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Comparative Analysis of the Chloroplast Genomes of a novel albino tea "Huabai 1" and the traditional albino tea "Huangjinya" for the revelation of the differences in the gene composition and their evolutionary relationship

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Abstract

The chloroplast genomes of albino tea variety "Huangjinya" and the newly discovered albino tea resource were analyzed comparatively, and DNA barcodes were used to construct phylogenetic trees between albino tea and other plants in *theaceae*, aiming to reveal the differences in the albino mechanism between the novel albino tea resource "Huabai 1" and the albino tea variety "Huangjinya" and to clarify the genetic evolutionary relationship between the novel albino tea resource "Huabai 1" and other plants in *theaceae* from the perspective of chloroplast genome. Studies result have shown that the chloroplast genome size of "Huangjinya" and "Huabai 1" is 158,916 bp, 157,028 bp respectively, LSC (large single-copy)、SSC (small single-copy) and IR (inverted repeat) are 86,654 bp, 86,590 bp、18,277 bp, 16,463 bp, and 27,900 bp, 26,081 bp respectively for "Huangjinya" and "Huabai 1", with a total of 113 genes annotated were obtained in the chloroplast genome of both albino tea, these include 84 protein coding genes, 24 tRNA genes and 5 rRNA genes. GC content of chloroplast genome of "Huabai 1" was 37.3%, slightly higher than that of "Huangjinya" (37.22%). The gene composition, classification and codon preference (preferred to use codons ending in A/U) of chloroplast genome in "Huabai 1" were basically the same as that in "Huangjinya", but the main difference was the length of *ycf1* fragment in IR region, the length of "Huabai 1" *ycf1* fragment was only 987 bp, while that of "Huangjinya" was 2889 bp. The construction of phylogenetic tree was based on the four DNA barcodes (*matK*, *rbcL*, *trnH-psbA*, and *ycf1*), and the results showed that *ycf1*, with the most taxonomic levels, was most suitable for the identification of tea varieties and the interpretation of the evolution of genetic relationships among varieties.

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Ycf1 cluster showed that the relationship between "Huangjinya" and "Longjing 43" was closer, and that of "Huabai 1" and "Fuding Dabai" was closer. It is speculated that the two tea varieties "Huangjinya" and "Huabai 1" may come from different ancestors, and thus led their quite different albino mechanism. This research will provide the basis for the revealing the albino molecular mechanism and phylogenetic development of the novel albino tea resource "Huabai 1".

Keywords: Chloroplast genome; DNA barcode; codon preference; cluster analysis

1. Introduction

The tea (*Camellia sinensis*) resources with abnormal colored new shoots and leaves often have good utilization value in production. Generally, there are several abnormal leaf colors such as purple, yellow and white. The purple shoots of purple bud tea tree usually with high anthocyanin content, the products made with the purple shoots have strong antioxidant effect (Liu *et al.*, 2018). The new shoots of albino tea trees usually have high amino acid content, which has an important effect on improving the fresh taste and other flavor quality of tea (Shu *et al.*, 2020). Therefore, it has attracted wide attention of academia and industry. At present, it has been bred into a series of tea varieties represented by "Baiye 1" and "Huangjinya" (Li *et al.*, 2016; Feng *et al.*, 2014). Recently, these varieties bred on the resource of albino mutant tea tree have become a kind of main varieties developed in tea gardens (Li *et al.*, 2016), and have been used on a large scale in production, resulting in good social and economic benefits.

Albino tea plants can be divided into two types: temperature sensitive and light sensitive types according to the albino mechanism. For example, "Baiye 1" belongs to low temperature sensitive albino tea plants (Xiong *et al.*, 2013). "Huangjinya" (a traditional albino tea variety), belonging to photosensitive albino tea plants, is characterized by yellow buds and yellow leaves on tender shoots, under the condition of light, the leaves change the green color to the yellow color and turn green under the condition of shade. In the albino leaves of "Huangjinya", the chlorophyll content is significantly lower than that of shading treatment and ordinary green tea variety "Fuding Dabai", and the contents of violaxanthin, lutein and total carotene increased and the formation of thylakoids and grana in chloroplasts was blocked (Li *et al.*, 2016). In order to further study the molecular mechanism of color change of "Huangjinya", our research group conducted transcriptome analysis on "Huangjinya" under shading treatment and non-shading treatment, and a total of 207 differentially expressed genes were obtained, and these genes were mainly involved in oxidation-

reduction reactions (especially in the flavonoid and carotenoid pathways related to antioxidant function), suggesting that the leaf color mutation of 'golden bud' may be related to the expression changes of these two pathway genes (Song *et al.*, 2017). In addition, Zhang *et al.* (2017) find that the increase of amino acid content in the lost green leaves of "Huangjinya" may be related to the acceleration of proteolysis or the slowing down of glycolytic metabolism and the reduction of the synthesis of other nitrogen-containing compounds except amino acids.

The newly discovered albino tea plants have been named as "Huabai 1" with such characters as white buds even at the beginning of germination, higher degree of albino, the white mesophyll and white vein of leaf, and the new young buds and leaves being beautiful jade white, at the same time, their albino period is more than 50% longer than that of "Huangjinya", according to the detection of the tea quality supervision, inspection and testing center of the Ministry of agriculture of the People's Republic of China, the processed green tea samples made of tender leaves from the newly discovered albino tea plants show excellent quality characteristics: the content of free amino acids for 8.4%, the content of tea polyphenols for 17.7%, the content of caffeine for 2.7%, the content of water extract for 48.6%, and with the high content of free amino acids in the main quality components, the appropriate content of tea polyphenols and caffeine, the high content of water extract, and fresh and refreshing tastes, to be a good prospect for breeding and application of new varieties. Therefore, "Huabai 1" is considered as an excellent germplasm resource of tea plants, but its albinism mechanism and its genetic relationship with other varieties have not been reported.

Chloroplast, an important organelle of green plants, provides necessary energy for plants through photosynthesis. Chloroplasts have their own genomes and are semi-independent organelles encoding 90-100 genes, which play roles in amino acid synthesis, fatty acid synthesis and immune response in plants (Yu *et al.*, 2021; Wang *et al.*, 2021). The main components of the chloroplast genome are protein-coding genes, transfer RNA (tRNA) and ribosomal RNA (rRNA). The chloroplast genome is a circular structure, with a length of 115-170 kb, including two mutually inverted repeat (IR) regions, a large single copy (LSC) region and a small single copy (SSC) region. LSC and SSC are just separated by the two IR sequences (Malte *et al.* 2018). Chloroplast genome is characterized by haploidy, maternal inheritance, gene number and highly conserved sequence structure, and its sequence variation can provide important information for plant classification and heredity. Therefore, chloroplast genome is often used as an important basis for studying the evolutionary relationship of plants (Wang *et al.*, 2021). For instance, most of the coding genes,

being single-copy genes except for the double-copy genes in the IR region, in the chloroplast genome are not affected by paralogous genes, so they can provide important analytical evidence for the evolution of higher plants (Saski *et al.*, 2005; Chumley *et al.*, 2006). Wambugu *et al.* (2015) effectively divided 19 rice varieties with AA genotypes into two groups based on differences of chloroplast genome sequence, one is the South American/African branch (containing two varieties: *O. glumaepatula* and *O. longistaminata*) and the other is the Australian branch (the largest branch) including 3 Australian varieties and other African and Asian varieties, which helps to further understand the evolutionary relationship between cultivated rice and wild rice, and also provides a reference basis for the utilization of wild rice in rice genetically improved varieties.

In this study, the chloroplast genome sequences of traditional albino tea "Huangjinya" and newly discovered albino tea "Huabai 1" were obtained by high-throughput sequencing. The composition and variation of chloroplast genome of albino varieties "Huangjinya" and "Huabai 1" were compared and analyzed by analyzing their sequence structure through the methods such as drawing the genome book genome map and analyzing its sequence structure, and the phylogenetic tree was constructed by using the intergenic sequences of the four common DNA bar code genes *matK*, *rbcL*, *ycf1* and *trnH-psbA* in the chloroplast genome and the sequences of other tea varieties and plants in camellia to analyze the genetic relationship between varieties in order to provide a theoretical basis for revealing the albinism mechanism of "Huabai 1" and its evolutionary relationship with other varieties.

2. Materials and methods

2.1 Plant materials

The two-year-old clonal cuttings "Huangjinya" and "Huabai 1" cultivated in the experimental field of Nanjing Agricultural University were selected as the test materials. The new shoots with one bud and two leaves were taken (the leaves phenotype of "Huangjinya" and "Huabai 1" showed in Fig.1), frozen with liquid nitrogen and stored at -80°C for DNA extraction.

2.2 DNA extraction and machine sequencing

DNA was extracted by using DN15-Plant Genomic DNA Rapid Extraction Kits (Adlai, Beijing) according to the instructions. The 1.2% agarose gel electrophoresis, Agilent 2100 biological analyzer (Agilent, USA) and NanoDrop ND-1000 spectrophotometer (NanoDrop, USA) were used to detect DNA quality and concentration. The range of OD260/280 between 1.7-1.9 indicated that the DNA quality was good.

After qualification of detecting the sample genome DNA, the DNA was segmented, then with a series of reactions including purification of the fragmented DNA fragments, the end repair, adding 3' A tail and joining ligation adapters and so on, and selection of the fragment size by 1.2% agarose gel electrophoresis, PCR amplification used to form a sequencing library. After the library preparation being qualified, the Illumina HiSeq 2500 platform was used to sequence.

2.3 Processing and analysis of sequencing data

Raw Data were filtered to remove joint sequences and low-quality Reads to obtain high-quality Clean Data. Clean Data were assembled using NOVOPlasty software according to the chloroplast genome sequence of the known variety "Longjing 43" (Genbank Accession Number: KF562708) to obtain the results of chloroplast sequence assembly. The chloroplast genome was annotated by CpGAVAS software and online website tool: GeSeq. CDS sequences were obtained by BLAST comparison of reference genomes or databases through the online website tool GeSeq, prediction of tRNA based on tRNAscan, prediction of single-copy regions and reverse repeat sequences using CpGAVAS software based on Vmatch, and, at last, chloroplast genome maps made by GeSeq based on OGDRAW (Wang *et al.*, 2020). The genome sequence was analyzed by SSR (through software and GRAMENE MISA online website (<http://archive.gramene.org/db/markers/ssrtool>)), and the number of SSR repeats set to 5.

2.4 Phylogenetic tree analysis

DNA barcode, a new technology for identifying species information proposed in recent years, can identify species quickly and accurately (Somaratne *et al.*, 2019). Because the chloroplast genome, relatively conservative, has certain variation, many genes or intergenic region sequences can be used as DNA barcodes to identify species and explore genetic relationships, such as *matK* and *rbcL*. In this study, based on the known chloroplast genome information of tea plant landed by NCBI, the sequences of four common DNA barcodes *matK*, *rbcL*, *ycf1* and *trnH-psbA* of chloroplast genome screened, CLUSTW used for sequence alignment, MEGA X was used to construct the phylogenetic tree. Neighbour-joining method as the statistical method, Maximum Composite Likelihood as the analysis model, *Camellia oleifera* was the root of the phylogenetic tree in this study.

3. Results

3.1 Chloroplast genome composition of "Huangjinya"

After sequencing and assembly, the chloroplast genome size of "Huangjinya" was 158,916 bp, of which the sizes of LSC, SSC and IR were 86,654 bp, 16,463 bp and 27,900 bp, respectively. A total

of 113 genes were obtained by annotation, including 84 protein-coding genes, 24 tRNAs and 5 rRNAs. The GC content of chloroplast genome in "Huangjinya" was 37.22%. The information distribution of gene, exon and intron in chloroplast genome of "Huangjinya" shown in Table 1, CLPP and ycf3 genes contained two introns, and other genes contained only one intron. As shown in Table 2,3, there were 29 high-frequency codons (relative synonymous codon usage RSCU>1) in the "Huangjinya" chloroplast genome with more preference to use codons ending in a / u, which indicated that the chloroplast genome in "Huangjinya" had an obvious preference for synonymous codon selection. The annotation results of chloroplast genome in "Huangjinya" showed that the genes constituting the chloroplast genome were mainly concentrated in photosystem I, photosystem II, cytochrome b / f complex, ATP synthase, RNA polymerase, etc. (Fig.2).

3.2 Chloroplast genome composition of "Huabai 1"

The chloroplast genome size of "Huabai 1", obtained by sequencing, was 157028 BP, and the length of LSC, SSC and IR were 86590 BP, 18277 BP and 26081 BP respectively. One hundred and thirteen genes were contained in the chloroplast genome of "Huabai 1", including 84 protein coding genes, 24 tRNAs and 5 rRNAs, which was consistent with the number and composition of the chloroplast genome in "Huangjinya". The GC content of chloroplast genome in "Huabai 1" was 37.3%, which was slightly higher than that in "Huangjinya". As shown in Table 1, the gene distribution and the composition of exon and intron in "Huabai 1" were consistent with that of chloroplast genome in "Huangjinya", and the other genes only had one intron except genes clpP and YCF3 with containing two introns distributed on LSC. Codon preference analysis showed that there were 29 high-frequency codons in the chloroplast genome of "Huabai 1", and the RSCU value was very close to that in "Huangjinya", which indicated that the codon use preference between the two varieties was relatively close, the chloroplast genome was relatively conservative and the difference was small (Table 2,4). The gene composition and classification of chloroplast genome in "Huabai 1" were basically the same as that in "Huangjinya", but the main difference was the length of ycf1 fragment in IR region, the length of "Huabai 1" ycf1 fragment was only 987 bp, while that of "Huangjinya" was 2889 bp (Fig. 3).

3.3 Analysis of chloroplast genomes of "Huangjinya" and "Huabai 1" via Simple Sequence Repeat (SSR)

In this study, 63 SSRs were found in the chloroplast genome of "Huangjinya", including 59 single base repeat SSRs (monomers) and 4 double base repeat SSRs (dimers). The SSR types of chloroplast

genome in "Huangjinya" was dominated by A/T (Table 5). Fifty-six SSRs were identified in the chloroplast genome of 'Huabai 1', including 52 single base repeat SSRs and 4 double base repeat SSRs. The SSR types of chloroplast genome in "Huabai 1" were also dominated by A/T, and the SSR location was highly consistent with that of "Huangjinya", indicating that the chloroplast genomes of the two tea varieties were highly conserved.

3.4 Analysis of genetic relationship among different varieties via chloroplast genome DNA bar code

As shown in Figure 4, *Camellia oleifera* was a single branch in the construction of the phylogenetic tree based on the four DNA bar code sequences, indicating that the four DNA bar codes can distinguish plants at the species level. However, for the identification of tea varieties or cultivated varieties, 10 tea varieties can only be divided into two groups by *rbc*, tea trees divided into three groups by the *matK* and *trnH-psbA* intergenic region, and the composition of each species is completely the same. Tea varieties were divided into multiple groups according to *YcfI* with the most grouping levels and being more specific. Among the four DNA bar code sequences, *ycfI* had the best classification effect. "Longjing 43" and "Huangjinya" clustered into one category was consistent with the classification results of *matK* and *trnH-psbA*, indicating that the genetic relationship between "Huangjinya" and "Longjing 43" was close; according to the phylogenetic tree of *ycfI* clustering, "Huabai 1" and "Fuding Dabai " were clustered into one category, while in the other three DNA bar code phylogenetic trees, these two varieties can't be separated from other varieties. Therefore, *ycfI* is most suitable for tea variety identification and explaining the evolution of genetic relationship among varieties.

4. Discussion

Chloroplast genome can provide important reference value for plant taxonomy and phylogenetic reconstruction. In this study, the whole chloroplast genomes of two albino tea varieties "Huangjinya" and "Huabai 1" were obtained by the method of the second-generation sequencing. The results showed that the chloroplast genome structures of the two albino tea varieties "Huangjinya" and "Huabai 1" were very similar, both contained LSC, SSC and two IR regions. The main difference between the chloroplast genome structures of the two albino tea varieties is in the length of IR region. The IR region length of the albino tea "Huangjinya" is longer by 1819 bp than that of the albino tea "Huabai 1", indicating that the IR region of chloroplast genome plays a less and less role in the evolution of the tea plants and gradually will be cut out.

Generally speaking, the genetic code of an organism consists of 61 codons, and each amino acid

is encoded by 2-6 synonymous codons, excluding Met and Trp. And codon preference widely existed in prokaryotic and eukaryotic systems, therefore it is of great significance to understand intraspecific or interspecific gene codon preference for studying species evolution (Ma *et al.*, 2015; Ma *et al.*, 2016). Mutation and natural selection as the main factors in the evolution of species affect codon preference in gene translation. Codons with high translation efficiency are generally chosen by highly expressed genes, so natural selection is the main factor affecting the use preference of codon of highly expressed genes. However, codon preference is mainly determined by mutation in the genes with low expression (Wang *et al.*, 2014). Codons ending in A/U are as the main codon preference in the chloroplast genome of tea tree and, both the number of codons and the codon usage parameters (RSCU value and CAI value) in the chloroplast genome of "Huangjinya" and "Huabai 1" are very close, indicating that the chloroplast genome of tea tree is relatively conservative.

DNA bar code is a species identification technology emerged in recent years, and a section of DNA region is used to identify species quickly, accurately and automatically (Bi *et al.*, 2018; Feng *et al.*, 2018). Due to chloroplast genome sequences with the advantages such as the simple and stable genetic structure, monogenic inheritance and unified amplification and obtainment of target sequences, chloroplast genome sequences are often used to study plant molecular phylogeny. At present, the commonly used chloroplast DNA barcode sequences include gene coding regions of *rbcL* and *matK* and so on (Parveen *et al.*, 2017). However, conserved coding regions with little variation contained in these gene sequences were often unable to distinguish closely related species, especially those species with more close relationship in evolution. In order to improve the resolution of plant phylogenetic analysis, some non-coding region or intergenic region sequences have been applied to phylogenetic reconstruction. In this study, tea trees can only be divided into two categories by the chloroplast coding gene *rbcL* as a DNA bar code to construct a phylogenetic tree: one is tea tree with the small and medium leaves represented by "Fuding Dabai" and "Longjing 43", and the other is tea tree with the large leaves represented by "Assam". Eleven tea varieties are divided into three categories by intergenic region sequence of another two encoding genes *matK* and *trnH-psbA*, "Huangjinya" and "Longjing 43" are clustered separately into a single group, indicating that the clustering effect of *matK* and *trnH-psbA* intergenic region sequence on tea varieties is better than that of *rbcL*. Eleven tea varieties can be divided into multiple different populations by gene *YcfI*, among them, albino tea "Huangjinya" and normal tea "Longjing 43" with the closest genetic relationship, and normal tea "Fuding Dabai" and albino tea "Huabai 1" with the

closest genetic relationship, and albino tea "Huangjinya" and "Huabai 1" are clustered into two different groups due to the relatively distant genetic relationships. The other two "Assam" species are clustered into one group, indicating that *ycf1* is the most suitable for DNA bar code to identify tea varieties.

5. Conclusion

The chloroplast genome sequences of two albino tea varieties "Huangjinya" and "Huabai 1" were obtained by high-throughput sequencing. The chloroplast genes of the two albino tea varieties are conservative, and the main difference lies in the length of IR region. Both tea varieties, preferred by using codons ending in a/u, have the strong codon preference. Cluster analysis shows that *ycf1* sequence located in IR region is the most suitable DNA bar code to identify different tea varieties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2 Codon usage of chloroplast genome of "Huangjinya" and "Huabai 1"

Amino acid	Codon		No. of codons		RSCU ^a		tRNA	
	HJY	HB	HJY	HB	HJY	HB	HJY	HB
Stop	UGA	UAA	0	0	0	0		
Stop	UAA	UGA	0	0	0	0		
Ala	GCC	GCU	150	456	0.63	1.91		trnA-UGC
Ala	GCG	GCC	87	152	0.37	0.64		
Ala	GCU	GCA	451	260	1.91	1.09	trnA-UGC	
Ala	GCA	GCG	257	86	1.09	0.36		
Cys	UGU	UGU	127	127	1.58	1.58	trnR-UCU	trnR-UCU
Cys	UGC	UGC	34	34	0.42	0.42	trnC-GCA	trnC-GCA
Asp	GAU	GAU	507	508	1.64	1.65		
Asp	GAC	GAC	110	109	0.36	0.35		
Glu	GAG	GAG	205	207	0.53	0.53		
Glu	GAA	GAA	566	571	1.47	1.47		
Phe	UUC	UUU	308	544	0.72	1.27	trnI-GAU	
Phe	UUU	UUC	544	312	1.28	0.73		trnI-GAU
Gly	GGA	GGG	441	180	1.57	0.64		
Gly	GGU	GGA	383	442	1.36	1.57		
Gly	GGG	GGC	180	118	0.64	0.42		trnG-GCC
Gly	GGC	GGU	120	386	0.43	1.37	trnG-GCC	
His	CAU	CAU	294	293	1.6	1.6		
His	CAC	CAC	73	73	0.4	0.4		
Ile	AUC	AUC	270	270	0.61	0.61		
Ile	AUU	AUA	652	400	1.48	0.91		
Ile	AUA	AUU	399	653	0.91	1.48		
Lys	AAG	AAG	175	176	0.5	0.5		
Lys	AAA	AAA	525	534	1.5	1.5		
Leu	UUA	UUA	517	522	1.91	1.91		
Leu	CUG	CUA	99	225	0.37	0.83		
Leu	CUA	UUG	226	324	0.83	1.19		trnM-CAU
Leu	CUC	CUC	111	113	0.41	0.41	trnH-GUG	trnH-GUG
Leu	CUU	CUU	351	355	1.3	1.3	trnL-UAG	trnL-UAG
Leu	UUG	CUG	320	97	1.18	0.36	trnM-CAU	
Met	AUG	AUG	371	373	1	1		
Asn	AAC	AAU	176	512	0.52	1.49		
Asn	AAU	AAC	507	176	1.48	0.51		
Pro	CCU	CCU	280	280	1.74	1.74	trnP-UGG	trnP-UGG
Pro	CCG	CCC	82	94	0.51	0.58		
Pro	CCA	CCG	189	82	1.17	0.51		
Pro	CCC	CCA	94	189	0.58	1.17		
Gln	CAA	CAA	396	402	1.49	1.48		
Gln	CAG	CAG	135	141	0.51	0.52		
Arg	CGC	AGA	55	274	0.36	1.77		

Arg	AGG	AGG	83	83	0.54	0.54		
Arg	CGG	CGG	63	63	0.41	0.41		
Arg	CGA	CGA	225	226	1.46	1.46		
Arg	CGU	CGC	225	55	1.46	0.36	trnR-ACG	
Arg	AGA	CGU	275	227	1.78	1.47		trnR-ACG
Ser	UCU	AGU	347	233	1.82	1.22	trnT-UGU	
Ser	UCG	UCG	92	92	0.48	0.48		
Ser	AGU	UCA	234	215	1.23	1.12		
Ser	UCC	UCU	190	349	0.99	1.82	trnS-GGA	trnT-UGU
Ser	UCA	UCC	214	190	1.12	0.99		trnS-GGA
Ser	AGC	AGC	69	69	0.36	0.36		
Thr	ACA	ACG	225	74	1.17	0.38		
Thr	ACU	ACC	333	140	1.73	0.72		
Thr	ACC	ACA	138	225	0.72	1.16		
Thr	ACG	ACU	75	335	0.39	1.73		
Val	GUU	GUG	339	114	1.55	0.52	trnE-UUC	
Val	GUC	GUA	88	336	0.4	1.54	trnV-GAC	
Val	GUG	GUU	112	335	0.51	1.53		trnE-UUC
Val	GUA	GUC	337	88	1.54	0.4		trnV-GAC
Trp	UGG	UGG	289	288	1	1	trnW-CCA	trnW-CCA
Tyr	UAU	UAU	450	449	1.63	1.62		
Tyr	UAC	UAC	103	104	0.37	0.38		

Notes: HJY, HB refer to "Huangjinya" and "Huabai 1" respectively; a: relative synonymous codon usage, RSCU.

Table 3 Codon usage indices of chloroplast genes in "Huangjinya"

Title	T3s	C3s	A3s	G3s	CAI	Nc	GC3s	GC
HJY_rbcL	0.5	0.18	0.39	0.16	0.28	47.97	0.27	0.44
HJY_rpl2	0.41	0.19	0.42	0.19	0.14	54.12	0.31	0.43
HJY_atpF	0.31	0.26	0.38	0.32	0.16	55.84	0.44	0.39
HJY_rps19-fragment	0.4	0.4	0.33	0.13	0.31	-	0.42	0.39
HJY_rpl33	0.47	0.19	0.44	0.21	0.16	52.09	0.29	0.36
HJY_petG	0.45	0.15	0.41	0.16	0.16	56.37	0.25	0.36
HJY_petD	0.35	0.27	0.32	0.42	0.17	54.63	0.48	0.41
HJY_rpl16	0.3	0.32	0.28	0.38	0.13	47.15	0.53	0.43
HJY_clpP	0.48	0.15	0.47	0.12	0.21	48.98	0.22	0.41
HJY_rpl36	0.43	0.11	0.58	0.12	0.12	41.35	0.17	0.36
HJY_psaC	0.53	0.12	0.45	0.15	0.18	55.76	0.21	0.42
HJY_petB	0.44	0.27	0.26	0.26	0.17	58.76	0.41	0.41
HJY_rpl32	0.53	0.03	0.55	0.13	0.22	39.4	0.13	0.32
HJY_rps3	0.42	0.19	0.59	0.09	0.17	47.42	0.2	0.34
HJY_rps4	0.47	0.17	0.46	0.15	0.16	48.06	0.25	0.38
HJY_ycf4	0.51	0.16	0.39	0.17	0.15	46.89	0.25	0.38
HJY_ndhK	0.5	0.13	0.46	0.13	0.16	48.06	0.21	0.37
HJY_rpoC1	0.47	0.16	0.46	0.18	0.15	50.07	0.26	0.39
HJY_psbK	0.55	0.24	0.22	0.17	0.15	43.69	0.33	0.37
HJY_psaB-fragment	0.48	0.17	0.38	0.18	0.2	49.69	0.27	0.41
HJY_rpoA	0.49	0.14	0.48	0.18	0.15	48.02	0.23	0.34
HJY_psbT	0.48	0.19	0.41	0.17	0.22	-	0.27	0.34
HJY_petN	0.4	0.16	0.5	0	0.11	30.7	0.16	0.41
HJY_rps8	0.42	0.17	0.47	0.15	0.12	41.47	0.25	0.37
HJY_rps15	0.45	0.12	0.52	0.22	0.19	49.14	0.25	0.32
HJY_rpl14	0.46	0.15	0.46	0.16	0.17	44.21	0.24	0.4
HJY_rps11	0.47	0.1	0.45	0.11	0.14	47.92	0.18	0.44
HJY_ycf2	0.45	0.21	0.4	0.26	0.16	53.26	0.34	0.38
HJY_psbZ	0.54	0.14	0.32	0.09	0.24	51.14	0.2	0.35
HJY_psbB	0.49	0.17	0.37	0.16	0.19	47.41	0.27	0.44
HJY_petL	0.52	0.1	0.35	0.15	0.12	61	0.2	0.33
HJY_rpoC2	0.45	0.16	0.46	0.19	0.14	49.26	0.27	0.37
HJY_ndhD	0.45	0.15	0.48	0.12	0.15	45.95	0.22	0.35
HJY_atpB	0.47	0.16	0.4	0.16	0.22	45.02	0.26	0.42
HJY_rps16	0.6	0.07	0.43	0.19	0.18	46.33	0.2	0.36
HJY_ndhJ	0.51	0.16	0.38	0.21	0.15	51.41	0.28	0.4
HJY_rpl20	0.45	0.11	0.46	0.21	0.1	46.82	0.23	0.35
HJY_psbM	0.57	0.13	0.42	0.05	0.24	47.17	0.15	0.31
HJY_ndhG	0.51	0.11	0.43	0.13	0.14	42.88	0.19	0.33
HJY_accD	0.58	0.17	0.35	0.2	0.2	48.22	0.27	0.35
HJY_ndhB	0.43	0.21	0.43	0.12	0.16	46.73	0.27	0.37
HJY_psbJ	0.53	0.13	0.27	0.15	0.16	36.11	0.24	0.4
HJY_pbf1	0.39	0.25	0.45	0.13	0.16	48.23	0.31	0.43

HJY_rpoB	0.46	0.12	0.46	0.21	0.15	48.21	0.25	0.39
HJY_rps19	0.43	0.14	0.53	0.14	0.17	44.8	0.21	0.35
HJY_psaA-fragment	0.51	0.14	0.38	0.18	0.17	48.74	0.26	0.41
HJY_psbI	0.47	0.28	0.44	0	0.22	24.1	0.26	0.37
HJY_psbC	0.46	0.19	0.4	0.13	0.19	43.49	0.27	0.44
HJY_atpE	0.5	0.13	0.39	0.2	0.16	46.05	0.25	0.39
HJY_ycf1	0.48	0.16	0.56	0.16	0.16	46.45	0.22	0.3
HJY_cemA	0.51	0.21	0.42	0.16	0.19	49.65	0.27	0.32
HJY_psbF	0.32	0.18	0.47	0.19	0.13	55.4	0.31	0.44
HJY_psaA	0.46	0.19	0.4	0.16	0.2	49.2	0.28	0.43
HJY_ndhE	0.56	0.12	0.36	0.15	0.17	41.09	0.21	0.32
HJY_rps2	0.48	0.14	0.44	0.17	0.18	47.47	0.24	0.38
HJY_psaJ	0.4	0.1	0.52	0.21	0.14	45.04	0.24	0.36
HJY_psbH	0.53	0.03	0.4	0.19	0.13	41.35	0.18	0.36
HJY_psaB	0.51	0.16	0.37	0.17	0.19	47.67	0.26	0.41
HJY_ndhC	0.54	0.1	0.43	0.14	0.2	45.99	0.18	0.35
HJY_rpl23	0.47	0.12	0.44	0.19	0.13	49.8	0.24	0.38
HJY_atpA	0.51	0.14	0.43	0.14	0.21	43.04	0.22	0.4
HJY_ndhF	0.55	0.1	0.42	0.14	0.14	41.78	0.18	0.32
HJY_ccsA	0.48	0.14	0.5	0.11	0.15	46.52	0.2	0.32
HJY_rpl22	0.47	0.15	0.54	0.12	0.17	43	0.2	0.35
HJY_petA	0.51	0.16	0.39	0.2	0.19	48.63	0.27	0.39
HJY_rps18	0.5	0.11	0.41	0.2	0.11	35.64	0.24	0.35
HJY_ndhA	0.47	0.12	0.48	0.1	0.13	41.33	0.18	0.34
HJY_infA	0.52	0.13	0.42	0.16	0.13	54.48	0.22	0.37
HJY_atpI	0.49	0.18	0.4	0.12	0.17	44.59	0.24	0.38
HJY_ndhI	0.5	0.18	0.46	0.16	0.18	49.59	0.25	0.36
HJY_psbA	0.53	0.25	0.31	0.07	0.32	40.6	0.28	0.42
HJY_rps7	0.43	0.13	0.52	0.12	0.19	45.81	0.2	0.4
HJY_psbE	0.45	0.25	0.37	0.11	0.16	45.58	0.3	0.42
HJY_atpH	0.52	0.08	0.33	0.18	0.18	49.16	0.23	0.46
HJY_ycf1-fragment	0.45	0.18	0.54	0.17	0.16	49.01	0.25	0.33
HJY_rps14	0.38	0.12	0.49	0.25	0.13	37.46	0.29	0.41
HJY_rps12-fragment	0.41	0.16	0.41	0.21	0.1	43	0.3	0.45
HJY_psbD	0.51	0.21	0.36	0.11	0.25	43.45	0.27	0.42
HJY_matK	0.5	0.16	0.45	0.18	0.16	47.2	0.25	0.33
HJY_ndhH	0.3	0.29	0.37	0.38	0.17	57.65	0.48	0.38
HJY_ycf3	0.47	0.16	0.47	0.21	0.15	56.67	0.27	0.39
HJY_psbL	0.44	0.28	0.42	0.13	0.19	47.54	0.33	0.33
HJY_rps12	0.33	0.28	0.39	0.29	0.12	54.93	0.44	0.44
HJY_psaI	0.54	0.14	0.4	0.09	0.27	35.75	0.19	0.35

Table 4 Codon usage indices of genes in chloroplast of "Huabai 1"

Title	T3s	C3s	A3s	G3s	CAI	Nc	GC3s	GC
HB_psbZ	0.54	0.14	0.32	0.09	0.24	51.14	0.2	0.35
HB_rpoA	0.49	0.14	0.48	0.19	0.15	48.47	0.24	0.34
HB_rps14	0.38	0.12	0.49	0.25	0.13	37.46	0.29	0.41
HB_psbM	0.57	0.13	0.42	0.05	0.24	47.17	0.15	0.31
HB_rps19	0.43	0.14	0.53	0.14	0.17	44.8	0.21	0.35
HB_ndhA	0.47	0.12	0.48	0.1	0.13	41.33	0.18	0.34
HB_matK	0.5	0.16	0.45	0.18	0.16	46.99	0.25	0.33
HB_rps8	0.42	0.17	0.47	0.15	0.12	41.44	0.25	0.37
HB_petD	0.35	0.27	0.32	0.42	0.17	54.63	0.48	0.41
HB_rpoC1	0.47	0.16	0.46	0.18	0.15	50.07	0.26	0.39
HB_ndhF	0.55	0.1	0.42	0.14	0.14	41.78	0.18	0.32
HB_rpl14	0.46	0.15	0.46	0.16	0.17	44.21	0.24	0.4
HB_ndhJ	0.51	0.16	0.38	0.21	0.15	51.41	0.28	0.4
HB_rps16	0.61	0.08	0.43	0.19	0.18	45.41	0.2	0.36
HB_atpA	0.51	0.14	0.43	0.14	0.21	43.04	0.22	0.4
HB_psbK	0.55	0.24	0.22	0.17	0.15	43.69	0.33	0.37
HB_ycf4	0.51	0.17	0.39	0.17	0.15	46.77	0.26	0.38
HB_ndhD	0.45	0.15	0.48	0.12	0.15	45.95	0.22	0.35
HB_rps11	0.47	0.1	0.45	0.11	0.14	47.92	0.18	0.44
HB_ndhE	0.56	0.12	0.36	0.15	0.17	41.09	0.21	0.32
HB_rpl20	0.45	0.11	0.46	0.21	0.1	46.82	0.23	0.35
HB_ndhK	0.5	0.13	0.46	0.13	0.16	48.06	0.21	0.37
HB_atpE	0.5	0.13	0.39	0.2	0.16	46.05	0.25	0.39
HB_ndhI	0.5	0.18	0.46	0.16	0.18	49.59	0.25	0.36
HB_ndhB	0.43	0.21	0.43	0.12	0.16	46.73	0.27	0.37
HB_rps7	0.43	0.13	0.52	0.12	0.19	45.81	0.2	0.4
HB_rps4	0.47	0.17	0.47	0.14	0.16	47.88	0.24	0.38
HB_atpB	0.47	0.16	0.4	0.16	0.22	45.02	0.26	0.42
HB_psaA-fragment	0.51	0.14	0.38	0.18	0.17	48.74	0.26	0.41
HB_psbF	0.32	0.18	0.47	0.19	0.13	55.4	0.31	0.44
HB_psbE	0.45	0.25	0.37	0.11	0.16	45.58	0.3	0.42
HB_psbL	0.44	0.28	0.42	0.13	0.19	47.54	0.33	0.33
HB_psbA	0.53	0.25	0.31	0.07	0.32	40.6	0.28	0.42
HB_rpl2	0.41	0.19	0.42	0.19	0.14	54.12	0.31	0.43
HB_petB	0.44	0.27	0.26	0.26	0.17	58.76	0.41	0.41
HB_rpl23	0.47	0.12	0.44	0.19	0.13	49.8	0.24	0.38
HB_psbD	0.51	0.21	0.36	0.11	0.25	43.45	0.27	0.42
HB_petG	0.45	0.15	0.41	0.16	0.16	56.37	0.25	0.36
HB_petN	0.4	0.16	0.5	0	0.11	30.7	0.16	0.41
HB_petL	0.52	0.1	0.35	0.15	0.12	61	0.2	0.33
HB_psbH	0.53	0.03	0.4	0.19	0.13	41.35	0.18	0.36
HB_rps12	0.33	0.28	0.39	0.29	0.12	54.93	0.44	0.44
HB_rps18	0.5	0.11	0.41	0.2	0.11	35.64	0.24	0.35

HB_psbB	0.49	0.17	0.37	0.16	0.19	47.41	0.27	0.44
HB_atpI	0.49	0.18	0.4	0.12	0.17	44.59	0.24	0.38
HB_ycf3	0.47	0.16	0.47	0.21	0.15	56.67	0.27	0.39
HB_psbT	0.48	0.19	0.41	0.17	0.22	*****	0.27	0.34
HB_psaC	0.53	0.12	0.45	0.15	0.18	55.76	0.21	0.42
HB_ccsA	0.48	0.14	0.5	0.11	0.15	46.52	0.2	0.32
HB_cemA	0.51	0.21	0.42	0.16	0.19	49.65	0.27	0.32
HB_infA	0.52	0.13	0.42	0.16	0.13	54.48	0.22	0.37
HB_petA	0.51	0.16	0.39	0.2	0.19	48.63	0.27	0.39
HB_psaB-fragment	0.48	0.17	0.38	0.18	0.2	49.69	0.27	0.41
HB_psbJ	0.53	0.13	0.27	0.15	0.16	36.11	0.24	0.4
HB_rbcL	0.5	0.18	0.39	0.16	0.28	47.97	0.27	0.44
HB_ndhH	0.3	0.29	0.37	0.38	0.17	56.39	0.48	0.38
HB_atpF	0.31	0.26	0.38	0.32	0.16	55.84	0.44	0.39
HB_psaJ	0.4	0.1	0.52	0.21	0.14	45.04	0.24	0.36
HB_rpl16	0.3	0.32	0.28	0.38	0.13	47.15	0.53	0.43
HB_rpl36	0.43	0.11	0.58	0.12	0.12	41.35	0.17	0.36
HB_ndhC	0.54	0.1	0.43	0.14	0.2	45.99	0.18	0.35
HB_ycf2	0.45	0.21	0.4	0.26	0.16	53.26	0.34	0.38
HB_psbC	0.46	0.19	0.4	0.13	0.19	43.49	0.27	0.44
HB_rps19-fragment	0.4	0.4	0.33	0.13	0.31	*****	0.42	0.39
HB_rpl33	0.47	0.19	0.44	0.21	0.16	52.09	0.29	0.36
HB_clpP	0.48	0.15	0.47	0.12	0.21	49.9	0.22	0.41
HB_rpl32	0.53	0.03	0.55	0.13	0.22	39.4	0.13	0.32
HB_pbf1	0.39	0.25	0.45	0.13	0.16	48.23	0.31	0.43
HB_psbI	0.47	0.28	0.44	0	0.22	24.1	0.26	0.37
HB_rps2	0.48	0.14	0.44	0.17	0.18	47.47	0.24	0.38
HB_ycf1-fragment	0.41	0.24	0.45	0.2	0.17	57.22	0.33	0.36
HB_rpoB	0.46	0.12	0.46	0.21	0.15	48.21	0.25	0.39
HB_psaI	0.54	0.14	0.4	0.09	0.27	35.75	0.19	0.35
HB_rpl22	0.47	0.15	0.54	0.12	0.17	43	0.2	0.35
HB_ycf1	0.48	0.16	0.56	0.16	0.16	46.55	0.22	0.3
HB_psaA	0.46	0.19	0.4	0.16	0.2	49.2	0.28	0.43
HB_rps15	0.45	0.12	0.52	0.22	0.19	49.14	0.25	0.32
HB_ndhG	0.51	0.11	0.43	0.13	0.14	42.88	0.19	0.33
HB_accD	0.58	0.17	0.35	0.2	0.2	48.21	0.27	0.35
HB_rpoC2	0.45	0.16	0.46	0.19	0.14	49.17	0.27	0.37
HB_rps12-fragment	0.41	0.16	0.41	0.21	0.1	43	0.3	0.45
HB_rps3	0.42	0.19	0.59	0.09	0.17	47.42	0.2	0.34
HB_atpH	0.52	0.08	0.33	0.18	0.18	49.16	0.23	0.46
HB_psaB	0.51	0.16	0.37	0.17	0.19	47.67	0.26	0.41

Table 5 SSR analysis of "Huangjinya" and "Huabai 1" chloroplast genome

Huangjinya						Huabai 1				
No.	Type	Size				Type	Size			
		SSR	(bp)	Start	End		SSR	(bp)	Start	End
1	Mon	(A) ₁₀	10	3791	3800	Mon	(A) ₁₀	10	3791	3800
2	Mon	(A) ₁₀	10	8904	8913	Mon	(A) ₁₀	10	65314	65323
3	Mon	(A) ₁₀	10	32930	32939	Mon	(A) ₁₀	10	73238	73247
4	Mon	(A) ₁₀	10	65330	65339	Mon	(A) ₁₀	10	128318	128327
5	Mon	(A) ₁₀	10	73302	73311	Mon	(A) ₁₀	10	129797	129806
6	Mon	(A) ₁₀	10	82858	82867	Mon	(A) ₁₀	10	138306	138315
7	Mon	(A) ₁₀	10	131686	131695	Mon	(A) ₁₁	11	8909	8919
8	Mon	(A) ₁₀	10	140204	140213	Mon	(A) ₁₁	11	17281	17291
9	Mon	(A) ₁₁	11	17271	17281	Mon	(A) ₁₁	11	32935	32945
10	Mon	(A) ₁₁	11	34201	34211	Mon	(A) ₁₁	11	34216	34226
11	Mon	(A) ₁₁	11	38261	38271	Mon	(A) ₁₁	11	38277	38287
12	Mon	(A) ₁₁	11	46229	46239	Mon	(A) ₁₁	11	46245	46255
13	Mon	(A) ₁₁	11	83627	83637	Mon	(A) ₁₁	11	83563	83573
14	Mon	(A) ₁₁	11	110415	110425	Mon	(A) ₁₁	11	110361	110371
15	Mon	(A) ₁₁	11	117891	117901	Mon	(A) ₁₁	11	116009	116019
16	Mon	(A) ₁₁	11	119067	119077	Mon	(A) ₁₁	11	117185	117195
17	Mon	(A) ₁₂	12	32596	32607	Mon	(A) ₁₂	12	38510	38521
18	Mon	(A) ₁₂	12	38494	38505	Mon	(A) ₁₃	12	129264	129275
19	Mon	(A) ₁₂	12	49310	49321	Mon	(A) ₁₃	13	356	368
20	Mon	(A) ₁₂	12	131153	131164	Mon	(A) ₁₃	13	32600	32612
21	Mon	(A) ₁₃	13	356	368	Mon	(A) ₁₃	13	46500	46512
22	Mon	(A) ₁₃	13	37621	37633	Mon	(A) ₁₃	13	49326	49338
23	Mon	(A) ₁₃	13	46484	46496	Mon	(A) ₁₄	14	37636	37649
24	Mon	(A) ₁₆	16	12554	12569	Mon	(A) ₁₇	17	12557	12573
25	Mon	(A) ₁₇	17	113487	113503	Mon	(T) ₁₀	10	27181	27190
26	Mon	(C) ₁₀	10	10968	10977	Mon	(T) ₁₀	10	37236	37245
27	Mon	(T) ₁₀	10	6390	6399	Mon	(T) ₁₀	10	56325	56334
28	Mon	(T) ₁₀	10	27177	27186	Mon	(T) ₁₀	10	65452	65461
29	Mon	(T) ₁₀	10	37221	37230	Mon	(T) ₁₀	10	65768	65777
30	Mon	(T) ₁₀	10	56308	56317	Mon	(T) ₁₀	10	70907	70916
31	Mon	(T) ₁₀	10	63155	63164	Mon	(T) ₁₀	10	80588	80597
32	Mon	(T) ₁₀	10	65468	65477	Mon	(T) ₁₀	10	82510	82519
33	Mon	(T) ₁₀	10	65784	65793	Mon	(T) ₁₀	10	105303	105312
34	Mon	(T) ₁₀	10	70971	70980	Mon	(T) ₁₁	11	19487	19497
35	Mon	(T) ₁₀	10	80652	80661	Mon	(T) ₁₁	11	52626	52636
36	Mon	(T) ₁₀	10	82574	82583	Mon	(T) ₁₁	11	58973	58983
37	Mon	(T) ₁₀	10	105357	105366	Mon	(T) ₁₁	11	133247	133257
38	Mon	(T) ₁₀	10	113875	113884	Mon	(T) ₁₂	12	8765	8776
39	Mon	(T) ₁₀	10	124960	124969	Mon	(T) ₁₀	12	13935	13946

40	Mon	(T) ₁₁	11	19477	19487	Mon	(T) ₁₂	12	15067	15078
41	Mon	(T) ₁₁	11	33233	33243	Mon	(T) ₁₂	12	15461	15472
42	Mon	(T) ₁₁	11	52609	52619	Mon	(T) ₁₂	12	33239	33250
43	Mon	(T) ₁₁	11	58959	58969	Mon	(T) ₁₂	12	56759	56770
44	Mon	(T) ₁₁	11	135145	135155	Mon	(T) ₁₂	12	127345	127356
45	Mon	(T) ₁₂	12	8760	8771	Mon	(T) ₁₂	12	128960	128971
46	Mon	(T) ₁₂	12	13931	13942	Mon	(T) ₁₃	13	85013	85025
47	Mon	(T) ₁₂	12	15063	15074	Mon	(T) ₁₃	13	115371	115383
48	Mon	(T) ₁₂	12	15451	15462	Mon	(T) ₁₄	14	53363	53376
49	Mon	(T) ₁₂	12	114406	114417	Mon	(T) ₁₄	14	60751	60764
50	Mon	(T) ₁₂	12	129234	129245	Mon	(T) ₁₄	14	121384	121397
51	Mon	(T) ₁₂	12	130849	130860	Mon	(T) ₁₅	15	83035	83049
52	Mon	(T) ₁₃	13	85077	85089	Mon	(T) ₁₇	17	130178	130194
53	Mon	(T) ₁₃	13	117253	117265	dimer	(AT) ₅	5	20854	20863
54	Mon	(T) ₁₄	14	53346	53359	dimer	(AT) ₅	5	84276	84285
55	Mon	(T) ₁₄	14	60737	60750	dimer	(TA) ₅	5	87246	87255
56	Mon	(T) ₁₄	14	123266	123279	dimer	(AT) ₅	5	156362	156371
57	Mon	(T) ₁₅	15	56742	56756					
58	Mon	(T) ₁₅	15	83099	83113					
59	Mon	(T) ₁₇	17	132067	132083					
60	dimer	(AT) ₅	5	20850	20859					
61	dimer	(AT) ₅	5	84340	84349					
62	dimer	(TA) ₅	5	87310	87319					
63	dimer	(AT) ₅	5	158250	158259					

Table 1 Position, exon and intron information of genes in chloroplast genome of "Huangjinya"and "Huabai 1"

Gene	Location		Exon I (bp)		Intron I (bp)		Exon II (bp)		Intron II (bp)		Exon III (bp)	
	HJY	HB	HJY	HB	HJY	HB	HJY	HB	HJY	HB	HJY	HB
trnA-UGC	IR	IR	36	36	41	41	27	27				
rps16	LSC	LSC	35	35	856	855	230	230				
atpF	LSC	LSC	143	143	718	718	409	409				
rps12	IR	IR	231	231	537	537	26	26				
clpP	LSC	LSC	70	70	794	794	291	291	597	597	227	227
ndhA	SSC	SSC	551	551	1086	1085	539	539				
ycf3	LSC	LSC	123	123	720	720	227	227	738	738	154	154
ndhB	IR	IR	776	776	680	680	755	755				
rpl2	IR	IR	390	390	671	671	433	433				
trnI-GAU	IR	IR	35	35	17	17	35	35				
rpoC1	LSC	LSC	431	431	745	745	1616	1616				

Notes: HJY, HB refer to "Huangjinya" and "Huabai 1" respectively.

Fig.1 The leaves phenotype of "Huangjinya" and "Huabai 1"

Fig. 2 Structure of chloroplast genome map of "Huangjinya"

Fig. 3 Structure of chloroplast genome map of "Huabai 1"

Fig. 4 Phylogenetic tree based on four DNA barcodes in chloroplast genome of tea plants