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Male sterility caused by cytoplasm of *Brassica oxyrrhina* in *B. campestris* and *B. juncea*

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Summary. Synthetic alloploid *Brassica oxyrrhina* (2n = 18, OO) × *B. campestris* (2n = 20, AA) was repeatedly backcrossed with *B. campestris* to place *B. campestris* nucleus in the cytoplasm of *B. oxyrrhina*. Alloplasmic plants, obtained in BC_5 generation, were stably male sterile but mildly chlorotic during initial development. Synthetic alloploid *B. oxyrrhina-campestris* was also hybridized with *B. juncea* to transfer *B. oxyrrhina* cytoplasm. Segregation for green and chlorotic plants was observed in BC_1 and BC_2 generations. By selection, however, normal green male sterile *B. juncea* was obtained in BC_3. Pollen abortion in both *B. campestris* and *B. juncea* is post-meiotic.

Key words: Cytoplasmic male sterility – *Brassica oxyrrhina* – *B. campestris* – *B. juncea* – Alien gene transfer

Introduction

Alloplasmics, obtained by combining cytoplasms and nuclei of different taxa, often express male sterility. In *Brassica*, cytoplasmic male sterility has been reported in three alloplasms: (1) *Raphanus* cytoplasm combined with nuclear genomes of *B. oleracea*, *B. napus* (Bannerot et al. 1974; Rousselle and Renard 1978), and *B. campestris* (Williams and Heyn 1981); (2) *B. campestris* nucleus in the cytoplasm of *Diplotaxis muralis* (Hinata and Konno 1979); and (3) *B. oleracea* nucleus placed in *B. nigra* cytoplasm (Pearson 1972). Outbreeding, promoted by such male sterility, can be utilized for producing commercial heterotic hybrids. It is desirable, however, to broaden the cytoplasmic base of male sterility to guard against vulnerability to diseases associated with certain cytoplasms. Male sterility in the alloplasmic combination of *B. oxyrrhina* cytoplasm with the nuclear genome of *B. campestris* has been reported in a short communication (Prakash and Chopra 1988). The *B. oxyrrhina* cytoplasm has now also been transferred to *B. juncea*. The deails are presented in this paper.

Materials and methods

Hybrids of *B. oxyrrhina* (2n = 18, OO), which grow wild in the sandy coast of Morocco, with *B. campestris* ssp. *oleifera* var. brown sarson cv *'Pusa Kalyani'* (2n = 20, AA) were obtained through embryo rescue. The synthetic alloploid *B. oxyrrhina-campestris* (2n = 38, OOAA) was obtained by doubling the chromosomes of the hybrid. The alloploid was repeatedly backcrossed with *B. campestris* to obtain cytoplasmic substitution. In BC_5, *B. oxyrrhina* chromosomes were eliminated, and *B. campestris* plants with *B. oxyrrhina* cytoplasm were obtained. Seed fertility was poor in BC_2, improved gradually at each backcross, and reached 96% in BC_5.

For developing alloplasmic *B. juncea*, synthetic alloploid *B. oxyrrhina-campestris* was hybridized with *B. juncea* cv *'Pusa Bold'* (Fig. 1). F_1 hybrids (2n = 37, AABO) were tall like *B. juncea*, but were intermediate between the parents in other morphological characters, particularly leaf shape.

The majority of PMCs in the F_1 had $10_{II} + 17_I$ at MI. However, 3.0% cells showed $16_{II} + 5_I$ or $1_{III} + 15_{II} + 4_I$. The hybrid was backcrossed twice to *B. juncea*. In the BC_3 generation, seven normal green, but completely male-sterile plants were obtained.

Results

The male-sterile *B. campestris* and *B. juncea* plants closely resembled normal fertile forms in general growth and morphology. In *B. campestris*, the leaves exhibited varying degrees of chlorosis during early growth but turned normal green later. In contrast, the leaves of *B. juncea* were perfectly green. Plant height was reduced

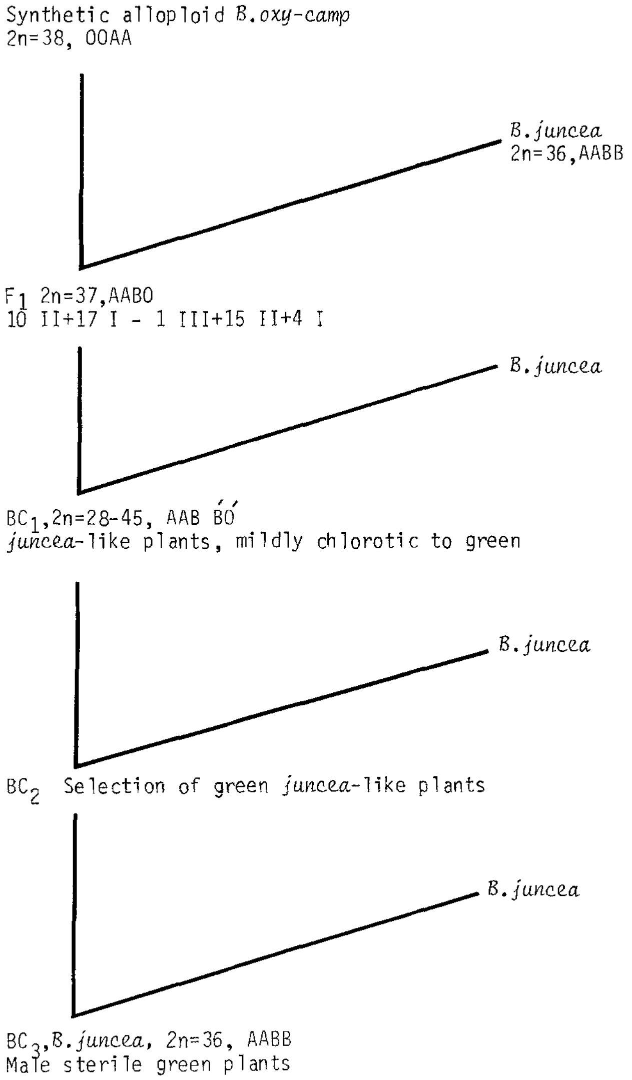

Fig. 1. The scheme employed for the development of alloplasmic male sterile *B. juncea*. B′ and O′ are hypodiploid genomes

in alloplasmic *B. campestris*. Flowering was delayed by about 10 days in both the alloplasmics. Male sterility of flowers could be identified by pollenless slender anthers.

Cytological analysis of male sterile *B. campestris* and *B. juncea* plants revealed that their somatic chromosome number was 20 and 36, respectively. The meiosis was completely normal with 10 and 18 bivalents at MI. Abortion of reproductive cells occurred after the formation of tetrads. Alloplasmic plants did not set any seed on selfing. However, good seed set was obtained on pollinations with maintainer parents. The plants so derived were male sterile. Female transmission of sterility confirmed its cytoplasmic nature.

Discussion

Many alloplasmics exhibiting cytoplasmic male sterility have been synthesized by substitution backcrosses in interspecific or intergeneric hybrids/alloploids, particularly in *Solanum, Nicotiana,* and *Triticum.* The report that cytoplasm of *Raphanus sativus* induces sterility (Ogura 1968) encouraged *Brassica* breeders to transfer this cytoplasm to the species *B. oleracea, B. campestris,* and *B. napus.* Subsequently, it was shown that cytoplasm of *Diplotaxis muralis* also induced male sterility (Hinata and Konno 1979). Wide hybridization was thus recognized as the procedure of choice for developing alloplasmic lines exhibiting cytoplasmic male sterility in *Brassica.*

In our study, it was observed that *B. oxyrrhina* cytoplasm affects morphological characteristics of the plant in addition to male fertility. Decreased height and chlorosis at early stages of growth was conspicuous in *B. campestris.* In *B. juncea* alloplasmics, height reduction and delay in flowering were observed. Morphological changes have been reported in many alloplasmics in *Brassica* earlier: linear narrow petals in (*D. muralis*)-*B. campestris* (Hinata and Konno 1979); ‘petaloid’ type of sterility and absence of nectaries in (*B. nigra*)-*B. oleracea* var. italica (Pearson 1972); and reduced floral organs and atrophied pollenless stamens in (*R. sativus*)-*B. napus* (Rousselle 1982).

Leaf chlorosis also occurs in alloplasmics of several crops, including wheat (Mukai and Tsunewaki 1976) and *Brassica* (Bannerot et al. 1977). Chlorosis limits the use of male sterility for hybrid seed production. Chlorosis in *B. napus,* caused by *Raphanus* cytoplasm, is thermosensitive (Bannerot et al. 1977; Rousselle 1982). Pelletier et al. (1983) and Jarl and Borman (1988) rectified this defect by replacing *Raphanus* chloroplasts with chloroplasts of *B. napus* through cytoplasmic hybridization. Chlorosis caused by *B. oxyrrhina* cytoplasm has also been attributed to its plastids. It should be possible to overcome leaf yellowing through plastid substitution.

We have observed that, while the synthetic alloploid *B. oxyrrhina-campestris* and its hybrid with *B. juncea* (2n = 37, AABO) were normal green, chlorotic segregants appeared in backcrosses with *B. juncea.* It is proposed that *B. oxyrrhina* possesses gene(s) that can rectify the cytoplasmically induced chlorosis. When *B. oxyrrhina* chromosomes are eliminated, this rectifying mechanism is lost. However, some green plants are obtained even when the *B. oxyrrhina* genome has been completely eliminated, and these are probably plants in which a segment of the *B. oxyrrhina* chromosome containing gene(s) for overcoming chlorosis has been introgressed into the *B. juncea* genome. Such a homoeologous recombinational event should have occurred in the F_1 or BC_1 generation. Cytological support for this comes from pairing behaviour in F_1 of *B. oxyrrhina-campestris* × *B. juncea.* Instead of the expected 10_{II}, 3.0% of PMCs had 16_{II} or $1_{III}+15_{II}$. The additional pairing can be attributed to allosyndetic pairing between *B. juncea* and *B. oxyrrhina* chromosomes, which could lead to genetic exchange.

Williams and Heyn (1981) and, more recently, Paulmann and Röbbelen (1988) have developed normal green CMS *B. campestris* and *B. napus* plants through introgression of genes from *Raphanus*. Because of male sterility of the alloplasm, the chlorosis-correcting segment will remain in the heterozygous condition until procedures are devised to obtain two doses of the chromosome-containing introgressed segment. It is proposed to achieve this by obtaining a homozygote from a doubled androgenetic haploid of the normal green plants in the alloplasmic population.

Brassica coenospecies comprises species and genera which are sufficiently related to the crop *Brassica*. It, therefore, constitutes a vast reservoir of genes of agronomic value and varying cytoplasms. The present investigation strongly suggests that cytosteriles of variable cytoplasmic origins can be synthesized.

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