MICROSPORE CULTURE OF INTERSPECIFIC HYBRIDS IN *BRASSICA*

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ABSTRACT

Embryos and regenerated plants were produced by microspore culture of the interspecific hybrids from reciprocal crosses between *Brassica napus* X *B. rapa* (*syn. campestris*) and *B. napus* X *B. juncea*. The optimum culture conditions for hybrids of *B. napus* X *B. rapa* were provided by the protocol for microspore culture of *B. rapa* (Baillie *et al.*, 1992) with 17% sucrose concentration of the NLN media and a pH of 6.2. Higher embryo yields and quality were achieved when the hybrid plants were grown under day/night temperature regime of 10 /5^{0}C. The optimum microspore culture conditions for *B. napus* X *B. juncea* hybrids were provided by the NLN media at 13% sucrose and a pH of 6.0. There was no correlation between pollen fertility of the hybrid as determined by pollen staining and embryo yield. Approximately 20% of the microspore-derived plants from reciprocal crosses of *B. napus* X *B. rapa* had chromosome numbers of n=19.

INTRODUCTION

The cultivated species of oilseed rape *Brassica napus*, oilseed turnip rape *B. rapa* and oilseed mustard *B. juncea* make up a significant portion of the world's edible oil production. The transfer of traits by interspecific hybridization between these species has been widely used in cultivar development programs (Downey *et al.*, 1975). Techniques which would reduce the time required to stabilize interspecific hybrids would be valuable in cultivar improvement.

It has been well documented that utilization of haploid production in plant breeding can accelerate the production of homozygous individuals (Keller *et al.*, 1987). Since the first success of microspore culture in *B. napus* (Lichter, 1982) extensive studies have established the protocols for high-frequency embryogenesis and plant regeneration in certain genotypes of this species (Choung and Beversdorf, 1985). More recently, successful microspore culture has been reported from cultivars of *B. rapa* (Baillie *et al.*, 1992).

The objective of this study is to examine the feasibility of applying microspore culture in the stabilization of interspecific hybrids between *B. napus* and *B. rapa* and between *B. napus* and *B. juncea*.

MATERIALS AND METHODS

F1 hybrids were produced in reciprocal crosses between two *B. napus* lines (S91-1186, S91-106) and two *B. rapa* lines (UM921, LGC) referred to as NR hybrids and in reciprocal crosses between the two *B. napus* lines and two *B. juncea* lines (85-991, 85-1336) referred to as NJ hybrids. All plants of the parent lines and hybrids used for microspore culture were grown in a controlled environment with day/night temperatures of 21/16^{0}C (except where specified) and a 16 hour photoperiod with light intensity of 340 μE m^{-2} s^{-1}. The plants were routinely managed.

Cytological examination of mitotic metaphase cells from root tips of the reciprocal NR and NJ hybrids were used to confirm hybridity. The pollen viability of the NR and NJ hybrids was determined by acetocarmine staining of a minimum of 300 pollen grains from each of the F1 donor plants used for microspore culture.

The cytological stage of the buds from the F1 donor plants was determined by acetocarmine staining. The method of microspore isolation followed Baillie *et al.*, (1992).

For plant regeneration, embryos were transferred onto B5 medium (0.8% agar, 2% sucrose, pH 5.8 or 6.0) and incubated at a constant 22^{0}C with a 16 hour photoperiod. Embryos were subcultured on B5 medium every three to four weeks if normal plantlets did not form. Colchicine treatment was applied to induce chromosome doubling.

Root tips were taken from either microspore-derived plants in soil or cultured plantlets with an established root system. The method of cytological examination of mitotic cells at metaphase followed Kao (1982).

RESULTS

Microspore culture

NR hybrids. There were significantly different responses to microspore culture between the parental lines and the reciprocal hybrids. The responsive parental lines S91-1186 and UM921 produced interspecific hybrids with a relatively high embryo yield. The difference between lines was significantly reduced by the growth of donor plants under the low temperature regime (10/5^{0}C) prior to sampling. The highest embryo yields occurred when the most productive NR hybrid S91-1186 X UM921 was cultured in NLN media at 17% sucrose and 0.1mg BA/l, at pH 6.2 for 48 hours at 32^{0}C in darkness followed by a change in NLN sucrose to 10%, pH 6.2 at 25^{0}C for the remainder of the 3-4 weeks. Before the embryos were transferred to the B5 solid medium for plant regeneration, approximately 40% of the embryos from the hybrid UM921 X S91-1186 and 30% in the reciprocal hybrid were scored as normal and the remainder as abnormal embryos.

NJ hybrids. Only NLN at 13% sucrose and pH 6.0 was used in the culture of these hybrids. Buds from hybrids of *B. napus* X *B. juncea* were taken from individual plants because of the low number of confirmed hybrids.

There was no correlation between pollen fertility (as measured by pollen stainability) and embryo yield for either NR or NJ hybrids though significant differences in pollen stainability were detected between some hybrids.

Characteristics of regenerated plants

Among the 57 microspore-derived plants from NR hybrid S91-1186 X UM921 and its reciprocal, 25 had n= 10 to n=19 chromosomes and 32 had chromosome numbers greater than 19. The six microspore-derived plants from NJ hybrid S91-1186 X 85-1336 had chromosome numbers ranging from 21 to 42. Two plants had 27 chromosomes possibly representing all the chromosomes from A(10), B(8) and C(9) genomes. The majority of plants are sterile but some seed has been obtained.

CONCLUSIONS

The study confirmed that the gametes produced from interspecific hybrids have embryogenic capacity under *in-vitro* culture conditions. The response of interspecific hybrids to microspore culture appears to be influenced by the embryogenic capacity of the parental lines, the physiological status of the donor plant and culture conditions and cytoplasmic types.

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