Intertribal crosses between *Brassica* species and *Capsella bursa-pastoris* for the improvement of oil quality and resistance to *Sclerotinia sclerotiorum* of *Brassica* crops

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Abstract:

Capsella bursa-pastoris is natural double-low germplasm and is highly resistant to *Sclerotinia sclerotiorum*. Intertribal crosses were made between two *Brassica* species (*B. napus*, 2n=38; *B. rapa*, 2n=20) and *C. bursa-pastoris* (2n=32) by a normal crossing procedure and embryo rescue, in order to improve the oil quality and resistance to *S. sclerotiorum* of *Brassica* crops. All F_1 plants except two *B. napus* haploids and three plants with 2n=29 were euploids with the same chromosome numbers as their female parents. Only when F_1 plants were intermediate between the parents, the others were resembled the female parent-type predominantly. Among three F_1 plants showing white petals of *C. bursa-pastoris*, one (2n=27-29) was from cross with *B. rapa*, two (2n=38) from crosses with *B. napus*. One to two chromosomes of *C. bursa-pastoris* were detected in PMCs of a few F_1 plants by genomic *in situ* hybridization (GISH), together with chromosome segments in ovary cells and PMCs of many F_1 plants. Amplifed fragment length polymorphisms (AFLP) bands of the male parent were found in F_1 plants, even in haploids. In some progenies, the oil quality and resistance to *S. sclerotiorum* was improved remarkablely. The mechanisms behind these results were discussed.

Key words: AFLP, *Brassica* species, *C. bursa-pastoris*, GISH, intertribal cross, oil quality, *S.sclerotiorum*

Introduction

Wide hybridization plays an important role in plant breeding and is considered to be a shortcut of introgression. It has been used successfully to transfer desired traits from wild species to cultivated species in a large number of species. *C. bursa-pastoris* has been used traditionally as vegetable and medicinal plant in China and some other countries (Zhou, 1987). It is natural double-low germplasm (0.68% erucic acid and 15.68 μmol glucosinolate /g). It is highly resistant to *A. brassicae* (Sigareva & Earle, 1999) and to *S. sclerotiorum* (in present study). So *C. bursa-pastoris* is a wild species with agronomic important traits potentially useful for introgression into *Brassica* crops. Intertribal somatic hybrids between *C. bursa-pastoris* and *B. oleracea* have been obtained and grown to flowering, but no progenies produced because of poor fertility (Sigareva & Earle, 1999). The present paper reports the first production of sexual hybrids between *Brassica* species and *C. bursa-pastoris*.

Materials and methods

Plant material and crosses: Cultivars used in the present study are *B. rapa* cv. Aijuehuang and *B. napus* cvs. Oro (the first *B. napus* cultivar with low content of erucic acid in the world), Huashuang No. 3 (double-low cultivar), Zhongyou 821 (with high content of erucic acid and glucosinolate, but high yield and resistance to *S. sclerotiorum*). *B. rapa* and *B. napus* had yellow petals and black seeds, while *C. bursa-pastoris* had little deep-green leaves, basal clustering branches, short plant, white petals and very small brown seeds. The crosses were performed in the fields by hand emasculation and pollination.

Cytological methods and GISH analyses: The cytological observation was made according to the methods of Li et al. (1995). GISH was performed according to the methods of Hua et al. (2006)

AFLP analysis: AFLP fingerprints were generated based on the protocol of Vos et al. (1995), and DNA bands were visualized by silver staining (Bassam et al., 1991). The bands with 80–800 bp were scored.

Fatty acid and glucosinolate analysis: Seed oil was extracted and the composition was analyzed on gas chromatography machine (HP 6890, Germany). The glucosinolate content was determined on near-infrared reflectance spectroscopy (Vector 22/N, Germzany).

Culture of *S. sclerotiorum* and infection: Fungal mycelia were cultured on solid Potato/Dextrose/Agar medium. Leaves excised from plants at the 9 to 12 leaf stage were inoculated according to the method of Zhao and Meng (2003). Plants in the field were inoculated 3 weeks before harvest according to the methods of Li et al. (2004).

Results

Crossability and phenotypes of F_1 plants

B. rapa × *C. bursa-pastoris*: After 7513 pollinations, 185 F_1 plants were produced (0.025seeds/silique) and 14 distinguished by their phenotypes and cytology and grouped by their chromosome numbers in two types, type I (one plant,

with 2n=27-29), type II (13 plants, with 2n=20). The plant of type I was morphologically intermediate between the two parents by expressing several traits of male origin, such as little deep-green leaves, nanism, basal clustering branches and white petals. Some plants exhibited its purple petiole and cleft leaves. Two plants had yellow seeds, similar to *C. bursa-pastoris*. Except plant of type I, all F_1 plants with variations of pollen fertility (47.6%-98.4%) had good seed-set after selfing. The plant of type I had very poor fertility for pollen (16.7%) and female, for no seeds were obtained after selfing and only several seeds after pollination by the female parent.

B. napus × *C. bursa-pastoris*: From 9248 pollinations, 169 F_1 plants obtained (0.018seeds/silique) and 22 selected and grouped into three types, type III (two plants, with 2n=19), type IV (three plants, with 2n=29), type V (17 plants, with 2n=38). Most of them resembled the female parent-type predominantly, only a few were intermediate between the parents and had some traits of *C. bursa-pastoris*, as cross with *B. rapa*. Plants of type III and IV had poor pollen fertility with no seeds produced after selfing and only one to three seeds in a silique after pollination by the female parent. Except one plant, all plants of type V had good seed-set after selfing. Four plants had red brown or yellow brown seeds; two had smaller seeds.

Cytogenetic and GISH analyses of F_1 plants

B. rapa × *C. bursa-pastoris* : 2n=29 was most frequent (85.3%) in ovary cells of plant type I. Majority of its PMCs at diakinesis had 13 II + 3 or 4 I, 14 II + 3 or 4 I, however, the chromosomes of PMCs at A I were 28-36, though 2n= 29 was still most frequent and 2n>29 appeared in 64.5% cells. One to five laggards were observed in most of A I PMCs. GISH investigations showed that one chromosomal arm in most ovary cells of the plant was fully covered by signals of the *C. bursa-pastoris* probe. One chromosome was fully labeled in many A I PMCs with various chromosome numbers, and two chromosomes in a few PMCs.

B. napus × *C. bursa-pastoris*: In plants of type IV, 60.5% PMCs at diakinesis had 1 III + 9 II + 8 I, the remaining had 10 II + 9 I. The chromosome pairing and segregation were normal in PMCs of type V. Only some chromosomal segments in ovary cells and PMCs of some plants were labeled by the probe of *C. bursa-pastoris*.

AFLP analyses of F_1 plants: The randomly selected fifteen pairs of AFLP primers displayed polymorphisms in the hybrids. Three kinds of bands, i.e., specific for *C. bursa-pastoris*, novel for two parents and deleted for *Brassica* species were detected in all F_1 plants except for three plants which had no specific bands, and the respective numbers of individual plants were 0~28, 39~168 and 32~80 for the cross with *B. rapa* and 0~11, 25~65 and 23~60 for the cross with *B. napus*. The numbers of the specific (28) and novel (168) bands of plant type I were the highest among F_1 plants with the percentage (55.9%) of polymorphic bands. The percentages of polymorphic bands in plants from the cross with *B. rapa* were all over 30% expect for one plant, being higher than in plants from cross with *B. napus* (about 20%). Loci of *C. bursa-pastoris* in F_1 plants were various (0-5.9%), in general the introgressions in cross with *B. rapa* were higher than with *B. napus*.

Fatty acid composition and glucosinolate content of F_1 plants and progenies

B. rapa × *C. bursa-pastoris* : The content of erucic acid was reduced at different levels (from 51.7% of *B. rapa* to 8.58%-37.78%) in F_1 plants of type II, and the contents of glucosinolate in seeds also decreased from 116.57 μmol/g of *B. rapa* to 41.53-85.39 μmol/g. However, none reached the level of *C. bursa-pastoris*. Most profiles of the selfed seeds of F_2 plants from one F_1 plant were similar to each other and to those of their F_1 plants.

B. napus × *C. bursa-pastoris* : The obvious changes of fatty acid profiles from those of *B. napus* parents were observed in some F_1 plants and progenies of type V. For the cross with *B. napus* cv. Oro, the content of glucosinolate was reduced in most F_1 plants and progenies, some ⩽30μmol/g. For the cross with Huashuang No. 3, the fatty acid profiles were nearly the same as those of female, and the content of glucosinolate was little lower in some F_1 plants and progenies. For the cross with Zhongyou 821, the contents of erucic acid and glucosinolate in most of F_1 plants were reduced remarkably, and one plant reached double-low standard. The content of glucosinolate of some F_2 plants was obviously deviated from those of F_1 plants, but erucic acid not.

Resistance to *Sclerotinia sclerotiorum* in progenies: Some lines (F_2 or F_3) derived from minority of F_1 plants showed significant higher resistance to *S. sclerotiorum* than female parents, there was a highly positive correlation between the results of leaf and stem infections. For the cross with *B. rapa*, some lines derived from four F_1 plants showed significantly ($P \leqslant 0.05$) lower damage on leaves and stems than female parent. For cross with *B. napus* cv. Oro, some lines derived from six F_1 plants showed significantly ($P \leqslant 0.05$) lower damage on leaves and stems than female parent. For crosses with Huashuang No. 3 and Zhongyou 821, only several lines showed significantly lower damage than female parents, probably due to the high resistance of female parents.

Discussion

Progenies from the intertribal sexual hybridizations between *Brassica* species and *C. bursa-pastoris* were examined for morphology, cytology and molecular characteristics, which enabled us to determine the hybridity status of each plant and to quantify the level of hybridization occurrence in these crosses.

Plant of type I from cross with *B. rapa* expressing more morphological characters of the male *C. bursa-pastoris* was a mixoploid with 2n=27-29 in somatic cells, while some PMCs had more chromosomes (28-36). GISH analysis demonstrated that only one or two *C. bursa-pastoris* chromosomes were included and chromosomal fragments translocated in partial cells. These results suggested that the hybrid cells (2n=26) underwent chromosome doubling one or two times during mitotic divisions of the zygotes or plants, eliminating of most male chromosomes, and extra duplicating of partial chromosomes

during meiotic synthesis phase. The similar results were observed in intergeneric cross between *B. rapa* and *O. violaceus*, where the genome doubling and successive elimination of *O. violaceus* chromosomes accompanied by the fragment recombination and introgression were responsible for producing *B. rapa*-type plants with modified genetic constitutions and phenotypes (Liu & Li, 2006). This mechanism would be valid for the explanation of the present results. AFLP analysis performed on 474 loci for plant type I indicated it contained 5.9% DNA fragments putatively derived from *C. bursa-pastoris*, however, 55.9% genomic loci were changed, suggesting that other reasons were also involved in these genomic variations. Extensive alteration in DNA methylation patterns, some mobile genetic elements, rapid sequence elimination in hybrids, and genomic rearrangements in the hybrids (Shaked et al., 2001) were the causes for the genomic variations.

Matroclinous plants of type II and V could be due to the complete elimination of the *C. bursa-pastoris* chromosomes but the introgression of its fragments and chromosome doubling. Plants of type IV had 2n=29 and PMCs at diakinesis had 10 II + 9 I and 1 III + 9 II + 8 I, suggesting that the genome of these plants consisted of 10 duplicated and 9 individual chromosomes. Cheng et al. (2002) obtained this kind of plants from *B. napus* × *O. violaceus* with the similar results of cytology and molecular markers. According to their pairing configurations, their genomic constitution was proposed as 20A + 9C and one C genome was lost from the complement of *B. napus*. One possible reason for this could be attributed to the dominance of rRNA genes from the two ancestors of *B. napus*, for the hierarchy of rRNA gene transcriptional dominance is *B. rapa* >*B. oleracea* and *B. rapa* rRNA transcripts are readily detected in natural *B. napus*, but *B. oleracea* not (Chen & Pikaard, 1997).

The combination of cytological and molecular techniques was successful to determine the chromosomal / genomic constitutions of partial / introgressive *Brassica* hybrids (Cheng et al., 2002; Hua et al., 2006; Liu & Li, 2006). In present study, the application of GISH and AFLP techniques better characterized the intertribal hybrids with very limited amount of alien genetic elements. In conclusion, the introgressive hybrids (type II and V) provided an opportunity to rapidly and successfully introduce useful traits of *C. bursa-pastoris* into *Brassica* species and to produce lines with better oil quality and higher resistance to *S. sclerotiorum*.

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