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Chloroplast DNA Polymorphism in Rice (*Oryza sativa* L.)

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Abstract

We analyzed the sequence alignment on 25 AA rice and 24 non-AA rice chloroplasts using two length diversity markers (ORF 100 and ORF29-TrnC^{GCA}) and four sequence markers existed in introns of rps16 gene and TrnT^{UGU}-TrnL^{UAA} spacer to explore the chloroplast diversity of different types of rice using PCR amplification and sequencing. Results showed that in terms of the length of ORF100 and ORF29-TrnC^{GCA}, chloroplast DNA (cp DNA) of Hainan ordinary wild rice, Dongxiang ordinary wild rice, Hepu ordinary wild rice and three-line cytoplasmic male sterile wild rice were indica-type, Chaling ordinary wild rice, Fusui ordinary wild rice, Niwara wild rice, Brazilian upland rice and Lemont were japonica-type among in AA genome. Besides, all non-AA wild rice was japonica-type. There were 4 indica-japonica markers utilizing introns of rps16 gene and TrnT^{UGU}-TrnL^{UAA}. We found that all the ordinary wild rice in Chaling and Fusui of AA genome presented as japonica specific sites, while the others owned two indica and japonica specific sites, respectively. There were two indica-japonica sites separately and a 6-base specific fragment in three-line cytoplasmic male sterile materials except Yuetai A, simultaneously, 2-base difference from Hainan wild rice. Moreover, Brazilian upland rice and Lemont were entire japonica specific sites. Result of three markers indicated that the cp DNA of non-AA wild rice was japonica-type and result of one marker showed indica-type. Sequencing results also suggested that wild rice existed many polymorphic base sites, CCDD genome, wart wild rice and malay wild rice had their own specific sites. In conclusion, significant differentiation trend of indica-japonica exhibits in chloroplast of ordinary wild rice, and non-AA wild rice is generally japonica-type. The cytoplasmic polymorphism level of three-line sterile lines is low. It is worth considering whether the cytoplasm of Honglian-type sterile

line Yuetai A comes from Hainan ordinary wild rice. Furthermore, genetic polymorphisms in wild rice are far more than in cultivar.

Keywords

Rice Chloroplast DNA, Three-Line Sterile Rice, Wild Rice, Polymorphism

1. Introduction

Cytoplasmic DNA is mainly maternal inheritance, which has higher genetic stability and lower mutation frequency than nuclear DNA. Therefore, cytoplasmic DNA is more suitable as a molecular marker for the origin and evolution of species and Chloroplast DNA has more variability than mitochondrial DNA [1]. Especially, analyzing the indica-japonica characteristic of chloroplast DNA (cp DNA) in rice is particularly useful for studying its origin and evolution, which is highly conserved [2]. Japonica-type cp DNA has a 69-bp repeat fragment while deleted in indica-type among in the ORF100 (open reading frame 100), so the band of japonica rice lags behind that of indica rice on the electrophoresis map. Based on this characteristic, Chen *et al.* discovered that the classification results of cultivated rice according to the 69bp deletion in ORF100 were effectively the same as indica-japonica discriminant function and isozymes [3] [4]. Sun *et al.* divided 151 cp DNA of ordinary wild rice into indica-japonica taking advantage of ORF100 marker [5]. Since then, it has been widely adopted as a marker for distinguishing the indica-japonica types [6]. Due to one 32-bp insertion between ORF29 and $TrnC^{GCA}$ (the intertranscriptional region ORF29 - $TrnC^{GCA}$) in typical indica rice but not to exist in japonica rice, Tang suggested that this fragment could be used as a marker for cpDNA indica-japonica typing [7]. In addition, Shaw recommended that the introns of rps16 (ribosomal protein S16) and the $TrnT^{UGU}$-$TrnL^{UAA}$ (Threonine and Leucine transfer RNA gene) intertranscriptional region were the two hypervariable fragments in plant cp DNA, which were vital for the study of rice cp DNA polymorphism [8].

Oryza is publicly recognized as owing 22 species, including A, B, C, D, E, F, G, H, J, and K [9]. The studied Asian cultivated rice and ordinary wild rice are AA genome type. There are 16 species of wild rice with chromosomes other than AA genome (referred to as non-AA wild rice), however, little research on indica-japonica differentiation. Zhu believed that the type of cpDNA variation was basically divided in accordance with the karyotype level of Oryza after analyzing 138 cp DNA in 14 species of Oryza and 2 species of Leersia, utilizing Restriction fragment length polymorphism (RFLP) [10].

In this paper, distinct 49 genomic types of rice were used as materials. We amplified the four polymorphic fragments of cp DNA including ORF100, ORF29-$TrnC^{GCA}$ intertranscriptional region, introns of rps16 and $TrnT^{UGU}$-$TrnL^{UAA}$ intertranscriptional region, but also sequenced the last two frag-

ments for comparison and analysis, in order to explore the cp DNA polymorphism and regulation in different genomes of rice.

2. Materials and Methods

2.1. Plant Materials

The test materials include 25 AA genome and 24 non-AA genome of Oryza (Table 1). There are 6 cultivated rice, 11 three-line sterile rice, 5 ordinary wild rice, 1 Nivara wild rice, 1 upland rice and 1 Javanese rice of AA genome, provided by the Plant Physiology Laboratory of Hunan Normal University. Non-AA genome materials cover 12 species, a total of 24 materials, provided by the National Germplasm Nanning Wild Rice Nursery.

2.2. Total DNA Extraction

Total DNA was extracted using CTAB method [11].

2.3. PCR and Electrophoresis

The primers were designed according to the chloroplast genome sequence of Asian cultivated rice 9311 for its' reliable genome (GenBank accession number AY522329) who was the parent of the first super hybrid rice, using the Primer Premier 5.0 (Table 2). We performed the PCR with a 25 μL reaction system: 1 × PCR Buffer, 2 mmol/L $MgCl_2$, 0.1 mmol/L dNTPs, 0.2 μmol/L primers, 60 - 100 ng DNA template, 2 U TaqDNA polymerase (Ferments, USA), under 94˚C for 5 min, followed by 32 cycles of 94˚C for 40 s, 50˚C for 40 s and 72˚C for 50 s, ultimately, 72˚C for 10 min. The amplified products were constantly electrophoresed on a 2% agarose gel containing 0.5 μg/mL EB and then imaged by UV.

2.4. DNA Sequencing

We directly sequenced the amplified products after recovered and purified under UV light using Ambio Biotechnology DNA Gel Recovery Kit.

2.5. Data Analysis

Sequencing results were analyzed to compare the differences and similarities between the two polymorphic fragments in various materials by MEGA 3.1.

3. Results

3.1. Comparison of the Length of ORF100 Amplification Fragments

Electrophoresis results of three typical indica rice and three typical japonica rice were in line with the indica-japonica character of ORF100 marker. Four types of three-line sterile materials were indica chloroplast. The bands of Hainan ordinary wild rice, Hepu ordinary wild rice and Dongxiang ordinary wild rice showed that they were consistent with typical indica rice. Fusui ordinary wild rice, Chaling ordinary wild rice, Nivara wild rice, Brazilian upland rice, and

Table 1. The materials and its nuclear genome type.

NO.	Material	nuclear genome type	NO.	Material	Nuclear genome type
1	9311	AA	26	*O. punctata*	BB
2	Nanjing 3 hao	AA	27	*O. minuta*[a]	BBCC
3	Guanglu ai si hao	AA	28	*O. minuta*[b]	BBCC
4	Nipponbare	AA	29	*O. rhizomatis*[a]	CC
5	Bai ri zao	AA	30	*O. rhizomatis*[b]	CC
6	Ai zi nuo	AA	31	*O. rhizomatis*[c]	CC
7	Jin 23A[1]	AA	32	*O. eichingeri*	CC
8	Guangye A[1]	AA	33	*O. officinalist*[a]	CC
9	V20A[1]	AA	34	*O. officinalist*[b]	CC
10	Chuanxiang 29A[1]	AA	35	*O. alta*[a]	CCDD
11	Zhenshan 97A[1]	AA	36	*O. alta*[b]	CCDD
12	T98A[1]	AA	37	*O. alta*[c]	CCDD
13	II-32A[2]	AA	38	*O. grandiglumis*[a]	CCDD
14	Zhong 9A[2]	AA	39	*O. grandiglumis*[b]	CCDD
15	You 1A[2]	AA	40	*O. grandiglumis*[c]	CCDD
16	K17A[3]	AA	41	*O. latifolia*[a]	CCDD
17	Yuetai A[4]	AA	42	*O. latifolia*[b]	CCDD
18	Hepu CWR	AA	43	*O. austrliensis*[a]	EE
19	Dongxiang CWR	AA	44	*O. eichingeri*[b]	EE
20	Hainan CWR	AA	45	*O. austrliensis*[c]	EE
21	Chaling CWR	AA	46	*O. brachyantha*	FF
22	Fusui CWR	AA	47	*O. meyeriana*	GG
23	Nivala wild rice	AA	48	*O. ridleyi*[a]	HHJJ
24	Brazil upland rice	AA	49	*O. ridleyi*[b]	HHJJ
25	Lemont	AA			

Note: 1 - cytoplasm source from hainan CWR, 2 - cytoplasm source from yinshui 6 hao, 3 - cytoplasm source from K52, 4 - cytoplasm source from hainan red awn CWR, a, b, c - the different species of the material.

Table 2. Primer sequence and target fragment.

Primer	Forword sequence 5'—3'	Reverse sequence 5'—3'	Target fragment
cp1	GTGGACCTGACTCCTTGAA	AGCCGAGGTCGTGGTAA	ORF100
cp2	GCAGCCCAAGCGAGACT	AAGGCTCGGCGATACTG	ORF29-TrnC^{GCA}
cp3	AGTGGGCTTACATAACAGAAA	ACCAAGGCTCAATACAATCA	rps16 gene intron
cp4	TTTTCTCCTCATACGGCT	TAGTCTGTTCTATTCGTCCC	TrnT^{UGU}-TrnL^{UAA}

Lemont were the same as typical japonica rice, and all other non-AA genome wild rice behaved similar to japonica rice characteristic (**Figure 1**).

3.2. Comparison of the Length of ORF29-TrnC^{GCA} Amplification Fragments

Amplification electrophoresis results of cp2 proved the primers cp1. Four types of three-line sterile rice, Hainan ordinary wild rice, Hepu ordinary wild rice and

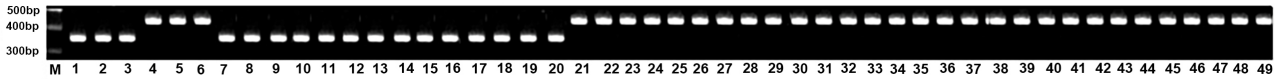

Figure 1. The fragments size of ORF100 in cpDNA. Note: The sequence of materials referring to **Table 1**.

Dongxiang ordinary wild rice were consistent with typical indica chloroplast. Fusui ordinary wild rice, Chaling ordinary wild rice, Nivara wild rice, Brazilian upland wild rice, Lemont, and all non-AA wild rice were the same astypical japonica chloroplast (**Figure 2**).

3.3. Polymorphism Analysis of Bases Sequence in Introns of Rps16 Gene Amplification Fragments

The primers cp3 were used to amplify a 677 - 684 bp fragment. After sequencing and comparison, results indicated that introns of rps16 were dominated by single base polymorphism with rare difference between longer fragments (**Table 3**). There was an indica-type specific site GTTTATC at 267 - 273 bp, interestingly, the sequences of 10 sporophytic sterile materials in rps16 fragments were completely identical, and a GTTGAG-specific sequence was existed at 220 - 225 bp. However, gametophytic sterile rice Yuetai A as well as others appeared deletion. Such as the base at 595 bp was G in Yuetai A and others were T, Hainan ordinary wild rice was G at 49 bp and others were C. Non-AA genome materials had no the same polymorphic fragments or bases. AA genome was deleted at 133 bp. Except for small grain wild rice was deleted and wart grain wild rice showed AA in non-AA genome, others were A. Apart from Australian wild rice was C at 332 bp and other genomes were T. At 369 bp, Australian wild rice and malay wild rice were T, others deleted. At 512 - 528 bp, wart grain wild rice and malay wild rice were deleted and others were TTATTTCGATTTCTATA. CCDD genome materials were TCAA at 603 - 606 bp, wart grain wild rice was -AA-, malay wild rice was -AAA, others were TAAAA.

3.4. Polymorphism Analysis of Bases Sequence in $TrnT^{UGU}$-$TrnL^{UAA}$ Amplification Fragments

An 813 - 824 bp fragment was amplified by the primers cp4 and it found three indica-japonica specific sites, which were 322 - 326 bp, 413 bp and 768 - 779 bp (**Table 4**). In this fragment, eleven three-line sterile rice had the same sequence structurally, and were the same as indica at three indica- japonica specific sites. Among ordinary wild rice, Fusui and Chaling were consistent with the two specific sites in typical japonica, two bases and one base deletion at japonica specific sites, respectively. Hainan and Hepu had two indica specific sites and 1 japonica specific sites, while Dongxiang had only two indica specific sites. Niwara wild rice had two specific sites for indica and one for japonica. Non-AA genome wild rice did not own indica specific sites but two japonica (Except for Australian wild rice, wart wild rice and malay wild rice which only had one japonica specific site). Simultaneously, there were many polymorphic sites. Such as wart grain wild rice was ------AA, malay wild rice was AAAAAGAAA but deletion in

Figure 2. The fragments size of ORF29-TrnCGCA. Note: The sequence of materials referring to **Table 1**.

Table 3. Sequence divergence of rps16 gene intron.

material	Base site No.								
	49	132 - 133	220.225	267 - 273	332	369	512 - 528	595	603 - 606
9311	C	--	--------	CTTTATC	T	-	TTATTTCGATTTCTATA	T	TAAA
Nanjing 3 hao	C	--	--------	CTTTATC	T	-	TTATTTCGATTTCTATA	T	TAAA
Guanglu ai si hao	C	--	--------	CTTTATC	T	-	TTATTTCGATTTCTATA	T	TAAA
Nipponbare	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Bai ri zao	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Ai zi nuo	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Jin 23A[1]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Guangye A[1]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
V20A[1]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Chuanxiang 29A[1]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Zhenshan 97A[1]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
T98A[1]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
II-32A[2]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Zhong 9A[2]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
You 1A[2]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
K17A[3]	C	--	GTTGAG	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Yuetai A[4]	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	G	TAAA
Hepu CWR	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Dongxiang CWR	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Hainan CWR	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Chaling CWR	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Fusui CWR	G	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Nivala wild rice	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Brazil upland rice	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
Lemont	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
O. *punctata*	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
O. *minuta*[a]	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
O. *minuta*[b]	C	--	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
O. *rhizomatis*[a]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
O. *rhizomatis*[b]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
O. *rhizomatis*[c]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA

Continued

O. *eichingeri*	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
O. *officinalis*[a]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
O. *officinalis*[b]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TAAA
O. *alta*[a]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TCAA
O. *alta*[b]	C	A	--------	----------	T	-	TTATTTCGATTTCTATA	T	TCAA
O. *alta*[c]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TCAA
O. *grandiglumis*[a]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TCAA
O. *grandiglumis*[b]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TCAA
O. *grandiglumis*[c]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TCAA
O. *latifolia*[a]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TCAA
O. *latifolia*[b]	C	A-	--------	----------	T	-	TTATTTCGATTTCTATA	T	TCAA
O. *austrliensis*[a]	C	A-	--------	----------	C	T	TTATTTCGATTTCTATA	T	TAAA
O. *eichingeri*[b]	C	A-	--------	----------	C	T	TTATTTCGATTTCTATA	T	TAAA
O. *austrliensis*[c]	C	A-	--------	----------	C	T	TTATTTCGATTTCTATA	T	TAAA
O. *brachyantha*	C	A-	--------	----------	T	T	TTATTTCGATTTCTATA	T	TAAA
O. *meyeriana*	C	AA	--------	----------	T		----------	T	-AA-
O. *ridleyi*[a]	C	A-	--------	----------	T	T	----------	T	-AAA
O. *ridleyi*[b]	C	A-	--------	----------	T	T	----------	T	-AAA

"-": the lack base or deletion.

others at 126 - 133 bp, which could be regarded as a specific site between wart and malay. 218 - 222 bp was as well as aspecific site for malay, but deletion in others. 479 bp, 520 bp and 599 bp were the specific sites respectively represented by Guangluai 4, Nanjing 3, and Hundred-day-old. This sequence reflected high genetic polymorphism and verified the view that it was a hypervariable region proposed by Shaw [7].

4. Discussion

Cultivated rice includes indica subspecies and japonica subspecies, previous studies on indica- japonica characteristics of wild rice have been widely recognized by breeders and geneticists. In this research, the length of fragments amplified by primers cp1 and cp2 of Dongxiang ordinary wild rice and Hepu ordinary wild were distinct with others, which were consistent with the typical indica rice and typical japonica rice, respectively. The same was true of cp3 and cp4 sequencing results and relatively supported the two-source origin theory of cultivated rice [12]. There were no differences in the two base sequence polymorphic fragments between introns of rps16 and $TrnT^{UGU}$-$TrnL^{UAA}$ intertranscriptional region for the tested wild abortive, Yinshui and K-type three-line sterile rice. Adversely, typical indica rice, typicaljaponica rice, ordinary wild rice and non-AA genome wild rice existed differences in various degrees. It demonstrated that the genetic

Table 4. Sequence divergence of TrnT^{UGU}-TrnL^{UAA} spacer.

material	Base site No.							
	126 - 133	218 - 222	322 - 326	413	479	520	599	768 - 779
9311	- - - - - - - -	-----	----	-	A	A	T	AGAAAA----------
Nanjing 3 hao	- - - - - - - -	-----	----	-	A	G	T	AGAAAA----------
Guanglu ai si hao	- - - - - - - -	-----	----	-	G	A	T	AGAAAA----------
Nipponbare	- - - - - - - -	-----	TATAT	T	A	A	T	AGAAAAAGAAAA
Bai ri zao	- - - - - - - -	-----	TATAT	T	A	A	C	AGAAAAAGAAAA
Ai zi nuo	- - - - - - - -	-----	TATAT	T	A	A	T	AGAAAAAGAAAA
Jin 23A[1]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
Guangye A[1]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
V20A[1]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
Chuanxiang 29A[1]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
Zhenshan 97A[1]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
T98A[1]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
II-32A[2]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
Zhong 9A[2]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
You 1A[2]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
K17A[3]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
Yuetai A[4]	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
Hepu CWR	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
Dongxiang CWR	- - - - - - - -	-----	----	--	A	A	T	AGAAA-AGAAAA
Hainan CWR	- - - - - - - -	-----	----	--	A	A	T	-GAAA-AGAAAA
Chaling CWR	- - - - - - - -	-----	TATAT	T	A	A	T	AGAAA-AGAAAA
Fusui CWR	- - - - - - - -	-----	TATAT	T	A	A	T	AGAAAAAGAAAA
Nivala wild rice	- - - - - - - -	-----	----	-	A	A	T	AGAAAAAGAAAA
Brazil upland rice	- - - - - - - -	-----	TATAT	T	A	A	T	AGAAAAAGAAAA
Lemont	- - - - - - - -	-----	TATAT	T	A	A	T	AGAAAAAGAAAA
O. punctata	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. minuta[a]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. minuta[b]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. rhizomatis[a]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. rhizomatis[b]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. rhizomatis[c]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. eichingeri	- - - - - - - -	-----	T---	T	A	A	T	AGAAAAAGAAAA
O. officinalis[a]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. officinalis[b]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. alta[a]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA

Continued

O. *alta*[b]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. *alta*[c]	- - - - - - - -	-----	T---	T	A	A	T	AGAAAAAGAAAA
O. *grandiglumis*[a]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. *grandiglumis*[b]	- - - - - - - -	-----	A---	T	A	A	T	AAAAAAAGAAAT
O. *grandiglumis*[c]	- - - - - - - -	-----	A---	T	A	A	T	AAAAAAAGAAAT
O. latifolia[a]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. latifolia[b]	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. austrliensis[a]	- - - - - - - -	-----	A---	-	A	A	T	AGAAAAAGAAAG
O. eichingeri[b]	- - - - - - - -	-----	A---	-	A	A	T	AGAAAAAGAAAG
O. austrliensis[c]	- - - - - - - -	-----	A---	-	A	A	T	AGAAAAAGAAAG
O. brachyantha	- - - - - - - -	-----	A---	T	A	A	T	AGAAAAAGAAAA
O. meyeriana	- - - - - -AA	-----	A---	-	A	A	T	AGAAAAAGAAAA
O. ridleyi[a]	AAAAAGAAA	AGAAT	A---	-	A	A	T	AGAAAAAGAAAA
O. ridleyi[b]	AAAAAGAAA	AGAAT	A---	-	A	A	T	AGAAAAAGAAAA

"-": the lack base or deletion.

background of these three sterile rice chloroplasts was relatively single, and the level of three-line sterile rice cp DNA polymorphism was lower than that in ordinary wild rice and non-AA genome, indicating the wild rice was the treasure of resources with great value.

Results of non-AA wild rice study showed that the length of fragments amplified by primers cp1 and cp2 was the same as japonica rice. There were three indica-japonica specific sites consistent with japonica of cp3 and cp4, suggesting non-AA genome wild rice was generally biased to japonica. Furthermore, it showed a certain tendency to indica. Ichikawa used molecular markers of the rice chloroplast genome for genetic analysis, and concluded that AA-type was relatively close to CCDD, BB, BBCC, CC, and FF, while EE might evolved from its own Oryza ancestors [13]. In our experiment, results of cp3 and cp4 amplification fragments manifested that Australian wild rice of EE genome did have significant base differences from others and it was relatively close to wart grain wild rice from EE and malay wild rice from HHJJ. At the same time, CCDD genome, wart grain wild rice and malay wild rice had their own specific sites in the two fragments but none in other genomes, which speculated that their kinship might be slightly farther than others.

In the three-line sterile materials, pollen abortion type of wild abortive, Yinshui and K-type was sporophytic sterility, further observed under microscope we defined they were typical, in addition, Honglian-type sterile linewas gametophytic sterility and also described as spherical. Among 11 lines of sterile materials utilized in this study, whether the two base fragments polymorphism in rps16 between Yuetai A and others at 220 - 225 bp and 595 bp could be used as its specific markers to identify the pollen abortion type, that is, the cytoplasmic

genetic characteristics of its cytoplasmic sterility. Especially the GTTGAG insertion of wild aborted materials at 220 - 225 bp in rps16, can it be used as a specific marker, which requires a large number of chloroplast genome sequence alignment results of wild aborted material and is worth further experimental proof. Besides, these two base sequence polymorphisms were also a characteristic marker to discriminate the Honglian-type sterile line Yuetai A from other 10 materials. The cytoplasmic donor of three-line sterile material Honglian-type is Hainan Hongmang wild rice [14], which belongs to the Hainan ordinary wild rice. The amplified electrophoresis images of ORF100 and ORF29-TrnC^{GCA} showed that the chloroplast genome of Hainan ordinary wild rice was japonica-type; Yuetai A was indica-type. Sequencing results of the products amplified by cp3 and cp4 declared that the single base sequence in rps 16 at 49 bp and 595 bp were different between Hainan ordinary wild rice and Yuetai A, revealing that a difference was existed in chloroplast genome between Hainan ordinary rice and Hongmang. According to the study on ORF100 fragment of Chinese ordinary wild rice cp DNA proposed by Sun Chuanqing that most in various regions (provinces) performed indica-japonica differentiation, but no research in Hainan ordinary wild rice [15], merely, our paper certified that there might be indica-japonica differentiation in its cp DNA.

5. Conclusions

Ordinary wild rice chloroplasts have obvious indica-japonica differentiation tendency; non-AA wild rice chloroplast DNA is generally japonica; cytoplasmic polymorphism of three-line sterile lines is low, and genetic polymorphism of wild rice is far more than that of cultivated rice.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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