Anther culture studies in *Brassica campestris*

G. SEGUIN-SWARTZ, D.S. HUTCHESON, and R.K. DOWNEY -- AGRICULTURE CANADA, 107 Science Crescent, SASKATOON, Canada S7N 0X2

Since the successful *in vitro* production of microspore-derived haploid plants in *Datura innoxia* (Guha and Maheshwari, 1966), there has been considerable interest in the application of this technique to crop plants. Although the anther culture technique in turnip rape, *Brassica campestris*, has been considerably improved in recent years (Keller and Armstrong, 1979), the embryonic response remains low, less than one embryoid per anther cultured. While the importance of environmental factors on microspore embryogenesis is relatively well documented, the possibility of a genetic basis for the phenomenon has received little attention. The present report describes the identification of a *B. campestris* genotype (7B3-10) which exhibits substantially greater embryoid yields than previously reported; preliminary results from studies designed to determine the heritability of the embryogenic characteristic are also presented.

Materials and Methods

The pedigree of plant 7B3-10 is summarized in Fig. 1. The initial object of the selection program was to develop a high erucic acid, low glucosinolate strain which would be useful in production of oil for industrial purposes. DF-314 is an experimental strain very similar to cultivar Candle.

Donor plants were grown in a growth chamber with a 16-hour day and a 25 C and 20 C day/night temperature. A mixture of incandescent and fluorescent sources provided an illumination intensity of approximately 284 $\mu EM^{-2}s^{-1}$, at 1 meter above bench surface.

Anthers were cultured on Keller et al. (1975) medium, with slight modifications (KI: 0.83 mgl^{-1}; sucrose: 8%; and Bacto-agar: 0.6%), and a serine supplement (100 mgl^{-1}). Only anthers containing uninucleate microspores were plated. Incubation was performed in darkness according to the following schedule: 24 hours at 35 C, 6 days at 30 C, and a further 30 to 35 days at 25 C.

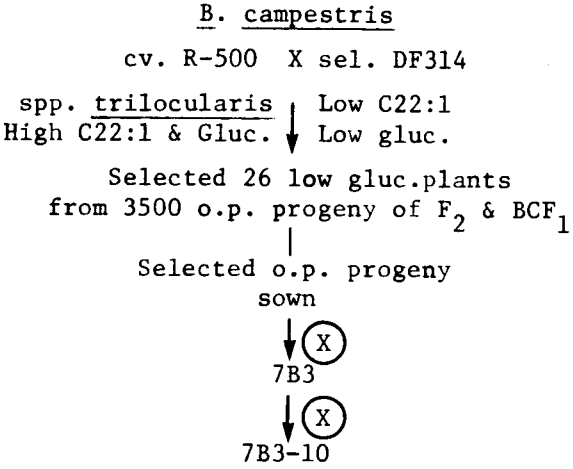

Fig. 1. Pedigree of high embryogenic *B. campestris* plant 7B3-10

When embryoids were observed, the plates were placed under continuous illumination (approx. 70 $\mu Em^{-2}s^{-1}$) at 25 C for 3 to 5 days to promote greening. Well developed embryoids were transferred to basal medium with 2% sucrose to encourage rooting. Rooted plantlets were transplanted into Jiffy-7 peat pellets or into pots containing a soil-free mixture and maintained in plastic-covered enclosures to prevent desiccation for about 6 days. The plantlets were repotted, when required, and grown under greenhouse conditions.

Selfed progeny was produced by the bud pollination technique developed by Kondra and Downey (1969).

Results and Discussion

Among the high erucic acid, low glucosinolate selections being examined for their suitability as anther donors, plant 7B3 was identified as having some potential to produce embryoids. Consequently, its S_1 progeny was chosen for further testing. Although three of the four progeny plants tested had an above-average embryoid frequency, the embryoid production of progeny 7B3-10 was truly remarkable (Table 1). The embryoids of 7B3-10 were detected after only 10 days of culture and produced vigorous plantlets within 2 weeks on the basal medium. The maximum number of embryoids recovered from one single embryogenic anther was 144. The plantlets were classified into two phenotypes: normal

(i.e. parent-like) or dwarf. Dwarf plants had been observed regularly in the material which gave rise to plant 7B3-10. Over 2,000 plants (normal phenotype) were grown and selfed seed was produced from colchicine-treated or spontaneously doubled plants. The embryogenic potential of this material remains to be determined.

Table 1. Embryoid production in four selfed progeny plants from *B. campestris* plant 7B3

Genotype	No. of anthers cultured	No. of embryoids observed	Embryoid frequency (%)
7B3-3	90	51	56.6
7B3-7	84	12	14.3
7B3-8	54	3	5.6
7B3-10	192	3449	1796.4

Embryoid production was examined in the selfed progeny of plant 7B3-10 (Table 2). Anthers from 21 of these S_3 progeny were cultured and embryoids were recovered from 13 of these plants, including two dwarf plants. All embryoids recovered from cultured anthers of the dwarf plants developed into dwarf plantlets.

Anther response in the 13 productive plants ranged from 0.2 to 29% of the anthers cultured. Embryoid frequency, i.e., the percentage of embryoids produced per cultured anther, varied from 0.2 to 661.4%. None of the 13 responsive plants showed a performance comparable to the parent plant 7B3-10. Anther response levels of 15% or more were observed in four plants, and five of the 21 progeny plants showed embryoid frequencies of over 100%. These data suggest that plant 7B3-10, despite its remarkable ability to produce embryoids, was not true breeding for this characteristic. It may also be that high embryoid-yielding plants, such as 7B3-10-24 and -27, have similar genetic embryoid capabilities as their parent, 7B3-10, but fail to express the characteristic due to inbreeding depression.

Self-pollinated seed from four embryogenic progeny of 7B3-10 were grown and anthers cultured. The frequency of embryoids was lower than expected, with the highest frequency of only 45%. The viability and vigor of the embryoids produced from these S_4 plants were also low. It is postulated that

inbreeding in this material over three generations has reduced donor plant vigor and adversely affected the ability of the anthers to produce large numbers of vigorous embryoids.

Table 2. Embryoid production in 7B3-10 and its inbred progeny

7B3 progeny	No.of anthers cultured	No. of embryoids observed	Anther response (%)	Embryoid frequency (%)
10	192	3449	37.0	1796.4
10-1	264	80	6.1	30.3
10-3	72	0	0.0	0.0
10-4	72	0	0.0	0.0
10-5	96	0	0.0	0.0
10-8	120	153	16.7	127.5
10-10	168	67	7.7	39.8
10-11	48	0	0.0	0.0
10-12	432	1	0.2	0.2
10-15	120	0	0.0	0.0
10-16	168	66	11.9	39.2
10-17*	84	2	1.2	2.4
10-18	24	0	0.0	0.0
10-20*	96	229	14.6	238.5
10-22	192	0	0.0	0.0
10-24	143	639	28.0	446.8
10-25	215	9	2.8	4.2
10-26	240	87	9.2	36.2
10-27	238	1574	29.0	661.4
10-28	406	1516	19.0	373.3
10-31	144	1	0.7	0.7
10-34	120	0	0.0	0.0

*Dwarf plants.

To investigate whether the embryogenic characteristic of plant 7B3-10 could be transferred through its inbred progeny to other turnip rape genotypes, F_1 hybrids were derived from reciprocal crosses between medium and low embryonic selfed progeny of 7B3-10 and cultivar Tobin, a low erucic acid, low glucosinolate genotype with a low embryoid frequency of about 1%.

Some variation was observed between the F_1's in embryoid production (Table 3). In every case, regardless of the direction of the cross, the level of embryoid frequency was higher than the low parent; only in the cross with 7B3-10-16, however, did the

F_1 plants exceed the high parent in frequency of embryoids. These results indicate that the embryogenic characteristic is transferable and that maternal effects are not evident.

Table 3. Embryoid production from parents and reciprocal F_1's from crosses between cv. Tobin and 7B3-10 selfed progeny

Parent & F_1 genotype	No. of plants tested	No. of anthers cultured	No. of embryoids observed	% anther response	Embryoid frequency (%)
Tobin					1.0
7B3-10-1					30.3
7B3-10-1 X Tobin	4	527	103	8.3	19.5
Tobin X 7B3-10-1	1	96	3	2.1	3.1
7B3-10-10					39.8
7B3-10-10 X Tobin	5	720	167	8.5	23.2
Tobin X 7B3-10-10	4	624	116	8.5	18.6
7B3-10-16					39.2
7B3-10-16 X Tobin	5	670	417	9.3	62.2
Tobin X 7B3-10-16	4	648	181	10.2	27.9
7B3-10-12					0.2
7B3-10-12 X Tobin	4	528	16	2.5	3.0
Tobin X 7B3-10-12	1	168	85	27.4	50.6

Conclusions

These results, although of a preliminary nature, clearly indicate that the genetic make-up of the donor plant can be a major factor in the efficient use of anther culture in a plant improvement program. It is also evident that this embryogenic characteristic is transferable. However, the inheritance of the characteristic in B. campestris appears to be complex and

greatly influenced by the vigor of the donor plant. Further studies are planned to more clearly determine the inheritance of the characteristic and to fix high embryogenic levels in line 7B3.

For anther culture to be effectively used in an ongoing breeding program, embryoid frequencies would need to be at least 100%, given the losses which can occur in embryoid propagation. For example, it would be much more efficient and equally powerful in terms of selection pressure to plate 150 F_1 anthers and obtain 1500 haploids than to grow some 780,000 F_2 plants from the same cross (Rajhathy, 1976). However, if embryoid frequencies are uncertain or fall much below 100%, then anther culture is not an attractive alternative. Plant 37B-10 and its inbred and doubled haploid progenies offer a potential solution to the uncertainty and low anther response in B. campestris.

Acknowledgement

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