PRODUCTION OF BRASSICA NAPUS HAPLOIDS THROUGH ANTHER AND MICROSPORE CULTURE

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ANTHER CULTURE

The production of B. napus haploids through induction of microspore embryogenesis in anther cultures has been documented by several investigators (Wenzel et al., 1977; Keller and Armstrong, 1978; Renard and Dosba, 1980; Lichter, 1981; Loh and Ingram, 1982). A number of factors are known to influence the frequency of microspore embryogenesis in Brassica spp. (Keller et al., 1982), however, anther culture temperature has been shown to be especially critical. Significant increases in embryo yield are obtained by culturing anthers at elevated temperatures for a limited time prior to maintenance at 25°C. Our early work with spring B. napus (cv Tower) revealed that the optimal elevated temperature was 30°C for 14 days (Keller and Armstrong, 1978; Table 1). In B. campestris, however, a 35°C treatment for two days was the most effective in stimulating embryogenesis (Keller and Armstrong, 1979). Studies with several winter B. napus cultivars have also shown that an initial culture period of 35°C for two days is superior to a 30°C treatment (Table 1). Recent work with three breeding lines of spring B. napus has shown that the highest embryo yields were obtained with an initial culture period of 35°C for one day followed by 30°C for six days (Table 1). These studies have provided evidence for specific genotype-temperature interactions. If a new genotype is to be utilized in haploid production, a range of culture temperature treatments should be tested.

We have demonstrated that liquid medium is superior to solid medium for induction of embryogenesis in B. napus anthers (Table 2). In addition to increased yields, embryos that are developmentally more advanced were obtained on liquid medium. Lichter (1981) has also been able to successfully employ liquid medium in B. napus anther cultures.

Anther density has been shown to be a critical factor influencing the production of microspore-derived callus in barley (Xu et al., 1981). In recent studies, we have been able to

demonstrate that increasing anther density from one anther per ml of liquid medium to four or six anthers per ml had a positive effect on embryo yield in winter x spring B. napus hybrids (Table 3). Increased embryo yields (although less dramatic than those in Table 3) have also been obtained with higher anther densities in other B. napus genotypes and other Brassica species.

Even though progress has been made in identifying factors influencing microspore embryogenesis, a great deal of variability still exists between experiments (i.e. note the embryo yield differences for the cultivar Jet Neuf in Tables 1 & 2). The basis for this variability could lie with the physiological condition of the donor plants and might include nutritional and water status, insecticide treatments, and gaseous environment.

ISOLATED MICROSPORE CULTURE

Lichter (1982) reported the successful production of embryos in isolated microspore cultures of B. napus cv Tower. We have recently induced embryogenesis in isolated microspores of B. napus cv Jet Neuf cultured in Lichter's medium. We have observed that a low temperature pretreatment used by Lichter was unnecessary and that maximal numbers of embryos ($\sim$ 2 per 1,000 microspores) were obtained after culture at 35°C for 18-36 h prior to continued incubation at 25°C.

PLANT REGENERATION

In order to regenerate plants from microspore-derived B. napus embryos, an embryo culture procedure is required (Keller and Armstrong, 1977, 1978). This involves reduction of sucrose from 10% to 2% (w/v) and elimination of the growth regulators and amino acid supplements used in the anther culture medium. In most cases more than 50% of the embryos survived, but more than 90% of these failed to develop a shoot system. Plants could be regenerated from such abnormal embryos through shoot induction in hypocotyl explants followed by rooting on hormone-free medium (Keller and Armstrong, 1977). The efficiency of this procedure varied from 50-90% for B. napus.

In B. napus, not all microspore-derived regenerates were haploids and the frequency of haploids varied with genotype (Table 4). The majority (>90%) of the non-haploids were diploids along with a few polyploids. Analysis of erucic acid levels in diploid regenerates has demonstrated that they were homozygous and

indicated that they originated from haploid microspores, possibly through endomitosis and/or nuclear fusion. These processes for increasing ploidy level have been identified in Datura anther cultures (Sunderland et al., 1974). Induction of shoot organogenesis in abnormal embryo explants also enhanced diploidization (Keller and Armstrong, 1981). Spontaneously-doubled haploids are, of course, valuable in breeding programs since they can be used directly without subjection to specific chromosome doubling procedures (see Keller and Stringam, 1978).

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Table 1. Influence of anther culture temperature treatments on microspore embryogenesis in Brassica napus genotypes*

Genotype**		Expected embryo yield/1000 anthers***			
		25°C (Continuous)	30°C 14 days	35°C for 2 days	35°C for 1 day: 30°C for 6 days
Jet Neuf	(W)	4(234)	17(234)	4068(321)	-
Herkules	(W)	0(114)	25(120)	2024(126)	-
Rafal	(W)	17(120)	125(120)	987(228)	-
Tower	(S)	37(296)	210(300)	130(638)	-
DI-820	(S)	5(204)	5(204)	39(204)	196(204)
DJ-63	(S)	10(203)	113(204)	54(203)	299(204)
ZN6-2836	(S)	5(204)	103(204)	10(204)	327(205)

*Anthers cultured according to procedures previously described (Keller and Armstrong, 1978); elevated temperature treatments followed by maintenance at 25°C.

** Winter types (W) received from Dr. J.G. Bowman, Nickerson RPB Ltd., Rothwell, UK.; spring types (S) received from Dr. K. Downey, Agriculture Canada, Saskatoon, Sask. Canada.

*** Values in brackets refer to total number of anthers cultured in three platings.

Table 2. Effect of physical composition of the culture medium on microspore embryogenesis in *Brassica napus* vs Jet Neuf

Medium*	No. of anthers cultured**	Expected embryo yield/1000 anthers
Agar (control)	201	1686
Liquid	202	2613
Liquid + 10% (w/v) Ficoll - 400	204	1392
Liquid - soaked filter paper on agar	194	361

*Composition of medium previously described by Keller and Armstrong (1977)

**Sum of three platings; six anthers/60 x 15 ml petri dish containing 5 ml of medium; anthers cultured at 35°C for 2 days prior to maintenance at 25°C.

Table 3. Effect of anther density on microspore embryogenesis in winter x spring F_1 hybrids of *Brassica napus**

Density (anthers/ml)	Expected embryo yield/1000 anthers**		
	Herkules x Altex	Jet Neuf x Altex	WRY445 x Altex
1	63(473)	131(282)	1000(252)
4	4663(178)	4672(125)	2865(466)
6	5621(430)	-	-

*Seed obtained from Dr. W. Beversdorf, University of Guelph, Guelph, Ontario, Canada; anthers cultured in liquid medium at 35 C for two days prior to maintenance at 25 C

**Figures in brackets represent total number of anthers cultured in three platings

Table 4. Frequency of haploids in microspore-derived regenerates of various *Brassica napus* genotypes

Genotype	No. of regenerates	Frequency* (%) Haploid	Frequency* (%) Diploid and polyploid
DI-820	22	23	77
DJ-63	46	37	63
ZN6-2836	49	47	53
Jet Neuf	91	63	37
Herkules	74	90	10
Rafal	80	39	61
Tower	50	20	80

*Calculations of frequencies were based on flower size and level of fertility; previous work (Keller and Armstrong, 1978) has shown a complete correlation of small sterile flowers with the haploid chromosome number.