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ORIGINAL PAPER

Development of a novel *Sinapis arvensis* disomic addition line in *Brassica napus* containing the restorer gene for *Nsa* CMS and improved resistance to *Sclerotinia sclerotiorum* and pod shattering

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Abstract An allo-cytoplasmic male sterile line, which was developed through somatic hybridization between *Brassica napus* and *Sinapis arvensis* (thus designated as *Nsa* CMS line), possesses high potential for hybrid production of rapeseed. In order to select for restorer lines, fertile plants derived from the same somatic hybridization combination were self-pollinated and testcrossed with the parental *Nsa* CMS line for six generations. A novel disomic alien addition line, *B. napus–S. arvensis*, has been successfully developed. GISH analysis showed that it contains one pair of chromosomes from *S. arvensis* and 19 pairs from *B. napus*, and retains stable and regular mitotic and meiotic processes. The addition line displays very strong restoration ability to *Nsa* CMS line, high resistance to *Sclerotinia sclerotiorum* and a low incidence of pod shattering. Because the addition line shares these very important agricultural characters, it is a valuable restorer to *Nsa* CMS line, and is named NR1 here (*Nsa* restorer no. 1).

Introduction

The *Sinapis arvensis* population was discovered in Xinjiang Autonomous Region of North-Western China, and was cataloged into *S. arvensis* based on genetic analyses (Guan 1996). This alien species possesses valuable agricultural traits such as enhanced resistance to *Sclerotinia sclerotiorum*, *Leptosphaeria maculans* and insects, greater tolerance to low temperatures and drought, as well as a low incidence of pod shattering (Qian and Guan 1988; Hu et al. 2002). *Sclerotinia* stem rot is one of the most devastating diseases of rapeseed in China. This disease causes yield loss of 10–80%, as well as a decline of oil quality (Oil Crops Research Institute, Chinese Academy of Agricultural Sciences 1975). Due to the lack of complete resistance resources in *Brassica* species, little progress has been achieved in genetic improvement of *Sclerotinia* resistance in rapeseed. Although germplasms with partial resistance have been identified by screening the *B. napus* genetic resources or mutants (Zhao and Meng 2003; Liu et al. 2005), quantitative trait locus (QTL) affecting the resistance only accounts for a small portion of the variance (6–22% of each) (Zhao and Meng 2003). Thus, more resources from different origins are needed for further improvement of *Sclerotinia* resistance in rapeseed.

Pod shattering is another ubiquitous negative factor which leads to seed yield loss of *B. napus*, especially for mechanized harvesting. It has been observed that yield decreased 11–25% as a result of unsynchronized maturation (Price et al. 1996), and yield loss of up to 50% was estimated in seasons when adverse weather conditions delayed harvesting (MacLeod 1981). Moreover, the released seeds fall to the ground where they germinate and become weeds (volunteers), hindering the crop rotation practice used by many farmers. Also due to the lack of resistant germplasm within *B. napus* and limited genetic variation in pod shattering resistance in commercial breeding lines (Roberts et al. 2002; Wen et al. 2008), the development and commercial release of resistant cultivars have

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been rarely reported. However, some related species of *B. napus*, including *B. carinata*, *B. juncea* and *S. alba* possess higher pod shattering resistance than *B. napus* does. And the pod shattering resistance of interspecific hybrids of *B. napus* with *B. rapa*, *B. carinata* and *B. juncea* (Prakash and Chopra 1988; Wang et al. 2007) or re-synthetic *B. napus* from *B. oleracea* and *B. rapa* crosses (Child et al. 2003; Summers et al. 2003) is better than that of common *B. napus* cultivars. Overexpression of a MADS box gene from *B. juncea* and *FRUITFULL* gene from *B. napus* resulted in an increase of pod shattering resistance in *B. napus* (Chandler et al. 2005; Østergaard et al. 2006). Exploitation of resistance traits for either the *Sclerotinia* stem rot or pod shattering in related species including the *S. arvensis* of Chinese origin is of great potential for the breeding of novel cultivars and for understanding the genetic basis of resistance.

Intergeneric hybridization of *B. napus* and *S. arvensis* through conventional crossing has rarely resulted in any viable hybrids (Hu et al. 2002). A number of symmetric hybrids between *B. napus* and *S. arvensis*, however, were successfully produced with either male sterile or male fertile character, which provides a further utilization of the valuable genes derived from parental *S. arvensis* (Hu et al. 2002). From these hybrids, a novel allo-*cytoplasmic male sterility* (*CMS*) *system*, *Nsa* CMS *system*, has been developed (Mei et al. 2003; Hu et al. 2004). *Nsa* CMS *system* is essentially different from other rapeseed CMS systems such as *ogu* (Ogura 1968), *nap* (Shiga and Baba 1971), *pol* (Fu 1981), *tour* (Mathias 1985) and *hau* (Wan et al. 2008), based on their origins and molecular characterization (Cheng et al. 2008). Furthermore, *Nsa* CMS line is more stable to temperature changes as observed at several locations in China (Hu et al. 2004), compared to *pol* and *nap*, which are the only two CMS *systems* used for hybrid production in China. Application of *Nsa* CMS *system* in hybrid seed production not only increases the genetic diversity of the cytoplasm of hybrid rapeseed, but also potentially improves hybrid seed purity due to the high sterility stability under different environment conditions.

The development of *Nsa* CMS hybrid varieties relies on the development of restorer lines. Testcrossing with the use of *B. napus* lines as pollen donors has failed to obtain any line which can restore the fertility of *Nsa* CMS line (Hu et al. 2004). However, partial restoration was observed from crosses of fertile somatic hybrids produced by protoplast fusion between the Chinese *S. arvensis* and *B. napus* cultivars (Hu et al. 2002) as the pollinator. Theoretically, the restorer genes encoding for CMS systems should have co-evolved along with the sterility genes (Budar and Pelletier 2001), such as those encoding for Ogura CMS system from *Raphanus sativus* in *B. napus* (Sakai et al. 1997), and for CMS systems with *Trachystoma ballii* or *Diplotaxis catholica* cytoplasm in *B. juncea* (Kirti et al. 1997; Pathania et al. 2003). Characterization of mitochondria from *Nsa* CMS line has indicated that this line mainly contains the *S. arvensis* cytoplasm (Wang 2008). Thus, the cytoplasmic sterile gene in *Nsa* CMS line is likely derived from the *S. arvensis* parent. Accordingly, the fertility restoration genes might be incorporated into the somatic hybrids from the parental *S. arvensis* parent as well. Therefore, selection from offsprings of the fertile somatic hybrids should allow the identification of restorer lines.

In this study, we have developed a restorer line, named NR1 here, from fertile somatic hybrid plants derived from protoplast fusion combination of a *B. napus* cultivar Zhongshuang 4 and a *S. arvensis* accession Yeyou 18 after continuously testcross with *Nsa* CMS lines and propagation by self-pollination. Further, we have assessed the resistance of NR1 to *S. sclerotiorum* and pod shattering.

Materials and methods

Plant materials

The sterile and fertile individual plants, derived from the somatic hybrids between *B. napus* (Zhongshuang 4) and *S. arvensis* (Yeyou 18), were planted in the trial field of Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (Wuhan, Hubei) from years 2002 to 2008. The sterile individual plants were maintained with the parental line *B. napus* (Zhongshuang 4) for at least four generations by continuous backcrossing. The fertile individual plants were propagated by self-pollination and testcrossed with sterile individual plants for the identification of restorer lines. At the same time, the restorer lines were screened for resistance to *S. sclerotiorum* and pod shattering in later generations. *B. napus* cultivar Zhongshuang 9 and the *B. napus* parent Zhongshuang 4 with moderate resistance to *S. sclerotiorum* were used as resistance controls. Eighteen *B. napus* cultivars or breeding lines including Zhongshuang 4 and another restorer for *Nsa* CMS lines, NR2, together with other 17 commonly used main parental lines in hybridization breeding programs, namely, R1-1, R1, 5899B, 1008B, 1055B, TR217, R3, R4, M1, 6098B, R6, 8908B2, R5, HenanR, R2, R6-1, 8908B1, were selected to perform the test of resistance to pod shattering in comparison with NR1.

Determination of pollen viability, fertility and quality

Pollen viability test was performed with the use of two staining procedures, one reported by Alexander (1969) and the other by Wei et al. (2007a). Newly opened flowers were sampled at 9–10 a.m. Anthers were squashed and pollen grains were stained with Alexander staining solution

containing 2% glacial acetic acid or with 1% aceto-carmine, and were observed under microscopy. Five individual plants of each material and five flowers of each plant were used and the calculation was based on 300 pollen grains of each flower. Pollen viability rate was calculated as the number of well-stained pollen grains/total pollen grains × 100%.

Fertility was assessed according to seed set after self propagation and crossing to ordinary lines. A fertile plant is defined as a plant that produces abundant pollen and yields seeds upon self-pollination. Compatibility index was calculated as the total number of seeds divided by the number of selfed or crossed flower buds. Female fertility was assessed using NR1 as female parent to cross with an ordinary *B. napus* line; male fertility was assessed using NR1 as the pollinator to cross with an ordinary *B. napus* line. Seed set rate was calculated as the total number of seeds divided by the number of siliques.

Erucic acid and glucosinolate contents in seeds were determined by Quality Inspection and Test Center for Oilseeds Products, Ministry of Agriculture, according to the Chinese national standards GB/T 17377-1998 (gas chromatography) and the international standard ISO 9167-1:1992 (E), respectively.

Evaluation of resistance to *S. sclerotiorum*

Fresh rapeseed isolate of *S. sclerotiorum* collected from field plants was maintained and cultured on solid potato/dextrose/agar (PDA, 20% potato, 2% dextrose and 1.5% agar) medium at 22°C for 3–4 days, after surface sterilization with 0.1% mercuric chloride for 10 min. Mycelial agar disks of 5 mm diameter punched from the growing periphery of the 3–4 day culture of *S. sclerotiorum* on PDA were used as inoculums to infect the plants (Godoy et al. 1990). The basal leaves were excised from each plant at early stage of flowering. Eight leaves, one for each plant, were placed into a box bedded with wet paper, and subsequently inoculated with two mycelial agar disks of 5 mm diameter separately (Godoy et al. 1990). The boxes were covered with plastic film, and kept at 23°C for 3 days. The lesion diameter was measured 72 h after inoculation to evaluate the level of resistance. Field assessment was carried out by surveying the mature plants for disease incidence and index before harvest. The disease grade of each plant was scored as 0–4 and disease index was calculated according to Zhou (1994). Ten plants from each line were scored. Three independent replicates were performed. Data were analyzed using DPS software based on Duncan's multi-comparison tests according to Tang and Feng (2007).

Evaluation of pod shattering

Random impact test developed by Kadkol et al. (1984) and modified by Wen et al. (2008) was adopted for the test of pod shattering resistance. In brief, pods harvested at fully mature stage were hang-dried indoors for 2 weeks, collected and sealed in plastic bags before test. Twenty pods were placed in a cylindrical container with an inner diameter of 19 cm and a height of 14 cm together with 12 steel balls of 13 mm diameter. The container was fixed on a horizontal shaker and shook with 280 rpm. The cracked pods were counted every minute 10 times and picked up from the container each time. The pod shattering resistance index was calculated as $SRI = 1 - \Sigma\chi_i \times (10 - i + 1)/200$, where $i = 1 - 10$, χ_i is the number of cracked pods at the ith minute. Five plants of each line were tested and each plant was sampled three times.

Chromosome preparation

The procedure described by Wei et al. (2005) was used with some modifications. Briefly, flower buds were fixed in a mixture of ethanol:acetic acid glacial (3:1) at 4°C overnight. They were washed 3–5 times with distilled water, digested in 1% (W/V) cellulase "Onozuka" R-10 (Yakult Honsha Co. Ltd) and 1% (W/V) pectolyase Y-23 (Yakult Honsha Co. Ltd) dissolved in distilled water at 28°C for 2.5–3 h. Then the samples were subjected to a hypotonic treatment in distilled water for 30 min before spread preparation by a flame-drying method.

Probes labeling and GISH (genome in situ hybridization)

GISH analysis was carried out as described by Wei et al. (2007b). Genomic DNA of *S. arvensis* (Yeyou 18) was labeled with a Biotin-Nick Translation Mix (Roche, Cat. No. 11745824910), and that of *B. napus* (Zhongshuang 4) was labeled with Dig-Nick Translation Mix (Roche, Cat. No. 11745816910), according to the instructions of the kits. The labeled DNAs were then used as probes for GISH. The labeling efficiency was evaluated by means of dot blotting. Chromosome preparations were pretreated with 100 µg/ml RNase (in 2× SSC) at 37°C for 1 h and rinsed briefly in 2× SSC. Chromosomal DNA was then denatured by immersing the slide in 70% deionized formamide at 70°C for 3 min. After dehydration of the preparation in an ice-cold 70, 95 and 100% ethanol series and air dried, 40 µl of denatured probe cocktail (5 ng/µl labeled probe DNA each, 0.5 µg/µl sheared salmon sperm DNA, 10% dextran sulphate, 50% deionized formamide, 0.1% SDS, 2× SSC) was mounted on the slide. Hybridization was carried out at 37°C overnight. Post-hybridization washes, including a stringent wash in 20% formamide, a wash in 2× SSC and a wash in 0.1× SSC at 42°C for 10 min, respectively, were performed to remove weakly bound probes. Signals were first detected with Streptavidin-Cy3 (Amersham, Cat. No. PA43001), followed by a wash in PBS for 10 min. After that, a sequential detection

was performed with Anti-Digoxigenin-Fluorescein (Roche, Cat. No. 1207741), followed by a wash in PBS for 10 min. Slides were counterstained with 2 μg/ml DAPI (4′,6-diamidino-2-phenylindole) and examined under a Leica DM IRB fluorescence microscope assembled with DFC300 CCD and FW4000 software.

Results

Selection of NR1

Thirty-six fertile individual plants derived from somatic hybrids between *S. arvensis* (Yeyou 18) and *B. napus* (Zhongshuang 4) were testcrossed with *Nsa* CMS plants in March 2002. In the next year (2003), the percentage of fertile F_1 hybrid plants from 18 testcross combinations was measured, ranging from 12.9 to 100%. The F_1 hybrids from eight combinations showed high fertility restoration, with an average pollen viability ranging from 38.89 to 99.22% and the number of seeds per pod upon self-pollination ranging from 4.98 to 19.01 (Table 1). The paternal plants of these eight combinations were used as primary restorer plants for selection and development of restorer lines through self propagation and further testcrossed to *Nsa* CMS line. In 2005, four restorer lines were obtained which showed high-restoration ability and yielded over 95% (ranging from 95.6 to 97.6%) fertile plants in their F_1 generation when crossed back to *Nsa* CMS line (Table 2). In 2006, in order to achieve the breeding objective for high-quality rapeseed varieties, these four restorer lines were used for quality determination that was able to select for lines with low erucic acid and low glucosinolate contents, which are nutritionally favorable for human and animal health (Downey and Rimmer 1993). In consequence, two double-low restorer lines, which both contained erucic acid levels lower than 1% in the oil and total glucosinolates lower than 30 μmol/g (meal), were identified (Table 3).

Individual plants of the two double-low restorer lines were continuously selected for high fertility and generous pollen. One ideal line (line 7196) showing better agronomic performance was obtained, and is therefore named NR1 here (*Nsa* restorer no. 1). Pollen viability test using Alexander staining solution or aceto-carmine showed that there was no significant difference between results from the two staining methods. The average pollen viability rate of NR1 was 96.07% from Alexander staining (Fig. 1a) and 95.99% from aceto-carmine staining (Fig. 1b). The average pollen viability rate of F1 hybrid plants derived from NR1 and *Nsa* CMS lines was over 90%. The development of flower organs of these F1 hybrid plants was normal (Fig. 1c). The seed number per pod was over 16 after self-crossing of NR1, and was not significantly different from that of ordinary *B. napus* lines such as Zhongshuang 4 (Fig. 1d).

The agronomic properties of NR1 are quite uniform despite individuals and generations, implying that this line

Table 1 Fertility segregation of F_1 hybrids between primary restorer plants and *Nsa* CMS plants in 2003

Code of combination	Number of fertile plants	Number of total plants	Fertile plant (%)	Pollen viability (%)	Self compatibility index	Seed per pod upon selfing
6829A-1 × 6988-2	6	16	37.5	99.2	16.6	19.0
6837A-3 × 6989-1	5	15	33.3	91.3	9.6	11.4
6834A-1 × 6992-1	4	15	26.7	95.9	10.1	11.6
6835A-2 × 6992-3	8	16	50.0	98.3	12.5	13.0
6833A-2 × 7029-1	4	4	100.0	38.9	4.9	6.7
6832A-80 × 7029-3	9	9	100.0	83.1	13.5	14.2
6832A-8 × 7031-5	5	6	83.3	84.5	4.5	5.0
6834A-1 × 7040-2	10	12	83.3	62.8	6.3	7.9
B. napus (Zhongshuang 4)				99.5	14.5	15.1

Table 2 Fertility restoration of four restorer lines derived from the primary restorer plants and their pedigree

Code of lines	Number of paternal plants	Number of F_1 plants	Rate of fertile plants	Pedigree
7244	3	158	95.6	[*B. napus* (Zhongshuang 4) + *S. arvensis* (Yeyou 18)]F_3
8367	3	163	97.6	{[*B. napus* (Zhongshuang 4) + *S. arvensis* (Yeyou 18)] × R_1-8B}F_3
7265	5	345	96.9	[*B. napus* (Zhongshuang 4) + *S. arvensis* (Yeyou 18)]F_3
7196	8	761	96.9	[*B. napus* (Zhongshuang 4) + *S. arvensis* (Yeyou 18)]F_4

Table 3 Fertility of two double-low restorer lines in 2006

Lines	Stainable pollen grains (%)	Self compatibility index	Selfed seed set rate	Female fertility		Male fertility	
				Compatibility index	Seed set rate	Compatibility index	Seed set rate
7196	93.9	6.5	10.9	10.2	11.4	7.0	8.9
8367	94.2	4.8	7.7	8.1	12.2	7.0	9.5
B. napus (Zhongshuang 4)	97.0	7.6	15.1	11.8	13.9	9.3	11.9

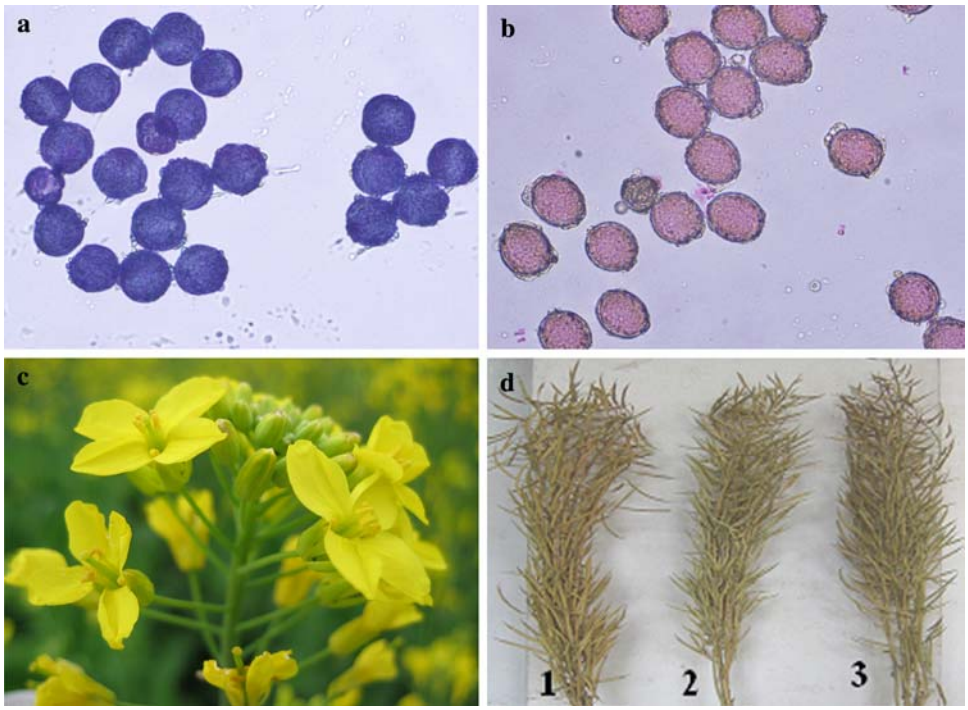

Fig. 1 Fertility of NR1 and its F_1 hybrids crossed with *Nsa* CMS line. **a** Pollen viability of NR1 by Alexander staining. **b** Pollen viability of NR1 by aceto-carmine staining. **c** Natural flowering of F_1 hybrid. **d** Seed set of different plants of NR1. *1* is *B. napus* (Zhongshuang 4), *2* and *3* are NR1 plants

has high genetic stability. However, a small ratio of segregation of fertility was always observed. In 2007, 515 plants of NR1 line were tested for their fertility, and 24 showed sterile. In the following year, 29 were sterile among 652 plants. The rate of sterile plants in different families that were derived from different paternal plants varied from 1 to 5%.

Resistance to *S. sclerotiorum*

Spreading speed of necrotic lesions in NR1 was obviously slower compared to that of currently cultivated resistant Chinese winter rapeseed cultivar *B. napus* (Zhongshuang 9) (Wang et al. 2004) and the original parent *B. napus* (Zhongshuang 4), as evaluated by the mycelial inoculation test of field plants. The lesion area of NR1 was significantly reduced compared to those of the two *B. napus* lines, with $P = 0.04$ (Table 4). Furthermore, field investigation also showed that NR1 is evidently lower in disease incidence and disease index compared to the two control cultivars, with $P = 0.016$ and $P = 0.003$, respectively (Table 4). Compared to Zhongshuang 9, the parent Zhongshuang 4 used in somatic hybridization seems more resistant, since the disease index of Zhongshuang 4 was significantly lower than that of Zhongshuang 9 (5% lower). However, NR1 is the most resistant one among the tested materials, with lesion area, disease incidence and disease index significantly different from those of the two controls (Fig. 2).

Table 4 Determination of resistance to *S. sclerotiorum* of NR1

Cultivar (or line)	Field determination		Mycelial inoculation
	Disease incidence (%)	Disease index	Lesion area (cm^2)
B. napus (Zhongshuang 9)	64.0 ± 16.1	23.0 ± 0.6	17.2 ± 3.0
B. napus (Zhongshuang 4)	52.9 ± 18.0	14.4 ± 5.5	14.5 ± 1.0
NR1	7.4 ± 2.7	1.9 ± 0.7	8.3 ± 1.2

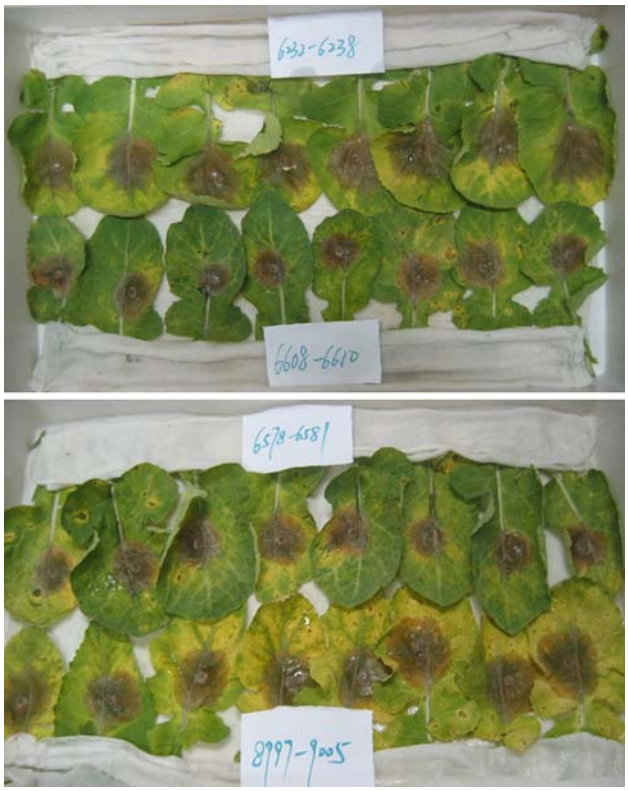

Fig. 2 Spread of lesions 3 days after inoculation in NR1 and current resistant cultivars *6578–6581* and *6608–6610*, NR1. *6232–6238*, resistant double-low cultivar *B. napus* (Zhongshuang 9). *8997–9005*, resistant double high cultivar *B. napus* (Zhongshuang 4)

Pod shattering resistance

Pod shattering resistance was assessed in the years of 2006 and 2007. This experiment also included a line selected from the other double-low restorer line, NR2. Both restorer lines, NR1 and NR2, showed very high pod shattering resistance. In 2006, both of them had a shattering resistance index (SRI) greater than 0.84, whereas the SRI of other tested lines ranged from 0.152 to 0.732. The SRI of the parental line Zhongshuang 4 was only 0.373 (Fig. 3). Similar results were obtained in 2007.

GISH analysis for genomic constitution of NR1

GISH analysis showed that NR1 has 40 chromosomes including 38 from *B. napus* (Zhongshuang 4) and 2 from *S. arvensis* (Yeyou 18). These data indicate that NR1 is a *B. napus–S. arvensis* disomic alien addition line (Fig. 4a). At mitotic anaphase, *S. arvensis* chromosomes were averagely distributed among two daughter cells (Fig. 4b). One bivalent from *S. arvensis* (Yeyou 18) could be detected at meiotic diakinesis of pollen mother cell (PMC), indicating that these two *S. arvensis* chromosomes in NR1 were homologous chromosome pair (Fig. 4c). At meiotic telophase II, each of the four daughter cells from a PMC has a single *S. arvensis* chromosome (Fig. 4d). These results prove that the chromosomes from *S. arvensis* in NR1 behave regularly both in mitosis and meiosis.

Discussion

In 7 years of our study, several approaches, including continuous self propagation, testcrossing and resistance selection, have been applied to develop a novel restorer line that could restore the fertility of *Nsa* CMS line and simultaneously possess high resistance to *S. sclerotiorum* and pod shattering. The restorer line, named NR1 here, was finally obtained. NR1 displays very strong restoration ability to *Nsa* CMS line, high resistance to *S. sclerotiorum* and a low incidence of pod shattering.

Since no restorer line for *Nsa* CMS line has been identified from *B. napus* germplasm, the evolution of the restorer gene might be cooperative with the sterile cytoplasm of *S. arvensis* (Budar and Pelletier 2001). Transfer of restorer genes from *S. arvensis* cannot be readily done with traditional approaches due to intergeneric crossing barrier.

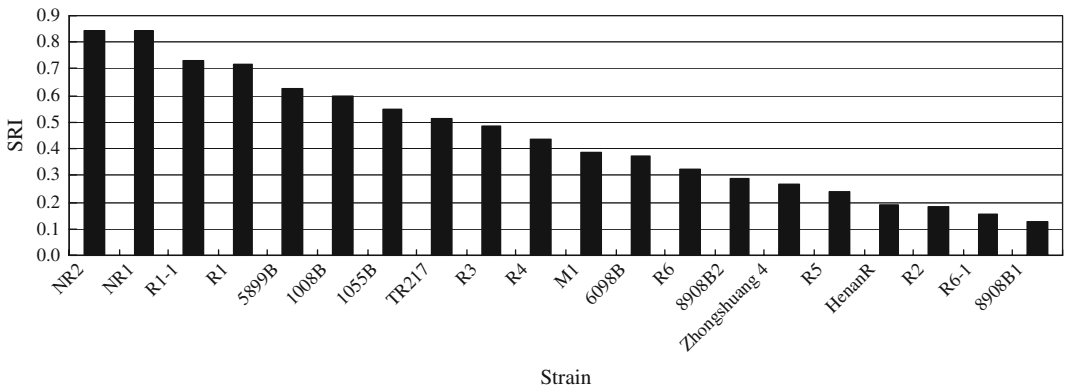

Fig. 3 Pod shattering resistance of *Nsa* CMS restorers and other breeding lines

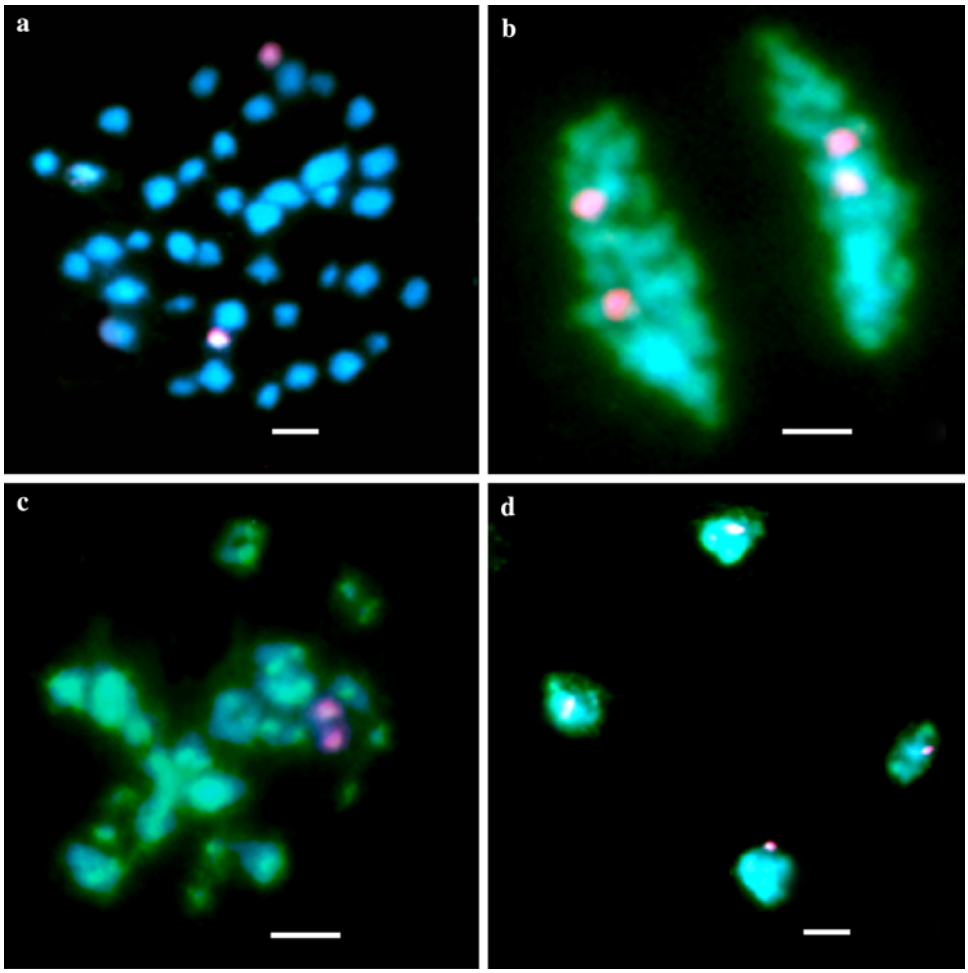

Fig. 4 GISH detected one chromosome pair in NR1 from *S. arvensis* (Yeyou18) genome. **a** A mitotic metaphase spread. **b** A mitotic anaphase. **c** A pollen mother cell showing one bivalent from *S. arvensis* (Yeyou18) at diakinesis. And in **d**, only one chromosome is detected in the four haploid meiotic cells at the end of meiosis. *Bar* 5 μm

Thus, the development of NR1 is a great breakthrough for the realization of hybrid seed production with the use of *Nsa* CMS system. GISH analysis demonstrated that NR1 is a *B. napus–S. arvensis* disomic alien addition line, contains 19 *B. napus* homologous chromosome pairs and one *S. arvensis* homologous chromosome pair, and displays regular mitotic and meiotic division and reliable genetic stability. Since the percent of fertile plants in F_1 generation produced by crossing NR1 to *Nsa* CMS plants was above 95%, direct use of the restorer line for hybrid production is possible. As almost all tested current *B. napus* cultivars (lines) were maintainers of *Nsa* CMS line (Hu et al. 2004), selection of combinations with high heterosis using NR1 as paternal and various *B. napus* lines as maternal parents is likely of success. Furthermore, development of new restorer lines using NR1 as the restorer gene donor should also be feasible. Thus, NR1 is of great potential for heterosis application in hybrid breeding of *B. napus* using the 3-line system.

NR1 has been identified to be resistant to *S. sclerotiorum* and pod shattering. Since the *B. napus* parent of the protoplast fusion combination did not show as high a resistance to either *S. sclerotiorum* or pod shattering as NR1 did, it is likely that the alleles related to these resistance properties from *S. arvensis* contributed to the enhancement of these resistance properties in NR1. The F_1 hybrid also showed increased resistance properties as NR1 did (unpublished data). It has been indicated that the restorer genes reside on the added chromosome since only fertile plants possess the added chromosome in fertility segregated populations derived from crosses between fertile plants and sterile plants (Huang et al. 2008). So do the genes coding for the *S. sclerotiorum* (Sun et al. 2009) and pod shattering resistances (data to be published). Recently, several studies have indicated that alien chromatin introgression may impose a mutator effect on the recipient genome by causing genetic and (or) epigenetic variations in plants (Liu et al. 2004; Zhang et al. 2008) and animals (Muller et al. 2001). Chen et al. (2006) reported that perhaps there is some correlative or causal relations between genetic and (or) epigenetic changes in the rice endogenous resistance gene analogs and the acquired disease-resistance phenotype in the introgression lines. Thus, another possible reason for the enhanced resistance of NR1 could be that the resistance is related to genetic and (or) epigenetic changes of the *B. napus* genome due to the addition of *S. arvensis* chromosome.

In theory, all of the handmade hybrids between NR1 and *Nsa* CMS lines should be completely fertile as the *Nsa* sterile line does not produce any pollen grains needed for self-pollination. The fact that less than 100% of the F_1 hybrids produced were fertile implies that the loss of added chromosomes might occur in the meiosis of NR1 considering the restorer gene(s) on the added chromosomes. Low percentages (1–5%) of sterile plants in NR1 observed in large populations (over 500 plants) are another indication for the loss of added chromosomes. Genetic instability is the major problem for plant addition lines to be practically used. For NR1 described in this paper, the percentage of fertile plants in F_1 hybrids made from NR1 crossed to *Nsa* CMS line significantly exceeds the purity rate specified in the national standard for rapeseed hybrid seed in China (90% for first grade and 83% for second grade). In addition, the agronomic characters of the F_1 plants are very uniform. Thus, it should be able to use NR1 as the restorer to produce F_1 hybrid seeds that meet the standard requirements for commercial use although the hybrid plants are monosomic. The breeding of new restorer lines using NR1 as restorer gene donor, however, is a difficult topic which needs to be addressed in the future since the fertility segregation in higher generations of crosses between NR1 and ordinary *B. napus* lines biased towards sterile plants. Thus, euploidization of NR1 without elimination of the above mentioned valuable traits, including fertility restoration, *Sclerotia* resistance and pod shattering resistance, is highly desirable and it is worthy to try using irradiation technology which has been very efficiently applied in wheat breeding (Tong et al. 2007).

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