The transfer of 'Polima' cytoplasmic male sterility from oilseed rape (Brassica napus) to broccoli (B. oleracea) by protoplast fusion

Stephen A. Yarrow, Laurie A. Burnett, Richard P. Wildeman, and Roger J. Kemble

Allelix Crop Technologies, 6850 Goreway Drive, Mississauga, Ontario, L4V 1P1, Canada

Received April 2, 1990/Revised version received June 1, 1990 - Communicated by F. Constabel

ABSTRACT

Protoplast fusion was utilised to transfer Polima type cytoplasmic male sterility (CMS) from Brassica napus, canola cv. Polima Karat (Pol-Karat) to B. oleracea, broccoli, var. "Green Comet". Southern and RFLP analysis confirmed that four cybrids possessed nuclear genomes of broccoli with Polima mitochondria and chloroplasts. A fifth cybrid was a nuclear hybrid between broccoli and Pol-Karat, with Polima mitochondria and chloroplasts of broccoli. The broccoli type cybrids were morphologically similar to "Green Comet", while the hybrid type was an intermediate of the two fusion parents. Flowers on the cybrids were distinctive in that although they possessed a morphology typical of Polima, they had very reduced petals. The broccoli type cybrids exhibited some female fertility, albeit low, establishing potential for F1 hybrid production.

INTRODUCTION

Commercial hybrid production of B. oleracea varieties relies largely on self-incompatibility (SI) systems to ensure high hybridity frequencies in a given seed batch. SI has a number of drawbacks such as breakdown of incompatibility, labour intensiveness (to maintain the inbred lines) and an inherent genetic complexity in the system itself. For these reasons, cytoplasmic male sterility (CMS), which is becoming increasingly popular in the fledging commercial hybrid B. napus canola industry, is seen as an attractive alternative.

B. oleracea and B. napus are not readily cross-compatible, although there have been reports of transferring desirable characters from one species to the other by conventional crossing and embryo rescue techniques [Chiang et al., 1977; Quazi, 1988]. A major disadvantage is that nuclear hybrids are produced, and extensive backcrossing is necessary to return to the normal B. oleracea phenotype. In addition, due to regular maternal inheritance, only whole cytoplasms can be transferred in alloplasmic substitutions. Protoplast fusion, however, offers the advantage of being able to transfer mitochondrial and/or chloroplast populations in one step, without nuclear fusion, and consequently without extensive backcrossing [Barsby et al., 1987a]. The transfer of the chloroplast encoded trait atrazine-resistance, from B. napus to B. oleracea (cauliflower), via fusion, has been described recently [Jourdan et al., 1989]. In this report, the production of cytoplasmic hybrids or cybrids through the transfer of Polima-type CMS from B. napus canola to B. oleracea broccoli is described.

MATERIALS AND METHODS

Plant material

Seed of B. oleracea Broccoli cv. "Green Comet" were obtained from Dominion Seed House, Georgetown, Ontario, Canada. Seed of B. napus (2n = 4x = 38) Polima-Karat (designated POL-Karat) were supplied by Dr. B.R. Stefansson, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada.

Protoplast manipulations

All conditions for plant growth and protoplast isolation were essentially as described by Barsby et al. (1986). POL-Karat protoplasts were isolated from dark-grown hypocotyls. Broccoli protoplasts were isolated from the leaves of 3-4 week-old light-grown seedlings as described by Yarrow et al. (1986). Following overnight (16 h) incubation in enzyme solution, protoplasts were isolated by sieving through cheesecloth and flotation in 0.35 M sucrose.

After isolation, POL-Karat protoplasts were subjected to 40 krad gamma-irradiation from a Cesium 35 source. Broccoli protoplasts were incubated with 3 mM iodoacetic acid (IOA) for 20 min at room temperature [Barsby et al., 1987b].

Equal proportions of broccoli and POL-Karat protoplasts were fused together by a sequential combination of polyethylene glycol and CaCl2 solutions [Barsby et al., 1984].

Offprint requests to: S. A. Yarrow
The density of the fusion-treated mixture was adjusted to 10^6 protoplasts/ml. Unfused controls were also prepared where gamma-irradiated and IOA-treated protoplasts were simply mixed and adjusted to the same density.

Fused and unfused control protoplast populations were cultured as detailed in Barsby et al. (1987b). Regeneration was induced by transferring the colonies to the shoot inducing 'E' and 'F' media of Pelletier et al. (1983). Regenerated shoots were transferred to 'grow-out' inducing medium and, thereafter, to the root induction procedure as described by Barsby et al. (1986).

**Whole plant manipulations**

Regenerated plants were transferred to growth chambers at 23°C, with a 16 h/day photoperiod of 160 μE m^-2 s^-1 of photosynthetically active radiation. Subsequently plants were transferred to the greenhouse for flowering and pollination, as females, with pollen from the original broccoli fusion parent.

**Cytoplasmic DNA analysis**

Chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) were extracted from leaves following the procedure of Kemble (1987). The DNAs were fragmented with Eco RI, subjected to electrophoresis on agarose gels and the organelle genomes classified according to the characteristic restriction fragment length polymorphism (RFLP) patterns produced.

**Total DNA analysis**

Total DNA was prepared from leaves by the procedure of Dellaporta et al. (1983). Restriction endonuclease digestions, agarose gel electrophoresis, transfer of DNA to nitrocellulose filters and hybridizations were performed according to Maniatis et al. (1982). The hybridization probe used was a polymerase chain reaction (PCR) amplified 166 base pair fragment from a conserved region of an acetolactate synthase (ALS) gene of *B. napus*.

**RESULTS**

Three colonies (NO-1, 2 and 3) were produced from the fusion cultured suspensions, but none from the unfused mixed control. Each colony regenerated shoots on the medium described by Pelletier et al. (1983) (Table 1). Earlier attempts at regenerating "Green Comet" protoplast derived colonies on media described for *B. napus* (Barsby et al., 1986) had failed. Southern hybridizations of total DNA (Fig.1.), confirmed that the plants from colonies NO-1 and NO-2 possessed broccoli nuclear DNA, and that the plant from NO-3 was a hybrid between the two parents. The ALS probe was selected because of its ability to distinguish cultivars of *B. napus* and *B. campestris* (Armagil pers comm). These results were reflected somewhat by the morphology of the plants; those from colonies NO-1 and NO-2, were virtually indistinguishable from "Green Comet" broccoli, whereas the NO-3A regenerant, although exhibiting the characteristic bluish, waxy leaf of broccoli, was generally smaller and less vigorous. This NO-3A plant also took longer to flower.

RFLP analysis of organelle DNA (Fig. 2 and Table 1.) revealed that all four shoots regenerated from colony NO-1 had mtDNA and cpDNA banding patterns the same as POL-Karat. The two surviving shoots from colony

<table>
<thead>
<tr>
<th>Colony</th>
<th>Shoot phenotype</th>
<th>Mitochondria/chloroplast type</th>
<th>Nuclear type</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO-1 A</td>
<td>sterile</td>
<td>POL/POL</td>
<td>broccoli</td>
</tr>
<tr>
<td>B</td>
<td>sterile</td>
<td>POL/POL</td>
<td>broccoli</td>
</tr>
<tr>
<td>C</td>
<td>sterile</td>
<td>POL/POL</td>
<td>broccoli</td>
</tr>
<tr>
<td>D</td>
<td>sterile</td>
<td>POL/POL</td>
<td>broccoli</td>
</tr>
<tr>
<td>NO-2 A</td>
<td>fertile</td>
<td>OLE/OLE</td>
<td>broccoli</td>
</tr>
<tr>
<td>B</td>
<td>did not survive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>fertile</td>
<td>OLE/OLE</td>
<td>broccoli</td>
</tr>
<tr>
<td>NO-3 A</td>
<td>sterile</td>
<td>POL/OLE</td>
<td>hybrid</td>
</tr>
</tbody>
</table>

*POL* designates Polima type organelles from *B. napus* and *OLE* designates organelles from *B. oleracea*.

![Figure 1](image-url)  
Analysis of total DNA, digested with Eco RI, electrophoresed on agarose gel, Southern transferred and hybridized with a 166 bp PCR amplified ALS probe. Lane P, standard POL-Karat pattern; lane O, standard "Green Comet" pattern; lane 1, regenerant NO-1A; lane 2, regenerant NO-1B; lane 3, regenerant NO-3A. The numbers at left indicate fragment size in kb of molecular weight markers derived from Hind III digest of lambda DNA.