Analysis of intergeneric sexual hybridization between transgenic *Brassica oleracea* and *Sinapis alba*

Jun Li · Chunlei Zhang · Chunyun Guan · Lixia Luo · Li Ren ·
Wenhui Wei · Guangyuan Lu · Xiaoping Fang

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Abstract *Sinapis alba* possesses a number of desirable traits that can be employed to broaden genetic variability in *Brassica oleracea* or *B. napus*. Using transgenic *B. oleracea* (CC, $2n = 18$) as paternal plants and non-transgenic *S. alba* (SS, $2n = 24$) as maternal plants, 11 hybrid plants were successfully obtained by using a combination of ovary culture and embryo rescue, while 2 seedlings of the reciprocal cross were generated but lost during the propagation. The hybridity of these plants was confirmed by flower color and other agronomic characteristics, chromosome counting, and pollen viability. Most of the

intergeneric F_1 plants showed only one set each of S and C chromosome (SC, $2n = 21$) and were male sterile, while a few plants were semi-fertile and had one set of S chromosomes but two sets of C chromosomes (CCS, $2n = 30$). PCR, genomic Southern blotting and qRT-PCR for *bar* gene revealed that among the F_1 hybrid plants only 9.1% was *bar* positive and could be forwarded to F_2 and F_3 generation. The majority of F_3 plants obtained sufficient resistance to *Alternaria brassicae*. Thus, the generation of disease (*A. brassicae*) and herbicide (Bastar) resistant intergeneric hybrids was of importance for breeding program. The existence of *bar* gene in these intermediate materials will facilitate the identification of hybridity and transfer of *S. alba* traits into target genetic background.

Keywords *Brassica oleracea* · *Sinapis alba* · Intergeneric hybridization · *Bar* gene · Disease resistance · *Alternaria brassicae*

J. Li · W. Wei · G. Lu (✉)
Key Laboratory of Biology and Genetic Improvement of
Oil Crops of the Ministry of Agriculture, Oil Crops
Research Institute, Chinese Academy of Agricultural
Sciences, Wuhan, China
e-mail: luwiz@163.com

J. Li · C. Zhang · L. Luo · L. Ren · X. Fang (✉)
Key Laboratory of Crop Cultivation and Physiology of the
Ministry of Agriculture, Oil Crops Research Institute,
Chinese Academy of Agricultural Sciences, Wuhan,
China
e-mail: fangxp@oilcrops.cn

C. Guan
College of Agriculture, Hunan Agricultural University,
Changsha, China

G. Lu
New Rural Development Institute, Hubei Engineering
University, Xiaogan, China

Introduction

Approximately 330 genera and 3700 species are included in the Brassicaceae family. Among these, the genus *Brassica* contains many economically important crops for oil, feed, condiment and vegetable such as *B. oleracea* (CC, $2n = 18$) (Kaneko and

Bang 2014). The *B. oleracea* is one of the most valuable crops and has provided humans with healthy vegetable for hundreds of years. *B. oleracea* comprises multiple, distinct types and is widely cultivated in China. However, *B. oleracea* has weak tolerance to frost, mildew, aphids, black spot, and black rot diseases and is also susceptible to abiotic stresses such as drought (Gribova et al. 2006; Wei et al. 2007), which may cause severe yield losses during production. Therefore, introduction of desired traits into *B. oleracea* from other related species is of great interest.

Sinapis alba (SS, $2n = 24$) is a species within Brassicaceae that is phylogenetically close to *Brassica* species and has many desirable agronomic traits, including tolerance to high temperatures and drought stress and resistance to a variety of diseases such as black spot (Hansen and Earle 1997), flea beetles (Bodnaryk and Lamb 1991) and clubroot (Lelivelt et al. 1993). However, *S. alba* has historically been developed only for condiment use because of its poor oil quality and high level of glucosinolates in the seed (Brown et al. 1997). Nevertheless, it is an attractive genetic stock for interspecific or intergeneric hybridization for germplasm enhancement, and a new variety with double low quality was recently developed in Poland (Pietka et al. 2014).

The fungal pathogen *Alternaria brassicae* can cause severe blackspot disease in the Brassicaceae, including three of the most important vegetable crops in the world, namely cauliflower, broccoli and cabbage. Moreover, if the disease continues to be aggravated on any part of a plant with spotting symptoms, the market value and production of vegetables will be greatly reduced. However, efforts to identify sources of high resistance to *A. brassicae* among related species that could be readily crossed with *B. oleracea* have failed (Hansen and Earle 1997).

Introduction of economically important traits from their wide relatives into Brassica crops via interspecific or intergeneric hybridization is recognized as a promising strategy. Successful hybridizations between *S. alba* and *B. napus* have been achieved by sexual or somatic hybridization (Lelivelt et al. 1993; Wang et al. 2005). Similarly, the crossability between *S. alba* and *B. rapa* has been confirmed (Gong et al. 1994). Likewise, Hansen and Earle (1997) carried out the somatic hybridization between *S. alba* and *B. oleracea* by protoplast fusion. However, somatic hybridization may generate chimeric lines and the plants obtained

always showed very poor pollen fertility (Chevre et al. 1994; Nothnagel et al. 1997; Chen et al. 2005; Wang et al. 2011). To avoid such shortcomings, we prefer to use sexual hybridization and embryo rescue techniques to produce intergeneric hybrid lines of *S. alba* and *B. oleracea*. In this study, we aimed to illustrate the sexual crossability between the transgenic *B. oleracea* and non-transgenic *S. alba* and further assess the potential utilization of such hybrids as intermediate genetic stock in breeding.

Materials and Methods

Plant materials

Transgenic cv. ‘Hong-Kong Zhonghua Jielan’ (*B. oleracea* var. *alboglabra*, genome CC, $2n = 18$) and non-transgenic accession ‘Xinjiang Baijie’ (*Sinapis alba* L., white mustard var. *Asta*, genome SS, $2n = 24$) plants were used in the intergeneric hybridization. Previously, the herbicide resistant *bar* gene was transferred into the genome of *B. oleracea* via agrobacterium mediated transformation (Li et al. 2006). A homozygous T₂ line (term ‘*B. oleracea-bar*’ hereafter) was chosen for this study. Seeds of the two lines along with their offspring were grown in pots in glass house or in field of Hanchuan Transgenic Experimental Station attached to Oil Crops Research Institute, CAAS (certificated by Chinese Government).

Intergeneric hybridizations

Reciprocal intergeneric hybridizations between *S. alba* and *bar* transgenic *B. oleracea* plants were conducted. The fully developed young buds were hand emasculated one day before flowering, and immediately pollinated with fresh pollens and topped with a paper bag to prevent cross pollen contamination. In total ~ 1200 crosses were made. Seven days after pollination, ovaries (young fruits) were excised from plants, surface sterilized (70% alcohol for 5–10 s, 1% DICA for 8–10 min, washed with sterile water 3–4 times) and cultured on MS medium supplemented with 500 mg/L hydrolyzed casein (with 0.8% agar and 3.0% sucrose) for approximately 35–40 days. Then, ovules, if any, were dissected from the fruits and

inoculated into MS medium until they grew into normal seedlings. Such seedlings were then cut off and treated in MS medium with 0.01% colchicine for 7–10 days for chromosome doubling and transferred into the rooting medium (MS + 0.2 mg/L NAA) for root initiation and multiplication. After robust roots developed, the plants were transplanted into pots and grown with appropriate shade and moisture.

Cytological test

The chromosome numbers of the hybrids was determined using young buds. Pistils of flower buds were peeled out, treated with 0.002 mol/L 8-hydroxyquinoline for 4 h, then fixed in Carnoy's solution for 24 h and stored in 70% ethanol at 4 °C until use. The pistils were hydrolyzed in 1.0 mol/L HCl at 60 °C for approximately 10 min, squashed in a drop of modified carbofuchsin and observed under a microscope.

Genomic in situ hybridization (GISH) assays were conducted according to the protocol described by Wei et al. (2007), with minor modifications. Briefly, chromosome preparations were treated with 80 µg/mL RNase at 37 °C for 1 h and then rinsed gently in 2× SSC. Chromosomal DNA was denatured by immersing the slide in 70% deionized formamide at 70 °C for 2 min. After dehydration of the preparation in an ice-cold 70, 95 and 100% ethanol series and air drying, 40 µL of denatured probe cocktail was placed on a slide and hybridization was performed at 37 °C for 12 h. Post-hybridization washes to remove weakly bound probe included a stringent wash in 20% formamide, a wash in 2× SSC and a wash in 0.1× SSC at 42 °C for 10 min to remove weakly bound probe. For double-color GISH, signals were first detected with Streptavidin-Cy3 (GE Healthcare Bio-Sciences, Pittsburgh, USA, Cat. No. PA43001) and then washed in PBS buffer for 10 min, followed by immediate sequential detection with Anti-Digoxigenin-Fluorescein (Roche, Penzberg, Bayern, Germany, Cat. No. 1207741) and another wash in PBS buffer for 10 min. For single-color GISH, only Streptavidin-Cy3 or Anti-Digoxigenin-Fluorescein was used. 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.

Pollen viability

To determine the pollen fertility, anthers from just-opening flowers were dissected and squashed in 1% acetocarmine and examined under a microscope. The pollen viability rate was calculated as the proportion of stained pollen grains in total pollen grains scored $\times 100$. Three fields of view per flower were examined for a total of approximately 800 pollen grains.

Seed weight and oil content assessment

The Marvin analyzer (GTA Sensorik GmbH, Neubrandenburg, Germany) was used for seed weight measurement. Around 0.3 g seeds were first weight by the electronic balance connected to the computer and then spread across the sample tray for a snapshot, from which the number of seeds and 1000-seed-weight were automatically calculated.

To measure the oil content of seeds in small seed quantity, we employed a method developed by Wei et al. (2008) rather than using the common Near Infrared Reflectance Spectroscopy method that required a large number of seeds. In brief, ~ 20 mg seeds were quantified and then crushed to the particle size < 0.5 mm and oils were extracted by petroleum ether for 60 min in ultrasound cleaning bath KQ-500DB (Ultrasound instrument Co., Ltd, Kunshan, China). KOH–methanol solution was added to the extract for the fatty acid methyl esters preparation. After centrifugation for 10 min at 4500 rpm, the supernatant of the solution was taken for gas chromatography analysis.

Herbicide resistance test and molecular detection

To detect herbicide resistance in field, the herbicide (13.5% Basta® at a 1:200 dilution) was sprayed on the leaves of hybrid lines, after 5 days the syndrome of phytotoxicity on the leaves were investigated. To confirm the resistance of herbicide in the lab, seeds were sown in culture medium amended with 15 mg/L L-Phosphinothricin (PPT, active ingredient of Basta®) and the growth status of plants was recorded after 10 days. Non-transgenic *B. oleracea* plants were used as negative controls.

Southern blotting analysis was conducted as follows: 25 µg of total genomic DNA was digested

completely with EcoR I and electrophoretically separated on 0.8% agarose gel. For probe labeling and detection, a digoxin labeling kit was used (Roche Diagnostics, Swiss). A PCR-generated 0.5-kb fragment of the *bar* gene was used as the template for probe labeling.

Real-time quantitative PCR was performed on a Roche LightCycler480 system by using the SYBR green PCR kit (TOYOBO, Japan). Based on previous research, gene *CruA* (Accession No. X14555) was selected as the endogenous reference according to previous research (Wu et al. 2010). qPCR was performed according to the following protocol: 10 s denaturation at 95 °C, 15 s annealing at 56 °C, and 20 s elongation at 72 °C in 42 cycles. Specific primers for the *bar* gene were the following: qbarF: 5'-ACAAGCACGGTCAACTTCC-3'; qbarR: 5'-ACTCGGCCGTCCAGTTGGTA-3'. Data were analyzed in triplicate using independent cDNA samples.

Assessment of resistance to *Alternaria brassicae*

Thirty-eight 4-week-old hybrid lines and ten each of the parents were analyzed for resistance to *A. brassicae* according to a previously described protocol, with minor modifications (Scholze et al. 2010). Briefly, *A. brassicae* was cultivated on the vegetable juice medium at 20 °C without light. The conidia were then suspended in sterile water and adjusted to approximately 7×10^4 spores/mL. Plants were inoculated with the conidia suspension and then incubated for approximately 6 days. The disease severity was assessed on a scale of 0–8, which was modified according to Hansen and Earle (1997) with 0 showing no disease symptoms, 1–2 indicating high resistance, 3–4 moderate resistance, 5–7 increasing susceptibility and 8 was a completely dead plant. The tests were repeated three times.

Results

Intergeneric sexual hybridization between transgenic *B. oleracea* and *S. alba*

In the glass house, the maternal non-transgenic *S. alba* plants were pollinated by the transgenic *B. oleracea* plants. A total of 410 ovaries were obtained, which were then further grew on MS medium. After

cultivation in vitro for 40 days, 17 embryos were rescued, which later grew into 13 putative intergeneric hybrid seedlings (Fig. 1). The estimated success rate was very low (3.17%, Table 1), indicating that it is difficult but possible to develop intergeneric hybrid between *S. alba* and *B. oleracea* by sexual hybridization. The seedlings were transplanted into the field, where 11 out of 13 F₁ plants survived (JF2601–2611, Table 2).

The Reciprocal crosses (i.e. *B. oleracea-bar* × *S. alba*) were also made, which yielded only 152 ovaries, mainly due to a lower fertilization frequency. Similarly, the post-pollinated ovaries were cultured on MS media. This procedure generated only 6 embryos and finally 2 hybrid seedlings. The percentage of seedling obtained was extremely low (1.32%, Table 1), meaning that it is even more difficult to generate intergeneric hybrid by using *B. oleracea* as female parent. Unfortunately, these two F₁ hybrid seedlings were both vitrified during media cultivation and could not grow into mature plants for further analysis.

Moreover, approximately 600 sexual crosses (both direct and reciprocal) were also performed under field conditions (without embryo rescue) in the Transgenic Experimental Station, but no seed setting was observed in any of the crosses, indicating that natural occurrence of intergeneric hybrid is unlikely to happen between *B. oleracea-bar* and *S. alba*, at least for the accessions tested in this study.

Transgene detection

To determine whether the *bar* gene has been incorporated into the intergeneric hybrid, PCR assays were performed using genomic DNAs from each of the 11 F₁ hybrid plants obtained. Compared with the result for the positive control, only one *bar* positive line (JF2608) was detected that had the same single DNA band (Fig. 2a), indicating that the frequency of intergeneric hybrid with *bar* gene was 9.1%. The results of Southern blot analysis indicated that only one copy of the exogenous *bar* gene inserted into the male parent *B. oleracea* genome and confirmed that the *bar* gene had been integrated into the genome of hybrid line JF2608 (Fig. 2b). Real-time quantitative PCR assay was further performed to quantified the expression level of *bar* gene, which revealed that it was highly expressed only in samples of the male parent *B. oleracea* and the positive line JF2608

Fig. 1 F₁ hybrid plant produced from intergeneric cross between *S. alba* × *B. oleracea-bar*. **a** Germination of ovules from *S. alba* × *B. oleracea-bar* cultured on MS media. **b** Seedling of *S. alba* × *B. oleracea-bar* cultured on MS media

supplemented with 0.2 mg/L NAA. **c** Phenotype of F₁ hybrid plant at flowering stage, which was treated with 0.01% colchicine for chromosomes doubling

Table 1 Frequencies of intergeneric hybrid between *S. alba* and *B. oleracea-bar* by sexual crossing and embryo rescue

Crosses	No. post inseminated ovaries	No. embryos obtained	No. seedlings	Percentage (%) ^b
<i>S. Alba</i> × <i>B. oleracea-bar</i> ^a	410	17	13	3.17
<i>B. oleracea-bar</i> × <i>S. alba</i>	152	6	2	1.32

^a*B. oleracea-bar* represents transgenic lines with *bar* gene

^bFrequencies of embryo obtained equal to the number of embryo obtained/number of ovary cultured × 100%, while hybrid production rate is equivalent to hybrid plantlets/number of ovary cultured × 100%

Table 2 Chromosome numbers and pollen stainability of F₁ intergeneric hybrid plants from *S. alba* × *B. oleracea-bar*

Plant code	No. chromosomes	No. samples	No. pollens	% Pollen stainability (mean ± SD)
JF2601	30	2	655	23.65 ± 10.89
JF2602	21	3	–	Sterile
JF2603	21	2	–	Sterile
JF2604	21	3	–	Sterile
JF2605	ND ^a	2	744	3.57 ± 2.27
JF2606	21	1	–	Sterile
JF2607	21	3	–	Sterile
JF2608	30	4	783	40.26 ± 11.22
JF2609	21	2	–	Sterile
JF2610	21	2	777	9.36 ± 3.60
JF2611	21	3	–	Sterile
<i>B. oleracea-bar</i>	18	3	969	98.87 ± 15.49
<i>S. alba</i>	24	3	864	91.35 ± 11.26

^aNot determined. The exact number of chromosomes was unknown due to poor GISH result

Fig. 2 Molecular characterization of the intergeneric F_1 hybrids. **a** PCR assay of *bar* gene in F_1 hybrids of *S. alba* $\times$ *B. oleracea-bar*; *M* marker, lane 1 negative control, lane 2 positive control, lane 3–13 F_1 plants of *S. alba* $\times$ *B. oleracea-bar*; among these, lane 5 indicates JF2608. **b** Southern blot assay of F_1 hybrid plants; a fragment of *bar* gene, generated by PCR

amplification of pCambia3300, was used as the probe (0.5 kb); *M* plasmid pCambia3300 as positive control, lane 1 negative control (non-transgenic *B. oleracea* cv. 'Zhonghua Jielan'), lane 2 the genomic DNA of hybrid JF2608, lane 3 genomic DNA of transgenic *B. oleracea*

Fig. 3 qRT-PCR analysis of *bar* gene in F_1 hybrid lines. P_1 *S. alba*, P_2 *B. oleracea-bar*, lanes 3–13 indicate hybrid lines JF2601 to JF2611, respectively (see also in Table 2)

(Fig. 3). Finally, when all hybrid lines were sprayed with herbicide (13.5% Basta at a 1:200 dilution), only JF2608 showed a resistance to herbicide, which was consistent with the PCR test and Southern blot analysis. Thus, the combination of results showed that the *bar* gene from transgenic *B. oleracea* was successfully integrated into the genome of the intergeneric hybrid; why the frequency was so low (given homozygosity of the donor) is not clear at the moment.

Cytological observations

Chromosome counting was performed using flower buds on 11 F_1 hybrid plants that could grow until flowering stage (Table 2; Fig. 4). As expected, 8 F_1 plants (JF2602–2604, JF2606–2607, and JF2609–2611) had 21 chromosomes, with 12 chromosomes from the *S* genome and 9 from the *C* genome (Fig. 4j). These plants were possibly the result of a failure of chromosome doubling induced by

colchicine because they possessed the exact number of chromosomes equal to the sum of the two parental species. Intriguingly, two F_1 hybrid plants (JF2601 and JF2608) showed 30 chromosomes, with 12 chromosomes from the *S* genome and 18 from the *C* genome (Fig. 4e). This result was unexpected and indicated that these plants had the entire sets of parental CC genomes, which might be caused by the generation of unreduced male gametes. In addition, one plant (JF2605) was failed to determine the chromosome constitution due to poor GISH result.

Pollen viability

Pollen fertility was also examined in the F_1 intergeneric hybrid plants. The pollen fertility of the 11 F_1 hybrid plants varied greatly, ranging from 0% (completely male sterile) to 40% (partially fertile (Table 2). Compared with the pollen fertility of the male parent *B. oleracea* (99%), most of the F_1 hybrid plants showing 21 chromosomes had a completely abortive phenotype (Table 2; Fig. 4h). However, notably, the *bar* gene positive line (JF2608, with 30 chromosomes) had higher pollen fertility than that of any other hybrid plants (Table 2; Fig. 4c).

Then, the semi-fertile plants (i.e. JF2601, 2605, 2608, and 2610) were further self-pollinated to generate F_2 seeds. A total number of 0, 2, 26 and 5 seeds respectively, were obtained from the above four F_1 plants, although some of them were poorly developed. The extremely low seed-setting rates indicated that there may exist self-incompatibility or pre-fertilization barriers.

Fig. 4 Morphological and cytogenetic characterizations of hybrid plants. **a–e** Morphology of *S. alba* × *B. oleracea-bar* F₁ hybrid with genome CCS = 30; *I* *S. alba*, *II* Hybrid, *III* *B. oleracea-bar*. **a** Leaves, **b** Siliques, **c** Flowers, **d** Whole plants at flowering stage, **e** Identification of mitotic spread of semi-fertile F₁ plants with 30 chromosomes consisting of 18 C chromosomes from *B. oleracea-bar* (green) and 12 S chromosomes from *S.*

alba (red or pale-red) by genomic in situ hybridization (GISH); **f–j** Morphology of *S. alba* × *B. oleracea-bar* F₁ hybrid with CS = 21. *I* *S. alba*, *II* Hybrid, *III* *B. oleracea*. **f** Seedling, **g** leaves, **h** flowers, **i** buds and flowers, **j** GISH for the sterile F₁ plants with 21 chromosomes consisting of 9 C chromosomes (Blue) and 12 S chromosomes (red)

Morphology of F₁ hybrids

All F₁ hybrid plants had considerable morphological features intermediate between the two parents during the process of development, which indicated that the phenotypic characters from the parents were inherited (Fig. 4).

The leaf of the hybrid plants was intermediate between those of the parents. The lower part of the hybrid leaf was similar to that of *S. alba*, with deeply pinnate lobes. However, the upper portion of the leaf was lanceolate and more similar to the leaf shape of *B. oleracea*. Additionally, trichomes were present on the leaf petioles and stem, which are not commonly found in *B. oleracea* (Fig. 4a, g).

The flowers also showed patterns similar to those of the parents. The flower buds of F₁ hybrid plants were numerous and widely spaced on the raceme, similar to those of *S. alba* but smaller than those of *B. oleracea*.

Compared with the flowers of the female parent *S. alba*, the flowers of F₁ hybrids were different colors, mostly white or milky (Fig. 4c, d, h, i). Moreover, notably, compared with the flowering time of either parent, the flowering time of F₁ hybrids was slightly earlier and longer than those of the parents.

The silique of F₁ hybrid plants was intermediate in length (Fig. 4b). Additionally, mature hybrid plants had more branches than either parent. Of note, although the genome sets were different, characters such as the shape of the leaf, stem, flower and silique of the F₁ hybrid plants with 21 chromosomes resembled those of the plants with 30 chromosomes (Fig. 4). The combination of the above observations indicated that the hybrid plants inherited characters of both parents effectively.

Agronomic trait performance

The F₂ seeds of JF2605, JF2608, and JF2610 were first sown in pots in greenhouse but only JF2608 could germinated. A total of five seedlings from JF2608 were obtained and pricked out to the field. At flowering stage, the JF2608 F₂ plants were bagged to produce F₃ seeds. Only two plants (JF2608-2, JF2608-5) could produce enough seeds (> 1.0 g) for the analysis of oil content and seed weight. Economically important agronomic traits were recorded at mature stage.

Each hybrid silique had from 2 to 6 seeds, with a mean seed size of 2.03 mm. The average seed size was 2.41 and 1.86 mm for *S. alba* and *B. oleracea*, respectively (Table 3). The results were similar for the thousand-seed weight and oil content of the F₂ plants and their parents. The hybrid had an average 1000-seed weight of 4.27 g, which was heavier than that of *B. oleracea* at 3.62 g but markedly lighter than that of *S. alba* at 5.59 g (Table 3). The seed oil content in hybrid was low (29.2%) close to the mean of its two parents (31%), indicating that there was no heterosis for this trait. However, hybrid vigor was observed for plant height. Compared with both parents, the hybrid plants were 19–63% taller, reaching a height of 74 cm (Table 3).

Alternaria brassicae resistance analysis

The disease assessment of JF2608 F₃ hybrid plants and the two parents inoculated with the pathogen for 6 days was shown in Fig. 5. Among the 38 JF2608 F₃ hybrid plants (14 derived from JF2608-2 and 24 from JF2608-5), almost half showed high resistance to *A. brassicae* (rating of 1–2), similar to that of the female parent *S. alba*. Some other lines had moderate resistance (rating of 3–4), while the remaining 4 lines

Fig. 5 Evaluation of *A. brassicae* resistance. Thirty eight F₃ hybrid plants (derived from two F₂ plants) and 10 plants of each parent were used to test the resistance to fungal pathogen *A. brassicae*. Scale 0 complete resistance, Scale 1–2 high resistance, Scale 3–4 moderate resistance, Scale 5–7 susceptibility; Scale 8, complete susceptibility

showed apparent susceptibility, with symptoms similar to those of the *B. oleracea* parent. For the F₃ hybrid plants, the average rating of disease severity was 3.1, whereas the rating was 1.4 and 6.4 for the female parents *S. alba* and *B. oleracea*, respectively (Fig. 5).

Discussion

By distant sexual hybridization coupling with embryo rescue, transgenic intergeneric hybrids between *S. alba* and *B. oleracea*-bar were obtained for the first time. The hybridity could be confirmed by both cytological investigation and morphological observation. Out of 410 pollinations, 13 F₁ hybrid seedlings were obtained from the direct cross '*S. alba* × *B. oleracea*', representing ~ 3% of the entire number of rescued ovaries. The frequency of the reciprocal crosses was much lower (Table 1), indicating that true hybrids would be more easily obtained with *S. alba* as the maternal parent. Our observation was consistent with the results from Wei et al. (2007) who

Table 3 Comparisons of agronomic traits in intergeneric hybrid F₂ plants and their parental lines

	Seed size (mm)	1000 seed weight (g)	Oil content (%)	Mature plant height (cm) (mean ± SD)
<i>S. alba</i>	2.41	5.59	25.3	62.1 ± 9.70
<i>B. oleracea</i> -bar	1.86	3.62	36.6	45.4 ± 7.60
<i>S. alba</i> × <i>B. oleracea</i> -bar (JF2608 F ₂)	2.03	4.27	29.2	74.2 ± 11.84

used non-transgenic *B. oleracea* and *S. alba* as plant materials. A pre-fertilization barrier might be the primary explanation for the lower success rate, as demonstrated by the limited growth of *B. carinata* pollen tubes in *B. oleracea* (Dey et al. 2015). However, our finding was contradict to those of Momotaz et al. (1998) who concluded that hybrids could be obtained from the combination of *B. oleracea* var. *alboglabra* $\times$ *S. alba* but not vice versa. This contradiction could be either due to different genotypes they used, or limited number of buds (only 33 ~ 45) they had pollinated. Generally, hybridizations are more successful when the maternal parent has a higher chromosome number (Justin and Schemske 1998; Cheung et al. 2015).

Theoretically, the somatic number of chromosomes of intergeneric sexual F_1 hybrid between *S. alba* and *B. oleracea* should be 21, or 42 if doubled. Indeed, our cytological investigations indicated that most of the F_1 hybrid plants had the expected 21 chromosomes (one set each of C and S chromosomes, Table 2), confirming the same observation by Wei et al. (2007) in non-transgenic *S. alba* $\times$ *B. oleracea*. However, we also obtained a few unexpected plants with 30 chromosomes, consisting of one S chromosome set and two C chromosome sets. These findings were consistent with those of previous research (Inomata 2002; Wei et al. 2007). Based on these results, we deduced that these plants were generated by unreduced male gametes from parental *B. oleracea*. Although the occurrence of unreduced male or female gametes is extremely low, this phenomenon has been reported in the family Brassicaceae (Ayotte et al. 1988; Fominaya et al. 2005). An alternative possibility is that the chromosome set was partially doubled by colchicine treatment (namely only C chromosome set was doubled).

We also observed the association between pollen viability and chromosome constitution of the intergeneric hybrid. Almost all F_1 plants with 21 chromosomes were highly male sterile, while those plants with more chromosomes tended to have higher level of pollen viability (Table 2). Male fertility could be further improved by backcrossing with *B. oleracea* (Wei et al. 2007), although Hansen and Earle (1997) argued that chromosome doubling should not help.

The generation of disease (*A. brassicae*) and herbicide (Bastar) tolerant intergeneric hybrids were of great interest, since they could be used as valuable intermediate materials to transfer genes from *S. alba* to

B. oleracea or *B. napus* via resynthesis of *B. napus* with *B. oleracea-bar* and *B. rapa*. The existence of *bar* gene will greatly facilitate the identification of hybridity and transfer of valuable traits into other genotypes. Basically, all F_1 offspring should carry a *bar* gene and thus was transgenic positive, if a homozygous *B. oleracea-bar* was used as parent. However in this study, based on both the PCR assay and Southern blot analysis, only one out of 11 hybrid F_1 was showed to be transgenic positive; therefore, the efficiency of inheritance of *bar* gene from parent to F_1 hybrids (~ 10%) was much lower than expected. In addition, our previous work showed that almost half the total offspring were transgenic positive in the hybridization of *B. oleracea-bar* $\times$ *B. rapa* (Li et al. 2006). The low proportion of transgenic plants in the hybrids described here is surprising. Low transmission rate of *bar* to subsequent might be partially a consequence of genome incompatibility between the parents *S. alba* and *B. oleracea* (Hansen and Earle 1997; Halfhill et al. 2002) or perhaps some gene silencing mechanism operating in an intergeneric hybrids.

Authors' contribution XF and GL conceived and designed the study. JL, CZ, CG, JL, LL, RL, and WW participated in the experiments. LJ and GL analyzed the data. JL and GL bred the material. All authors drafted the manuscript and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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