DEVELOPMENT OF METHODOLOGY AND APPLICATIONS OF DOUBLED HAPLOIDS IN BRASSICA RAPA.

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ABSTRACT

Haploid and doubled haploid plants have been developed in a number of species including the genus *Brassica*. The protocol is different for each of the *Brassica* species and there are genotypic differences within the species. Genotype screening, identification of an embryogenic line, and an efficient microspore culture system are necessary for the application of haploid technology. Microspores, haploid embryos, and haploid and doubled haploid plants can be used in biochemical mutagenesis and transformation studies as well as in plant breeding programs. Genotype screening, optimization of culture protocol and applications of this technique will be discussed for *Brassica rapa*.

INTRODUCTION

Isolated microspore culture techniques have been used to generate haploid and doubled haploid plants in many species (Ferrie et al. 1994) including the genus *Brassica* (Lichter 1982, Keller et al. 1987). In the case of oilseed *Brassicas,* most of the research has been with *B. napus* where techniques are well established. Haploid technology has been utilized in varietal development (Unpublished data), mutant selection (Swanson et al. 1988), biochemical analysis of lipids (Taylor et al. 1990, Taylor et al. 1993) and genetic engineering (Swanson and Erickson 1989) studies. Comparatively limited research has been conducted with *Brassica rapa* L. var. *oleifera* as it is more recalcitrant in cell and tissue culture than *B. napus* (Baillie et al. 1992, Burnett et al. 1992). A number of factors influence embryogenesis in the species including donor plant conditions, genotype, developmental stage of the pollen, media constituents and culture conditions.

EXPERIMENTAL

Genotype Evaluation.

Twenty-three genotypes consisting of 21 Canadian *B. rapa* cultivars, one Winter X Spring (W x S) cross and one Agriculture and Agri-Food Canada breeding line (DSC-3) were evaluated using the protocol of Baillie et al. (1992) (Table 1). Two experiments were conducted, replicated three times.

In the first experiment, differences in microspore embryogenesis were observed between 17 *B. rapa* genotypes when using the standard protocol. Embryos were produced from all genotypes except the cultivar Horizon which did not respond. The number of embryos/100 buds ranged from 2.4 for Arlo to 70.2 for DSC-3. In the second experiment with newly registered *B. rapa* genotypes, genotypic differences were again observed. Frequency of embryogenesis ranged from 34 to 2006 embryos/100 buds.

Table 1. Microspore embryogenesis of *B. rapa* genotypes (Average number of embryos/100 buds)

Cultivar	Em/100	Cultivar	Em/100	Cultivar	Em/100
Arlo	2.4	Eldorado	31.5	Reward	70.0
Candle	2.6	Goldrush	32.3	R500	14.4
Cash	255.0	Horizon	0.0	Sunshine	34.0
Chinook	61.0	Hysyn 100	270.0	Span	11.4
Colt	10.8	Hysyn 110	225.2	Torch	22.7
DSC-3	70.2	Maverick	2004.0	Tobin	10.8
Echo	4.3	Parkland	27.1	W X S*	13.9
Eclipse	16.5	Polish	17.9		

*Winter x Spring

As observed in this study and others, there are genotypic differences. Previous studies (unpublished) have shown also that there is plant to plant variation for microspore culture response within the genotype. Selection studies within DSC-3 resulted in the identification of a highly embryogenic *B. rapa* line. This line consistently yields 2000 embryos/100 buds but yields of 16 000 embryos/100 buds have been obtained depending on the treatment. Seed of this embryogenic *B. rapa* line (CV-2) is available from: Dr. Kevin Falk, Agriculture and Agri-Food Canada Research Station, 107 Science Place, Saskatoon, SK, Canada, S7N 5E1, FAX (306) 956-7247.

Optimization of culture protocol.

A number of factors affect embryogenesis, regeneration, and chromosome doubling. Factors include donor plant conditions, genotype, developmental stage of the pollen, pretreatments, media, and culture conditions. Experiments to optimize the haploid technology in *Brassica* have evaluated media, culture conditions, and chromosome doubling techniques. Colchicine and trifluralin have been used to double the chromosome number.

Applications of haploid technology

A highly embryogenic genotype is beneficial for varietal development, genetic, biochemical, mutagenesis and transformation studies.

Mutagenesis: Microspore mutagenesis has been used in *B. napus* to develop lines resistant to herbicide (Swanson et al. 1988). Development of a *B. rapa* microspore mutagenesis system to develop lines with novel fatty acid profiles is in progress. The highly embryogenic *B. rapa* line (CV-2) was crossed to a self-fertile line (available from Dr. Don Woods, Beaverlodge Agriculture and Agri-Food Canada). Lines that were self-fertile and highly embryogenic were selected for the mutagenesis studies. Mutagen is applied to the microspores and embryos are recovered.

Transformation: A highly embryogenic line would also be useful for microspore transformation. A system is being developed.

Desiccation: Desiccation studies have been conducted using *B. napus* and *B. rapa* microspore-derived embryos.

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