FUSION-MEDIATED SYNTHESIS OF INTERSPECIFIC BRASSICA OLERACEA/ B. NAPUS HYBRIDS WITH NOVEL MALE-STERILE CYTOPLASMS

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INTRODUCTION

Brassica oleracea comprises many important vegetable crops, for example, cabbage, cauliflower, broccoli and Brussel sprouts. The species tends naturally to be hybrid because of the prevalence of self-incompatibility (SI) genes found within the species. Nevertheless, production of B. oleracea seed that is entirely composed of a desired hybrid is problematic, since the SI conferred by many alleles is incomplete and because maintenance of parental lines requires a method for circumventing the SI. Cytoplasmic male sterility (CMS) as a pollination control mechanism could be used to augment the weaker, more easily maintainable SI genes of B. oleracea and thereby increase the purity of the hybrid seed produced.

CMS has not been extensively investigated in B. oleracea. The Ogura or ogu CMS of radish has been introgressed through sexual crosses into B. oleracea but the ogu CMS B. oleracea plants express chlorosis in seedlings and perform poorly (Bannerot et al. 1977). More recently, protoplast fusion technology has been used to introduce the Polima or pol CMS of B. napus into broccoli (Yarrow et al. 1990). The performance of these plants, however, has not been reported.

We report here the results of fusions of broccoli hypocotyl protoplasts with mesophyll protoplasts of three B. napus lines. Seven plants with unique organelle DNA compositions and phenotypes have been recovered from these experiments. Some of these may prove to be useful sources of CMS for B. oleracea and/or B. napus.

MATERIALS AND METHODS

Plant materials

Brassica oleracea spp. italica cvs. Paragon and Premium Crop (Stokes Seeds Ltd., St. Catharines, Ont.) were used as sources of hypocotyl protoplasts. Three lines of B. napus were used as sources of mesophyll protoplasts: (1) NFP26-6, a Westar line carrying an ogu CMS, triazine-tolerant hybrid cytoplasm (Kao et al. 1991); (2) ogu CMS Westar, carrying the ogu CMS cytoplasm and (3) P, a pol CMS B. napus line. Plants were maintained as described by Kao et al. (1990).

Protoplast isolation and inactivation

Mesophyll protoplasts were isolated according to Kao et al. (1991), except that Driselase was omitted from enzyme solution E27. During enzymatic incubation, the digesting leaf tissue was subjected to 100 Krad of γ-irradiation from a Caesium 137 source.

Hypocotyl protoplasts were isolated as described (Kao et al. 1990). For the purpose of inactivation, a 1% (v/v) protoplast suspension was incubated for 30 min in 1 mM iodoacetic acid (IOA) dissolved in wash solution. Protoplasts were fused as described (Kao et al. 1991) and cultured through to plant regeneration as described (Kao et al. 1990).

Organelle DNA analysis and cytology

Mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) analyses and cytological analysis were conducted as described (Kao et al. 1991).

RESULTS

Plant regeneration

Seventeen shoots regenerated from the fusion experiments and 12 shoots produced roots and survived to maturity. We report on the analyses of 3 plants (OFPI-OFPIII) from experiment 1, 4 plants (OFPV-OFPVIII) from experiment 2 and 1 plant (OFPX) from experiment 3 (Table 1). Morphologically, the regenerated plants resembled the broccoli more than the B. napus fusion partner in that they had short internodes and large, dark green leaves. OFP1 had a central compact inflorescence typical of broccoli while the other plants had semi-compact inflorescences.

Table 1. Characterization of fusion products between Brassica oleracea and B. napus.

Plant	Chromosome number	Male fertility(1)	Stamen morphology	mtDNA	cpDNA
Paragon	18	F	normal	ole	ole
Premium Crop	18	F	normal	ole	ole
NFP26-6	38	S	pistillate	ogu	ctr
ogu CMS Westar	38	S	pistillate	ogu	ogu
P	38	S	short	pol	pol
OFPI(2)	36	F	normal	ole	ole
OFPII	56	S	pistillate	ogu	ctr
OFPIII	56	S	pistillate	ogu	ctr
OFPV(3)	58	S	reduced	ogu/ole(4)	ole
OFPVI	56	S	reduced	ogu/ole	ole
OFPVII	56	S	reduced	ogu/ole	ole
OFPVIII	56	S	degenerate	ogu	ole
OFPX(5)	48,56	S	short	pol/ole(6)	pol

(1) F - fertile, S - sterile
(2) Fusion products from experiment 1 - OFP I, II, III
(3) Fusion products from experiment 2 - OFP V, VI, VII, VIII
(4) Recombinant ogu/ole mtDNA
(5) Fusion product from experiment 3 - OFPX
(6) Recombinant pol/ole mtDNA

Fusion experiment 1

The protoplast fusion experiment employed Paragon and NFP26-6. Regenerant OFPI produced flowers with well-developed anthers that shed abundant pollen, while OFPII and OFPIII produced flowers that showed the ogu pistillate male-sterility that is characteristic of NFP26-6.

Restriction analysis with SalI indicated that the mtDNA of OFPI resembled that of the B. oleracea parent while OFPII and OFPIII had the ogu mtDNA SalI fragment pattern characteristic of NFP26-6. Mitochondrial DNA preparations of NFP26-6 contained a linear DNA plasmid and double-stranded RNAs (Kao et al. 1991). Analysis of undigested mtDNA preparations showed that the dsRNAs but not the DNA plasmid were present in OFPII and OFPIII. This suggests that the mitochondrial genome of NFP26-6 was transferred to OFPII and OFPIII independently of the mtDNA plasmid, as has been observed in other B. napus protoplast fusion experiments (Vedel et al. 1987; Kemble et al. 1988; Rosen et al. 1988). EcoRI analysis of mtDNA was consistent with the SalI analysis.

EcoRI analysis of cpDNA showed that OFPI had the chloroplast genome of B. oleracea (ole) cytoplasm, while OFPII and OFPIII had the chloroplast genome of the ctr cytoplasm. Cytological analysis showed that OFPI had 36 chromosomes while OFPII and OFPIII each had 56 chromosomes.

Fusion experiment 2

The protoplast fusion experiment employed Paragon and ogu CMS Westar. All regenerants expressed male-sterility, but to varying degrees. Stamen development in regenerants OFPV, OFPVI and OFPVII appeared normal in young buds, but arrested upon bud maturation and resulted in small sterile anthers. Stamen development in regenerant OFPVIII was arrested early in bud development.

SalI digests of mtDNA preparations indicated that the mitochondrial genomes of OFPV, OFPVI and OFPVII contained restriction fragments characteristic of each of the fusion partners while OFPVIII had an unaltered ogu mtDNA fragment pattern. OFPV mtDNA had all but 3 of the ole mtDNA fragments, 4 ogu-specific fragments and 2 novel fragments. OFPVI had all but the same 3 ole fragments missing in OFPV, 4 ogu-specific fragments and 2 novel fragments. OFPVII had all but 1 of the ole fragments, 8 ogu-specific fragments and 3 novel fragments.

The presence of restriction fragments characteristic of each fusion partner together with novel fragments not found in either partner is thought to indicate that the mitochondrial genome of a regenerated plant has been formed through recombination of the parental mtDNAs (Belliard et al. 1979; Boeshore et al. 1985). The results of EcoRI and PstI analysis were consistent with the view that OFPV, OFPVI and OFPVII all possessed recombinant mitochondrial genomes. Chloroplast DNA analysis showed that the 4 regenerants had the ole chloroplast genome.

Cytological analysis showed that OFPV had 58 chromosomes while OFPVI, OFPVII and OFPVIII each had 56 chromosomes.

Fusion experiment 3

Regenerant OFPX in the protoplast fusion experiment employing Premium Crop and P was male-sterile and had short stamens that did not produce pollen. SalI digests of the mtDNA showed that OFPX had a fragment pattern that resembled P except it lacked 1 pol-specific band and possessed 1 novel high molecular weight band. OFPX also had double-stranded RNA molecules presumably resulting from the transfer via protoplast fusion from Premium Crop. RNA molecules have been observed in some but not all Premium Crop plants (Kao et al. 1990). Transfer of these RNAs via protoplast fusion experiments in B. napus has been observed (Kemble et al. 1988). In addition, EcoRI analysis of mtDNA showed that OFPX had a fragment pattern resembling the pol mtDNA plus a couple of ole-specific bands. EcoRI analysis of cpDNA showed that OFPX had the pol CMS cpDNA genome.

Plant OFPX was divided into 2 cuttings, having each 56 and 48 chromosomes, respectively. The observation suggests that OFPX was an unstable B. oleracea-B.napus hybrid and partial chromosome elimination occurred.

DISCUSSION

Plants OFPII and OFPIII, regenerated from the first fusion experiment, are judged to be B. oleracea/B. napus somatic hybrids on the basis of their chromosome number and morphological characteristics. They carry the ogu mtDNA and the ctr cpDNA. Correspondingly, the plants have the characteristic ogu CMS flower phenotype and are expected to show tolerance to triazine herbicides. To our knowledge, this experiment is the first example in the Brassica of the use of a previously synthesized cybrid as a fusion partner with protoplasts of a different species. It should be possible to use these plants as bridging genotypes for the development of CMS, triazine tolerant B. oleracea.

The fertile plant OFPI had 36 chromosomes and resembled broccoli and most probably is a tetraploid B. oleracea resulting from a B. oleracea-B. oleracea fusion event.

In the second fusion experiment, regenerants OFPV, OFPVI and OFPVII were found to possess recombinant mtDNAs and regenerant OFPVIII to possess an unaltered ogu mtDNA. The 4 regenerants were cytoplasmic hybrids since they also possessed the ole cpDNAs. As a result, the plants were a dark green color and did not suffer from the chlorosis and poor nectary development that characterize ogu CMS B. oleracea (Bannerot et al. 1977). Pelletier et al. (1983) have previously observed that the replacement of the ogu radish chloroplast with nap chloroplasts in ogu CMS B. napus remedied the incompatibility of the ogu CMS. The plants are most probably B. oleracea/B. napus fusion products judging from their chromosome counts (≈56) and morphological traits.

In the third fusion experiment, plant OFPX was found to possess a rearranged mtDNA and is probably a somatic hybrid containing all or parts of the B. oleracea and B. napus genomes. To our knowledge, this is the first report of the pol CMS mtDNA genome undergoing rearrangement. Previous somatic hybridization studies with the pol CMS mitochondria in B. napus did not detect any mtDNA rearrangements (Chuong et al. 1988; Kemble and Barsby 1988), whereas the ogu CMS mtDNA has been reported to undergo rearrangement (Vedel et al. 1987; Morgan and Maliga 1987; Jourdan et al. 1989).

The regenerants with recombinant mtDNAs may prove useful in identifying the mtDNA determinants that contribute to the Ogura or Polima male sterile phenotypes. Moreover, these plants may represent new and potentially useful forms of CMS. The expression of the CMS trait in B. napus plants should prove particularly interesting. One of these cytoplasms has recently been backcrossed into B. napus and will be further analyzed. Nuclear restoration of male-sterility is required for a workable CMS system in oilseed crops such as B. napus. It may be that the male sterility conferred by these recombinant mtDNAs is altered such that some of the difficulties associated with the development of restorer lines are circumvented. Evidence for mtDNA recombinations associated with easier restoration of the CMS trait has been observed in B. napus nap/ogu cybrids (Pelletier et al. 1988).

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