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Genetic and non-genetic factors affecting anther culture of Brussels sprouts (*Brassica oleracea* var. *gemmifera*)

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Summary. Eight inbred lines of Brussels sprouts and ten F_1 hybrids derived from them were tested for their response to anther culture. From 5–19 plants per genotype were tested, and each plant was tested on 3–6 separate occasions. Results from the inbred lines were broadly similar to those from the F_1 hybrids, despite the inbreds producing fewer buds and having a higher frequency of anther deformities. The maximum embryo yield from an inbred line was 215 embryos per 100 anthers, and from a hybrid was 275. From estimation of the variance components it was calculated that, for both inbreds and hybrids, about half the total variation was genetic whereas variation due to plants within genotypes and to occasions within plants were each about 13% of the total. The narrow sense heritability of responsiveness to anther culture (estimated by the proportion of variation between inbred lines which was genetic) was 0.48, and there was partial dominance for this character. In three cases the hybrid outyielded the better inbred, and this heterosis may well be due to dispersed dominant genes.

Key words: Brassica – Anther culture – Embryos – Heritability – Responsiveness

Introduction

Genotype often has a major effect on the success of anther culture, and this is documented for *Brassica napus* (Dunwell et al. 1985), *B. campestris* (Sovari 1985) and for several types of *B. oleracea,* namely broccoli (Orton and Browers 1985), Brussels sprouts (Ockendon 1985) and cabbage (Lelu and Bollon 1985; Chiang et al. 1985). For experimental work, genotypes which respond well to anther culture are commonly chosen for study, but the plant breeder is likely to want to use anther culture on all his breeding material, which may vary greatly in its responsiveness. Previous work on seven F_1 hybrid genotypes of Brussels sprouts showed a very wide range of responsiveness, and a suggestion that the high responsiveness of two of the F_1 hybrids might be associated with the inbred parent common to both of them (Ockendon 1985). This work has been extended by testing the inbred parents of F_1 hybrids already tested, and by testing four more F_1 hybrids involving the apparently responsive inbred parent. In view of the lack of vigour of the inbreds, it is important to know whether they are in general as responsive to anther culture as the F_1 hybrids. Study of the inheritance of responsiveness to anther culture is made difficult by the large amount of uncontrollable and environmentally-induced variation in the results (Ockendon 1984). Some reduction in the latter source of variation has been achieved by raising the donor plants under carefully controlled conditions. An objective of this work was to separate the total variation into genetic and non-genetic components, the latter consisting of variation between plants within a genotype, and between occasions on which a plant was anther cultured. It was also hoped such data would allow estimates to be made of the heritability of responsiveness to anther culture in Brussels sprouts.

Materials and methods

The material consisted of eight inbred lines of Brussels sprouts (*Brassica oleracea* var *gemmifera*) and ten F_1 hybrids derived from them. Six of the F_1 hybrids constituted a half diallel involving lines A-D, while the other four F_1 hybrids had line A

(thought to be highly responsive) as a common parent as follows:

Inbred	Hybrids
A	
B	A×B
C	A×C B×C
D	A×D B×D C×D
E	A×E
F	A×F
G	A×G
H	A×H.

The inbreds were all well-established lines which were morphologically uniform and had been inbred for at least six generations. Some of the F_1 hybrids are commercial cultivars: A×B = cv Gower, A×C = cv Nym, B×D = cv Hal.

Due to the work involved in testing each genotype thoroughly, the genotypes were divided into three groups and tested sequentially in separate experiments. Inbred A and the hybrid A×B were included as a control in each group. The aim was to test at least five plants of each genotype and culture 300 anthers from each plant.

The plants were grown in John Innes compost in 15 cm pots and kept in a glasshouse until they were at least 5 months old, prior to being vernalized for 10 weeks at 3°–6°C. After vernalization, the plants were transferred to a growth room with a temperature regime of 16°C day/10°C night, a 14 h day and a light intensity of approximately 100 W/m².

The inbred plants produced many fewer buds than the F_1 hybrids, so in order to culture the number of anthers required, each plant was cultured on at least three occasions, with 90 anthers per occasion. Culturing was started as soon as sufficient buds of the right size were available and was usually completed by about the time the first flowers opened. Buds with malformed anthers were rejected, and as these occurred mainly in the terminal shoot of the inflorescence, buds were taken from the side shoots as far as was possible. The buds used for anther culture had a petal/anther ratio in the range 2:3 to 7:8.

The culture medium used was Gamborg's B5, as modified by Keller et al. (1975), with 10% sucrose, 0.1 mg/l 2,4-D and 0.1 mg/l NAA, solidified with 0.8% agar; 30 anthers were plated on culture medium in a 9 cm Petri plate sealed with Nescofilm and incubated in the dark. Immediately after plating, the anthers were given a thermal shock at 35°C for 16 h, prior to culture at 25°C. Then 5 to 6 weeks after the start of anther culture, the number of anthers responding and the number of embryos produced were recorded.

The embryo yields were logarithmically transformed, ln (no. of embryos per 100 anthers cultured +1), and the number of anthers responding were expressed as proportions and logistically transformed: ln (p/(1-p)) where p = (no. responding +0.5)/(no. tested +1). A model was fitted in order to estimate the variance components due to genotypes, plants within genotypes, occasions within plants (which showed no systematic differences and were therefore treated as random effects) and plates within occasions. An analysis of variance was performed as shown in Table 1 and the components estimated by equating the resulting mean squares to their expectations (Henderson 1953). No systematic differences were found between the three experiments, and so the results of these were pooled.

Table 1. Expected mean squares in the analysis of variance and variance component estimate

Source of variation	*df*	Expected mean squares
Inbred		
Genotype	7	$\sigma_e^2 + 3.39\,\sigma_o^2 + 12.79\,\sigma_p^2 + 77.09\,\sigma_g^2$
Plants	48	$\sigma_e^2 + 3.30\,\sigma_o^2 + 11.65\,\sigma_p^2$
Occasions	150	$\sigma_e^2 + 3.18\,\sigma_o^2$
Between plates (error)	662	σ_e^2
Hybrid		
Genotypes	9	$\sigma_e^2 + 4.34\,\sigma_o^2 + 12.41\,\sigma_p^2 + 78.31\,\sigma_g^2$
Plants	57	$\sigma_e^2 + 4.33\,\sigma_o^2 + 12.08\,\sigma_p^2$
Occasions	173	$\sigma_e^2 + 3.03\,\sigma_o^2$
Between plates (error)	814	σ_e^2

Results

One of the inbred lines (G) was much more responsive than the other seven, both in terms of embryogenic anthers and embryo yield (Table 2). Lines A and H gave a moderately good response and were not statistically different. Lines B and F gave a low response; that from E was very low, while C and D gave no response at all, although the differences between these lines were not significant in most cases.

The F_1 hybrids tested (Table 3) can be grouped as follows: very highly responsive (A×G), highly responsive (A×B, A×F and A×H), moderately responsive (A×C, A×D and A×E) and almost or totally non-responsive (B×C, B×D and C×D). In most cases the differences between hybrids from different groups were significant.

Despite the fact that the inbred plants were substantially less vigorous than the hybrid plants, had fewer buds available for anther culture and produced a higher frequency of deformed anthers, the levels of response were broadly similar to those from the F_1 hybrids. Three of the hybrids involving line A gave higher embryo yields than the better parent and one exceeded the parental mean but was not significantly greater than the better parent (Table 4). This heterosis for embryo yield, especially where more responsive inbreds were involved, may well be due to dominant genes dispersed between the parents. The narrow sense heritability of embryo yield can be estimated as 0.48, being the proportion of variation between the inbred lines (Table 5). This result is not very accurate because the number of inbreds used was small. It is an estimate of the expected efficiency of selection of inbred lines on the basis of their performance in this experiment. The broad heritability will probably exceed the narrow heritability due to dominance. It is not possible to estimate broad heritability with any confidence from the results of the diallel with lines A, B, C and D

Table 2. Response of eight inbred lines of Brussels sprouts to anther culture. *SED:* SE of the difference, calculated from an analysis of variance excluding C and D

Line	Plants tested	Occasions	Anthers cultured	Mean % embryogenic anthers		Mean embryo yield per 100 anthers	
				Untransformed	Transformed	Untransformed	Transformed
A	19	66	6,930	7.2	−2.85	32.4	2.08
B	5	13	1,470	1.1	−3.81	2.5	0.48
C	5	14	1,620	0.0	−4.11	0.0	0.00
D	5	12	1,350	0.0	−4.11	0.0	0.00
E	5	22	1,980	0.3	−4.03	0.4	0.12
F	6	25	2,280	3.1	−3.37	3.9	0.98
G	6	27	2,430	36.1	−0.84	214.6	4.50
H	5	20	1,800	7.1	−3.11	37.6	1.58
SED (40 *df*) Comparisons with line A					0.29		0.46
Other comparisons					0.37		0.57

Table 3. Response of ten F_1 hybrid Brussels sprouts to anther culture. *SED:* SE of the difference, calculated from an analysis of variance excluding B×C and C×D

F_1 hybrid	Plants tested	Occasions	Anthers cultured	Mean % embryogenic anthers		Mean embryo yield per 100 anthers	
				Untransformed	Transformed	Untransformed	Transformed
A×B	17	64	6,570	13.2	−2.25	51.4	2.80
A×C	6	6	1,380	7.5	−2.92	18.5	1.65
A×D	5	21	1,860	4.9	−3.21	8.1	1.23
A×E	7	31	2,760	4.2	−3.43	10.8	1.11
A×F	6	27	2,430	19.2	−1.87	52.0	3.02
A×G	6	23	2,160	42.3	−0.38	275.4	5.08
A×H	5	20	1,890	11.6	−2.35	46.7	2.71
B×C	5	21	1,890	0.0	−4.11	0.0	0.00
B×D	5	5	1,500	0.5	−3.99	0.7	0.17
C×D	5	22	1,980	0.0	−4.11	0.0	0.00
SED (49 *df*) Comparisons with A×B					0.34		0.48
Other comparisons					0.42		0.59

Table 4. Embryo yields (ln transformed) of seven F_1 hybrid Brussels sprouts involving line A compared with that of the corresponding inbred lines and the calculated parental means. Line A had a yield of 2.08; *ns*: non significant

Line	Inbred	F_1 (A×B–H)	Parental mean	F_1 performance exceeds:	
				Parental mean	Better parent
B	0.48	2.80	1.26	***	*
C	0.00	1.65	1.04	ns	
D	0.00	1.23	1.04	ns	
E	0.12	1.11	1.10	ns	
F	0.98	3.02	1.53	***	*
G	4.50	5.08	3.29	***	ns
H	1.58	2.71	1.83	*	*

* $P<0.05$
*** $P<0.001$

Table 5. Variance components and percentages of total variance for the percentage of anthers responding (after logistic transformation) and number of embryos per 100 anthers cultured (after logarithmic transformation)

Component	Inbred		Hybrid	
	Anthers	Embryos	Anthers	Embryos
Between plates	0.54 25%	1.28 29%	0.54 23%	1.05 23%
Occasions	0.37 17%	0.55 12%	0.33 14%	0.59 13%
Plants	0.16 7%	0.47 11%	0.31 13%	0.61 13%
Genotype	1.10 51%	2.15 48%	1.19 50%	2.32 51%
Total variance	2.17	4.45	2.37	4.57

because two of the inbreds and three of the hybrids gave almost zero response.

The results of the variance component model were very similar, both for the inbreds compared with the hybrids and for the frequency of embryogenic anthers compared with the embryo yield (Table 5). In each case the genotype accounted for very nearly half the total variation, while plant to plant variation and occasion to occasion variation each accounted for about one-eighth of the total variation. The genetic components of inbreds and hybrids are not strictly comparable, since they include different sources of genetic variation. That of the inbreds includes only additive variation while that of the hybrids includes dominance variation but less additive variation because most of them shared a common parent (Mather and Jinks 1982). The error component includes bud to bud and anther to anther variation, which are considerable but were not tested for in this experiment. Visual assessment indicated that floral abnormalities were more common and varied more between plants in inbreds than hybrids. However, this did not affect the anther culture results, as indicated by the slightly lower plant to plant variation of the inbreds compared with that of the hybrids.

Discussion

There is little published information on the genetic basis of responsiveness to anther culture and no good indication of the number of loci involved. Most of the data apply to cereals, where it is necessary to distinguish between the frequency of responsive anthers (giving callus or embryos) and the frequency of calli which give green plants. These two characters are separately inherited in both barley (Foroughi-Wehr et al. 1982) and wheat (Lazar et al. 1984). In hexaploid triticale an important heterotic effect on embryogenesis was reported by Bernard (1977), but Charmet and Bernard (1984) found predominantly additive gene action for this character. For both triticale (Charmet and Bernard 1984) and wheat (Lazar et al. 1984), some of the F_1 hybrids were superior to both inbred parents in their embryo yields and the broad sense heritability of this character was 0.66 for triticale and 0.6–0.7 for wheat. These results are similar to those we have obtained for Brussels sprouts.

In rape, Thurling and Chay (1984) suggested that buds taken from the inflorescence at an early stage of development gave better results than those taken at a later stage. We examined the occasion to occasion variation but no general trend was apparent for the majority of plants, which were cultured on only three occasions. However, 18 plants were tested on 6 occasions, and on the last occasion the plants were usually in full flower. For six of those plants there was a tendency for the embryo yield to drop markedly after the third, fourth or fifth occasion, and to remain low, but for five other plants the yield on the sixth occasion was just as high as on the first. As embryo yields may be reduced as flowering proceeds, it is best if buds are taken as early as possible during the course of flowering. In our work, results from fourth and subsequent occasions were omitted from the general analysis.

Dunwell et al. (1987) emphasize the need for experimental designs which allow the separation of heritable and non-heritable variation. Although they were able to demonstrate heritable variation in responsiveness to anther culture in barley, the major source of variation in their results was due to differences between spikes from a single plant, which accounted for 46% to 95% of the total variation in the experiment. This is a rather higher level of non-heritable variation than the 49% to 52% which we have found in Brussels sprouts.

It is possible that some of the plant to plant variation resulted from residual genetic variation within the inbred lines, but this is unlikely because all the lines were highly uniform morphologically and were at least at I_7. In a majority of genotypes which gave a moderate or good response, one or two plants were found to give a very low response or none at all. For example, of 19 plants tested in inbred A, 13 plants gave embryo yields in the range 16–59 (per 100 anthers cultured), 4 plants gave 6–12 embryos, and two gave no embryos at all. Wherever there is substantial plant to plant variation within a genotype, the results from the most responsive plants are the most informative as they indicate the potential responsiveness of that genotype, even though not all the plants of that genotype attain that particular level. One approach to this problem is to test more plants of each genotype, but this may mean that fewer genotypes can be tested and can cause difficulty in accommodating large numbers of plants in limited controlled environment facilities. In any case, it is desirable to test at least five plants of each genotype. From the breeder's point of view, it may be desirable to test the responsiveness to anther culture of a large number of genotypes. To do this accurately with existing methods would involve a large amount of work, but if the methods can be made more robust then the plant to plant variation could well be reduced.

The question arises from the breeder's point of view as to whether it is more efficient to test the responsiveness of hybrids or of inbreds. Hybrids have the advantages of more buds per plant, fewer bud deformities and that the material generated from anther culture of hybrids may be useful for breeding purposes, whereas that from inbreds cannot. However, for a given set of inbreds, a much larger number of hybrid combinations are possible, and it would be impractical to test

all of these. One compromise is to test a few hybrids with common parents. In this way it may be possible to identify responsive inbreds and then eventually to predict the responsiveness of hybrids based on that of their parents.

Anther culture is already being used in commercial breeding programmes for Brussels sprouts, despite some genotypes being non-responsive. If a method could be found to improve substantially the general level of responsiveness to anther culture of Brussels sprouts this could well reduce the variability in the results, as well as improving the responsiveness of genotypes which are currently regarded as non-responsive. In the meantime, the relatively high heritability of responsiveness means that it should be fairly easy to introduce this character into material which is non-responsive.

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