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Identification of Cytoplasmic Male Sterility in Chinese Radish Following PCR Analysis of Mitochondrial DNA

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Abstract In order to understand the molecular characteristics of the Chinese radish, the mitochondrial DNA structure and sequence were analyzed in Chinese wild radish and cultivated varieties. A total of four male-sterile lines, four maintainer lines, 17 cultivars, and a single Chinese wild radish were used, along with 25 male-sterile individuals and 159 fertile plants. We found that the cytoplasm of Chinese radishes could be classified into two types: the normal type and the Ogura type. The Ogura-type cytoplasm was detected in 25 male-sterile plants. The 159 fertile plants had normal cytoplasm. Both the Ogura cytoplasm and the normal cytoplasm were detected in the male-sterile "RA". The *orf138* gene in mitochondrial DNA was detected in cultivated Chinese radish cultivars but not in the wild radish. The Chinese radish *orf138* nucleotide sequence was determined in four male-sterile lines and displayed complete homology to the known *orf138* type A nucleotide sequence. Three types of mitochondrial *orfB* (type 1, type 2 and type 3) were found in Chinese radishes. Type 1 was only present in the male-sterile lines. Chinese cultivated radishes were divided into type 2 and type 3, while the Chinese wild radish only had type 3 cytoplasm.

Keywords *Raphanus sativus* · Mitochondrial DNA · *orf138* · *orfB*

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Abbreviations

CMS	Cytoplasmic male sterility
MS	Male sterility
NMS	Nuclear male sterility
Rf	Restorer-of-fertility
SI	Self-incompatibility

Introduction

The radish (*Raphanus sativus* L.) is cultivated worldwide but mainly in eastern Asia where it is used as a vegetable, fodder, grass and oilseed crop (Lu et al. 2008). The radish has a long history of cultivation in China, with an approximate planted acreage of 1,200,000 ha. Radish is the third most consumed vegetable domestically, and the edible organs include the leaves, pod and root (Wang and He 2005). The genetic resource of radish is very abundant in China. To date, more than 2,100 radish accessions have been conserved in the National Mid-term Genebank for Vegetables located in the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (Kong et al. 2011). Radish breeding research started in the 1950s in China. Before the 1980s, open pollination radish cultivars were dominant; however, since 1990, Chinese breeders have released more than 100 hybrid cultivars, which widespread use has improved yield, quality and disease resistance (He 1993).

Male sterility (MS) and self-incompatibility (SI) are two common methods used to produce hybrid seeds. Compared with SI, MS can improve the purity of hybrids and has been widely employed in commercial production of F_1 hybrid radish seed. MS conveys the advantage of plants whose anthers cannot produce functional pollen but retain normal

female fertility. MS is classified into two types: nuclear male sterility (NMS) and cytoplasmic male sterility (CMS). In the radish, pollen sterility occurs via CMS as a result of to gain-of-function mutations in the plant mitochondrial genome (Chase and Gabay-Laughnan 2004; Hanson and Bentolila 2004). The CMS is determined by the mitochondrial genome and is associated with a pollen sterility phenotype that can be suppressed or counteracted by nuclear genes known as restorer-of-fertility genes (*Rf* genes) (Chase 2006, 2007; Chase et al. 2010; Budar and Berthomé 2007; Wise and Pring 2002; Gulyas et al. 2010). The mitochondrial genome is maternally inherited in most plant species (Hagemann 2004). Maternally inherited CMS genes do not segregate; therefore, uniform populations of male-sterile plants can be obtained by simple cross pollination. However, the transcription of plant mitochondrial genes is complex and occasional paternal inheritance can occur in some plants (Azhagiri and Maliga 2007; Elansary et al. 2010; McCauley and Olson 2008).

Radish CMS was first discovered by Dr. Ogura in a Japanese wild radish in 1968 (Ogura 1968). The Ogura CMS is characterized by a mitochondrial male-sterile gene and a nuclear chromosomal gene to restore fertility, which has proved useful in hybrid radish breeding. The Ogura CMS has now been introduced to other cruciferous crops including rapeseed, cabbage, Chinese cabbage, cauliflower and broccoli by intergenetic crosses and protoplast fusion (Bannerot et al. 1977; Sakai and Imamura 1990). Several other CMS types including 77-01A (He et al. 1981), Kos CMS (Ikegaya 1986), NWB-CMS (Nahm et al. 2005) and DCGMS (Lee et al. 2008), have been discovered in China, Japan and South Korea where they have been utilized in the production of F_1 hybrid seeds. The molecular and genetic basis of Ogura CMS has been further characterized. The examination of fertile and sterile radish cybrids, a fusion of the *Brassica* nuclear genome and radish cytoplasm, revealed a region of Ogura cytoplasmic mtDNA that contained a unique transcribed gene, termed *orf138*, upstream of the *orfB* (or *atp8*, encoding ATP synthase subunit 8), which correlates with CMS (Bonhomme et al. 1991,1992; Krishnasamy and Makaroff 1993). Although strong evidence has identified the role of *orf138* in pollen abortion, the Ogura-related cytoplasm of wild plants in European natural populations carries an *orf138* locus that has lost its ability to induce male sterility due to processing of its transcript, which disrupts the *orf138* coding sequence (Giancola et al. 2007). In contrast to many sterility-inducing proteins, the ORF138 protein is not a chimeric polypeptide composed of fragments of conventional mitochondrial proteins. Rather, it has recently been shown to reside in the inner membrane of mitochondria where it is likely assembled as a homopolymer. The mechanism by which it interferes with pollen production is still unclear (Duroc et al. 2005). Nevertheless, because the expression of *orf138* has been shown to strongly inhibit bacterial growth, ORF138 is presumed to produce a certain level of toxicity to mitochondrial activity in the tapetum of anthers (Duroc et al. 2005).

Furthermore, *orfB*, a homolog of *atp8* is found in both Ogura and normal cytoplasms. The sequence comparison of *orfB* showed that the structure in this region can be classified into three types, two of which occur in normal cytoplasm (Terachi et al. 2001). Recently, molecular markers for specific amplification of the *orf138* and *orfB* genes have been used to classify diverse radish germplasms based on their cytoplasmic type (Yamagishi and Terachi 1994; 1996; Yamagishi 2004). Ogura-CMS can be suppressed or counteracted by nuclear *Rf* genes. Several groups recently succeeded in cloning the Ogura CMS restorer locus, *Rfo*, in radish by positional cloning (Brown et al. 2003; Desloire et al. 2003; Koizuka et al. 2003). The *Rfo* locus contains three genes that are organized in tandem, arbitrarily named *PPR-A*, *PPR-B*, and *PPR-C*. These genes are predicted to encode highly similar proteins. *PPR-B* was genetically defined as the restorer gene and is predicted to encode a pentatricopeptide repeat (*PPR*) protein that belongs to the *P* subfamily of *PPR* genes and comprises 17 *PPR* repeats (Lurin et al. 2004). The primary role of *PPR-B* in restoring fertility is to inhibit ORF138 synthesis in the tapetum of young anthers (Uyttewaala et al. 2008).

CMS in Chinese radish is known to consist of male-sterile cytoplasm and two recessive nuclear alleles (Zhang et al. 1999). However, few molecular studies have investigated the CMS of Chinese radish in detail. In this study, we report the identification of Chinese radish cytoplasm types using a PCR assay of mitochondrial DNA. The results will contribute to the breeding of new male-sterile lines and also provide a better understanding of the genetic origin of the radish.

Materials and Methods

Plant Materials

A total of four male-sterile lines, four maintainer lines, 17 cultivars, and a single Chinese wild radish were used in this study, as shown in Table 1. The lines RA, RB, WA, WB, SA and SB were created by Zhang. QA and QB were developed by He et al. (1981). The 17 cultivars are traditional types that are widely grown in China. The wide radish was gifted from Dr. Liu of Nanjing Agricultural University. The population of materials included 25 male-sterile individuals and 159 fertile individuals. The plants were grown in growth chambers with a 16-h photoperiod, at 20°C day and 15°C

Table 1 Radish cultivars used in this study

Name	No. of plants tested	Fertility
Male-sterile lines		
RA	4	S
WA	8	S
QA	7	S
SA	6	S
Total	25	
Maintainers		
RB	8	F
WB	8	F
QB	4	F
SB	6	F
Chinese cultivated radishes		
Miyunhong	7	F
Dongsheng Dahongpao	8	F
Beijing Dahongpao	8	F
Tangshanhong	7	F
Jiangsu Wuying	8	F
Jinzhou Wujinhong	8	F
Huangtugang Xinlimei1	5	F
Huangtugang Xinlimei2	8	F
Beitaipingzhuang Xinlimei	6	F
Beijing Xinlimei1	8	F
Beijing Xinlimei2	4	F
Beijing Xinlimei3	8	F
Xinuoqing	7	F
Yanqing No. 2	8	F
Anhui-lvyuan	8	F
Nan Pan Zhou	4	F
Short-leaf 13	2	F
Chinese wild radish		
Cuan Lanhuazi	19	F
Total	159	

during the night. The fresh leaves were harvested in liquid nitrogen before use for DNA extraction.

Isolation of Total DNA

Total genomic DNA was extracted from fresh leaves of individual plants using a commercial DNA extraction kit (DNeasy Plant Mini Kit, QIAGEN, Germany) according to the manufacturer's instructions. The quantity and the quality of DNA extraction were determined by 1.0% agarose gel electrophoresis in 1× TAE buffer using λ-*Hind*III as a marker (Invitrogen, USA)

Table 2 Primers used in this study

Primer	5′–3′sequence	Usage
138-5′KM	GTCGTTATCGACCTCGCAAGG	P
138-3′KM	TCACGGTCCATTTTCCACCTC	P
NorfBF	TTGCGGAAGATGTCTTATCACG	P
orfBR	CCACCCATGGTACAGAGTGT	P
138,158/fw	TTTCATTCTGCATCACTCTCCC	P,S
OrfBrv-3	CTACCAGAGGTATCTATAGAATG	P,S
orfB5′RKM	TCCCACCAGACAGCTTCA	S
138,158/fw	TTTCATTCTGCATCACTCTCCC	S
138/xbaI/rv	CTTCTCTAGATGTCTCATGGTTCG	S
138 F07	ATCAAATGTACCTAACGATCAAG	S

P indicates the PCR primers, *S* indicates the sequencing primers

Cytoplasm Type Identification Using PCR

Two primer pairs (138-5′KM/138-3′KM and NorfBF/orfBR) were used for the identification of the diverse cytoplasm types (Table 2). 'MS-Gensuke' was used as a control for the Ogura cytoplasm and 'Comet' as a normal cytoplasm (Yamagishi 2004). PCR amplification was performed by GeneAmp PCR system 9700 (Applied Biosystems, USA). The reaction systems were 25-μl reaction mixtures containing 40 ng template DNA, 2.5 μl of 2 mM 10× TAE buffer, 2.5 μl 2 mM dNTPs, 2.5 μl 25 mM MgCl, 0.2 μl *Taq* polymerase (Invitrogen, USA), 1.25 μl 10 μM forward

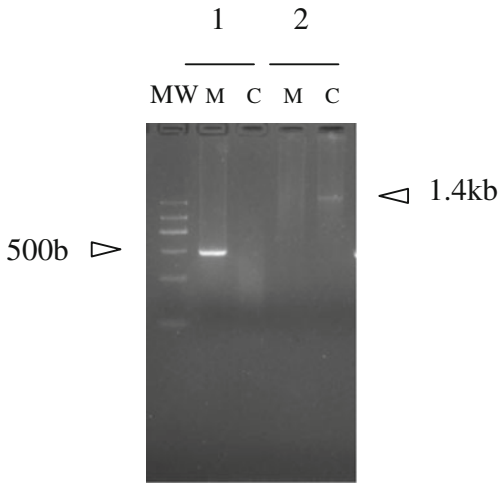

Fig. 1 PCR amplification of *orf138* and normal PCR products from cytoplasmic mtDNA in "MS-Gensuke" and "Comet". The 500-bp DNA of 'MS-Gensuke' was amplified with the primer pair 1385′KM/1383′KM. The 1.4-kb DNA of 'Comet' was amplified with the primer pair NorfBF/orfBR. (1) PCR with 1385′KM/1383′KM; (2) PCR with NorfBF/orfBR. *M* MS-Gensuke, *C* Comet, *MW* molecular marker (Marker II, Tiagen, China)

and reverse primer solutions and 13.8 μl MilliQ water. PCR was performed with an initial denaturation at 94°C for 3 min followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min. The amplified products were analyzed by 1.5% agarose gel electrophoresis in 1× TAE buffer and stained with ethidium bromide.

PCR-RFLP of the Mitochondrial *orfB* Region

According to the *orfB* nucleotide sequence (Terachi et al. 2001), normal and Ogura cytoplasm can be classified into three types (types 1, 2 and 3). Ogura cytoplasm contained only the type 1 sequence, whereas plants with normal cytoplasm had either type 2 or type 3. A nucleotide substitution in *orfB* sequence at +782 bp gives a type 3-specific *Sna*BI restriction site. The PCR products amplified using the NorfBF/orfBR primers were digested with *Sna*BI (NEB, Hertfordshire, UK) following the manufacturer's instructions and the products were examined by 1% agarose gel electrophoresis.

Male-Sterile Cytoplasm Nucleotide Sequencing Analysis

The *orf138* locus was directly sequenced from the PCR product. PCR amplification was performed with the

Table 3 Detection of *orf138* and normal cytoplasm in Chinese radish populations

Population	Number of plants tested	Number with Ogura mtDNA	Number with normal mtDNA
Male-sterile lines			
RA	4	4	4
WA	8	8	0
QA	7	7	0
SA	6	6	0
Total	25	25	4
Maintainers			
RB	8	0	8
WB	8	0	8
QB	4	0	4
SB	6	0	6
Chinese cultivated radish			
Miyunhong	7	0	7
Dongsheng Dahongpao	8	0	8
Beijing Dahongpao	8	0	8
Tangshanhong	7	0	7
Jiangsu Wuying	8	0	8
Jinzhou Wujinhong	8	0	8
Huangtugang Xinlimei1	5	0	5
Huangtugang Xinlimei2	8	0	8
Beitaipingzhuang Xinlimei	6	0	6
Beijing Xinlimei1	8	0	8
Beijing Xinlimei2	4	0	4
Beijing Xinlimei3	8	0	8
Xinuoqing	7	0	7
Yanqing No. 2	8	0	8
Anhui-lvyuan	8	0	8
Nan Pan Zhou	4	0	4
Short-leaf 13	2	0	2
Chinese wild radish			
Cuan Lanhuazi	19	0	19
Total	159	0	159
MS-Gensuke (control)	10	10	0
Comet (control)	7	0	7

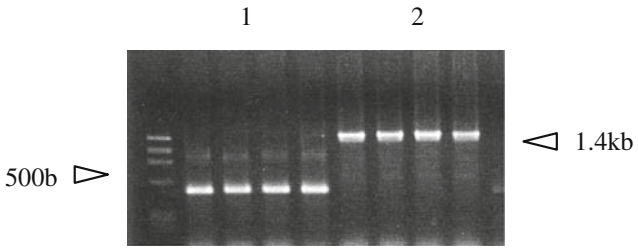

Fig. 2 PCR amplification of *orf138* and N*orfBF* PCR products from cytoplasmic mtDNA in four different individual plants of the Chinese radish "RA" cultivar using (1) primer pair 1 (138-5′KM/138-3′KM) to amplify a 500-bp region of the Ogura *orf138* gene and (2) primer pair 2 (NorfBF/orfBR) to amplify a 1.4-kb region of the *orfB* gene. Marker: *Φ*X174/*Hind*III (Invitrogen, USA)

138,158fw and orfB,rv3 primers (Table 2) and the products were purified to remove unincorporated primers and dNTPs using the PCR-M™ Clean Up System (Viogene-Biotek Corp., Sijhih City, Taiwan). The products were sequenced using the orfB5′RKM, 138,158/fw, 138/xbaI/rv and 138Fo7 primers (Table 2) using the GenomeLab™ Dye Terminator Cycle Sequencing with the Quick Start Kit (Beckman Coulter, Inc., Brea, CA). The sequencing results were analyzed using the Gene Work 2.5.1 software (Beckman Coulter, Inc., Brea CA).

Results

Chinese Radish Cytoplasm Type Detection

Using specific primers for the *orf138* mtDNA gene, associated with Ogura cytoplasm in radish, we observed a 500-bp PCR product in "MS-Gensuke". Conversely, a 1.4-kb DNA fragment corresponding to amplification of the normal cytoplasm mtDNA sequence was observed in "Comet" (Fig. 1). The two PCR primers were used to identify the diverse cytoplasm in Chinese radish. The 500-bp PCR fragment was found in 25 Chinese radish male-sterile individual plants. The *orf138* amplification product was not detected in any of the 159 fertile individuals. Conversely, a 1.4-kb DNA fragment corresponding to amplification of the normal cytoplasm mtDNA sequence was observed in 163 individuals including 159 fertile plants and four sterile plants. The normal-specific band was also absent from 21 individuals from three male-sterile lines (Table 3). These results indicate that all the fertile Chinese radish cultivars tested in this study had normal cytoplasm. Additionally, male-sterile cytoplasm in the Chinese radish is linked to the same mitochondrial gene as Ogura CMS in radish, and the *orf138* gene is distributed in Chinese radishes.

Interestingly, we discovered that the normal cytoplasm-specific band was observed in the male-sterile line RA, a population from the northeast of China (Table 3, Fig. 2). The presence of both the normal cytoplasm and *orf138* CMS bands in one individual cultivar suggests the mitochondrial DNA of RA is heterogeneous.

Distribution of Three Types of orfB in Chinese Radish

To identify the three types (types 1, 2 and 3) of *orfB* sequence in cytoplasmic mtDNA, PCR amplification was performed in Chinese radishes with normal cytoplasm using the NorfBF/orfBR primer pair and *Sna*BI PCR-RFLP analysis. Type 2 mitochondrial *orfB* PCR product does not contain the *Sna*BI restriction site and produces a single 1.4-kb DNA fragment, whereas type 3 mitochondrial *orfB* is cleaved by *Sna*BI to produce two bands at 1.2 kb and 200 bp (Fig. 3). Type 1 (male-sterile cytoplasm) is not amplified using the NorfBF/orfBR primer pair (Fig. 1, Table 2).

Table 4 summarizes the distribution of *orfB* types in Chinese radish, in which all three types of *orfB* were observed. Among the four male-sterile lines, type 1 was detected in three male-sterile lines, WA, QA and SA, with a frequency of 84.0%, while type 2 was in RA

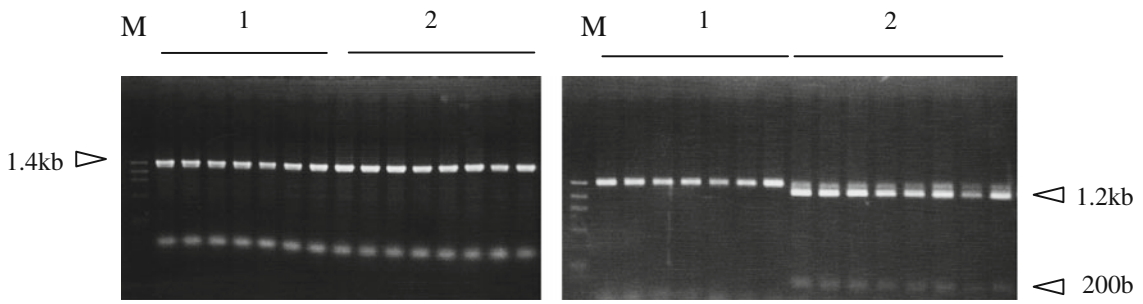

Fig. 3 PCR amplification and PCR-RFLP of *orfB* in Chinese radishes with normal cytoplasm. *Left*: PCR amplification profile showing the 1.4-kb *orfB* PCR amplification product. *Right*: PCR-RFLP profile using *Sna*BI digestion to distinguish between type 2 mitochondrial *orfB* which does not contain the *Sna*BI restriction site and type 3 mitochondrial *orfB* which is cleaved by *Sna*BI to produce 1.2-kb and 200-bp bands. Type 1 (male-sterile cytoplasm) is not amplified using the NorfBF/orfBR primer pair. (1) Maintainer line 'WB'; (2) Chinese cultivated radish 'Beijing Dahongpao'

Table 4 Mitochondrial *orfB* types in the Chinese radish

Population	Number of plants			
	Plants tested	Type 1	Type 2	Type 3
Male-sterile lines				
RA	4	0	4	0
WA	8	8	0	0
QA	7	7	0	0
SA	6	6	0	0
Frequency ratio		84.0%	16.0%	0
Maintainers				
RB	7	0	2	5
WB	8	0	8	0
QB	4	0	4	0
SB	6	0	0	6
Frequency ratio		0.0%	56.0%	44.0%
Chinese cultivated radish				
Miyunhong	7	0	0	7
Dongsheng Dahongpao	8	0	0	8
Beijing Dahongpao	8	0	0	8
Tangshanhong	7	0	0	7
Jiangsu Wuying	8	0	0	8
Jinzhou Wujinhong	8	0	0	8
Huangtugang Xinlimei1	5	0	0	5
Huangtugang Xinlimei2	8	0	0	8
Beitaipingzhuang Xinlimei	6	0	0	6
Beijing Xinlimei1	8	0	0	8
Beijing Xinlimei2	4	0	0	4
Beijing Xinlimei3	8	0	0	8
Xinuoqing	7	0	0	7
Yanqing No. 2	8	0	0	8
Anhui-lvyuan	8	0	0	8
Nan Pan Zhou	4	0	3	1
Short-leaf 13	2	0	0	2
Frequency ratio		0.0%	2.6%	97.4%
Chinese wild radish				
Cuan Lanhuazi	19	0	0	19
Frequency ratio		0.0%	0.0%	100.0%

with a frequency of 16.0%. The maintainers possessed type 2 and type 3 with a frequency of 56% and 44%, respectively. In the Chinese cultivated radishes, type 2 and type 3 *orfB* types was detected and most of the plants (97.4%) possessed type 3. The Chinese wild radish was classified as type 3.

In contrast to the other three male-sterile lines, the populations of the male-sterile line "RA" tested were all type 2. Its maintainer line, the "RB" population, was composed of two *orfB* types, with two plants having type 2 and five plants having type 3. Most of the Chinese radish cultivars were of only one type, while polymorphisms of both type 2 and type 3 genes were observed in the "RB" maintainer and "Nan Pan Zhou" cultivar (Table 4).

orf138 Nucleotide Sequence in Cytoplasmic Male-sterile Chinese Radishes

A large-scale sequence analysis has previously indicated the *orf138* sequence can be classified into nine types, termed A–I (Yamagishi and Terachi 2001). To confirm the *orf138* types in Chinese radishes with CMS, the nucleotide sequences of the PCR-amplified fragments from the four male-sterile lines were analyzed. One

plant from each male-sterile line was sequenced and the *orf138* sequence in each was identical to the published type A *orf138* (Fig. 4).

Discussion

Since the first report of Ogura male-sterile cytoplasm (Ogura 1968), mitochondrial genomes in the radish have been classified into Ogura or normal cytoplasm. Many molecular markers, based on the presence or absence of gene-specific PCR products, have previously been used to distinguish Ogura and normal cytoplasms in different radish cultivars (Yamagishi and Terachi 1994; 1996; Lee et al. 2005). In this study, the cytoplasm type was distinguished using specific markers, and in contrast to the previous studies in radish, we observed two types of cytoplasm in Chinese radish: the Ogura type and the normal type.

Heterogeneous cytoplasm, which was identified by the presence of both Ogura and normal cytoplasm-type PCR amplification products, was found only in the "RA" male-sterile line. The nucleotide sequence of the PCR-amplified fragments from "RA" was identical to *orf138*, which indicated that the normal PCR-amplified fragment in "RA" was not due to the presence of normal mitochondrial sequences. The heterogeneous cytoplasm may have had a copy of the normal *orfB* that was introduced in the mt genome. Further investigations should be performed to identify the normal cytoplasm type PCR amplification products in "RA".

The original "RA" male-sterile plants were found in a single cultivar from the northeast region of China. Using the male-sterile plants as female parents, the male-sterile line was developed by continuous backcrossing of the fertile plants as male parents. Chimeric individuals have been observed in this variety, and the male sterility ratio varies from 97% to 100% in different seasons (data not shown). It may be possible that the heterogeneous cytoplasm observed in this variety can lead to unstable fertility, or that the environmental conditions may influence the expression of mitochondrial genes associated with fertility.

Orf138, the Ogura-specific mitochondrial gene, was found in the four male-sterile lines of Chinese cultivated radish, which have different morphologies and originated from different regions of China. For example, the RA male-sterile line was transferred from the Chinese cultivar 'Dinglonghong' which has a large spherical root, red skin, white flesh and is grown in the northeast of China. WA, which has a long cylindrical root, white skin and flesh, was from the south of China. QA was bred by backcrossing from the 'Qingyuancui' cultivar

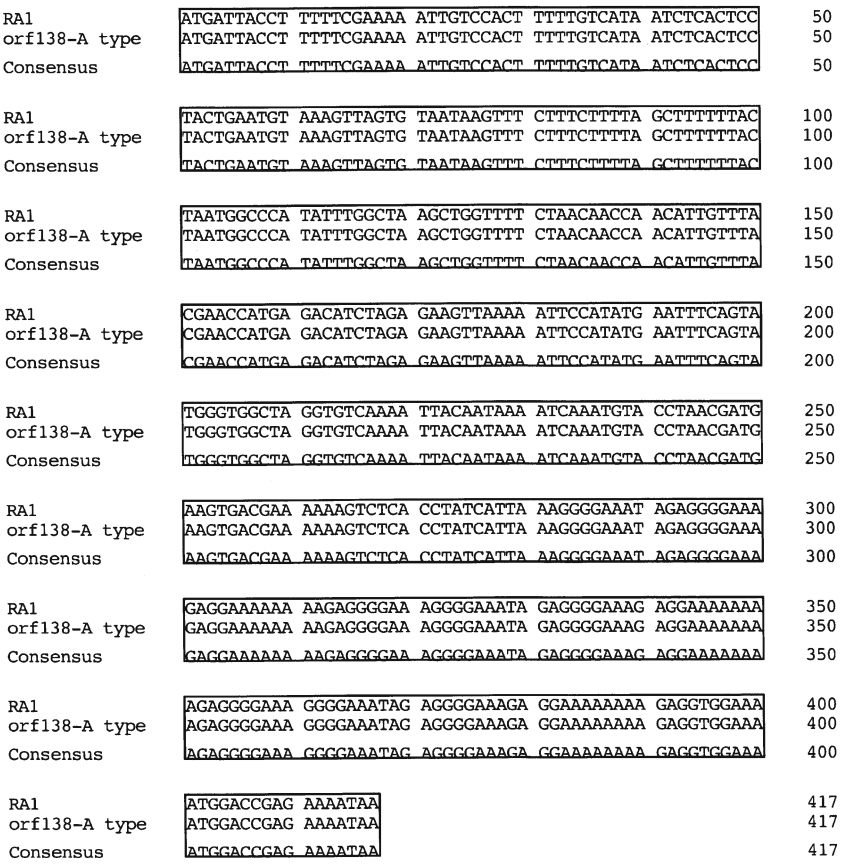

Fig. 4 Sequence alignment showing complete homology between the *orf138*-A type nucleotide sequences and *orf138* sequences in the Chinese radish *RA* cultivar

and SA arose from the 'Xiaowuying' cultivar. The *orf138* gene sequence in the cytoplasm of these four male-sterile lines is identical to the *orf138* type A sequence with no detected polymorphisms. It was identical to the 'Chibetto Kei Daikon' cultivar from the southwest of China. However, a previous study showed that five different Chinese radish cultivars from southern China contained the type H *orf138* sequence (Yamagishi and Terachi 2001). It is notable that cultivars with Ogura mtDNA have been found in diverse geographical regions in south China and Japan, and they have very different morphologies (Yamagishi and Terchi 1996). It is difficult to assume that these cultivars with Ogura mtDNA had a common ancestor.

Terachi et al. (2001) described three types of cytoplasm in wild and cultivated radishes that differed in their *orfB* 5′ flanking sequences. One cytoplasm type was found to be strictly linked to *orf138* and found in Ogura and Ogura-related cytoplasm, while the other two types were present in normal cytoplasm. We analyzed the mitochondrial *orfB* locus using PCR-RFLP in Chinese radish Ogura-related cytoplasm and normal-related cytoplasm, and investigated the classification of *orfB* in these populations (Table 5). The majority of Chinese radishes were type 3 and type 2, with these types distributed in several cultivated radishes including a male-sterile line, three maintainers and one cultivated radish from southern China. Two cultivars, *RB* and Nan Pan Zhou, showed polymorphic type 2 and 3 cytoplasms. Most Japanese radishes and Japanese cultivars are type 2, with the other types of *orfB* present less frequently (Yamagishi 2004). Three types of *orfB* have been described in cultivated Chinese radishes, and our study indicates that the frequency of the three types of cytoplasm varies in Chinese radishes. Additionally, these findings suggest that the mtDNA of cultivated radishes in China and Japan is highly similar but that a significant amount of variation has occurred in the course of evolution.

Male-sterile plants are very useful in hybrid radish seed production, and consequently, radish breeders have produced a significant amount of experimental data on the process of obtaining and maintaining CMS. The identification of their cytoplasm will help to identify the initial material for the new CMS lines.

Table 5 Classification of 25 Chinese radish varieties by *orfB* type

Type	Population			
	Male-sterile line	Maintainer	Chinese cultivated radish	Chinese wild radish
Type 1	WA[a]			
	QA[a]			
	SA[a]			
Type 2	RA[a]	RB[b,c]	Nan Pan Zhou[b,c]	
		WB[b]		
		QB[b]		
Type 3			RB[b,c]	Cuan Lanhuazi[b]
			SB[b]	
			Miyunhong[b]	
			Dongsheng Dahongpao[b]	
			Beijing Dahongpao[b]	
			Tangshanhong[b]	
			Jiangsu Wuying[b]	
			Jinzhou Wujinhong[b]	
			Huangtugang Xinlimei1[b]	
			Huangtugang Xinlimei2[b]	
			Beitaipingzhuang Xinlimei[b]	
			Beijing Xinlimei1[b]	
			Beijing Xinlimei2[b]	
			Beijing Xinlimei3[b]	
			Xinuoqing[b]	
			Yanqing No. 2[b]	
			Anhui-lvyuan[b]	
			Nan Pan Zhou[b,c]	
			Short-leaf 13[b]	

[a]Ogura-type cytoplasm

[b]Normal cytoplasm

[c]Polymorphic normal and Ogura cytoplasm

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