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Introgressing Cold-tolerant Ogura Cytoplasm from Rapeseed into Pak Choi and Chinese Cabbage

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Abstract. Cold-tolerant, Ogura male-sterile, somatic hybrid rapeseed *(Brassica napus* L.) lines were used as maternal parents in two independent introgression experiments. In one experiment, an atrazine-sensitive *B. napus* (aacc) somatic hybrid was crossed directly with a male-fertile pak choi *(B. campestris* Chinensis Group, aa) accession. Allotriploid (sac) progeny were then backcrossed to the recurrent pak choi parent. Forty-five percent of the progeny from the first backcross were determined to be diploids (aa). In the other experiment, an atrazine-resistant *B. napus* somatic hybrid was crossed first to a bridge line. Three additional backcross generations to Chinese cabbage *(B. campestris* Pekinensis Group) resulted in Chinese cabbage resistant to black rot *(Xanthomonas campestris* pv. campestris). These materials may be useful for production of *B. campestris* hybrid vegetable seed.

Cytoplasmic male sterility (ems) has contributed to efficient production of F_1 hybrid seed in many crops (Hanson and Conde, 1985) and shows promise for *Brassica* breeding. The "Ogura" male-sterile cytoplasm was discovered in a variety of Japanese radish *(Raphanus sativus* L.; *n* = 9) (Ogura, 1968) and was subsequently transferred to *B. oleracea* Botrytis Group *(n* = 9) and *B. napus (n* = 19) by backcrossing (Bannerot et al., 1974). Three problems existed in the initial *B. napus* material: 1) low-temperature chlorosis; 2) low nectar production; and 3) lack of good male fertility restoration (Rousselle, 1982). The low-temperature chlorosis is probably caused by an incompatibility between the *Raphanus* chloroplasts and the *Brassica* nucleus (Jarl et al., 1989). The chlorosiscan lead to a reduction in seed yield, making such material unacceptable.

Problems with low-temperature chlorosis and poor nectary development have been circumvented through protoplasm fusion. *Brassica napus* plants with *Brassica* rather than *Raphanus* choroplasts were produced by fusion and exhibited no low-temperature chlorosis (cold tolerance). Fusion strategies included 1) resynthesis of *B. napus* by combining Ogura male-sterile *B. oleracea* with either atrazine-resistant *B. campestris* (Jourdan et al., 1989) or atrazine-sensitive *B. campestris* (Heather, 1993); and 2) the production of *B. napus* cybrids (e.g., Pelletier et al., 1983; Stephenson, 1991).

Transfer of traits between *B. napus* (aacc) and *B. campestris* (aa) is often done by introgression. In Japan, such introgression is a standard rapeseed breeding technique (Shiga, 1970). Because *B. campestris* is generally recalcitrant in culture (Jourdan and Earle, 1989), introgression of traits into *B. campestris* is more efficient than protoplasm fusion. The objectives of this study were to 1) transfer cold-tolerant Ogura cms from somatic hybrid *B. napus* lines into pak choi and Chinese cabbage; and 2) incorporate black-rot resistance through introgression of selected *B. napus* and *B. campestris* lines with Chinese cabbage. In both cases, traits were introgressed from *B. napus* rather than *B. campestris* because only *B. napus* materials with the desired cytoplasm were available.

Materials and Methods

Plant materials and culture. Cold-tolerant, male-sterile *B. napus* somatic hybrid 903025 (atrazine sensitive) was produced through protoplast fusion of *B. oleracea (cc)* cauliflower line (NY7642A), which carries the Ogura or R1 cms cytoplasm (Dickson, 1985), with *B. campestris* 'Candle' by Le Thi Xuan (Heather, 1993). Cold-tolerant, male-sterile *B. napus* somatic hybrid 893149 (atrazine resistant) was produced through protoplasm fusion of NY7642A with the atrazine-resistant isoline of *B. campestris* 'Candle' (BC_5) by Stephenson (1991). The *B. campestris* 'Candle' seeds were obtained from W. Beversdorf, Univ. of Guelph, Ont., Canada. Pak choi PI 418988 *(n* = 9; aa; origin China) was obtained from the U.S. Dept. of Agriculture's North Central Regional Plant Introduction Station, Ames, Iowa. Black-rot-resistant Chinese cabbage was produced by Guo et al. (1991). Line 15 originated as a rescued embryo from a cross between ***B. oleracea*** and *B. napus* (Quazi, 1988). This line was originally reported to be a hexaploid (aacccc). However, the DNA content of F, seed is the same as *B. napus*, suggesting elimination of the extra c genome. The bridge line was used to improve seed recovery, as direct introgression into Chinese cabbage proved difficult. In this study, all plants received weekly fertilization with a complete soluble fertilizer (15N-16P–17K plus micronutrients) at the rate of 5 g·liter^{-1} and 16 h of supplemental fluorescent light (225 µmol·m^{-2}·s^{-1}).

Introgression. For introgression into pak choi, *B. napus* (aacc) somatic hybrid 903025 was crossed directly with pak choi (aa) PI 418988. All controlled pollinations were made between O to 2 days after anthesis in a greenhouse at a constant 21 ± 3C. Allotriploid (aac) F_1 progeny were then backcrossed to the same pak choi pollen parent, and seed set and ploidy data were recorded. Mean seed set was determined by counting the number of seeds from 20 randomly selected BC_1 pods. Ploidy was based on nuclear DNA content, which was analyzed using flow cytometry (Arumuganathan and Earle, 1991).

In the introgression into Chinese cabbage, *B. napus* (aacc) somatic hybrid 893149 was initially hand-pollinated with bridge line 15 (Quazi, 1988) in a greenhouse. These F, progeny were then open-pollinated with several Chinese cabbage selections carrying black-rot resistance. Two additional backcross generations to the Chinese cabbage selections were carried out in the field using natural pollination vectors.

In both experiments, cold tolerance was assessed by exposing young plants to air at 10C for 2 to 4 weeks. All progeny were nonchlorotic, indicative of the presence of cold-tolerant *Brassica* chloroplasts that were shown to be present in all parents in previous organellar DNA analyses (Heather, 1993; Stephenson, 199 1). Atrazine resistance was assessed in *B. napus* somatic hybrid 893149 by swabbing young true leaves with a 1% atrazine solution. Lack of bleaching indicated the presence of atrazine-resistant *Brassica* chloroplasts, which agreed with a molecular analysis of the progeny (Stephenson, 1991). Black-rot resistance was assessed by pricking young true leaves with a needle dipped in a 2-day-old culture of *Xanthomonas campestris* pv. campestris grown at 28C. The infected plants were incubated for 7 days at 28C. Male sterility was confirmed by microscopic in-

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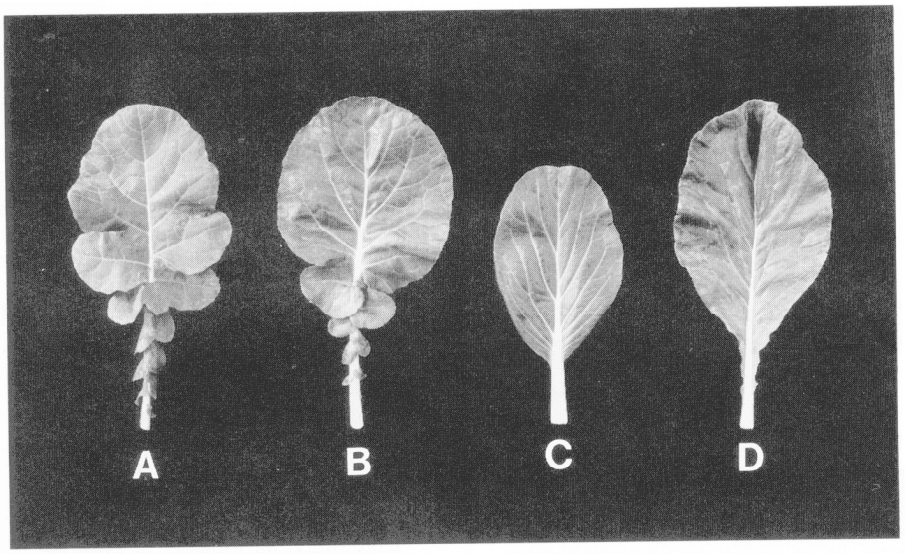

Fig. 1. Mature leaves of **(A)** *Brassica napus* L. parent (somatic hybrid 903025; aacc); **(B)** allotriploid F_1 progeny (sac); **(C)** *B. rapa* Chinensis Group (PI 41 8988; aa); and **(D)** diploid BC_1 progeny (aa).

spection of the anthers, which were short, shriveled, and did not shed pollen. Observations were also made on flower morphology and nectary development. Good nectary development was indicated by beads of nectar exuded at the base of the flowers.

Results and Discussion

Pak choi. When pak choi (aa) was crossed directly with *B. napus* (aacc) somatic hybrid 903025, allotriploid (sac) F_1 progeny were recovered. Leaves of allotriploid plants were morphologically intermediate between the *B. napus* and *B. campestris* Chinensis Group parents (Fig. 1 a-e). Flowers were completely male sterile due to shriveled anthers, which produced no pollen. Other flower parts, including nectaries, were observed to be normal. Diploid *B. campestris* Chinensis Group plants bearing a closer resemblance to the pak choi parent (Fig. 1d) were recovered after a single backcross of the F_1 progeny with pak choi. According to flow cytometry (Table 1), 45% of the BC_1 progeny were diploid, while 35% remained triploid, and 20910 showed reductions in DNA content to levels between triploid and diploid values. The diploid progeny all remained male sterile and cold tolerant. Plants with DNA contents intermediate between diploid and triploid exhibited some abnormal characters, including albino leaf margins, rugose leaf surface, and lack of floral initiation. Allotriploid F_1 progeny produced an average of 11.2 ± 3.3 seeds/pod. Seed set increased significantly in the second backcross generation {[(903025 x 418988) x 418988] × 418988), which produced 15.9 ± 1.8 seeds/pod.

Table 1. Nuclear DNA content of BC_1 progeny from the cross between Ogura male-sterile allotriploids (sac) and pak choi (aa) *Brassica.*

Plants (%)[y]	DNA content[z] (pg/2c ± SE)	Interpretation[x]
7 (35)	1.69 ± 0.03	Triploid (aac)
4 (20)	1.41 ± 0.13	Partial c genome elimination
9 (45)	1.07 ± 0.04	Diploid (aa)

[z]Nuclear DNA content (c = haploid, 2c = diploid) determined by flow cytometry using chicken red blood cells [2.33 picograms (pg)] as an internal control.

[y]Number of plants in BC_1 population and percentage of total population in parentheses.

[x]*Br-assica napus* parent (aacc) of initial cross= 2.32 pg, *B. chinensis* (aa) = 1.09 pg, and F_1 triploids (aac) = 1.71 pg.

Chinese cabbage. For introgression into Chinese cabbage, line 15 (Quazi, 1988) provided a bridge for hand pollinations of *B. napus* somatic hybrid 893149 with Chinese cabbage breeding lines carrying resistance to black rot (Guo et al., 1991). Based on flow cytometric analysis performed on BC_3 plants, an average of 30% of the BC_3 population was allotriploid (sac), while 30% showed partial c genome elimination, and 40% was diploid (aa). Despite the range of ploidy observed, all progeny exhibited normal vegetative and reproductive development with high female fertility. The BC_3 progeny also exhibited stable male sterility with no pollen detected, lack of low-temperature chlorosis, and complete immunity to black rot as evidenced by no lesion formation on infected leaves after 7 days incubation.

In summary, cold-tolerant Ogura cms was successfully incorporated into pak choi and Chinese cabbage from somatic hybrid rapeseed using two strategies; both resulted in rapid transfer of the desired traits with maintenance of female fertility. In the case of introgression into pak choi, flow cytometry allowed rapid identification of aneuploids, increasing breeding efficiency. The Ogura cms system is an attractive method for hybrid seed production in that it appears stable from observations made by us and from previous work (Bartkowiak-Broda et al., 1979). Further, the system is especially useful for all *Brassica* vegetable species because no fertility restoration is required.

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