ESTABLISHMENT OF A NEW CMS-SYSTEM IN *BRASSICA NAPUS*

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ABSTRACT

Male sterility was introduced into *B. napus* by protoplast fusion with *B. tournefortii*, the putative donor of the cms juncea system. This line (25-143) is carrying the nucleus and chloroplasts of the *B. napus* recipient, but recombined mitochondrial DNA of both parents. Restorer genes for both cms juncea and line 25-143 were found in the *B. napus* varieties 'Mangun' and 'Yudal'. Such genes were also introduced into *B. napus* by interspecific crosses from *B. tournefortii* using *B. tournefortii / B. campstris* amphidiploid hybrids as a bridge species. Attempts for the identification of a molecular marker for this restorer gene by RAPD analysis were made.

INTRODUCTION

Many cms-systems have been described in *B. napus*, but only the Ogura cms-restorer system is ready for hybrid seed production under European weather conditions. The cms juncea is known to impart stable male sterility in *B. napus*, too. It occurred spontaneously in a field trial of *B. juncea* in India (Rawat and Anand 1979) and it was transferred into rapeseed by Mathias (1985). Recently, restriction analyses of the mt- and cp-DNAs showed that cms juncea is of alloplasmic origin and *B. tournefortii* is the probable source of this cytoplasm (Pradhan et al. 1991). As a result of protoplast fusion experiments, a stable male sterile line (25-143) has been derived with a recombined mitochondrial DNA and chloroplasts contributed by *B. napus* (Stiewe and Röbbelen 1994).

In this paper, we report the establishment of a new cms-restorer system using *B. tournefortii* as a source of both cytoplasmic male sterility and restorer genes. In addition, the cms juncea and the newly developed cms line 25-143 were crossed to restorer genotypes described by Sodhi *et al.* (1994).

MATERIALS AND METHODS

The male sterility forms cms juncea and line 25-143 were available in the *B. napus* variety 'Duplo' and crosses were made using these cms lines as female parents. The F_1 seeds were obtained by crossing cms lines to 8 'Mangun', 10 'Yudal', and 4 'Asahi natane' plants as pollen parents. Ten descendants of each specific F1 combination from cms lines crossed to 'Mangun', 'Yudal', and 'Asahi natane' were scored for male sterility / fertility. The transfer of restorer genes from *B. tournefortii* has been initiated by crossing cms juncea to amphidiploid plants derived from *B. tournefortii* TT / *B campestris* AA crosses with TTAA genomic configuration (developed at Tata Energy Research Institute, New Delhi, India., unpublished). The resultant male fertile AATC plants were crossed to cms juncea and line 25-143. The next generation plants were scored for male sterility /

fertility. Sterility / fertility observations were taken during flowering three times once a week using the scale 0 (sterile) to 9 (fertile). The average of the three values was calculated as a percent value.

Plants of AACT genomic configuration were subjected to RAPD analysis. DNA isolation was performed as described by Stiewe and Röbbelen (1994); RAPD analysis was carried out according to standard protocols using decamer primers (Operon).

RESULTS

All F_1 hybrid plants raised from a cross involving cms juncea and line 25-143 with four different 'Asahi natane' plants turned out to be male sterile. However, the F_1 hybrid plants raised from the crosses with 'Mangun' and 'Yudal' showed segregation into male sterile / male fertile plants. The extent of male fertility ranged from 0 to 100 %. Table 1 presents percent male fertility in F_1 generation plants.

TABLE 1: Percent of male fertility restoration in the F_1 generation obtained from cms lines crossed with 'Mangun' and 'Yudal'

Seed parent	Pollen parent	Plants corresponding to the extent of male fertility restoration		
		0-33 %	34-66%	67-100%
cms juncea 'Duplo'	'Mangun'	5	38	15
cms juncea 'Duplo'	'Yudal'	12	82	10
line 25-143 'Duplo'	'Mangun'	11	58	7
line 25-143 'Duplo'	'Yudal'	13	80	5

In a parallel programme the intent was to transfer the restorer function from *B. tournefortii* to *B. napus*. 17 differrent plants were raised from the cross cms juncea x TTAA amphidiploid. Three of them showed a male fertile phenotype and 9 out of 17 were identified as true hybrids by RAPD analysis. 150 RAPD primers were tested in AATC hybrids. 55 of them showed polymorphisms between TTAA and AACC genomes. With 3 different primers polymorphic bands could be observed which occurred only in the 3 fertile plants. The AATC hybrid with the best male fertility was pollinated to CMS juncea and pollinated with 'Duplo'. The results are given in Table 2.

TABLE 2: Percent of male fertility restoration in the BC_1 generation obtained from cms juncea crossed with TTAC hybrids

Seed parent	Pollen parent	Plants corresponding to the extent of male fertility restoration		
		0-33 %	34-66%	67-100%
cms juncea AACC (male sterile)	AATC	4	5	5
cms juncea AATC (male fertile)	'Duplo'	14	4	0

In both directions male fertile plants could be observed. However, plants with nearly normal fertility were found only in the BC_1 generation derived from cms juncea AACC as seed parent and AATC male fertile plants as pollen parent (Table 2).

DISCUSSION

The stable male sterile cms line 25-143 with normal chloroplast function was isolated from fusion experiments conducted between *B. tournefortii* and *B. napus* (Stiewe and Röbbelen 1994). The cms juncea and cms line 25-143 were crossed to 'Asahi natane', 'Mangun', and 'Yudal' in order to test for restoration of male fertility in F_1 plants. Only the F_1 plants from crosses with 'Mangun' and 'Yudal' segregated into male sterile / fertile plants. Most of the F_1 plants scored during the present study were of intermediate male fertility (Table 1). This fact is also discussed by Sodhi *et al.* (1994). However, this reduction in pollen formation does not impair the pollen viability and male fertile F_1 plants could easily be selfed to generate F_2 seeds. The restorer genes from 'Mangun' and 'Yudal' were also backcrossed to different *B. napus* genotypes to study the genetic background. Restoration of male fertility in cms juncea and cms line 25-143 by 'Mangun' and 'Yudal' confirmed the similar mechanism of male sterility operating in the two cms lines. So *B. tournefortii* is identified as the source of the cms juncea cytoplasm. This was already proposed by Pradhan et al. (1991) and Stiewe and Röbbelen (1994) based on mt- and cp-DNA restriction patterns. Male fertility has also been restored by nuclear genes of *B. tournefortii* (Table 2). The selected plants with high male fertility were now backcrossed to cms juncea and line 25-143 to transfer the T genome restorer locus into the C genome of *B. napus*. These crosses are followed by RAPD analysis to find selectable markers for the restoration and to identify plants containing the restorer gene with a minimum amount of T genome background.

The stable male sterility in line 25-143 without any limitations of nuclear chloroplast incompatibilities and its restoration by 'Mangun' and 'Yudal' establishes a new cms-restorer system for hybrid seed production in rapeseed.

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