Plant Cell Rep (2017) 36:901–909
DOI 10.1007/s00299-017-2121-4

ORIGINAL ARTICLE

Identification of different cytoplasms based on newly developed mitotype-specific markers for marker-assisted selection breeding in *Brassica napus* L.

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Received: 1 December 2016 / Accepted: 15 February 2017 / Published online: 6 March 2017

Abstract

Key message **Different mitotype-specific markers were developed to distinguish different cytoplasms in *Brassica napus* L.**

Abstract Mitotype-specific markers have been developed to distinguish different mitotypes in plant. And use of molecular markers to identify different mitotypes in *Brassica napus* would enhance breeding efficiency. Here, we comparatively analyzed six sequenced mitochondrial genomes in *Brassica napus* and identified collinear block sequences and mitotype-specific sequences (MSSs) of these mitochondrial genomes. The collinear block sequences between mitochondrial genomes of *nap, cam*, and *pol* cytoplasmic male sterility (CMS) lines were higher than those of other lines. After comparative analysis of the six sequenced mitochondrial genomes (*cam, nap, ole, pol* CMS, *ogu* CMS, and *hau* CMS), 90 MSSs with sizes ranging from 101 to 9981 bp and a total length of 103,756 bp (accounting for 6.77% of the mitochondrial genome sequences) were identified. Additionally, 12 mitotype-specific markers were developed based on the mitochondrial genome-specific sequences in order to distinguish among these different mitotypes. Cytoplasms of 570 different inbred lines collected across scientific research institutes in China were identified using the MSS markers developed in our study. In addition to confirming the accuracy of the cytoplasmic identification, we also identified mitotypes that have not been reported in *Brassica napus*. Our study may provide guidance for the classification of different mitotypes in *B. napus* breeding.

Keywords Mitotype-specific sequence · Mitochondria · MAS (marker-assisted selection) · *Brassica* · *Brassica napus*

Communicated by Kinya Toriyama.

Electronic supplementary material The online version of this article (doi:10.1007/s00299-017-2121-4) contains supplementary material, which is available to authorized users.

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Introduction

Plant mitochondria have several important roles, including providing a location for cell respiration and ATP-synthesizing machinery and regulating cellular oxidative stress, programmed cell death, and even male sterility. Manipulation of mitochondrial function may lead to enhanced tolerance to both biotic and abiotic stresses (Hossain et al. 2012; Jones et al. 2007; Livaja et al. 2008; Taylor et al. 2009). Since the sequencing of the first mitochondrial genome of the model plant *Arabidopsis thaliana* (Unseld et al. 1997), researchers have made great progress in mitochondrial genome sequencing and identified different mitotypes in various crop plants, such as in rice, maize, *Brassica*, etc., (Chang et al. 2011; Horn et al. 2014; Hu et al. 2014; Touzet and Meyer 2014). Moreover, identification and efficient use of these different mitotypes, particularly cytoplasmic male sterility (CMS)

mitotypes are crucial. Collinear block sequences are the sequences of collinear homologous segments among two or more genomes due to recombination and sequences gain and loss. Extensive rearrangement in the mitochondrial genomes accounts for the vast majority of mitotype-specific sequences (MSSs) (Havey 2001). Even in the same plant species, large amounts of different specific mitochondrial DNA (mtDNA) had been detected (Xie et al. 2014). Handa (2003) compared rapeseed and *Arabidopsis* mitochondrial genomes and found that large parts of mitochondrial genomes in higher plants are species specific. Multiple reorganizations of genome structure occurred during the evolution of higher plants (Sugiyama et al. 2005). Molecular markers were developed for the classification of diverse germplasms and found two different normal mitotypes in radishes (Kim et al. 2007). The mitochondrial genomes of *ogu* CMS lines were shown to be highly rearranged, with one large repeat and multiple short repeats, when compared with the normal-type genomes (Tanaka et al. 2012). Comparative analysis of the mtDNA of several angiosperms, including wheat, rice, maize, *A. thaliana*, and rapeseed, showed that non-coding sequences of higher plants had undergone multiple reorganizations events during the evolution of mtDNA in higher plants (Liu et al. 2011). Novel mitochondrial genomic rearrangements unique to the CW-CMS cytoplasm have been found when compared to the Nipponbare (*Oryza sativa* L. ssp.*japonica*) mitochondrial genome in rice (Fujii et al. 2010). Mitotype-specific sequence (MSS) markers have been used to differentiate different cytoplasms, even the gametophytic CMS and sporophytic CMS lines in rice (Xie et al. 2014). Sequence characterized amplified region (SCAR) markers have also been used to identify S-cytoplasms in pepper plants (Ji et al. 2014).

The allotetraploid *Brassica napus* (genome AACC), which is one of the world's most important oilseed crops has two progenitor species, *B. rapa* (Asian cabbage or turnip, genome AA) and *B. oleracea* (Mediterranean cabbage, genome CC) (Nagaharu 1935). And recent studies have described the complete genomes of *B. rapa* (Wang et al. 2011), *B. oleracea* (Liu et al. 2014), *B. napus* (Chalhoub 2014) and *B. juncea* (Yang et al. 2016). Additionally, many different mitotypes (autoplasmic and alloplasmic cytoplasms) have been reported in *B. napus*, including *pol* CMS (Tanaka 1998), *ogu* CMS (Ogura 1968), *nap* CMS (Thompson 1972), *tour* CMS (Rawat and Anand 1979), *Moricandia arvensis* CMS (Bhat et al. 2006), *Nsa* CMS (Hu et al. 2003), and *hau* CMS (Wan et al. 2008) etc. To date, the mitochondrial genome of *nap* (Handa 2003) and *pol* CMS (Chen et al. 2011) had been sequenced by Sanger sequencing. And the mitotypes of *cam* (*B. rapa*) (Chang et al. 2011), *ole* (*B. oleracea*) (Chang et al. 2011), *ogura* CMS (Tanaka et al. 2012), *Ogura*-cms-cybrid (*oguC*) (Wang et al. 2012), *hau* CMS (Heng et al. 2014) and *ole* (*B. oleracea* L. var. botrytis) (Grewe et al. 2014) have been sequenced through next generation sequencing technology. The cytoplasms of *pol* CMS (Tanaka 1998) and *ogu* CMS (Ogura 1968) are the most widely used cytoplasms in *Brassicaceae*. Additionally, the *hau* CMS cytoplasm is a newly identified cytoplasm that has been characterized in *B. juncea* and transferred to *B. napus* (Wan et al. 2008). The *ole* mitotype (Chang et al. 2011) is the largest because of the presence of a duplication of a 141.8-kb segment. But the *ole* (*B. oleracea* L. var. botrytis) mitochondrial genomes was different from it may be due to size and structure variable among *B. oleracea* (Grewe et al. 2014). Till now, molecular markers had been used widely to distinguish different cytoplasms in *Brassica* crops. It was very useful for sterility identification needed in breeding program for F1 hybrid development and can also be used to identify the materials with unknown mitotypes. PCR markers based on different CMS causative genes had been developed for rapidly identifying cytoplasm in *B. napus* (Zhao et al. 2010). But with the number of cytoplasms increased, numbers of MSS markers can be usefully applied to *Brassica* breeding. However, no comprehensive analysis of MSSs based on sequenced mitochondrial genome sequences in *B. napus* has been performed to date.

Collinear block sequences and MSSs were found after comparatively analyzed 6 sequenced mitochondrial genomes in *Brassica*. Additionally, MSS markers were developed to distinguish among the six sequenced mitochondrial genomes. Using these MSS markers, we identified mitotypes of 533 lines through analysis of 570 inbred lines and found some lines that may possess new cytoplasms not previously reported in *B. napus*.

Materials and methods

Plant materials

The plant materials analyzed in our study are listed in Table 1. Among them, Wester was a *nap* cytoplasm

Table 1 Plant materials representing six different mitochondrial genomes used for mitotype-specific marker development in *B. napus*

Materials	Cytoplasm type	Male fertility
Wester (*B. napus*)	*nap*	MF
Suzhongqing (*B. cam*)	*cam*	MF
08C717 (*B. ole*)	*ole*	MF
6-100A (*B. napus*)	*pol* CMS	MS
6-101A (*B. napus*)	*oguC* CMS	MS
6-102A (*B. napus*)	*hau* CMS	MS

donor; Suzhongqing and 08C717 harbored the *cam* and *ole* mitotypes sequenced by Chang et al. (2011); 6-100A, 6-101A, and 6-102A were *pol* CMS, *Ogura*-cms-cybrid (*oguC*), and *hau* CMS mitotype donors, respectively. The mitotype of 14RA1 was *ogu* CMS from *R. sativus*. *OguC* CMS was also named as *ogu*-INRA CMS in *B. napus*. Detailed information on the 570 inbred lines used in our study is listed in Table S2. These materials were planted from September 2014 to May 2015 in the research fields, located at Huazhong Agricultural University (Wuhan, China, latitude 30°N, longitude 114°E).

Isolation of mitochondrial DNA and total genomic DNA

Highly purified mitochondria were isolated based on discontinuous percoll gradient centrifugation according to the method reported by Heng et al. (2014). Total genomic DNA and mtDNA were extracted from the fresh leaves of different plants by the CTAB method (Doyle 1990).

Comparative analysis of the six sequenced mitochondrial genomes in *Brassica napus*

Six sequences of mitochondrial genomes were downloaded from GenBank, EMBL and the DDBJ Database. The accession numbers of these analyzed mitochondrial genomes are listed in Table 2. *pol* CMS (accession no. FR715249), *ogu* CMS (accession no. AB694744), *hau* CMS (accession no. KF736092) and *nap* (accession no. AP006444) exhibited the CMS mitotype, while the other two lines were fertility line, i.e., *cam* (accession no. JF920285) and *ole* (accession no. JF920286). But the *nap* mitotype only exhibits male sterility in the "Bronowski" background (L'Homme et al. 1997). Progressive Mauve (Darling et al. 2010) and BLASTN (Altschul et al. 1990) with *e* value cutoffs at 1e−5 were used for multiple alignments among the six sequenced *B. napus* mitochondrial genomes. MSSs larger than 100 bp that were not shared by all of the six sequenced mitochondrial genomes were extracted for further analysis.

Development of MSS markers and PCR amplification

Mitotype-specific sequences (MSS) markers were developed based on the extracted MSSs in the specific regions. MSS primers were designed with Primer 3 (Untergasser et al. 2012). WebSNAPER (http://pga.mgh.harvard.edu/cgi-bin/snap3/websnaper3.cgi) was used to develop single nucleotide polymorphism (SNP) markers specific to the *ole* cytoplasm. Detailed information of the MSS markers is listed in Table S3. PCR was performed in a total reaction volume of 10 μL, including 0.2 mM dNTP mix, 1 unit *Taq* DNA polymerase, 2.0 mM $MgCl_2$, 2 μL of 10× *Taq* buffer with $(NH_4)_2SO_4$, and 0.5 μM of each primer, (Sangon Biotech, China). The PCR protocol was as follows: 94 °C for 5 min; 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s; and a final extension at 72 °C for 10 min. The PCR products were detected by electrophoresis on 1% agarose gels, followed by staining with ethidium bromide.

Results

Characterization of the six sequenced mitochondrial genomes in *B. napus*

The genome sizes of the six sequenced mitochondrial genomes varied from 219,747 bp in *cam* to 360,271 bp in *ole*, with the gene number ranging from 54 in both *cam* and *nap* to 95 in *ole* (Table 2). Collinear block sequences identified among the six sequenced mitochondrial genomes in *B. napus* are shown in Fig. 1. The homology sequences between *cam* and *pol* CMS genomes were 98%, suggesting that they shared the most collinear block sequences among these six mitochondrial genomes. Apart from the 4.5-Kb CMS-associated region in *pol* CMS, the *cam* and *pol* CMS mitochondrial genomes are the most collinear of these six mitochondrial genomes (L'Homme et al. 1997). The similarity reached 96% when comparing the *nap* genome with either the *cam* or *pol* CMS genome sequences. The lowest were 80–83% similar between *nap* mitochondrial genome and the alloplasmic mitochondrial genomes of *ogu* CMS and *hau* CMS. The *ogu* CMS and *hau* CMS both had large

Table 2 Analysis of six mitochondrial genome sequences in *B. napus*

Mitotype	Size (bp)	Gene content	Material	GenBank AN	References
nap	221,853	54	Wester	AP006444	Handa (2003)
cam	219,747	54	Suzhouqing	JF920285	Chang et al. (2011)
ole	360,271	95	08C717	JF920286	Chang et al. (2011)
pol CMS	223,412	55	NH12A	FR715249	Chen et al. (2011)
hau CMS	247,903	63	6-102A	KF736092	Heng et al. (2014)
Ogu CMS	258,426	61	MS-Gensuke	AB694744	Tanaka et al. (2012)

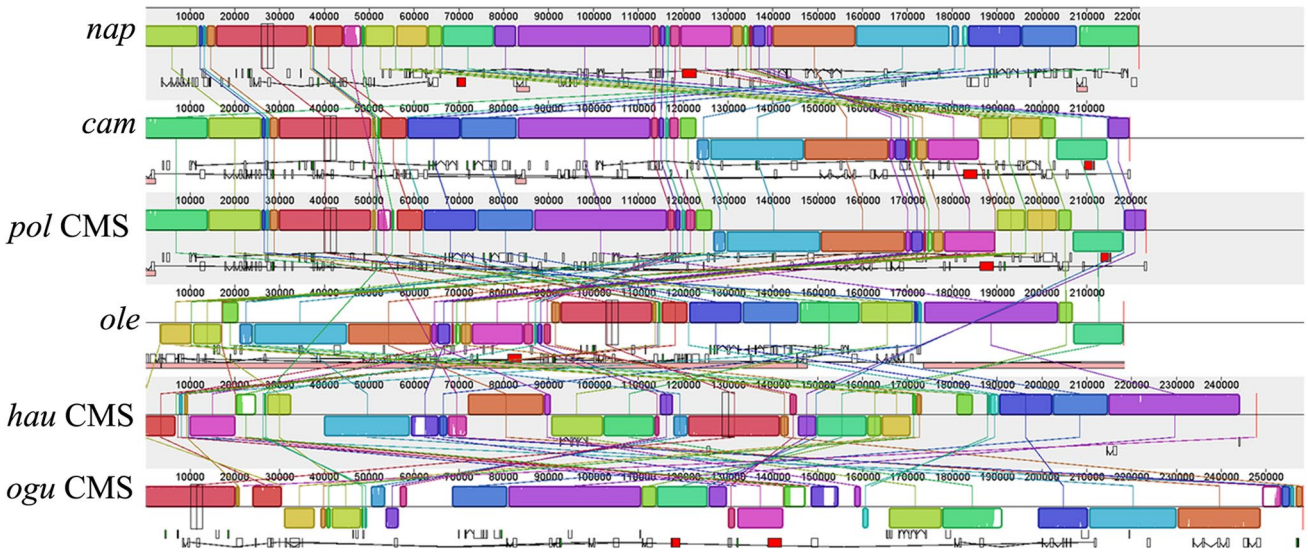

Fig. 1 Collinear block sequences identified among the six sequenced mitochondrial genomes in *Brassica*. Mauve visualization of locally collinear block sequences identified among the six sequenced mitochondrial genomes (including: *nap, cam, pol* CMS, *ole, hau* CMS and *ogu* CMS) in *Brassicas*. The 141.8 kb segment from 173,638 to 315,446 bp in *ole* mitochondrial genome was deleted for the reason that Tandem repeats >10 kb in total length without an anchor are ignored during this alignment by MAUVE. And contiguously *colored* region is a locally collinear block (LCB) region without rearrangement of homologous sequence. *Lines* between genomes trace each orthologous LCB through every genome. LCBs in reverse orientation to the reference genome (*nap*) are shown below the *line*

rearrangements when compared with other mitochondrial genomes sequenced in *B. napus*. These values are in good agreement with the phylogenetic relationship among these six mitochondrial genomes (Heng et al. 2014).

Identification of MSSs among these different mitochondrial genomes used in *Brassica napus*

In addition to the collinear block sequences identified in these six mitochondrial genomes, the MSSs (>100 bp) not shared by all six mitochondrial genomes were also extracted using Progressive Mauve and BLASTN. Detailed information of these MSSs is given in Fig. 2 and Table S1. In total, we identified 90 MSSs (from MSS1 to MSS90) ranging from 101 to 9981 bp over all six mitochondrial genomes, with a total size of 103,756 bp. Among these, five MSSs (MSS1–MSS5) were unique to *nap* mitochondrial genomes, MSS8 and MSS9 were unique to *pol* CMS mitochondrial genomes, 13 MSSs (MSS10–MSS22) were specific to *ogu* CMS mitochondrial genomes, 9 MSSs (MSS23–MSS31) were specific to *hau* CMS mitochondrial genomes and MSS7, which was developed by WebSNAPER, was specific to the *ole* mitochondrial genome. The other MSSs were shared by more than two different mitotypes. There was no MSS for the *cam* cytoplasm and only MSS6 was shared by *cam* and *pol* CMS mitotypes. And 13 MSSs (MSS32–MSS44) were shared by the alloplasmic cytoplasm (*ogu* CMS and *hau* CMS), 6 MSSs (MSS60–MSS65) were shared by *nap, cam, ole*, and *pol* CMS cytoplasms. The number of MSSs from *ogu* CMS and *hau* CMS mitotypes was apparently increased compared with that of other mitotypes.

Development of MSS markers to distinguish different mitotypes in *B. napus*

Here, based on the identified MSSs, MSS markers were developed to identify and differentiate different cytoplasms in *B. napus*. Some MSSs for different mitotypes were chosen randomly, and a total of 12 MSS markers were developed. Detailed information on these MSS markers is shown in Table S3. MSS2 and MSS4 were specific to the *nap* cytoplasm; MSS6 was specific to the *cam* and *pol* CMS cytoplasm; MSS7 was specific to the *ole* cytoplasm; MSS8 and MSS9 were specific to the *pol* CMS; MSS13, MSS14 and MSS21 were specific to the *ogu* CMS; MSS26 was specific to the *hau* CMS; MSS61 was detected in the *nap, cam, ole*, and *pol* CMS mitotypes and MSS67 was detected in the *nap* and *pol* CMS mitotypes.

Next, 12 MSS markers were used to distinguish among the known mitotypes shown in Table 1. The mitochondrial genomes from Wester, Suzhongqing, 08C717, and 6-102A were the reference mitochondrial genomes sequences of the *nap, cam, ole*, and *hau* CMS mitotypes. 6-100A and 6-101A (*pol* CMS and *oguC* CMS lines) were used as CMS lines by breeders in *B. napus*. Validation of

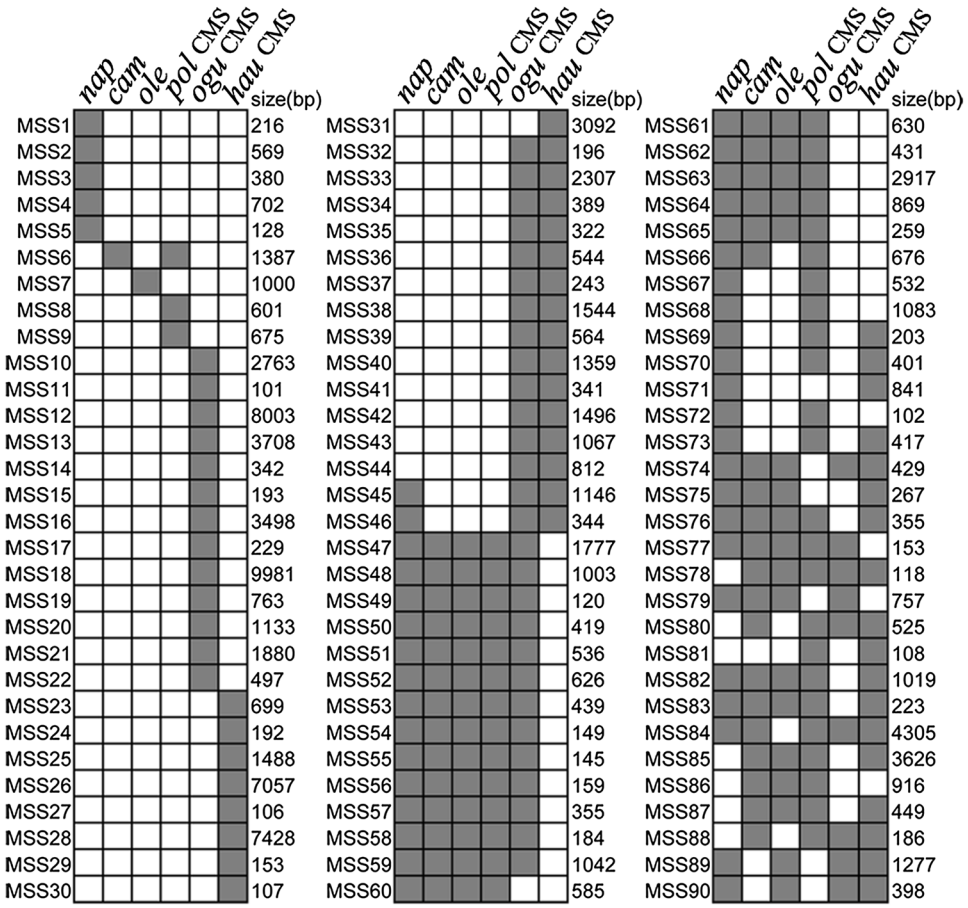

Fig. 2 Distribution of MSSs among six mitochondrial genomes in *Brassica*. The *gray* and *white boxes* indicate the analyzed mitochondrial sequences that are with or without MSS. All MSS are larger than 100 bp

these 12 MSS markers in mtDNAs extracted from fresh leaves of the known six mitotypes are shown in Fig. 3. Interestingly, marker specific to the *nap* cytoplasm (i.e., MSS2 and MSS4) could be amplified in *oguC* CMS *B. napus*. And MSS61 and MSS67 could also be amplified in the *oguC* CMS in *B. napus*. To validate the specificity of MSS2 and MSS4, the DNA of the *oguC* CMS cytoplasm from *B. napus* was replaced with *ogu* CMS DNA from *Raphanus sativus* and the *nap* cytoplasm-specific marker *orf222* developed by Wei et al. (2005) and Zhao et al. (2010) were used to test the MSS markers in the *nap* cytoplasm. When *ogu* CMS DNA from *R. sativus* was used as a template, all the three markers (*orf222*, MSS2 and MSS4) were specific to the *nap* cytoplasm (Fig. 4). This result confirmed the specificity of these MSS markers to the *nap* mitochondrial genome. Other MSS markers were consistent with the MSSs showed in Fig. 2 and Table S1. When the mtDNA of different mitotypes was replaced with total DNA, PCR pattern of these MSS markers were identical. Thus, these MSS markers developed in our study could be used to identify and differentiate different mitotypes in *Brassica*.

Identification of mitotypes in 570 different inbred lines

Six MSS markers were chosen to identify 570 different inbred lines which are representing a diverse cytoplasm background in China. As showed in Fig. 3, MSS4 was specific to the *nap* cytoplasms, MSS6 was specific to the *cam* and *pol* CMS cytoplasm, MSS7 was specific to the *ole* cytoplasm, MSS8 was specific to the *pol* CMS cytoplasm, MSS13 was specific to the *ogu* CMS cytoplasm, and MSS26 was specific to the *hau* CMS cytoplasm. MSS4 combined with MSS13 can distinguish *nap* and *oguC* CMS cytoplasm effectively.

Among the 570 inbred lines tested in our study, 356 inbred lines possessed the *nap* cytoplasm, 102 inbred lines possessed the *pol* CMS cytoplasm, 52 inbred lines possessed the *cam* cytoplasm, 15 inbred lines possessed the *oguC* CMS cytoplasm, five inbred lines possessed the *ole* cytoplasm, and three inbred lines possessed the *hau* CMS cytoplasm (Table 3 and Table S2). Additionally, the cytoplasm types of 37 inbred lines did not belong to any of the six mitotypes when identified using these MSS markers. These results confirmed the accuracy of the MSS markers developed in our study.

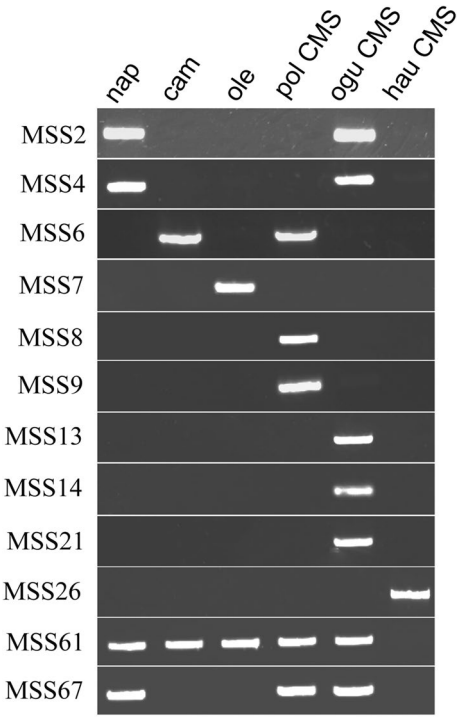

Fig. 3 Pooled PCR products of different MSSs markers. Six mitotype-specific lines were used to confirm the effective of Different MSSs markers. The mtDNA from Wester (*nap*), Suzhongqing (*cam*), 08C717 (*ole*), 6-100A (*pol* CMS), 6-101A (*oguC* CMS) and 6-102A (*hau* CMS) were used to distinguish different MSSs markers

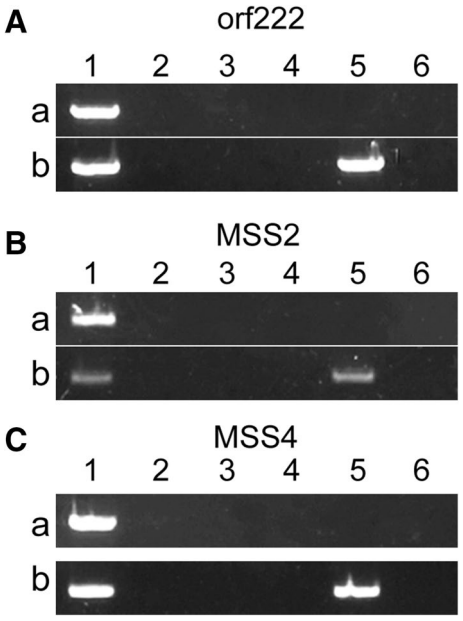

Fig. 4 Validation of the *nap* cytoplasm-specific MSS markers. *orf222* (**a**), MSS2 (**b**), MSS4 (**c**) were markers specific to *nap* cytoplasm. **a** 1–6 were mitochondrial DNA of *nap* (*B. napus*), *cam* (*B. rapa*), *ole* (*B. oleracea*), *pol* CMS (*B. napus*), *ogu* CMS (*R. sativus*) and *hau* CMS (*B. napus*) mitotype. **b** 1–6 were mitochondrial DNA of *nap* (*B. napus*), *cam* (*B. rapa*), *ole* (*B. oleracea*), *pol* CMS (*B. napus*), *oguC* CMS (*B. napus*) and *hau* CMS (*B. napus*) mitotype

Discussion

To date, more than nine different mitotypes have been reported in *B. napus*, the normal mitotypes confer male fertility in a normal environment include *cam*, and *ole* (Chang et al. 2011) cytoplasms. CMS mitotypes, which is associated with male sterility, include *pol* CMS (Tanaka 1998), *ogu* CMS (Ogura 1968), *nap* (Thompson 1972), *tour* CMS (Rawat and Anand 1979), *Moricandia arvensis* CMS (Bhat et al. 2006), *Nsa* CMS (Hu et al. 2003), *hau* CMS (Wan et al. 2008), etc. With the development of next-generation sequencing technology, an increased number of mitochondrial genomes has been reported in plants, particularly in *Brassica*. To date, more than six different mitotypes mainly used in *B. napus* have been sequenced (Table 2). However, few MSS primers have been developed; *orf222* (specific for the *nap* cytoplasm), *orf224* (specific for the *pol* CMS cytoplasm), *orf138* (specific for the *ogu* CMS) (Zhao et al. 2010) have been widely used in cruciferous crops to distinguish different cytoplasms, particularly CMS lines. Mitotype-specific open reading frames (ORFs) have been used to develop molecular markers to discriminate different mitotypes between the *hau* CMS line and its iso-nuclear maintainer line in *B. juncea* by simple PCR amplification (Heng et al. 2014). However, with the increased number of different cytoplasms identified and used in *B. napus*, these MSS markers may not have the capacity to distinguish different cytoplasms effectively.

In this study, through comparative analysis of the sequenced mitochondrial genomes used in *B. napus*, we analyzed collinear block sequences and 90 MSSs of these mitochondrial genomes. More MSSs were detected in alloplasmic cytoplasms (*ogu* CMS and *hau* CMS) than autoplasmic cytoplasm (*nap, cam, pol* CMS, and *ole* cytoplasm). The mitochondrial genomes from alloplasmic cytoplasms exhibited more diversity when compared with mitochondrial genomes from autoplasmic cytoplasms. Theoretically, all of the 90 MSSs can be used to develop different MSS markers to identify and distinguish different mitotypes in *B. napus*. The *oguC* CMS in *B. napus* is an alloplasmic cytoplasm that originated from *R. sativus* by protoplast fusion (Pelletier et al. 1983), is a heterogeneously composed mitochondrial genome (Wang et al. 2012). The MSS markers for the *nap* cytoplasm could also be detected in the *oguC* CMS line of *B. napus* in our study. Although the co-amplification of *nap* mitotype-specific markers and *ogu* CMS mitotype-specific markers in the *oguC* CMS background, it does not affect these MSS markers used in our study to discriminate different mitotypes in *B. napus*. Either MSS2 or MSS4 combined with MSS13,

Table 3 Classification and distribution of mitotypes among 570 *B. napus* inbred lines

Cytoplasm type	Number	Percentage (%)
nap	356	62.46
cam	52	9.12
ole	5	0.88
pol CMS	102	17.89
oguC CMS	15	2.63
hau CMS	3	0.53
Unknown	37	6.49
Total	570	100.00

MSS14 and MSS21 can distinguish *nap* and *oguC* CMS cytoplasm effectively.

Six MSS markers were used to identify and distinguish among 570 inbred lines collected from different scientific research institutes in China. Our work showed that the *nap* cytoplasm was the most prevalent cytoplasm used in *B. napus* and that most hybrid lines used in China possessed the *pol* CMS system. These results were consistent with previous results reported by Handa (2007) and Zhao (2010). The *ole* cytoplasm was identified in only five inbred lines, three of which were resynthesized rapeseed lines using *B. oleracea* as the female parent. Besides the cytoplasm types identified in *B. napus* in this study, the cytoplasm types of some inbred lines could not be detected by MSS markers or by the markers reported by Zhao (2010). Thus, the cytoplasms of these inbred lines may have not been reported previously. No2127 is a resynthesized rapeseed line derived from an interspecific hybridization between *B. oleracea* var. *alboglabra Bailey* and *B. rapa* var. *Yellow Sarson* (Chen et al. 2010). However, MSS7, specific to the *ole* line, was not detected in this inbred line. The sequence and structure of *B. oleracea* mitochondrial genome sequenced by Grewe et al. (2014) disagreed with a previous assembly reported by Chang et al. (2011). There may be more than two cytoplasms present in *B. oleracea*. Among the 37 unidentified inbred lines, there were unidentified cytoplasms existing in them.

From our data, the most prevalent cytoplasms in *B. napus* were the *nap* and *pol* CMS mitotypes, accounting for 62.46 and 17.89% of the inbred lines surveyed in our study, respectively. However, the *ole, ogu* CMS, and *hau* CMS cytoplasms in *B. napus* have not been widely used in China. Therefore, it is important to broaden the cytoplasm resources used for *B. napus* breeding. Recently, the *hau* CMS non-heading Chinese cabbage was obtained by interspecific crosses between *hau* CMS line of *B. juncea* and *B. rapa*. Three different mitochondrial genes specific markers had been used to distinguish *hau* CMS cytoplasm with *pol* CMS and *ogu* CMS cytoplasm in *B. rapa* (Heng et al. 2015). These MSS markers developed in our study may be used to identify different mitotype in them. It can be usefully applied to other *Brassica* crops breeding. With next-generation sequencing technology, an increasing number of uncharacterized mitochondrial genomes will be sequenced, and more MSS markers will need to be developed and used to for application in the identification and differentiate different mitotypes in *B. napus*. Such newly developed MSS markers will not only contribute to identifying different mitotype variations within and among *Brassica* species, but also provide convenience for breeding based on MAS. The results of the current study have demonstrated an efficient method for identification of different cytoplasms used in *B. napus*.

Author contribution statement SH, FC, ZY, CW, and KH carried out the experiments and performed the sequence analysis. SH analyzed the data and wrote the manuscript. JW, BY, CM, JT and PS contributed new reagents, materials, analysis tools and helped draft the manuscript. JS and TF designed the experiments and directed the manuscript writing. All authors read and approved the final manuscript.

Acknowledgements We sincerely thank Professor Rongzhan Guan for his kindly provide the seeds and mtDNAs of Suzhongqing (*B. rapa*) and *B. oleracea*. This research was financially supported by the National Natural Science Foundation of China (NSFC Grant Number 31271761), the National Key Research and Development Program of China (Grant Number 2016YFD0101300), the Program for Modern Agricultural Industrial Technology System (nycytx-00501) and Nanhu Scholars Program for Young Scholars of XYNU.

Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interests.

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