s, recycled around 35 times, and at last 72 ℃ for 4 min. The total DNA samples and PCR products were checked by electrophoresis through 0.8% agarose gel. The PCR products were sent to Shanghai Sonny Biological Technology Co., Ltd to purify and sequence. The raw sequences were checked using Chroms 2.13, and some individual sites were corrected manually via Lasergene. These sequences were then aligned using UltraEdit. The phylogeny reconstruction was

Table 1 PCR primers used in gap closures and verification of the *C. sinensis* cv. Longjing 43 cp genome

Primer names	Primer locations/bp	Primer sequences (5′—3′)
GAP1-F	33 140 - 33 161	AACGCCATGGTAAGGCGTAAGT
GAP1-R	34 116 - 34 095	TCCACGGGTCGAAGAAAGAAAG
GAP2-F	60 382 - 60 404	ATTGCCGAACCCAATGCCTAC
GAP2-R	60 974 - 60 954	TCCCATCCAACAGGGATTCTT
GAP3-F	60 959 - 60 978	TCCCTGTTGGATGGGATTCT
GAP3-R	62 256 - 62 237	TCGTAGGAAGACACGACGAT
GAP4-F	112 473 - 112 494	GAACAAGAGGGATCCACCGAAG
GAP4-R	113 752 - 113 731	GGTTATATTCACCGATTTTCGC
GAP5&6-F	113 778 - 113 798	GGACCAAAAACAAGCAAGGGG
GAP5&6-R	114 986 - 114 967	GCTCCACTTCCAGTTCCTGT
GAP7-F	114 898 - 114 920	TGAAAAGCCCACATACGACGAAG
GAP7-R	116 377 - 116 356	GATTGGGTTGAAAGCGGCAAAT
ycf1-F	127 294 - 127 316	TGTTCGGTTGAGGGAACAGATAC
ycf1-R	128 407 - 128 386	TTCTTCAATATCACGGAACGTC
psbA-trnH-F	156 771 - 156 791	CCGGATCTAAGCGTTGGCTAG
psbA-trnH-R	554 - 533	CGTGCTAACCTTGGTATGGAAG
psbK-psbI-psbI-F	7 802 - 7 821	TATGCTCTGGGACGGAAGGA
psbK-psbI-psbI-R	9 126 - 9 107	TGGCTGAGTGGACTAAAGCG

performed using neighbor-joining (NJ) method[19] in Mega 5.0. The confidence of each branch was tested by bootstrap 1 000 times.

2 Results

2.1 Chloroplast genome assembly of Longjing 43

Using the cp genome of Korean *Camellia* as a reference, Longjing 43 cp genome-related reads were collected. After assembly and gap closure, the complete cp genome sequences were obtained.

The 23 G cp genome data for Longjing 43 were obtained with the average sequencing depth of 10. Compared with the reference cp genome sequence, there was 99.98% coverage. After assembly by the CLC Genomics Workbench, the contig N50 was 26 kb and the longest contig was 41 kb. The complete consensus sequence was 157 085 bp after filling the gaps using GapCloser. We designed six pairs of PCR primers (Table 1, Gap1 – Gap7) for gap validation. The results showed that five gaps were the same as the assembly results. When the Gap7 site was sequenced using the Sanger method, it turned out that the length of gap was 30 bp longer than the assembly result. The final cp genome of *C. sinensis* cv. Longjing 43 has been annotated and deposited in GenBank under the accession number KF562708.1.

2.2 Characteristics of Longjing 43 cp genome

The entire length of the cp genome of Longjing 43 was 157 085 bp (Fig.2), including the large single-copy

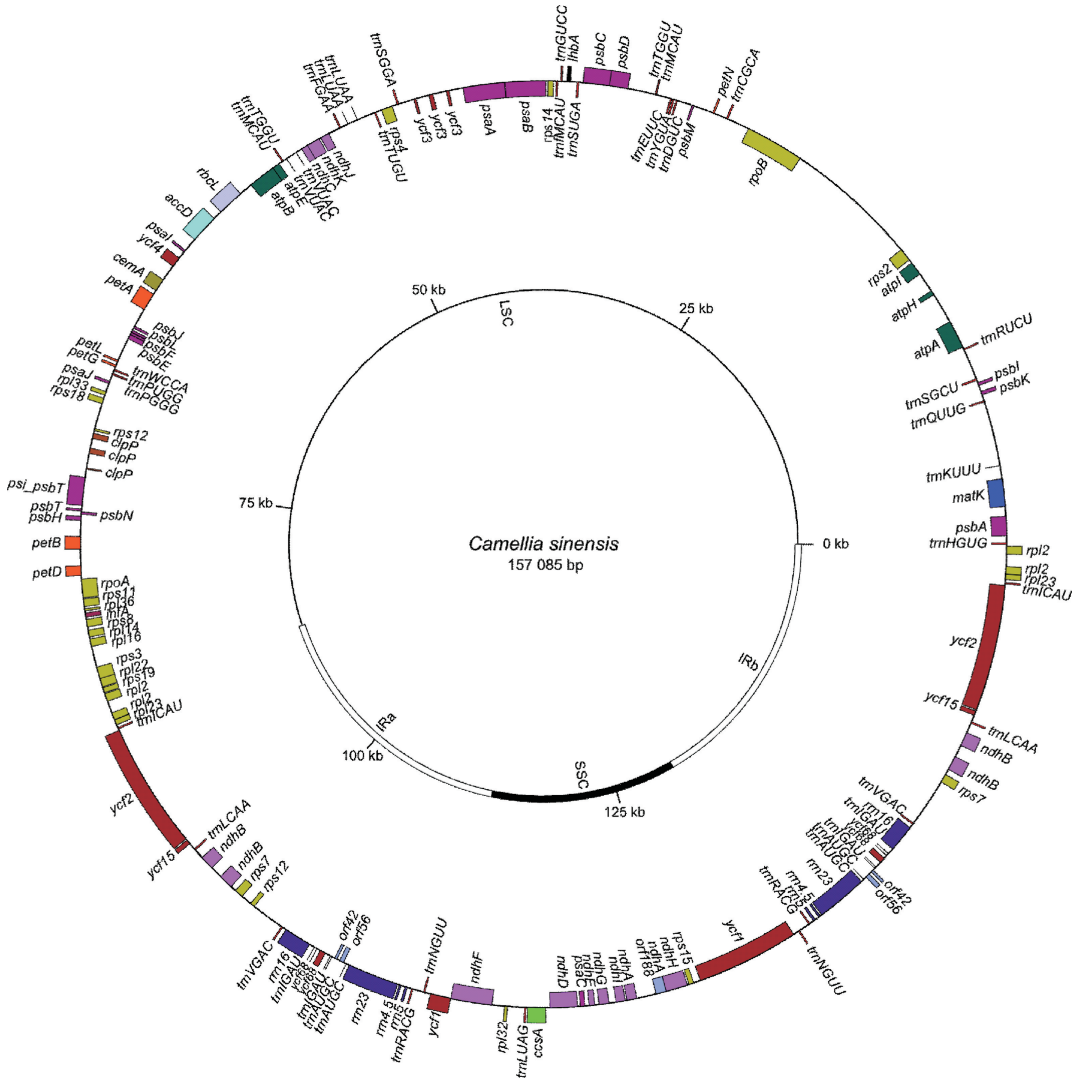


Fig.2　Gene map of the *C. sinensis* cv. Longjing 43 cp genome

(LSC, 86 642 bp) region, the small single-copy (SSC, 18 283 bp) region, and the inverted repeat (IR, 26 080 bp) region. This cp genome consisted of 58% coding region and 42% non-coding region. There were 134 genes divided into 75 self-replication genes, 47 photosynthesis genes, 3 other protein genes and open reading frame, 2 pseudogenes and 7 unknown functional genes (Table 2).

Table 2 Genes of the cp genome of *C. sinensis* cv. Longjing 43

Category for genes	Group of genes	Name of genes
Self replication	tRNA gene	*tRNA.UGC**(x2), *tRNA.ACG*(x2), *tRNA.UCU*, *tRNA.GUU*(x2), *tRNA.GUC*, *tRNA.GCA*, *tRNA.CAU*, *tRNA.UUG*, *tRNA.UUC*, *tRNA.GCC*, *tRNA.UCC**, *tRNA.CAU*(x2), *tRNA.GUG*, *tRNA.CAU**(x2), *tRNA.CAA*(x2), *tRNA.UAA**, *tRNA.UAG*, *tRNA.UUU**, *tRNA.CAU*, *tRNA.GAA*, *tRNA.UGG*, *tRNA.GCU*, *tRNA.GGA*, *tRNA.UGA*, *tRNA.GGU*, *tRNA.UGU*, *tRNA.CCA*, *tRNA.GUA*, *tRNA.GAC*(x2), *tRNA.UAC**
	rRNA genes	*rrn4-5*(x2), *rrn5*(x2), *rrn16*(x2), *rrn23*(x2)
	Ribosome gene	*rpl12**(x2), *rpl14*, *rpl16**, *rpl20*, *rpl22*, *rpl23*(x2), *rol32*, *rpl33*, *rpl36*, *rps2*, *rps3*, *rps4*, *rps7*(x2), *rps8*, *rpsl1*, *rpsl2-5′end*, *rpsl2-3′end*, *rpsl4*, *rpsl5*, *rpsl6**, *rpsl8*, *rpsl9*
	RNA polymerase gene	*rpoA*, *rpoB*, *rpoC1**, *rpoC2**
	Translation initiation factor gene	*infA*
Genes for photosynthesis	Photosynthesis proteins gene	*psaA*, *psaB*, *psaC*, *psaI*, *psaJ*, *psbA*, *psbB*, *psbC*, *psbD*, *psbE*, *psbF*, *psbH*, *psbI*, *psbJ*, *psbK*, *psbL*, *psbM*, *psbN*, *psbT*, *psbZ*
	ATP synthase subunit genes	*atpA*, *atpB*, *atpE*, *atpF**, *atpH*, *atpI*
	NADH subunit gene	*ndhA**, *ndhB**(x2), *ndhC*, *ndhD*, *ndhE*, *ndhF*, *ndhG*, *ndhH*, *ndhI*, *ndhJ*, *ndhK*
	RuBP carboxylase subunit gene	*rbcL*
	Protease subunit P gene	*clpP***
	Cytochrome-related gene	*ccsA*, *petA*, *petB**, *petD**, *petG*, *petL*, *petN*
Other genes	Membrane protein gene	*cemA*
	Acetyl-CoA carboxylase subunit gene	*accD*
	Maturase gene	*matK*
	Pseudo gene	*ψyef1*, *ψrpsl9*
Unknown functional genes		*yef1*, *yef2*(x2), *yef3***, *yef4*, *yef5*(x2)

*, ** Represent one and two introns, respectively; (x2) indicates the genes duplicated in the cp genome.

2.3 Genetic variation between Longjing 43 and Korean cultivar

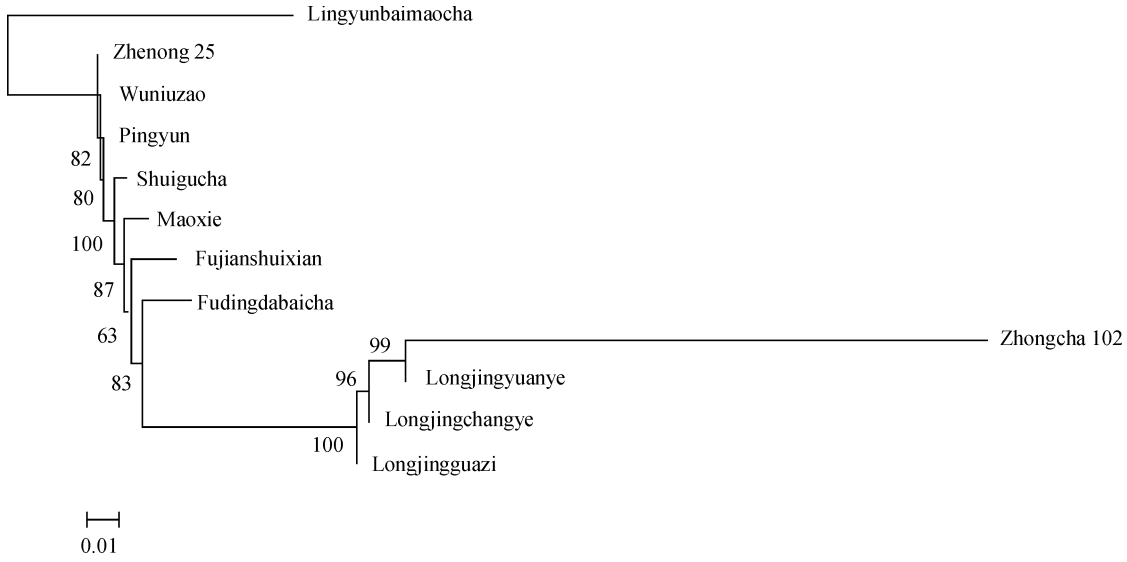
The results showed that the length of Longjing 43 cp genome sequence was 18 bp shorter than the Korean cultivar. There were 15 genes with non-synonymous mutation in the coding region (Table 3) of the Longjing 43 cp genome, including gene *rpl2*, which was on the inverted repeat region. Genes *psbT*, *rpl2*, and *rpoC2* of Longjing 43 had frameshift mutations, among which the protein sizes of *psbT* and *rpoC2* were not changed. The mutation of *rpl2* was caused by some insertion, which resulted in adding eight amino acids to the final protein. There were 16 bases mutated on partial regions of the *ycf1* gene in Longjing 43, which could be a great characteristic among different varieties based on such concentrated single-nucleotide polymorphisms. In comparing the coding regions of the cp genome sequences between Longjing 43 and Korean cultivar, there were more than 100 polymorphic sites found. There especially existed 30 bases inserted in 61 266 sites on the large single-copy of Longjing 43, which could be a DNA marker to identify the different varieties of *C. sinensis*.

Table 3　Gene loci with variations between Longjing 43 and Korean cultivar cp genomes

Gene	Longjing 43 (site; base)	Korean cultivar (site; base)	Differences of Longjing 43 and Korean cultivar
psbT	76 633 - 76 634 76 672 - 76 673	76 641; A 76 680 - 76 681; AG	13AA - 14AA
rpl2(×2)	86 845(IRA); T 156 877(IRB); A	86 848 - 86 849(IRA) 156 896 - 156 897(IRB)	19AA - 24AA
rpoC2	20 084; T 20 165 - 20 166	20 113 - 20 114 20 194; C	28AA - 28AA
matK	2 367; T 3 362; A	2 368; C 3 363; G	N(AAU)-D(GAU) I(AUU)-T(ACU)
rps16	5 205; G	5 208; T	T(ACG)-K(AAG)
rpoC2	18 307; C 20 910; T	18 336; T 20 939; C	A(GCU)-T(ACU) Q(CAA)-R(CGA)
rpoB	26 807; A	26 836; C	V(GUA)-G(GGA)
ycf3	44 549; T	44 587; C	E(GAA)-G(GGA)
accD	59 112; A 59 184; A	59 148; G 59 220; G	K(AAA)-E(GAA) N(AAU)-D(GAU)
clpP	72 526; A	72 532; C	S(UCU)-A(GCU)
rps8	82 856; A	82 866; T	I(AUU)-N(AAU)
ndhD	118 331; G	118 345; T	P(CCA)-T(ACA)
ndhH	124 958; G	124 965; T	Q(CAA)-K(AAA)
rps15	126 018; T	126 025; G	N(AAC)-T(ACC)
ycf1	127 579; G	127 586; A	S(UCG)-L(UUG)
	127 589; G	127 596; A	R(CGU)-C(UGU)
	127 643; T	127 650; G	N(AAU)-H(CAU)
	127 676; T	127 683; A	T(ACG)-S(UCG)
	127 679; G	127 686; A	L(CUU)-F(UUU)
	127 681; T	127 688; A	Y(UAU)-F(UUU)
	127 688; G	127 695; A	L(CUU)-F(UUU)
	127 720; T	127 727; A	Y(UAU)-F(UUU)
	127 754; T	127 761; G	K(AAG)-Q(CAG)
	127 757; T	127 764; G	N(AAC)-H(CAC)
	127 802; G	127 809; A	L(CUU)-F(UUU)
	129 923; A	129 930; G	F(UUU)-L(CUU)

2.4 Phylogenetic analysis of 12 varieties of *C. sinensis* in China

The study used the three fragments (ycf1, psbA-trnH, and psbK-psbI-psbI) with condensed variations from 12 varieties of *C. sinensis* to build the phylogenetic tree. The result showed that these varieties were divided into two groups with Lingyunbaimaocha in one group and the other 11 in another group. Among the other 11 varieties, the Longjingchangye, Longjingyuanye, Longjingguazi, and Zhongcha 102 had a closer relationship and were formed into one cluster with 100% support rate (Fig.3).



The scale indicates the genetic distance.

Fig.3 Phylogenetic tree of 12 tea cultivars based on neighbor-joining method

3 Discussion

The second generation sequencing technologies, namely the high-throughput sequencing technologies[20], which preserve the high accuracy of the first generation technologies and can dramatically reduce sequencing costs and increase sequencing speed, serve as a milestone in the field of genomics. A key step in traditional technologies to get cp genome is the preparation of sequencing templates, while the extraction of cpDNA is the limiting factor of the preparation of sequencing templates. Up till now, no general method of extraction was existed. Based on the maternal inheritance and high conservation of cp genome, we collected the cp-related reads using an evolutionarily closed species (Korean *Camellia*) as the reference and then assembled Longjing 43 cp genome. Purification of cpDNA prior to sequencing is not required in this method. However, if there is a lack of efficient reference sequences or some cp genome fragments are inserted into the nucleic genome, the cp genome is hard to be assembled. In this study, we successfully obtained Longjing 43 cp genome directly from the whole genome sequencing data. It fully demonstrated the efficiency of this method.

Previous results showed that the structure of the cp genome sequences is highly conservative[21] and cp genome has important value to the research on the evolution of species and the genetic relationship between different varieties. In study on phylogenetic genomics, we should use the proper DNA sequences with different origins (cp and nucleic genomes), and combine with traditional physiological characters. Then we can obtain the distinctive characters at the different classification levels. This study analyzed the genetic relationship among 12 *C. sinensis* varieties

in China based on the characters of the Longjing 43 cp genome. The results generally matched to the identification theories of species resources[22], which demonstrated the reliability of the method that used the cp genome sequences to investigate the genetic relationships. Recently, DNA molecular markers such as RAPD, AFLD, and RFLP have been used for the determination of *C. sinensis* varieties and the analysis of the genetic polymorphism. Comparing those DNA molecular markers in identifying the relationship among different species, RAPD has a lower reliability, while AFLP and RFLP are more difficult to utilize and expensive. The method presented in this study was based on condensed variant regions of the cp genome, which is easier to be processed and thus has more reliable information.

China was the first country to discover and utilize *C. sinensis*. Korean *C. sinensis* originated from China[23]. It can be seen that there are no major differences between the complete cp genome of Longjing 43 and that of Korean *C. sinensis*. There were 15 genes with non-synonymous mutations in the coding region of Longjing 43 cp genome. *psbT* and *rpoC2*, which had been frameshifted, could express proteins in the same size, while *rpl2* expressed the protein with eight more amino-acids. While in the non-coding area, we detected more than 100 polymorphic sites. These mutations may be the result of the genetic changes caused by the environmental changes, which is a possible result of species evolution.

Camellia sinensis is an important economic crop in China. Due to the complex chromosome structures, the genomic research on *C. sinensis* is not conducted as much as conducted in other crops. This research took advantages of the high-throughput sequencing technologies to obtain the complete Longjing 43 cp genome, which provided sequence information for further genomic research on *C. sinensis*.

Acknowledgements　We thank Prof. Chen Liang of Tea Research Institute, Chinese Academy of Agricultural Sciences for offering several materials.

References:

[1] Hiratsuka J, Shimada H, Whittier R, *et al*. The complete sequence of the rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. *Molecular and General Genetics*, 1989,217:185-194.

[2] Neuhaus H E, Emes M J. Nonphotosynthetic metabolism in plastids. *Annual Review of Plant Physiology and Plant Molecular Biology*, 2000,51:111-140.

[3] 王玲,董文攀,周世良.被子植物叶绿体基因组的结构变异研究进展.西北植物学报,2012,32(6):1282-1288.
Wang L, Dong W P, Zhou S L. Structure mutations and reorganizations in chloroplast genomes of flowering plants. *Acta Botanica Boreali-Occidentalia Sinica*, 2012, 32(6): 1282-1288. (in Chinese with English abstract)

[4] Shinozaki K, Ohme M, Tanaka M, *et al*. The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. *The EMBO Journal*, 1986,5(9):2043-2049.

[5] OhyamaK, Fukuzawa H, Kohchi T, *et al*. Structure and organization of *Marchantia polymorpha* chloroplast genome. I. Cloning and gene identification. *Journal of Molecular Biology*,1988,203(2):281-298.

[6] Zhang Y J, Ma P F, Li D Z. High-throughput of six bamboo chloroplast genomes: phylogenetic implications for temperate woody bamboos (Poaceae: Bambusoideae). *PLoS ONE*, 2011,6(5):e20596.

[7] Wang W, Messing J. High-throughput sequencing of three Lemnoideae (duckweeds) chloroplast genomes from total DNA. *PLoS ONE*, 2011,6(9):e24670.

[8] Clarke J L, Daniell H, Nugent J M. Chloroplast biotechnology, genomics and evolution: current status, challenges and future directions. *Plant Molecular Biology*, 2011,76:207-209.

[9] 姬生国,潘胜利,王峻,等.基于叶绿体 *matK* 基因序列探讨 10 种石杉科石杉属植物的系统关系及分子鉴定.中国中药杂志,2007,32(19):1971-1975.
Ji S G, Pan S L, Wang J, *et al*. Phylogeny relationship and molecular identification of ten Huperzia species (Huperziaceae) based on *matK* gene sequences. *China Journal of Chinese Materia Medica*, 2007, 32(19): 1971-1975. (in Chinese with English Abstract)

[10] Nock C J, Waters D L E, Edwards M A, *et al*. Chloroplast genome sequences from total DNA for plant identification. *Plant Biotechnology Journal*, 2011,9(3):328-333.

[11] Whitney S M, Andrews T J. Plastome-encoded bacterial ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) supports photosynthesis and growth in tobacco. *Proceeding of the National Academy of Sciences of the United States of America*, 2001,98:14738-14743.

[12] O'Neill B M, Mikkelson K L, Gutierrez N M, *et al*. An

exogenous chloroplast genome for complex sequence manipulation in algae. *Nucleic Acids Research*, 2012,40(6):2782-2792.

[13] 马春雷,陈亮.茶树功能基因分离克隆研究进展.分子植物育种,2006,4(增刊3):16-22.
Ma C L, Chen L. Research progress on isolation and cloning of functional genes in tea plant. *Molecular Plant Breeding*, 2006,4(Suppl. 3):16-22. (in Chinese with English abstract)

[14] Langmead B, Trapnell C, Pop M, *et al*. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology*, 2009,10(3):R25.

[15] Li R Q, Zhu H M, Ruan J, *et al*. *De novo* assembly of human genomes with massively parallel short read sequencing. *Genome Research*, 2010,20:265-272.

[16] Wyman S K, Jansen R K, Boore J L. Automatic annotation of organellar genomes with DOGMA. *Bioinformatics*, 2004, 20(17):3252-3255.

[17] Conant G C, Wolfe K H. GenomeVx: simple web-based creation of editable circular chromosome maps. *Bioinformatics*, 2008,24(6):861-862.

[18] Tel-Zur N, Abbo S, Myslabodski D, *et al*. Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera *Hylocereus* and *Selenicereus* (Cactaceae). *Plant Molecular Biology Reporter*, 1999,17:249-254.

[19] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 1987,4:406-425.

[20] Ansorge W J. Next-generation DNA sequencing techniques. *Nature Biotechnology*, 2009, 25:195-203.

[21] Bock R. Genetic engineering of the chloroplast: novel tools and new applications. *Current Opinion in Biotechnology*, 2014,26:7-13.

[22] 白堃元,杨亚军,方嘉禾,等.中国茶树品种志.上海:上海科学技术出版社,2001:38-133.
Bai K Y, Yang Y J, Fang J H, *et al*. *China Tea Varieties*. Shanghai: Shanghai Scientific & Technical Publishers, 2001: 38-133. (in Chinese)

[23] 金惠淑,梁月荣,陆建良.中、韩两国主要茶树品种基因组DNA多态性比较研究.茶叶科学, 2001,21(2):103-107.
Kim H, Liang Y R, Lu J L. Comparative study on genomic DNA diversity between Korean and Chinese tea cultivars by RAPD technique. *Journal of Tea Science*, 2001,21(2):103-107. (in Chinese with English Abstract)

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Xiang Y J, Li X Z, Wang X D. Preliminary study on cold resistance of five different lianas. *Journal of Zhejiang University: Agriculture and Life Sciences*, 2011,37(4):421-424. (in Chinese with English abstract)

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浙江大学学报
（农业与生命科学版）
（双月刊　1956年创刊）
第40卷　第4期（总第185期）　2014年7月20日

Journal of Zhejiang University
(Agriculture & Life Sciences)
(Bimonthly, Started in 1956)
Vol.40 No.4 (General Serial No. 185) July 20, 2014

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