Theor Appl Genet (1990) 79:33–38

Genotype, plant, bud size and media factors affecting anther culture of cauliflowers (*Brassica oleracea* var. *botrytis*)

C. Phippen* and D. J. Ockendon

AFRC Institute of Horticultural Research, Wellesbourne, Warwick, CV35 9EF, UK

Received August 3, 1989; Accepted September 18, 1989
Communicated by J. W. Snape

Summary. Eleven F_1 hybrid cultivars of cauliflower, representing a range of maturity types, were examined for their responsiveness to anther culture. Embryos were produced from each of the cultivars tested, and the mean embryo yield varied from 82.2 embryos per 100 anthers cultured for cv Dova to 0.6 embryos for cv Serrano. Variation between genotypes and between plants within a genotype was significant, both in terms of embryo yield and percentage responsive anthers. Autumn and winter maturing cauliflowers were generally more responsive than summer types. Embryo yields were enhanced by culturing anthers on solid rather than on liquid media. An increase in concentration of 2,4-Dichlorophenoxyacetic acid (2,4-D) from 0.1 to 0.3 mg/l also increased embryo yield. Embryo yield was doubled when anthers were cultured on solid media containing 0.3 mg/l 2,4-D compared to liquid media containing 0.1 mg/l 2,4-D. Although bud size alone did not have a significant effect on embryo production, genotype × bud size and plant × bud size (within genotype) interactions were significant. Estimation of the variance components demonstrated that, apart from the residual plate-to-plate variation, variation between plants was the largest source of variation, accounting for approximately 30% of total variance. Plant × bud size (within genotype) interaction accounted for 18% of total variance and genotypic differences for approximately 8%.

Key words: Cauliflowers – Anther culture – Responsiveness – Variance components of embryo yield

Introduction

Anther culture has been successfully carried out on several *Brassica* species (Keller et al. 1983). Within *Brassica oleracea,* microspore-derived embryos have been obtained from kale (Keller and Armstrong 1981), broccoli (Keller and Armstrong 1983; Orton and Browers 1985), cabbage (Chiang et al. 1985; Lillo and Hansen 1987) and Brussels sprouts (Ockendon 1984, 1985). Little work has been carried out on cauliflower. Bagga et al. (1982) produced embryos from cultured anthers of cauliflower, although the cultivars used and embryo yields obtained were not mentioned. More recently, Ockendon (1988) obtained embryos from four F_1 hybrid cultivars of cauliflower. One of these (cv Nedcha) was highly responsive, one showed a low response and the other two were virtually non-responsive.

The objective of the present study was to test a number of F_1 hybrid cauliflowers of differing maturity type for their responsiveness to anther culture, and to identify responsive and non-responsive genotypes. F_1 hybrid cultivars were chosen in order to minimise genetic variation between plants of the same cultivar. At the time, few F_1 hybrids were commercially available, and the majority of these were autumn cauliflower types. However, three of the four main maturity groups of cauliflower have been tested.

Materials and methods

Ten commercial F_1 hybrid cultivars of cauliflower (*Brassica oleracea* var. *botrytis*) and an experimental F_1 hybrid (Roscoff) were used, representing summer, autumn and winter types. In order of their maturity, the cultivars were: Montano (3), Serrano (3), Plana (3.5), Siria (3.5), Stella (4), Surfrider (4.5), Woomera

* Present address: ICI Agrochemicals, Jealotts Hill, Bracknell, Berks. RG12 6EY,UK

(5), Dova (5), White Dove (5), Arbon (5.5), Roscoff (6.5). The approximate times to maturity, in months, are given in brackets.

Seed of each cultivar was sown at intervals throughout 1986 and 1987, so that material was available for anther culture throughout the year. The plants were grown in John Innes No. 3 compost, initially in 12-cm pots, and then transferred to 20-cm pots after 12–15 weeks. The plants were maintained in a cool glasshouse at temperatures between 10° and 20 °C. Supplementary lighting in the form of high pressure sodium lamps was provided between October and March. The summer and autumn types formed curds spontaneously in the glasshouse, but the Roscoff plants had to be vernalised for 10 weeks at 4°–7 °C to ensure good curd formation.

Bud size was used as a guide to the optimum stage of anther development for culture. Five bud sizes within the range of 3.75 to 5.25 mm were selected for each genotype. The actual sizes used varied, depending on the cultivar being cultured, and were determined by preliminary tests. The buds were removed just before or during early flowering, and had a petal: anther ratio in the range of 0.8 to 1.5. Buds were taken from 11 plants of each genotype – except for Roscoff, for which only 9 plants were available – and each plant was sampled once.

The method used for anther culture was similar to that described by Ockendon (1984). Buds were surface sterilized for 6 min in a freshly made solution of sodium dichloroisocyanurate containing 1% available chlorine, and then rinsed three times in sterile distilled water. Anthers were carefully removed without the filaments and placed onto culture media contained in 5-cm petri dishes. Fifteen anthers were cultured per dish and 300 were cultured per plant. The media were based on Gamborg's B5 medium as modified by Keller et al. (1975), with 10% sucrose and 0.1 mg/l napthalene acetic acid (NAA). The four variants of the basic medium involved liquid or solid medium (8 g/l agar) containing 0.1 or 0.3 mg/l 2,4-D (Table 3). The plates were sealed with Nescofilm. Immediately after plating, the anthers were exposed to 35 °C for 16 h, prior to incubation at 25 °C in the dark. After 6 weeks, the number of embryos was recorded for each anther separately (solid media only) or for the whole plate (liquid media).

Results for all media were calculated in terms of embryo yield per 100 anthers cultured. For solid media only, the percentage of anthers responding and embryo yield per 100 responsive anthers was also estimated. These figures could not be calculated for anthers cultured on liquid media because the embryos became detached from their 'parent' anthers and floated away.

The data for the percentage anthers responding were logistically transformed [ln $(P/(1\text{-}P)$ where $P=$(no. of anthers responding $+0.5$)/(no. of anthers cultured $+1$)] and embryo yield was logarithmically transformed [ln (no. of embryos per 100 anthers cultured $+1$)]. Statistical analysis in the form of analysis of variance was carried out on the transformed data using a model incorporating the terms listed in Table 6 and including bud size, medium and their interaction as fixed effects. Variance components for the terms were estimated by equating the mean squares from the analysis of variance to their expected values.

Results

Analysis of variance of the number of embryos per 100 anthers cultured showed that all the sources of variation tested were significant (Table 1), except for bud size alone, although bud size interactions were significant. The same was true for the analysis of variance of percentage anthers responding (data not presented).

Table 1. Analysis of variance for the number of embryos per 100 anthers cultured (logarithmic transformation)

	Source of variation	*df*	Mean square	Tested against	F. Probability
a	Genotype	10	81.46	d	0.001
b	Bud size	4	8.32	e	N.S
c	Medium	3	31.70	f	0.001
d	Plants within genotypes	102	20.11	h	0.001
e	Genotypes × bud size	40	7.41	g	0.001
f	Genotype × medium	30	2.63	h	0.001
g	Plants within genotypes × bud size	408	3.49	h	0.001
h	Residual	1595	1.28	–	–

Embryos were produced from each of the 11 genotypes tested. Considerable variation existed between genotypes both in terms of embryo yield and in percentage of anthers responding (Table 2). Summer and early autumn maturing cauliflowers were generally less responsive to anther culture than mid- and late autumn and winter types. The highest yielding genotype on the untransformed scale was cv Dova, with a mean yield of 82.2 embryos per 100 anthers cultured and, on the transformed scale, cv Woomera, with an untransformed mean yield of 38.6 embryos per 100 anthers cultured. This compares with only 0.6 embryos per 100 anthers cultured for cv Serrano, the least responsive cultivar.

The range of yields presented in Table 2 shows that plants of the same genotype varied greatly in their response to anther culture. Dova was the most variable cultivar, with yields in the range of 0 to 591.7 embryos per 100 anthers cultured and 0%–54% embryogenic anthers. Plants of cv Surfrider varied in response from 0 to 141.5 embryos per 100 anthers cultured and 0% to 25% embryogenic anthers. All plants of the genotypes Roscoff, Stella and White Dove produced some embryos.

Significantly higher embryo yields were obtained from anthers cultured on solid than on liquid media, irrespective of hormone concentration (Table 3). A concentration of 0.3 mg/l 2,4-D was more effective in stimulating embryogenesis than 0.1 mg/l 2,4-D, both in terms of embryo yield per 100 anthers cultured (Table 3) and in anther response (Table 4). However, the effect of hormone concentration on embryo yield per 100 responsive anthers cultured was not significant (Table 4). A two-fold increase in embryo yield was obtained when anthers were cultured on solid media containing 0.3 mg/l 2,4-D, compared to liquid media containing 0.1 mg/l 2,4-D (Table 3).

Table 2. Response of 11 F_1 hybrid cauliflowers of differing maturity type to anther culture

Genotype	Maturity type	Percentage anthers responding (solid media only)			No. of embryos per 100 anthers cultured		
		Mean	Range between plants	Transformed mean [a]	Mean	Range between plants	Transformed mean [b]
Arbon	Late-autumn	7.2	0 –18.7	–2.61	18.9	0 – 45.6	1.42
Dova	Late-autumn	11.3	0 –54.0	–2.41	82.2	0 –591.7	1.79
Montano	Mid-summer	2.8	0 –10.0	–3.03	3.8	0 – 12.3	0.67
Plana	Late-summer	2.2	0 – 4.7	–3.11	1.6	0 – 3.7	0.37
Roscoff	Winter	8.4	2 –16.0	–2.45	33.1	5.3– 79.3	1.71
Serrano	Late-summer	0.7	0 – 2.7	–3.32	0.6	0 – 2.3	0.13
Siria	Early-autumn	3.3	0 – 8.7	–2.99	6.1	0 – 16.1	0.76
Stella	Early-autumn	5.8	0.7–16.0	–2.74	11.0	1.4– 26.0	1.10
Surfrider	Mid-autumn	6.1	0 –25.0	–2.75	29.3	0 –141.5	1.19
White Dove	Late-autumn	8.7	0.7–21.3	–2.47	17.5	0.4– 80.0	1.38
Woomera	Mid-autumn	11.5	0 –27.3	–2.27	38.6	0 – 85.7	2.06

[a] LSD ($P=0.05$) 0.51
[b] LSD ($P=0.05$) 0.85

Table 3. Effect of media type and hormone concentration on the mean number of embryos per 100 anthers cultured

Media type	Concentration of 2,4-D mg l^{-1}			
	0.1		0.3	
	Mean	Transformed mean	Mean	Transformed mean
Solid	25.3	1.16	30.3	1.43
Liquid	14.9	0.87	20.1	1.11

LSD ($P=0.05$) 0.19

Table 4. Effect of hormone concentration on the number of embryos per 100 responsive anthers and percentage anthers responding. Data for solid media only

Concentration of 2,4-D mg/l	Percentage anthers responding		No. of embryos per 100 responsive anthers	
	Mean	Transformed mean [a]	Mean	Transformed mean [b]
0.1	5.5	–2.81	284.3	5.16
0.3	7.0	–2.67	311.3	5.22

[a] LSD ($P=0.05$) 0.15
[b] LSD ($P=0.05$) 0.12

Bud size alone did not have a significant effect on embryo yield when tested against the genotype × bud size interaction, but the genotype × bud size interaction itself was significant (Table 1). Genotypes differed in their response to bud size, both in terms of embryo yield and percentage anthers responding. Plants within a genotype also varied with respect to bud size. For six plants of cv Woomera, the optimum bud size differed (Table 5). Plants one and four produced higher yields when small to medium-sized buds were used, whereas plant five was more responsive when larger buds were used. The plant × bud size (within genotypes) interaction had a significant effect on embryo yield per 100 anthers cultured and on the percentage anthers responding ($P \leq 0.001$), but not on yield per 100 responsive anthers (Table 6).

A variance component model was fitted to the data. The amount of variation attributed to each component for embryo yield and the percentage of responsive anthers is presented in Table 6. Similar results were obtained for each type of yield estimation. Apart from a

Table 5. Relationship between bud size and embryo yield in a sample of six plants of F_1 hybrid Woomera. Figures are for embryo yield per 100 anthers cultured, untransformed (above), transformed (below)

Plant no.	Bud size (mm)				
	4.25	4.5	4.75	5.0	5.25
1	106.7	90.0	186.7	8.3	0
2	3.3	148.3	88.9	26.7	155.0
3	3.3	65.0	146.7	186.7	26.7
4	56.7	18.3	53.3	1.7	0
5	25.0	40.0	6.7	103.3	76.7
6	3.3	33.3	5.0	25.0	1.7
1	4.17	4.24	4.89	1.43	0
2	0.67	4.99	4.24	3.12	4.96
3	0.67	3.57	3.69	5.09	2.58
4	3.17	1.08	3.20	0.51	0
5	1.86	2.67	1.33	4.52	3.79
6	1.02	3.44	1.18	3.20	0.51

LSD ($P=0.05$) 1.57

Table 6. Variance components and percentage of total variance of components for percentage of anthers responding (logistic transformation), embryo yield per 100 responsive anthers (logarithmic transformation) and embryo yield per 100 anthers cultured (logarithmic transformation)

Variance component	Responsive anthers[a]		Embryos per 100[a] responsive anthers		Embryos per 100 anthers cultured	
	Variance	% Total Variance	Variance	% Total Variance	Variance	% Total Variance
Genotype	0.08	8	0.06	8	0.28	9
Plants within genotypes	0.33	32	0.21	27	0.95	30
Genotype × bud size	0.02	2	0.04	5	0.09	3
Plants within genotypes × bud size	0.18	18	0	0	0.54	17
Genotype × medium	0.01	1	0.02	3	0.03	1
Genotype × medium × bud size	0	0	0.06	8	0	0
Residual plate to plate	0.40	39	0.38	49	1.27	40
Total variance	1.02	100	0.77	100	3.16	100

[a] Data for solid media only

high residual plate-to-plate variance in all cases, plant-to-plant variation was the largest source of variation, accounting for approximately 30% of total variance. The second largest source of variation (for embryo yield per 100 anthers cultured and percentage responsive anthers) was the plant × bud size (within genotype) interaction, which accounted for approximately 17% of total variance. Genotypic differences accounted for only approximately 8% of total variance.

Discussion

Embryos were successfully produced from cultured anthers of cauliflower. The maximum yield for a single plant was 592 embryos per 100 anthers cultured. This compares with maximum yields of 357 per 100 anthers cultured in Brussels sprouts (Ockendon 1985) and 270 per 100 anthers cultured in brocolli (Keller and Armstrong 1983).

A wide spread of responsiveness between cauliflower genotypes was observed. Due to the variability in yield between plants of the same genotype, however, it was difficult to classify the genotypes into groups according to their response. Variation between cauliflower genotypes in their responsiveness is a common feature of *Brassica* anther culture and, indeed, of many other species. Large differences in embryo yield and anther response have been observed in cultivars of spring and winter rape (*Brassica napus*) (Dunwell et al. 1983; Loh and Ingram 1982) and various forms of *Brassica oleracea*, namely broccoli (Orton and Browers 1985) and Brussels sprouts (Ockendon 1984, 1985). It happened that the eight F_1 hybrid cultivars of Brussels sprouts tested by Ockendon (1985) fell into three groups, two of the cultivars being highly responsive, two moderately responsive and four virtually non-responsive. The cauliflower genotypes tested here could not be grouped in such a simple way, partly because of the great variation between plants within a genotype. Cardy (1986) compared the embryo yield of spring and winter cultivars of rape. Although wide variation in yield was found in both maturity types, spring cultivars were generally less responsive than winter cultivars. In cauliflower it appears that mid- and late autumn and winter cultivars are generally more responsive than are summer and early autumn cultivars.

Variation in response to anther culture between plants of the same genotype is a major problem in cauliflower. Many of the genotypes which produced high embryo yields also contained plants which gave no embryos at all. Plant-to-plant variation has also been observed in broccoli (Keller and Armstrong 1983) and in Brussels sprouts (Ockendon 1984). This variation was greater in cauliflower than in Brussels sprouts. In this experiment, variation between plants accounted for approximately 30% of total variation, compared to only 13% in Brussels sprouts (Ockendon and Sutherland 1987). Sovari (1985) demonstrated in turnip rape that embryoid formation fluctuated, depending on the time of year when the anthers were cultured, spring and autumn being more suitable for anther culture than winter and summer. The variation in response with season may be due to fluctuations in temperature and light intensity. Plants in the present experiment were grown in a glasshouse and cultured at different times throughout the year, which may account for some of the plant-to-plant variation observed. The data were examined for correlations between embryo yield and time of culture, but no consistent pattern was seen. It was quite common to find

that plants of the same genotype cultured on consecutive days showed big differences in embryo yield. Although plants raised in a growth room gave more consistent results than those grown in a glasshouse, variation still occurred (Ockendon 1985).

F_1 hybrids were chosen in this study in the expectation that the plants of each cultivar would be genetically identical. In the glasshouse, plants within a cultivar were uniform in appearance. However, self-incompatibility tests indicated that there were two types of cv Dova, one possessing the S-allele S_{50} and one with an unidentified S-allele. Similarly, some plants of cv Surfrider were self-compatible, whereas others were self-incompatible and contained S_{12}. It is, therefore, apparent that commercial F_1 hybrid cultivars of cauliflower are not totally genetically uniform. This within-cultivar heterogeneity may partly explain the relatively high level of plant-to-plant variation found in the present study.

Embryo production in cauliflower was greater on solid than on liquid media, with yield increases of up to 100%. This is in contrast to previous work on *Brassica,* in which a beneficial effect of liquid media was demonstrated (Lichter 1981; Keller et al. 1983). The difficulties encountered with embryo regeneration are generally less if solid media is used rather than liquid (Ockendon 1986). As embryos sink below the surface of liquid media, they may encounter anaerobic conditions which inhibit the growth of the embryo (Dunwell 1985).

In broccoli (Keller and Armstrong 1983) and cabbage (Lillo and Hansen 1987), a large increase in embryo yield was demonstrated when the concentration of 2,4-D was increased from 0.1 to 1.0 mg/l. Ockendon (1986) also found an increase in the number of embryos produced in Brussels sprouts when they were cultured on 0.3 mg/l 2,4-D compared to 0.1 mg/l. The effect of 2,4-D concentration on embryo production in cauliflower was similar to that found in Brussels sprouts. Thus, a significant increase in embryo yield per 100 anthers cultured and percentage anthers responding was obtained when anthers were cultured on 0.3 mg/l 2,4-D compared to 0.1 mg/l.

The effect of hormone concentration on embryo yield per 100 responsive anthers was not significant. This suggests that 2,4-D influences embryo production by determining the number of anthers that respond, rather than embryo yield per anther. Of the media tested, solid media containing 0.3 mg/l 2,4-D was the best for anther culture of cauliflowers.

In rape, a narrow developmental window of less than 8 h exists during which microspores can be induced to produce embryos (Pechan and Keller 1988). Several criteria may be used to choose buds with anthers at the optimum stage of development for culture. These include estimation of the petal: anther length ratio, and measurement of bud size. In cauliflower, the petals are generally longer than the anthers and so become folded. This makes estimation of the petal: anther length ratio difficult. In this experiment, therefore, bud size was used as the criterion for choosing anthers for culture.

Bud size overall did not have a significant effect on embryo production in cauliflower. This implies that the bud sizes chosen were appropriate for the genotypes being tested. However, a significant genotype × bud size interaction was observed. Variation in the optimum bud size for each genotype occurred, both in terms of embryo yield and percentage of responsive anthers. This suggests that the same bud sizes in different genotypes correspond to different developmental stages. Plants within a genotype also varied in their response to bud size. Plant × bud size (within genotype) interactions had a significant effect on embryo yield per 100 anthers cultured, and the percentage of anthers responding, but not embryo yield per 100 responsive anthers. This implies that bud size affects embryo production by determining the number of anthers that respond and not embryo yield per anther.

A disadvantage of using bud size as a developmental marker is that buds from different plants and different genotypes vary in their shape. This makes estimation of size difficult. Bud size, therefore, can only be used as an approximate guide to development stage. An alternative to measuring bud size would be to determine the stage of development of a single anther from each bud cultured, using cytological techniques. This, however, is very time-consuming and would mean that far fewer anthers could be cultured in a given time.

A number of difficulties were encountered in cauliflower anther culture which do not occur with other *Brassica* species. Prior to bud formation, cauliflowers must first initiate a curd. Cauliflowers of different maturity types have different vernalisation requirements for curd production. During the cooler parts of the year, the summer and autumn cauliflowers we used curded spontaneously in the glasshouse. However, during the warmer periods of the year, the curding of the late autumn types was delayed, and this sometimes gave poor quality curds. This, in turn, may affect the uniformity and quality of the buds. Whereas Brussels sprouts produce buds fairly steadily over a period of 2–3 weeks, in cauliflower large numbers of buds are produced in a period of a few days. This may lead to competition between buds for nutrients, resulting in abnormal buds and poor quality anthers. The production of a large number of buds at the same time also means that cauliflower anthers can only be cultured over a short period of time, generally 5–10 days. It may be possible to improve the uniformity and quality of buds by regulating the way in which plants are grown to produce small, uniform, early maturing curds.

Genotype, plants within a genotype, media and bud size are all important in their effect on embryo yield in cauliflowers. From the partitioning of variance, it ap-

pears that plants within a genotype are the largest factor effecting embryo yield, followed by plant × bud size (within genotype) and then genotype. In Brussels sprouts, genotype is more important than plants within a genotype as a source of variation (Ockendon and Sutherland 1987).

The production of embryos from each of the 11 genotypes tested indicates the potential value of anther culture as a breeding tool in cauliflowers. However, the large variability in embryo yield between and within genotypes may mean that its immediate application is limited. Further work is required to improve the overall responsiveness of genotypes and reduce plant-to-plant variation. This may be achieved by the addition of silver nitrate to the culture medium, as has already been shown for Brussels sprouts by Biddington et al. (1988). The effect of silver nitrate on cauliflower anther culture is currently being investigated and will be the subject of a further paper. By culturing anthers from doubled haploids of cauliflower, it should be possible to obtain a clearer distinction between the genetic and non-genetic factors affecting embryo yield.

Acknowledgements. Thanks are due to Dr. R. A. Sutherland for help with the statistical analysis. Financial support from the Horticultural Development Council and the Ministry of Agriculture Fisheries and Food (Open Contracting Scheme) is gratefully acknowledged.

References

Bagga S, Bhalla-Sarin N, Sopory SK, Guha Mukherjee S (1982) Comparison of in vitro plant formation from somatic tissues and pollen grains in *Brassica oleracea* var. *botrytis*. Phytomorphology 32:152–156

Biddington NL, Sutherland RA, Robinson HT (1988) Silver nitrate increases embryo production in anther culture of Brussels sprouts. Ann Bot 62:181–185

Cardy BJ (1986) Production of anther-derived doubled haploids for breeding oilseed rape (*Brassica napus* L.). PhD Thesis, University of Guelph

Chiang MS, Frechette S, Kuo CG, Chong C, Delafield ST (1985) Embryogenesis and haploid plant production from anther culture of cabbage. Can J Plant Sci 65:1033–1037

Dunwell JM (1985) Haploid cell cultures. In: Dixon RA (ed) Plant cell culture – A practical approach. ILR Press, Oxford, pp 21–36

Dunwell JM, Cornish M, DeCourcel ACL, Middlefell-Williams JE (1983) Induction and growth of 'microspore-derived' embryos of *Brassica napus* ssp. *oleifera*. J Exp Bot 34:1768–1778

Keller WA, Armstrong KC (1981) Production of anther-derived dihaploid plants in autotetraploid marrow stem kale (*Brassica oleracea* var. *acephala*). Can J Genet Cytol 23: 259–265

Keller WA, Armstrong KC (1983) Prodcution of haploids via anther culture in *Brassica oleracea* var. *italica*. Euphytica 32:151–159

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 de la I (1983) The production and utilization of microspore-derived haploids in *Brassica* crops. In: Sen SK, Giles KL (eds) Plant cell culture in crop improvement. Plenum Press, New York, pp 169–183

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

Lillo C, Hansen M (1987) Anther culture of cabbage. Influence of growth temperature of donor plants and media composition on embryo yield and plant regeneration. Norw J Agric Sci 1:105–109

Loh CS, Ingram DS (1982) Production of haploid plants from anther cultures and secondary embryoids of winter oilseed rape *Brassica napus* spp. *oleifera*. New Phytol 91:507–516

Ockendon DJ (1984) Anther culture in Brussels sprouts (*Brassica oleracea* var. *gemmifera*). I. Embryo yields and plant regeneration. Ann Appl Biol 105:285–291

Ockendon DJ (1985) Anther culture in Brussels sprouts (*Brassica oleracea* var. *gemmifera*. II. Effects of genotype on embryo yields. Ann Appl Biol 107:101–104

Ockendon DJ (1986) Utilization of anther culture in breeding Brussels sprouts. In: Horn W, Jensen CJ, Odenbach W, Scheider O (eds) Genetic manipulation in plant breeding. de Gruyter, Berlin, pp 265–272

Ockendon DJ (1988) The ploidy of plants obtained from anther culture of cauliflowers. Ann Appl Biol 113:319–325

Ockendon DJ, Sutherland RA (1987) Genetic and non-genetic factors affecting anther culture of Brussels sprouts (*Brassica oleracea* var. *gemmifera*). Theor Appl Genet 74:566–570

Orton TJ, Browers MA (1985) Segregation of genetic markers among plants regenerated from anthers of broccoli (*Brassica oleracea* var. *italica*). Theor Appl Genet 69:637–643

Pechan PM, Keller WA (1988) Identification of potentially embryogenic microspores in *Brassica napus*. Physiol Plant 74:377–384

Sovari S (1985) Production of haploids from anther culture in agriculturally valuable *Brassica campestris* L. cultivars. Ann Agric Fenn 24:149–160