Development of a restorer line for Nsa CMS and molecular marker linked to the fertility restoration

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ABSTRACT

A novel alloplasmic male sterility system (Nsa CMS) derived from somatic hybrids of *Brassica napus* and *Sinapis arvensis* was used to screen for restorers using test cross with B. napus breeding lines and lines derived from somatic hybrids of *B. napus* and *S. arvensis*. Restorer lines were identified only 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 that it is a disomic additional line with 40 chromosomes. GISH showed that the restorer contained one pair of *S. arvensis* chromosomes and 19 pairs from *B. napus*, which kept stable and regular mitotic and meiotic processes. In view of the fact that most of the cloned Rf genes for plant CMS systems encoding PPR（pentatricopeptide repeat）protein, a pair of primers designed based on PPR genes of *Arabidopsis thaliana* and the Rf gene for Ogura CMS in *Raphnus sativa* were identified to be specific for the restoration and tightly linked to the fertility restoration in fertility segregated F2 population.

Key words: Alloplasmic male sterility - Restorer screen - Aneuploid-MAS

INTRODUCTION

The *Nsa* allo-***cytoplasmic male sterility system (Nsa*** CMS) was produced by symmetric somatic hybridization between *Brassica napus* and *Sinapis arvensis* of Chinese origin (Hu et al. 2002; 2004). *Nsa* CMS system was different from other rapeseed CMS systems based on its cytoplasmic origin and characterization of mitochondria genome (Cheng et al. 2008). Compared to *pol* CMS and *nap* CMS which are the only two CMS systems used in China, *Nsa* CMS is more stable to temperature change, possessing high potential in hybrid seed production of oilseed rape in China (Hu et al. 2004). A prerequisite of using Nsa CMS to make fertile hybrids is the development of parental lines including maintainers and restorers. Test cross using *B. napus* lines as the pollen donor did not reveal any line which could restore *Nsa* CMS, all lines tested maintained the sterility of Nsa CMS (Hu et al. 2004). Theoretically, the restore genes for cytoplasmic male sterility should have co-evolved along with the sterility gene, as those for Ogura CMS system from *R. sativa* in *B. napus* (Sakai et al. 1997; Budar and Pelletier 2001). The cytoplasmic sterile gene in *Nsa* CMS is likely from the *S. arvensis* parent (Wang 2008) and the fertility restoration genes might also be incorporated into the somatic hybrids from the *S. arvensis* parent. Thus, selection of restorers from offspring lines of the fertile somatic hybrids should be possible. This paper reports the identification of restore lines and development of a molecular marker lined to the fertility restoration trait in a restore line.

MATERIALS AND METHODS

Fertile somatic hybrids were self-propagated and directionally selected for fertility restoration ability based on the fertility performance of their F1 plants after crossed to CMS line. F_2 population derived from the CMS line and a restorer was used for marker identification. The parental lines of somatic hybridization which yielded the Nsa CMS line, *B. napus* cv. Zhongshuang 4 and *S. arvensis* cv. Yeyou 18, as well as restorers and maintainers for Polima CMS or Ogura CMS were used for marker analysis. GISH analyse was carried out as described by Wei et al. (2007) using Genomic DNA of Yeyou 18 as a probe.

RESULTS

Identification of restorer lines

Among thirty-six individual plants derived from somatic hybrids of Yeyou 18 and Zhongshuang 4 with partial or nearly normal male fertility that were test-crossed with CMS plants, 18 gave rise to F_1 hybrid plants with the percentage of fertile plants ranging from 12.9% to 100%. Further selection for high restoration rate upon self propagation and testcross resulted in 4 lines that yielded F1 plants with >95% were fertile. Two of these lines contained erucic acid levels lower than 1% in the oil and total glucosinolate lower than 30 μmol/g (meal), which meet the high quality criterion of canola type, were studied further to investigate if they were good enough for using as restorers.

The fertility of the two restore lines were high, with >95% of the offspring plants produced by either self propagation or test cross to CMS line were fertile from two years' continuous observation (Table 1). Sterile plants, however, were also found in the offspring population, indicating the aneuploidy nature of the restore lines. The two lines with code numbers of 7196 and 8367 were then designated as NR1 and NR2, respectively.

Table 1 Fertility of Restore lines

Year	Code of restore line	Fertility of F1 after self propagation			Fertility of F1 after cross to CMS line		
		No. of plants	No. of F1 plants	% of fertile plants	No. of plants	No. of F1 plants	% of fertile plants
2006	8367	3	75	100%	3	201	98.27%
	7196	9	852	95.16%	9	204	100%
2007	8367	5	136	100%	12	477	95.05%
	7196	12	1706	96.3%	44	2004	97.78%

Cytological analysis of NR1

Cytological analysis showed that NR1 consisted of 40 chromosomes, which in most cases, behavior normally in meiosis with 20 bivalents (Fig.1a). At mitotic anaphase, chromosomes were averagely distributed to two daughter cells, proving that the chromosomes from wild mustard in NR1 behave regularly.

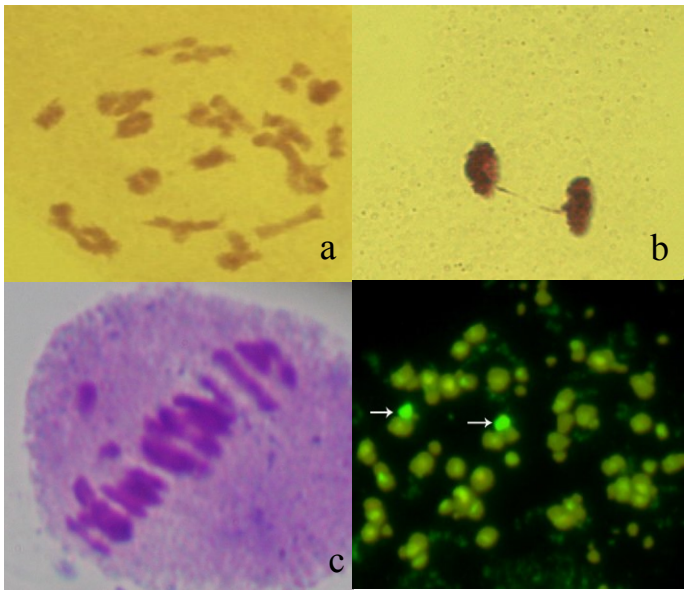

Fig.1 Cytological analysis of NR1

Lagged chromosomes or chromosome bridges were observed occasionally indicating lose of the additional chromosome also occurred (Fig.1b, c). GISH results showed that among the 40 chromosomes, two were from *S. arvensis* cv. Yeyou 18 (Fig. 1d), confirming that NR1 is a *B. napus* – *S. arvensis* disomic alien addition line.

Identificatin of a molecular marker linked to the fertility restoration trait

As the restore line was confirmed to be an aneuploid, it is difficult to develop new restore lines with traditional cross because lose of the additional chromosome occur more frequently in offsprings of crosses. Euploidization of the restore is necessary to make good use of Nsa CMS. As most of the restore genes (Rf) were revealed to encode PPR (pentatricopeptide repeat) proteins, primer pairs were designed based on the conserved regions of PPR genes in *Arabidopsis thaliana* and the Rf gene of Ogura CMS in *Raphanus sativa*. One pair of the primers could amplified a single band only in fertility restored plants of Nsa CMS system or S. arvensis cv Yeyou 18 or the restorers (Fig. 2), indicating a tight link of this marker to fertility restoration.

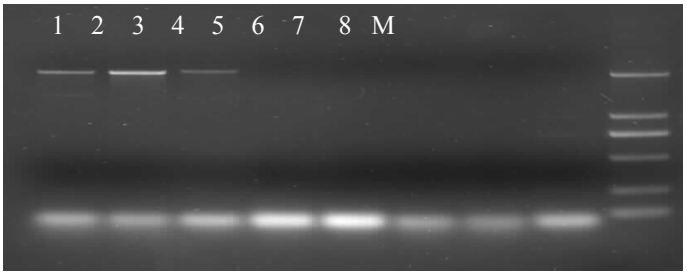

Fig 2 Specific amplification of the marker in Nsa CMS restorer lines. Lanes 1, 2, 3 are restorers of Nsa CMS; Lanes 4, 5 are restorers of Polima CMS; Lanes 6, 7 are maintainers of Polima CMS; Lane 8 is restorer of Ogura CMS.

To confirm the linkage of this marker with fertility restoration, an F2 population of Nsa CMS×NR1 was established.42 fertile plants and 41 sterile plants were used for the amplification of this band. The marker existed in all fertile plants whereas only one sterile plant contained this marker (Fig.3), indicating a tight link of this marker to the restore genes.

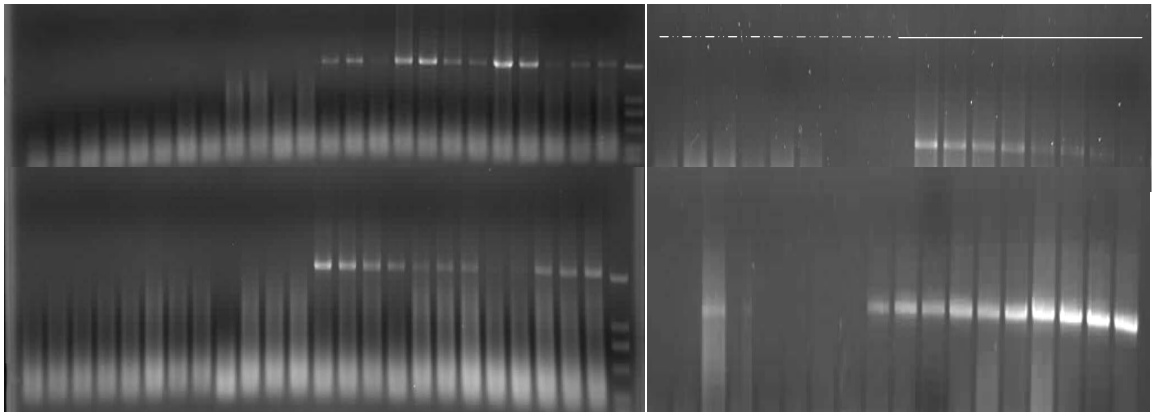

Fig. 3 Marker investigation of F2 population

DISCUSSION

The development of such aneuploid restore lines is of importance concerning the difficulty of find restore material in *B. napu*. However, the instability on fertility of these lines themselves as well as the offsprings derived from crosses of these line with euploid lines of *B. napus* renders the practical use of the restore lines problematic. Euploidization of the restore line is the next step towords the use of Nsa CMS in hybrid production. Irradiation might be a choice for this purpose. But selection of restore lines is always time consuming and labor intentsive. The molecular marker which is tightly linked to fertility restoration of Nsa CMS system can be used to increase the selection efficiency.

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