Development of three-line system with a novel alloplasmic male sterility in *Brassica napus* L.

HU Qiong, LI Yunchang, MEI Desheng, LI Yinde, XU Yusong

Institute of Oil Crops Research, Chinese Academy of Agricultural Science, Wuhan, Hubei, 430062
Email: q0701@hotmail.com;huqingy@public.wh.hb.cn

Abstract

Alloplasmic male sterility has the advantages of less sensitivity to temperature and high stability, thus helps to increase the safety of using hybrid seeds in oilseed rape production. A novel alloplasmic male sterility system (Nsa CMS) containing cytoplasm from *Sinapis arvensis* with Chinese origin has been established using protoplast fusion technology combined with backcross, test cross and fertility determination. Test of maintainer-restorer relationship and analysis of specific mitochondrial DNA fragment confirmed that Nsa CMS has different cytoplasm from CMS systems in *Brassica*s previously identified, namely Polima, nap, Nca, Ogura CMS. All rapeseed lines including the restorers of other CMS systems tested are maintainers of Nsa CMS. Restorer lines were identified from offspring lines of fertile somatic hybrids derived from the same protoplast fusion combination as those hybrids from which the CMS lines developed. Cytologic analysis of one restorer line revealed it is a disomic additional line with 40 chromosomes. The aneuploidy nature, however, did not cause severe abortion of the fertility of the restorer line and it F_1 hybrid with the CMS lines. All three lines with canola quality have been set up and the application of these lines in the development of hybrid variety with high yield and safety is discussed.

Key words: Nsa CMS； Alloplasmic; Somatic hybrids; Restorer

Introduction

Cytoplasmic male sterility (CMS) is a main pollination control system for oilseed rape hybrid seed production in China (Fu, 2004). Alloplasmic male sterility has the advantage of less sensitivity to temperature, thus increases the safety of using oilseed rape hybrid seeds in oilseed rape production. Interspecific and intergeneric hybridization are efficient approaches for the induction of alloplasmic male sterility (Delourme and Budar, 1999). Somatic hybrids between *Brassica napus* and *Sinapis arvensis* with Chinese origin displayed male sterility with high female fertility (Hu et al., 2002). From these hybrids, a cytoplasmic male sterile line was established after backcross to the *B. napus* parental line (Hu et al., 2004). This CMS line was named Nsa represents napus, sinapis and arvensis, respectively)CMS, which is the first alloplasmic male sterile line developed in China by intergeneric somatic hybridization. In order to use this sterile system for oilseed hybrid variety development, it is necessary to confirm the novelty of this cytoplasm and to develop restorer lines for hybrid production. This paper reports the results of cytoplasmic determination and the development of the three lines for hybrid production.

Materials and methods

Fertile somatic hybrids were self-propagated and sterile ones were backcrossed to the *B. napus* fusion parent. Over 200 *B. napus* breeding lines/varieties were used for test cross. Test cross and specific PCR primers were used for maintainer-restorer relationship determination and analysis of specific mitochondrial DNA fragments. F_2 population derived from the CMS line and a restorer was used for marker identification.

Results

The sterility of Nsa CMS is complete and more temperature stable

The flowers of Nsa CMS plants are much smaller than normal *B. napus* plants, with non-extended stamens, wedge shaped and brownish anthers which do not release any pollen (Fig 1a). Selfing by bag-isolation of Nsa CMS plants did not result any seeds, whereas some seeds were set on Shaan 2A plants maintained by the same line after self ing (Fig 1b, Table 1).

Restorer-maintainer test and PCR of specific fragments showed Nsa is distinct from other CMS

Two hundred and twenty-two rapeseed varieties or breeding lines, including restorers of Pol CMS, nap CMS and Nca CMS, were test crossed with the CMS line. Fertility determination of the F_1s showed all *B. napus* lines are maintainers of this CMS. PCR primers which can amplify specific bands from Ogu CMS and Pol CMS did not amplify Ogu CMS or Pol CMS specific bands (Fig.2a,b). A pair of Rep-PCR primers can amplify an Nsa CMS specific band, thus distinguishs Nsa CMS from other CMS systems (Fig. 2c).

Fertility restorer lines were identified among lines derived from fertile somatic hybrids

Six lines which restored the fertility of Nsa CMS were identified after test cross with a restoration rate ranged from 93.7%-100% (Table 2). They were all derived from partially fertile somatic hybrids either by self-propagation or backcross with *B. napus*. The restored plants released stainable pollen grains and have normal flowers (Fig. 3a,b,c). Selfing of the F_1

plants yielded seeds as normal as ordinary fertile lines.

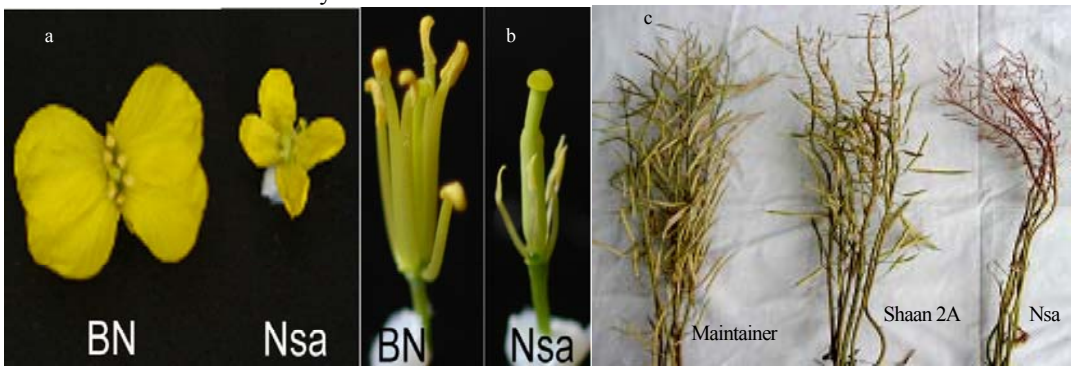

Fig. 1 Morphology of Nsa flower and seed set after selfing

BN: *B. napus*, Nsa: Nsa CMS; a: Flower. b: Stemens. c: Siliques after bag isolation

Table 1 Seed set of lines with a same nuclear background

Lines	Compatibilty index	**Seed/pod**
Maintainer	10.28	14.06
Shaan 2ACMS	2.51	8.02
Nsa CMS	0	0

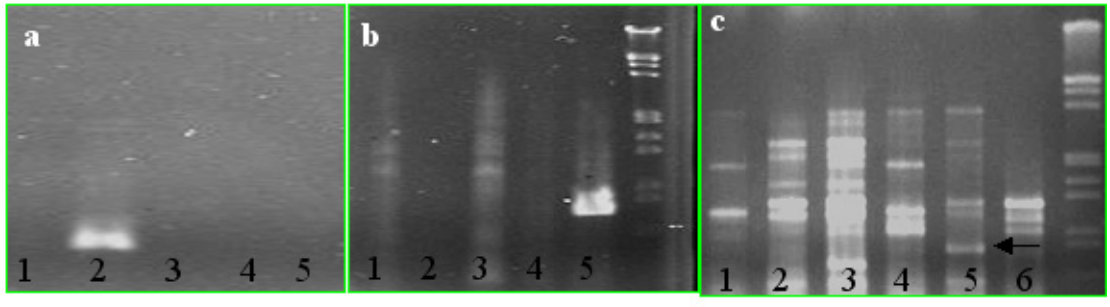

Fig. 2 Molecular distinction of Nsa CMS from other CMS

a: Amplified by Ogu CMS specific primers. b: Amplified by Pol CMS specific primers. 1. Nca CMS. 2. Ogu CMS. 3. Nsa CMS. 4. No CMS. 5. Pol CMS. c: Amplified by a pair of Rep-PCR primers, 1. nap CMS. 2. AT CMS. 3. Ogu CMS. 4. No CMS. 5. Nsa CMS. 6. Pol CMS. Arrow shows Nsa CMS specific band.

Table 2 Fertility performance of F_1 pollinated by restorer lines

Line code	No. of F_1 plants	% of fertile plants	Pedigree
7244	64	100	(Zhong4+W4296)F_3
8401	70	100	(Zhong8+W4296)F_5
8367	47	98.4	(Zhong4+W4296)BC_1F_3
7265	342	95.5	(Zhong4+W4296)F_3
7196	228	94.8	(Zhong8+W4296)F_4
7199	166	93.7	(Zhong4+W4296)BC_1F_3

Fertility abortion of the CMS started from meiosis and the restorer contains mainly cells with 40 chromosomes

Ultrastructure and microstructure observations showed that the fertility abortion started from the beginning of meiosis. The pollen mother cells started to degrade and vacuolated under electro-microscopy (Fig. 4a). Abnormality could be observed throughout the whole meiosis process, such as irregular division of the chromosomes at anaphase I and hexad formation (Fig. 4c,e,g). Finally, spores could not be developed and no functional pollen grains could be produced.

Cytological analysis of one of the restorer lines showed that the line had 40 chromosomes, indicating it is a disomic addition line with two extra chromosomes probably come from the alien species *S. arvensis* (Fig. 5a). Meiosis of the restore line showed most of the cells had 20 bivalents, and tetrads with spores of 20 chromosomes were most frequently observed, implying the high possibility of a homo-disomic addition line of the restorer (Fig. 5b,c).

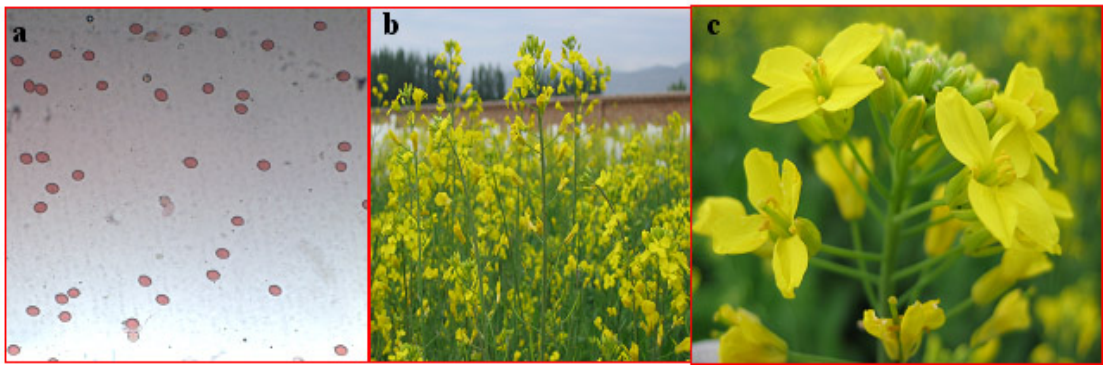

Fig. 3 Fertility of F_1 hybrids

a: Pollen stained by aceto-carmine. b: Fertile F_1 population. c: Fertile F_1 florescence.

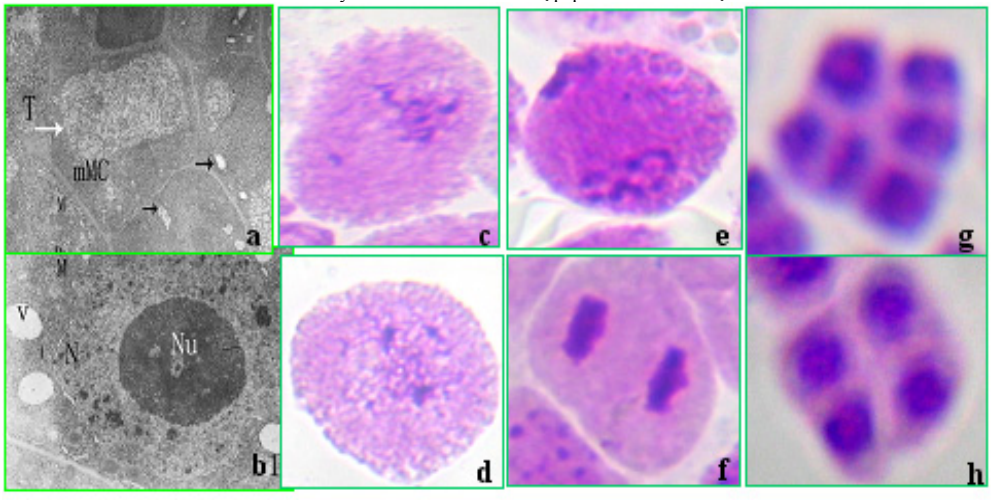

Fig. 4 Cytological observation of CMS line.

a,b: Ultrastructure of pollen mother cells (a: CMS, b: maintainer. c-h: Meiosis of CMS (c,e,g) and maintainer (d,f,h) .

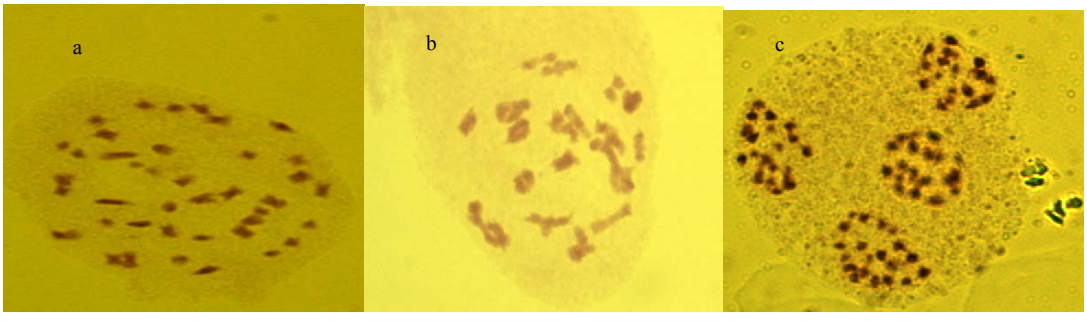

Fig. 5 Cytological observation of the restorer line.

a: Mitosis of a somatic cell. b: Metaphase I with 20 bivalents. c: Telophase II with spores containing 20 chromosomes.

Discussion

Due to the stability of Nsa CMS, the perspective of this CMS system for hybrid variety development is promising. However, the restorer line which contains two more additional chromosomes may render the development of stably inherent restorer lines more difficult. And it is likely that the restorer genes reside on the additional chromosomes. Preliminary results also showed that the restorer lines possess high resistance to *Sclerotinia* stem rot and pot shattering. Further efforts should be paid on facilitating the introgression of restorer genes into *B. napus* chromosomes in order to obtain more useful restorer lines.

References

[1] Fu T D. The present and future of rapeseed quality improvement. Journal of Huazhong Agricultural University, 2004, 34(Suppl): 1-6. (in Chinese)

[2] Hu Qiong, LI Yun-chang, MEI De-sheng, FANG Xiao-ping, Lise N Hansen, Sven B Andersen 2003, Establishment and Identification of Cytoplasmic Male Sterility in *Brassica napus* by Intergeneric Somatic Hybridization. Agricultural Sciences in China, 2(12): 1321-1328.

[3] Delourme, R., Budar F., Male Sterility, In Gomez-Campo C.(Ed): Biology of *Brassica* Coenospecies, 1999, Elsevier, Amsterdam, PP185-21