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A comparison of anther and microspore culture as a breeding tool in *Brassica napus*

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Summary. A direct comparison of microspore culture and anther culture was made in *Brassica napus* using F_1 crosses of Regent (canola) by Golden (rapeseed), and their reciprocals, as well as a hybrid between Reston and a highly embryogenic, canola-quality breeding line (G231) as donor plants. The study confirmed that microspore culture can be ten times more efficient than anther culture for embryo production. Embryo yields from cultures initiated from the Reston × G231 were four-fold greater than those initiated from the Regent × Golden crosses, and significant differences were also detected among cultures initiated from the different Regent × Golden crosses. These results illustrate the influence that donor plant genotype has on embryo production. However, superior embryogenic potential among donor material was not always coincident with superior plant production. The average haploid-to-diploid ratio in microspore-derived regenerates was 2:1 for the population obtained from the Regent × Golden crosses but 1:1 for the Reston × G231 cross. For both types of material, the frequency of diploids increased upon repeated cycles of explanting. A field study showed that there were no differences between the populations of anther-derived and microspore-derived spontaneous diploid and doubled haploid lines, with respect to the days required for them to flower or to mature. The information is valuable for canola breeding programs considering the use of haploidy.

Key words: *Brassica napus* – Microspore and anther culture comparison – Androgenic lines – Days to flower – Days to maturity

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Introduction

The first successes with the production of microspore-derived embryos from *Brassica* anther cultures were reported by Keller et al. (1975) and Thomas and Wenzel (1975). In the early studies, the embryo production frequencies were low and embryo production was sporadic. Since that time there have been significant improvements in the anther culture technique that have made it more productive and reliable. These include preconditioning the donor material at low temperatures (Keller et al. 1982; Keller and Armstrong 1983), using liquid culture media (Lichter 1982), and incubating the cultures continuously at 30 °C or 35 °C prior to maintenance at 25 °C (Keller and Armstrong 1978, 1979). With the best genotypes, 100–900 embryos per 100 cultured anthers have been obtained (Keller and Stringam 1978; Dunwell and Cornish 1985).

The development of a culture system for isolated *B. napus* microspores by Lichter in 1982 was a major advance in *B. napus* haploid production technology. Using the isolated microspore cultures, yields in excess of 150,000 embryoids per 100 anther equivalents have been reported (Swanson et al. 1987; Kott et al. 1988). Only one comparison of the two techniques with the same genotypes has been reported (Lichter et al. 1988). Since the genotype and the physiological state (Keller 1984; Thurling and Chay 1984) of the donor plant affect the productivity of the androgenic cultures initiated from them, the relative efficacy of the two techniques can only truly be evaluated by isolating microspores and anthers from the same donor plants.

In the present study, the embryo production frequencies and plant production frequencies in anther and microspore cultures initiated from three F_1s obtained from crosses between Regent and Golden, and their recipro-

cals, as well as a hybrid between Reston and a canola-quality breeding line, G231, were compared. The study also contributes to the very limited information that is available on the effects of subculturing on the ploidy of regenerates from microspore culture. Furthermore, the days to flower and days to reach maturity of the adrogenic lines from the Regent × Golden crosses were compared in the field. This information is of value to plant breeders considering the use of these techniques to rapidly produce homozygous lines.

Materials and methods

Plant material

The donor plants for microspore and anther culture were F_1 hybrids obtained from crosses between individual plants of the spring *Brassica napus* L. cultivars Regent (canola) and Golden (rapeseed) and their reciprocals. Plants of the same cultivar were chosen to have similar erucic acid and glucosinolate levels. The levels were determined by gas liquid chromatography, in extracts from half cotyledons of imbibed seeds as described by Downey and Harvey (1963). A cross between the spring *Brassica napus* cultivar Reston (high erucic, low glucosinolate) and a highly embryogenic, canola breeding line, G231, was also used to initiate androgenic cultures.

Donor plants for anther and microspore culture were grown from the F_1 hybrid seed. Six randomly chosen seeds of each cross were planted in Jiffy-7 peat pellets, and were transferred after 2 weeks to 15-cm (6″) plastic pots containing MetroMix (W.R. Grace and Co., Ajax, Ontario) potting medium. The plants were grown in an environmentally controlled growth room under a 16-h photoperiod and a 22 °C day/18 °C night temperature regime. Grolux Wide Spectrum and Cool White fluorescent lamps (Sylvania Canada, Drummondville, Quebec) supplied a photosynthetic photon flux density of 250 μM m^{-2} sec^{-1} at bench level. The plants were watered as required and 20:20:20 (N:P:K) fertilizer was applied twice weekly at a rate of 1 g l^{-1}.

Microspore and anther techniques

Buds 4–5 mm in length were removed from the terminal and the upper two axillary racemes of donor plants that had approximately five open flowers on the terminal raceme. The buds were surface-sterilized in a 5.7% hypochlorite solution for 10 min and were subsequently rinsed three times (for 5 min) with autoclaved, deionized-distilled water. Intact anthers were removed from buds which had petal-to-anther ratios between 1:2 and 2:3. From each bud, two of the six anthers were randomly chosen for microspore culture and the remaining four were used for anther culture.

Anthers for anther culture were floated on 2.5 ml of M41 liquid medium (Keller et al. 1975) with 10% sucrose, 0.1 mg l^{-1} 2,4-D, and 0.1 mg l^{-1} naphthalene acetic acid (NAA) in 60 × 15 mm Falcon plates (Becton Dickinson, Labware, Oxnard/CA). Twelve intact anthers were placed into each plate and these were incubated, in the dark, at 35 °C for 2 days followed by 28 days at 25 °C to induce embryogenesis.

Anthers for microspore culture were bulked in the liquid B5 medium and macerated with a teflon rod to release the microspores. The resulting suspension was filtered through 63 μm and 44 μm Nytex filters, and the filtrate was centrifuged at 1,000 rpm for 3 min to pellet the microspores. The supernatant was removed and the microspores were resuspended in B5 wash and centrifuged at 1,000 rpm for 3 min. This step was repeated three times. The final microspore pellet was resuspended in a modified Nitsch and Nitsch (1967) liquid NLN medium (Lichter 1981, 1982), to give a microspore density of approximately 204,000 microspores/ml^{-1}. One milliliter of microspore suspension was added to 60 × 15 mm Falcon plates which already contained 1.5 ml of liquid NLN, to give a final microspore density of 81,600 microspores ml^{-1}. Microspore cultures were incubated in the dark at 32 °C for 3 days followed by 19 days at 25 °C (Chuong and Beversdorf 1985).

Embryo culture and regeneration

After 22 days for microspore cultures and 30 days for anther cultures, the cotyledonary embryos (Chuong and Beversdorf 1985) that were produced were counted and transferred to solid B5-H medium, containing 2% sucrose and 0.8% agar without hormones (Gamborg et al. 1968). Additional cotyledonary embryos were transferred from the microspore culture plates at 25 days and from the anther culture plates at 33 days onto solid B5-H, to rescue embryos that had previously been too small.

After 4 weeks, the number of plants that had developed normal shoots and roots were counted, individually labelled, and transferred to flats containing MetroMix and covered with a clear plastic lid. Abnormal regenerates, lacking either a well-developed shoot apical meristem or root system, were explanted onto solid B5-H to induce normal plantlet development. After 6 weeks, the normal regenerates which had developed were transferred to MetroMix in the growth room, as described above, and a second explant onto fresh B5-H was performed with recalcitrant material. After 4 weeks, the normal regenerates which developed were transferred to the growth room, while the remainder of the material was discarded.

Colchicine doubling and seed production

At flowering, the ploidy of the regenerates was assessed by examining the floral morphology. Spontaneous diploids were bagged to produce selfed seed, while haploid plants underwent colchicine treatment. The roots of the haploid plants were washed and immersed in a 0.1 mg l^{-1} colchicine solution containing a few drops of a surfactant (Tween 20) for 5 h. The treated plants were rinsed, potted in styrofoam cups containing MetroMix, and placed in the growth room. Doubled flowers or sectors of the treated plants were bagged to produce selfed seed from these doubled haploids.

Field observations

A total of 185 spontaneous diploid and doubled haploid lines (160 microspore-derived and 25 anther-derived) from the Regent × Golden crosses were produced. These lines were hand-planted in 1.5-m rows with an intra-row spacing of 10 cm and inter-row spacing of 50 cm in early May of 1987. Notes on days to flower and days to maturity were recorded for each individual line. A days-to-flower value was given to rows when each plant in the row had at least one flower, and a days-to-maturity value was given to rows when a pod taken from the terminal raceme of each plant contained 75% brown seeds.

Results

Embryo production for microspore and anther culture

Embryo production frequencies in microspore and anther cultures initiated from six Regent × Golden crosses are shown in Table 1. Microspore cultures initiated from

Table 1. Embryo and plant production frequencies from microspore and anther cultures initiated from six Regent × Golden crosses

Cross	Normal embryos per 100 anthers		Normal primary regenerates per 100 embryos		Normal secondary regenerates per 100 explants	
	m/c[a]	a/c[b]	m/c	a/c	m/c	a/c
R2 × G2[c]	190.1 ab	3.3 b	2.9	0.0	13.2	22.5
G2 × R2	224.9 ab	8.0 b	1.1	4.7	11.9	25.1
R8 × G6	56.5 b	9.0 b	0.0	0.8	22.7	21.3
G6 × R8	32.4 b	3.6 b	1.8	0.0	15.6	22.8
R14 × G20	418.3 a	44.5 a	2.4	1.1	23.2	17.2
G20 × R14	336.5 a	43.0 a	1.8	0.0	21.0	20.6
Average	209.8	18.6	1.7	1.1	17.9	21.6

[a] m/c, microspore culture
[b] a/c, anther culture
[c] R, Regent, G, Golden
– means within a column followed by the same letter are not significantly different ($P > 0.05$), as determined by a Duncan's New Multiple Range Test

Table 2. Embryo and plant production frequencies from microspore and anther cultures initiated from the Reston × G231 cross

Cross	Normal embryos per 100 anthers		Normal primary regenerates per 100 embryos		Normal secondary regenerates per 100 explants	
	m/c[a]	a/c[b]	m/c	a/c	m/c	a/c
Reston × G231	1,325.0	804.5*	6.2	1.0*	11.2	3.9**

[a] m/c, microspore culture
[b] a/c, anther culture
*, ** mean yields from anther culture and microspore culture are significantly different at the 5% and 1% level, respectively

Regent-8 × Golden-6 and its reciprocal produced the least embryos, Regent-2 × Golden-2 and its reciprocal were intermediate in their response, and Regent-14 × Golden-20 and its reciprocal produced the most embryos. In comparison, anther cultures initiated from Regent-2 × Golden-2 and Regent-8 × Golden-6, and their reciprocals produced fewer embryos than Regent-14 × Golden-20 and its reciprocal. Microspore cultures were on average ten times more productive than anther cultures.

An analysis of variance of embryo production frequencies in androgenic cultures initiated from the six Regent × Golden crosses indicated that the mean number of normal embryos produced per 100 anthers among microspore cultures was not significantly different at the 5% level of confidence. However, a Duncan's New Multiple Range Test detected significant differences among the embryo production frequencies in microspore cultures initiated from the different crosses (Table 1). An analysis of variance of the embryo production frequencies for anther cultures initiated from the same crosses indicated that highly significant differences existed among crosses, and this was supported by a Duncan's New Multiple Range Test (Table 1). In addition, there appeared to be an interaction between culture technique and the source of the microspores, particularly for Regent-2 × Golden-2 and its reciprocal.

Table 2 shows that high frequencies of embryo production were achieved in microspore and anther cultures initiated from the Reston × G231 cross. Embryo production frequencies were on average four times higher than those observed for cultures initiated from the Regent × Golden crosses. An analysis of variance of embryo production frequencies in the cultures initiated from the Reston × G231 cross showed a significant ($P = 0.05$) difference between microspore and anther culture, with the former being superior.

Plant production frequencies from primary embryos and hypocotyl explants

Plant production frequencies from primary embryos obtained from cultures initiated from the Regent × Golden crosses were less than 5% (Table 1). The analysis of variance indicated that the source of the embryo did not have a significant effect on the plant production frequen-

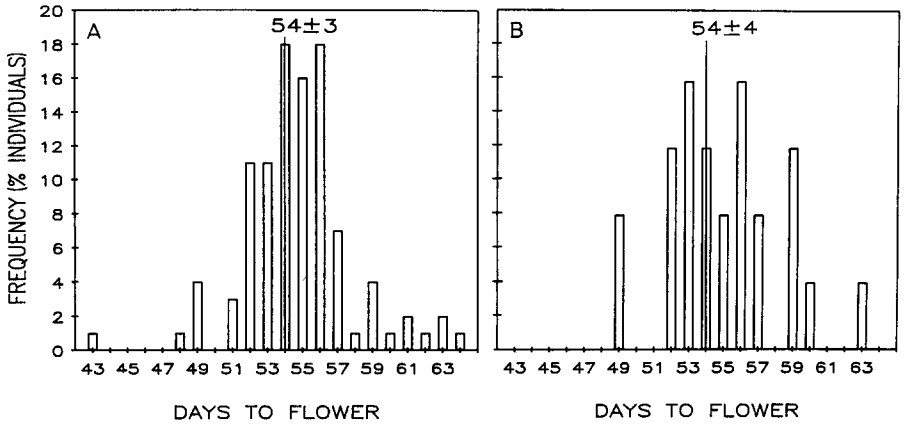

Fig. 1 A and B. Days to flower for **A** microspore-derived and **B** anther-derived androgenic lines obtained from cultures initiated from the Regent × Golden crosses. The means ± standard errors for the populations are indicated; n = 160 for microspore culture; n = 25 for anther culture

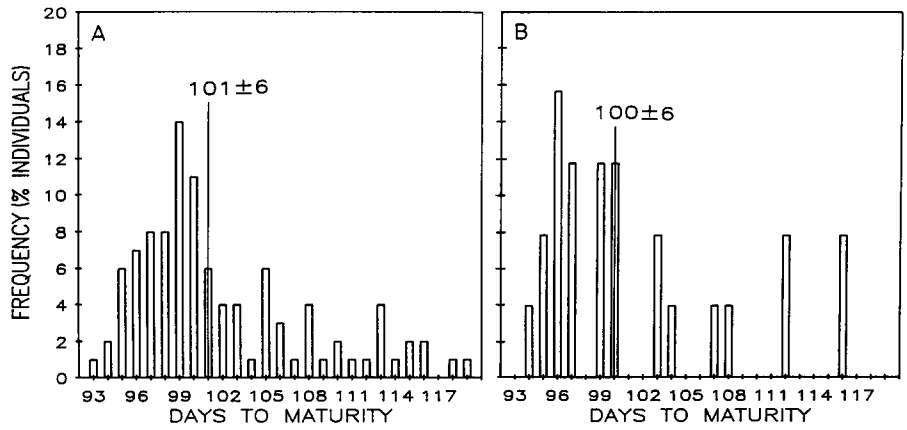

Fig. 2 A and B. Days to maturity for **A** microspore-derived and **B** anther-derived androgenic lines obtained from cultures initiated from the Regent × Golden crosses. The means ± standard errors for the populations are indicated; n = 160 for microspore culture; n = 25 for anther culture

Table 3. Frequencies of haploid and diploid lines regenerated from androgenic embryos and explants of embryos obtained from microspore cultures initiated from Regent × Golden and Reston × G231 crosses

Cross	Primary regenerates				Explant I				Explant II				Total regenerates			
	Hap[a]		Dip		Hap		Dip		Hap		Dip		Hap		Dip	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
R2 × G2[b]	5	56	4	44	5	56	4	44	23	74	8	26	33	67	16	33
G2 × R2	47	82	10	18	45	88	6	12	25	76	8	24	117	83	24	17
R8 × G6	1	100	0	0	6	75	2	25	5	83	1	17	12	80	3	20
G6 × R8	0	–	0	–	4	67	2	33	1	33	2	67	5	56	4	44
R14 × G20	37	74	13	26	41	76	13	24	47	60	31	40	125	69	57	31
G20 × R14	13	87	2	13	27	75	9	25	29	60	19	40	69	70	30	30
Regent × Golden total no. of regenerates	103	–	29	–	128	–	36	–	130	–	69	–	361	–	134	–
Regent × Golden avg. (%)	–	80	–	20	–	73	–	27	–	64	–	36	–	71	–	29
Reston × G231	97	55	78	45	24	47	27	53	–	–	–	–	121	54	105	46

[a] Hap – Haploid; Dip – Diploid
[b] R – Regent; G – Golden

cy, since no significant cross, culture, or interaction effects were detected. Direct plant production frequencies obtained from cultures initiated from the Reston × G231 cross were similar to those observed for the Regent × Golden embryos (Table 2). However, an analysis of variance showed that microspore-derived embryos from the Reston × G231 cross were significantly more likely to develop directly into plants than anther-derived embryos.

In total, 12%–25% of the Regent × Golden embryo-derived hypocotyl explants developed into plants after two rounds of subculturing onto fresh media (Table 1). No significant cross, culture, or interaction effects were detected by an analysis of variance of this data. Plant production from hypocotyl explants was substantially less for embryos obtained from androgenic cultures initiated from the Reston × G231 cross (Table 2) than that from the Regent × Golden crosses (Table 1). The analysis of variance of the secondary regeneration frequencies from Reston × G231 embryos showed that plant regeneration from hypocotyl explants of microspore-derived embryos was significantly ($P=0.01$) more frequent than that from anther-derived embryos.

Ploidy of lines regenerated from microspore culture

Because the number of anther-derived lines was low, only the microspore-derived lines were compared with respect to their ploidy. Table 3 shows that 80% of the lines regenerated directly from embryos obtained from cultures of the Regent × Golden crosses were haploid. However, the frequency of haploids declined to 73% and 64% in the regenerates obtained from the first and second explant cultures, respectively, of the same material.

In contrast, haploid-to-diploid frequencies in the population of regenerates obtained from the primary embryo from microspore cultures initiated from the Reston × G231 cross were nearly equal (Table 3). As with the Regent × Golden material, the frequency of haploids was lower in the explant-derived regenerates than in those obtained from the primary embryo.

Days to flower and days to maturity for microspore- and anther-derived plants

Figure 1 illustrates that the distribution of 160 microspore-derived and 25 anther-derived androgenic lines for the character days to flower are very similar. Both populations appeared to be normally distributed about a mean value of 54 days. Figure 2 shows the distributions of the same populations of plants for the parameter days to maturity. As for the days-to-flower parameter, the populations from microspore culture and anther culture were very similar, with mean values of 101 and 100 days, respectively. In addition, both populations tended to be skewed to include a larger proportion of early lines.

Discussion

The results of the present study using F_1 hybrids of Regent × Golden and Reston × G231 demonstrated that microspore culture is two to ten times more efficient in embryo production than anther culture. The direct comparison, thus, confirms what can be inferred from the results obtained from separate tests of these two techniques, namely, that microspore culture is the superior technique. It has been suggested that the low productivity of anther cultures may be caused by severe competition among microspores within the confines of the anther (Hoffmann 1980; Hoffmann et al. 1982) or by inhibitory leachates from the anther wall (Heberle-Bors 1984).

The genotype of the donor plant is a strong determinant of the embryogenic capacity of microspores isolated from them (Nitsch 1969; Guha et al. 1970; Seguin-Schwartz et al. 1983; Heberle-Bors 1984; Dunwell et al. 1985; Chuong et al. 1988a; Kott et al. 1988). A four-fold greater embryo yield from cultures initiated from the Reston × G231 cross compared to those initiated from the Regent × Golden crosses illustrates the influence that donor plant genotype has on embryo productivity. Since most *B. napus* varieties are somewhat heterogeneous, individuals in an F_1 population would be expected to be different. Therefore, differences in the embryogenic responses observed among cultures initiated from the three Regent × Golden crosses also reflect the effect of plant genotype on embryogenesis. The nucleus appears to be the site of the genes controlling embryogenesis, since no differences in productivity between cultures initiated from reciprocal Regent × Golden crosses were noted. This lack of a reciprocal effect agrees with results obtained by Seguin-Schwartz et al. (1983).

Several studies have indicated that androgenic embryogenesis can be improved through hybridization with highly embryogenic genotypes (Wenzel 1979) and that the embryogenic character is transferable to the F_1 (Seguin-Schwartz et al. 1983). The G231 cultivar has been shown to be highly embryogenic (Kott et al. 1988). Cosequently, the superior embryogenic potential of the Reston × G231 hybrid may be due to the characteristics of the G231 parent.

The results of the present study agree with previous studies (Keller and Armstrong 1983; Keller 1984; Chuong et al. 1988a) that concluded that the frequency of plant production from microspore-derived embryos is under genetic control. Furthermore, the genes that promote plantlet production do not appear to be the same as the ones that are involved in embryogenesis, since the Reston × G231 cross showed superior embryo production than the Regent × Golden crosses, but the plantlet production frequencies per embryo were very similar. In addition, there was a genotype-dependent influence of androgenic culture technique on plant production. Em-

bryos obtained from microspore and anther cultures, initiated from the Regent × Golden crosses, were not different with respect to their plant production frequencies both directly from the primary embryo or from hypocotyl explants. However, microspore-derived embryos from the Reston × G231 cross were superior in plant regeneration to anther-derived embryos for both primary and secondary plant regeneration. Possibly the 40-fold greater productivity of the Reston × G231 versus Regent × Golden anther cultures resulted in embryos that were deficient or more stress-sensitive in the former than the latter. The embryo production frequency in androgenesis is usually inversely related to the size and quality of embryos produced (Chuong and Beversdorf 1985).

A marked difference between the haploid to diploid ratios observed for the populations of microspore-derived lines from the Regent × Golden versus the Reston × G231 crosses suggests that this characteristic is also under genetic control. Previous reports of genotype-dependent effects on this ratio have been made by Chuong et al. (1988 b). It is of interest that this ratio changed to favour diploids the longer the material remained in culture. This observation is consistent with previous reports of polyploidization of long-term tissue cultures and, in particular, chromosome doubling in haploid lines (Sacristan 1971). An understanding of the process that leads to chromosome doubling in the microspore-derived cultures may ultimately allow the ploidy of the regenerates to be manipulated. This would be particularly advantageous for plant breeding programs, because it would reduce or eliminate the need for colchicine doubling and, thus, substantially reduce pure line production times and costs.

The field studies indicated that the populations of plants produced by microspore culture or anther culture from the Regent × Golden crosses did not differ with respect to days to flower or days to maturity. These results suggest that the androgenic procedures are equivalent with regard to the population of gametes that are induced to develop into plantlets. All the populations are continuous, which is in agreement with studies of flowering and maturity in *Brassica* (Thurling and Das 1979) that indicate that these are quantitatively inherited traits. The skewness of the populations to early maturity may represent in vitro selection in the tissue culture systems, as has been suggested for glucosinolate content in androgenic populations of *Brassica napus* by Wenzel (1979), or may reflect environmental effects or segregation patterns of genes controlling this trait in the material.

Very little information about the field characteristics of populations of *Brassica* plants obtained from anther or microspore culture is available. However, a recent comparison of microspore-derived and single-seed descent populations shows that they are equivalent with respect to their fatty acid profiles (Chen and Beversdorf 1987), and analyses of androgenic populations for a number of simply inherited and complexly inherited traits (Siebel and Pauls 1987) and for glucosinolate levels (Lichter et al. 1988) suggest that fears that haploidy leads to the production of inferior lines are largely unfounded.

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References

Chen J, Beversdorf WD (1987) Comparison of fatty acid variation in spring rapeseed (*B. napus*) derived from microspore culture and single seed descent. Proc Crucifer Genet Workshop IV, University of Wisconsin, Madison

Chuong PV, Beversdorf WD (1985) High frequency embryogenesis through isolated microspore culture in *Brassica napus* L. and *Brassica carinata* Braun. Plant Sci 39:219–226

Chuong PV, Deslauriers C, Kott LS, Beversdorf WD (1988 a) Effects of donor genotype and bud sampling in microspore culture of *Brassica napus*. Can J Bot 66:1653–1657

Chuong PV, Pauls KP, Beversdorf WD (1988 b) High frequency embryogenesis in male sterile plants of *Brassica napus* through microspore culture. Can J Bot 66:1676–1680

Downey RK, Harvey BL (1963) Method of breeding for oil quality in rape. Can J Plant Sci 43:271–275

Dunwell JM, Cornish M (1985) Influence of preculture variables on microspore embryo production in *Brassica napus* ssp. *oleifera* cv Duplo. Ann Bot 56:281–289

Dunwell JM, Cornish M, Courcel AGL de (1985) Influence of genotype, plant growth temperature and anther incubation temperature on microspore embryo production in *Brassica napus* ssp. *oleifera*. J. Exp Bot 36:679–689

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50:151–158

Guha S, Iyer RD, Gupta N, Swaminathan MS (1970) Totipotency of gametic cells and the production of haploids in rice. Curr Sci 39:174–176

Heberle-Bors E (1984) Genotypic control of pollen plant formation in *Nicotiana tabacum* L. Theor Appl Genet 68:475–479

Hoffmann F (1980) Pflanzliche Zellkulturtechniken als Züchtungsschritt am Beispiel Raps. Naturwissenschaften 67: 301–306

Hoffmann F, Thomas E, Wenzel G (1982) Anther culture as a breeding tool in rape. 2. Progeny analyses of androgenetic lines and induced mutants from haploid cultures. Theor Appl Genet 61:225–232

Keller WA (1984) Anther culture of Brassica. In: IK Vasil (ed) Cell culture and somatic cell genetics of plants, vol 1. Academic Press, New York, pp 302–310

Keller WA, Armstrong KC (1978) High frequency production of microspore-derived plants from *Brassica napus* anther culture. Z Pflanzenzuecht 80:100–108

Keller WA, Armstrong KC (1979) Stimulation of embryogenesis and haploid production in *Brassica campestris* anther culture by elevated temperature treatments. Theor Appl Genet 55:65–67

Keller WA, Armstrong KC (1983) Production of *Brassica napus* haploids through anther and microspore culture. Proc 6th Int Rapeseed Conf, Paris

Keller WA, Stringam GR (1978) Production and utilization of microspore-derived plants from *Brassica napus* anther culture. In: TA Thorpe (ed) Proc IAPTC: Frontiers of plant tissue culture, International Association for Plant Tissue Culture, University of Calgari, pp 113–122

Keller WA, Rajhathy T, Lacapra J (1975) In vitro production of plants from pollen in *Brassica campestris*. Can J Genet Cytol 17:655–666

Keller WA, Armstrong KC, Roche AI de la (1982) The production and utilization of microspore-derived haploids in *Brassica* crops. In: SK Sens; KL Giles (eds) Plant cell culture in crop improvement. Plenum Press, New York, pp 169–183

Kott LS, Polsoni L, Beversdorf WD (1988) Pre- and postmitotic cytological evens in isolated microspore culture of *Brassica napus*. Can J Bot 66:1658–1664

Lichter R (1981) Anther culture of *Brassica napus* in liquid culture medium. Z Pflanzenphysiol 103:229–237

Lichter R (1982) Induction of haploid plants from isolated pollen of *Brassica napus*. Z Pflanzenphysiol 105:427–434

Lichter R, Groot E de, Fiebig D, Schweiger R, Gland A (1988) Glucosinolates determined by HPLC in seeds of microspore-derived lines of rapeseed (*Brassica napus* L.). Plant Breed 100:201–221

Nitsch JP (1969) Experimental androgenesis in *Nicotiana*. Phytomorphology 19:389–404

Nitsch C, Nitsch JP (1967) The induction of flowering in vitro in stem segments of *Plumbaga indica* L. I. The production of vegetative buds. Planta 72:355–370

Sacristan MD (1971) Karyotypic changes in callus cultures from haploid and diploid plants of *Crepis capillarii*. Chromosoma 33:273–283

Sequin-Schwartz G, Hutcheson DS, Downey RK (1983) Anther culture studies in *Brassica campestris*. Proc 6th Int Rapeseed Conf, Paris

Siebel J, Pauls KP (1987) The use of haploids in *Brassica napus* breeding. Proc Crucifer Genet Workshop IV, University of Wisconsin, Madison

Swanson EB, Coumans MP, Wu SC, Barsby TL, Beversdorf WD (1987) Efficient isolation of microspores and the production of microspore-derived embryos from *Brassica napus*. Plant Cell Rep 6:94–97

Thomas E, Wenzel G (1975) Embryogenesis from microspores of *Brassica napus*. Z Pflanzenzuecht 74:77–81

Thurling N, Das LDV (1979) The influence of donor plant genotype and environment on the production of multicellular microspores in cultures of anthers of *Brassica napus* ssp. *oleifera*. Ann Bot 54:681–693

Wenzel G (1979) Recent progress in microspore culture of crop plants. In: Davies DR, Hopwood DA (eds) The plant genome. The John Innes Charity, Norwich