PRODUCTION OF HAPLOIDS IN CMS PLANTS AND THEIR USE FOR THE MOLECULAR ANALYSIS OF MALE STERILITY IN RAPESEED (*B. NAPUS* L.)

Y. S. SODHI, G. STIEWE, C. MÖLLERS

Institute of Agronomy and Plant Breeding, University of Göttingen, Von-Siebold-Str.8, D-37075 Göttingen, Germany

ABSTRACT

Embryoids have been derived from male sterile plants of cms juncea and cms Tokumasu in *B. napus*, through isolated microspore culture technique. Regenerated plants have been analysed by flow cytometry for ploidy level. The cms cytoplasm of regenerated plants was confirmed by RFLP analysis of total DNA and hybridisation to mitochondrial specific gene probes. The regenerated plants are being multiplied to be used further in fusion experiments as a source of haploid protoplasts.

INTRODUCTION

Haploid induction through isolated microspore cultures in several *Brassica napus* genotypes is one of the most efficient systems for plant regeneration in-vitro (Lichter 1982; Möllers *et al.* 1994). The haploids were also successfully induced in microspore cultures from Polima cytoplasmic male sterile (Polima CMS) and *Diplotaxis muralis* male sterile (Mur-MS) lines of rapeseed (Chuong *et al.* 1987). The flower buds taken from Polima CMS and Mur-MS plants showed normal microspore development. However, the normal microspore development is negligibly small in *B. napus* cms juncea (Mathias 1985) and cms Tokumasu (Paulmann and Röbbelen 1988).

Here, we report the efficient induction of haploid plants from cms juncea and cms Tokumasu in rapeseed. Their subsequent use in protoplast fusion and molecular analysis of male sterility in *B. napus* is also discussed.

MATERIALS AND METHODS

Both cms juncea in *B. napus* genotype 'Duplo' and cms Tokumasu in *B. napus* genotype 'Topas' were taken from the Institute's germplasm collection. The plants were kept in a growth chamber maintained at 15°C day / 10°C night temperature with 80 % relative humidity and 16 h photoperiod.

A total of 90 flower buds was collected from cms juncea and cms Tokumasu plants. The length of each bud was approximately 2mm. The further isolation of microspores and culture conditions were followed as earlier described by Iqbal *et al.* (1994). The experiments were repeated 7-8 times. The first embryoids appeared after 14 days of culture. The 28 days old embryoids were given two weeks abscisic acid treatment as explained by Iqbal *et al.* (1994). The embryoids were regenerated on a modified S1 medium, *i.e.* S1 + 0.5 mg/l gibberellic acid. The fully grown plants are being multiplied and maintained in culture.

Ploidy level analysis was conducted making use of a Partec CA II flow cytometer. Each DNA histogram was compared to the DNA histograms of diploid *B. napus* as standard. The cms cytoplasm of selected regenerants was confirmed by hybridization of digested total DNA with mitochondrial gene probes following standard protocols.

RESULTS

43 embryoids of cms juncea and 10 of cms Tokumasu were regenerated to plants. The ploidy analysis of these is given in Table 1. 26 plants from cms juncea have been clearly identified as haploids. The remaining 17 are spontaneous diploids. Only one regenerated plant has been identified of diploid nature from cms Tokumasu. The remaining 9 plants are haploid.

TABLE 1: Ploidy level of the microspore derived plants from cms juncea and cms Tokumasu in rapeseed

B. napus genotype	Total	Spontaneous diploids	Haploids
cms juncea 'Duplo'	43	17	26
cms Tokumasu 'Topas'	10	1	9

Total DNA of 36 cms juncea and nine cms Tokumasu regenerants was digested with Hind III and Eco RI. Hybridization with the mitochondrial specific gene probes cox III and atp 9, respectively, showed the banding pattern of the corresponding cms cytoplasms thereby, the presence of the respective cms cytoplasm has been confirmed.

DISCUSSION

Microspore development is inhibited at premeiotic stage in cms juncea and at postmeiotic stage in cms Tokumasu (Theis and Röbbelen 1990). Haploid plants have been obtained from these cms lines despite of low concentration of potential microspores. All the embryoids were regenerated to plants on a modified solid medium + 0.5 mg/l gibberellic acid (GA3). It is also effective to induce regeneration without secondary embryogenesis in the *B. napus* genotypes 'Andor', 'Duplo', and 'Janetzky' (unpublished results).

Both cms juncea and cms Tokumasu are known to have a nuclear-chloroplast incompatibility, which causes yellowing of leaves at a low temperature. Therefore, haploid protoplasts from normal *B. napus* have been fused with haploid protoplasts from each of these cms lines to select for stable male sterile plants without any chloroplast disfunction (Pelletier *et al.* 1983; Stiewe and Röbbelen 1994).

Also experiments have been initiated to fuse haploid protoplasts from cms juncea and cms Tokumasu. Fusion of two different cytoplasmic male sterile lines has been reported to restore male fertility in *Nicotiana tabacum*. This restoration of male fertility has been assigned to recombination of the mitochondrial genomes from two cms lines (Kofer *et al.*

1992). An attempt will be made to search for male fertile diploid (2n) plants. RFLP analysis will be conducted on mitochondrial DNA from male fertile plants and compared to the parental male sterile cms lines. It should be possible to ascertain the mitochondrial DNA fragment associated with male sterility in *B. napus* .

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