RECENT PROGRESS IN BRASSICA NAPUS RESYNTHESIS BY MEANS OF IN-OVULE EMBRYO CULTURE

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INTRODUCTION

Since the pioneering work of U (1935) the resynthesis of *Brassica napus* has gained increasing scientific and economic importance. The early work in this field was focused on evidence for Morinaga's (1934) classical theory on the evolution of *Brassica* amphidiploids. Later on, first attempts on introgression of economic important genes into *Brassica napus* from its diploid ancestors were carried out. The traits of interest were disease resistance, earliness, cold hardiness and more recently cytoplasmic male sterility and quality traits like fatty acid and glucosinolate patterns (see Chen & Heneen 1989 for review). The aim of the present work is the introgression and combination of S-alleles from self-incompatible *B. campestris* and *B. oleracea* genotypes in synthetic rapeseed lines. This material could serve as an excellent source for studies on inter- and intraspecific interactions of S-alleles in the genus *Brassica* and their use as an outbreeding mechanism for commercial hybrid rapeseed production.

The present paper reports on efficient resynthesis of *B. napus* from a wide range of self-incompatible genotypes of its diploid ancestors, mostly homozygous for a known S-allele. Problems of self-incompatibility interactions in amphihaploid and amphidiploid rapeseed originating from this investigation are discussed in an accompanying paper (Beschorner & Odenbach 1991).

MATERIALS AND METHODS

Seeds of *B. oleracea* and *B. campestris* were provided by Dr. D.J. Ockendon (Wellesbourne) and Dr. T. Hodgkin (Invergowrie). The *B. oleracea* lines are homozygous for a known S-allele and the *B. campestris* genotypes derived from selfing single plants. An accession of self-compatible Yellow Sarson and the commercial F_1-cultivar "Tokyo-King" were also incorporated in the crossing programme. Origin, taxonomy and, if known, the S-allele status of all genotypes are given in Table 1.

The plants were grown in greenhouse under natural light regime. The temperature during the crossing period ranged from 15° to 25° C. Buds were emasculated 1 -3 days prior to anthesis, directly pollinated and bagged to prevent selfing. Developing pods were removed 24-28 days after pollination. If the rapid growing subspecies B. camp. 4 and B. camp. 6 were used as female parents, this time was reduced to 14-16 days.

Ovule culture was according to Sacristan & Gerdemann (1986). Ovules were transfered to petri dishes with MS-medium containing 1% sucrose but no hormones and cultured under cool fluorescent light at 25°C. Developing embryos were grown on the same medium or rooted on a Gamborg-medium supplemented with 0,2 mg/l NAA. The plants were transferred to soil and hybrid nature of the plants was verified by cytological examination.

In order to improve the culture medium the effect of sucrose concentration (1, 3, and 8%), caseinhydrolysate (0, 200 and 500 mg/l) and gelling agents (8g/l Difco-Bacto Agar, 2,5 g/l Gelrite) on the efficiency of resynthesis was tested. Ovules of one pod were equally distributed between the different concentrations or gelling agents respectively to avoid single pod effects.

Table 1. Origin of the parental lines

Code	Line-No.	Taxonomy	S-Allele
a. *B. oleracea*			
B.ol. 1	I 85 - 241		S 8
B.ol. 2	W S 2	subvar. laciniata L. x var. gemmifera DC.	S 2
B.ol. 3	W S 4	subvar. medullosa	S 4
B.ol. 4	W S 14	subvar. medullosa	S 14
B.ol. 5	W S 15	subvar. medullosa x var. gemmifera	S 15
B.ol. 6	W S 32	subvar. millecapitata	S 32
B.ol. 7	W S 45	var. gemmifera DC.	S 45
B.ol. 8	I 85 - 349	ssp. alboglabra	S 2
B.ol. 9	I 85 - 362	ssp. alboglabra	S 29
b. *B. campestris*			
B.camp. 1	I 85 - 326		
B.camp. 2	I 85 - 382	ssp. rapifera	
B.camp. 3	I SD-87-320	ssp. chinensis	
B.camp. 4	I	ssp. nipposinica	
B.camp. 5	F_1 - Hybrid	ssp. pekinensis cv. "Tokyo-King"	
B.camp. 6		ssp. trilocularis	self-compatible

5 cross combinations were selected to compare the efficiency of ovule culture to the ovary culture technique, which also proved to be suitable for *B. napus* resynthesis. 4 to 6 days after pollination ovaries were transferred to culture vessels with a Nitsch & Nitsch medium, modified according to Inomata (1985), supplemented with 5% sucrose and 300 mg/l caseinhydrolysate. Developing seeds were taken from the ovaries 40 days after pollination and cultured in the same way as the ovule culture technique described above.

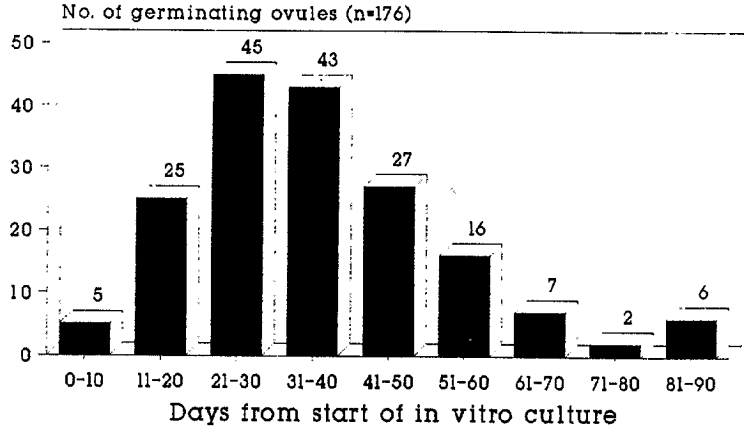

Fig. 1. Time course of ovule germination during *in vitro* - culture

RESULTS

1. Results of interspecific hybridization

Out of 583 pollinated buds from 104 different cross-combinations nearly 5000 ovules were transfered to *in vitro* conditions. The first ovules started to germinate 4 days from the start of *in vitro*-culture. Even 3 months later, some ovules germinated and gave raise to hybrid embryos. Figure 1. illustrates the time course of ovule germination.

56 % of the embryos exhibited an abnormal, teratomous growth habit. Several subcultures on solid or liquid hormone-free MS-medium enabled the regeneration of normal plants from these embryos. Embryos developing late during *in vitro* culture showed a significantly lower rate of abnormal growth. This indicates, that the formation of teratomous structures is due to early germination of the ovules *in vitro*.

A total number of 433 interspecific hybrids from 71 different cross combinations were transfered to soil. The efficiency of resynthesis was strongly influenced by the genotype of the female parent. The results of the interspecific crosses are given in Table 2.

Table 2. Results of the interspecific crosses

♀- line	No. of pollinated buds (A)	No. of paternal lines	No. of hybrids (B)	B/A
a. *B. oleracea*				
B.ol. 1	40	6	6	0.15
B.ol. 2	42	6	19	0.45
B.ol. 3	38	5	12	0.32
B.ol. 4	42	6	17	0.41
B.ol. 5	39	6	14	0.36
B.ol. 6	37	6	3	0.08
B.ol. 7	33	6	41	1.24
B.ol. 8	18	4	19	1.06
B.ol. 9	25	6	19	0.76
Σ	314		Σ 150	0.48
b. *B. campestris*				
B.camp. 1	50	9	20	0.40
B.camp. 2	64	9	2	0.03
B.camp. 3	43	9	70	1.63
B.camp. 4	33	8	53	1.61
B.camp. 5	43	9	113	2.63
B.camp. 6	36	9	25	0.69
Σ	269		Σ 283	1.05
Σ	**583**		**Σ 433**	**0.74**

Hybrid plants exhibited intermediate morphology. Their anthers were shrivelled and completely sterile. Cytological examination showed the expected chromosome number of 2n=19. Compared to their diploid parents most amphihaploid hybrids exhibited a vigorous growth, indicating a strong interspecific heterosis.

2. Variation of the culture medium

The effect of some exogenous factors on the efficiency of resynthesis of *B. napus* by means of in-ovule embryo culture is demonstrated in Table 3. Sucrose

concentrations higher than 10 g/l were of no advantage in ovule culture. Casein hydrolysate, often found useful for ovary culture of interspecific *Brassica* hybrids, proved to be ineffective in the range of the tested concentrations. Compared to Difco-Bacto Agar, the use of Gelrite as a gelling agent significantly increased the rate of hybrid plants.

Table 3. The effect of exogenous factors on the efficiency of resynthesis

	No. of buds pollinated (A)	No. of ovules cultured	No. of hybrid plants (B)	B/A
a. sucrose concentration (16 cross-combinations)				
10 g/l	49	243	42	0.86
30 g/l	49	249	35	0.71
80 g/l	49	245	25	0.51
b. casein hydrolysate (17 cross-combinations)				
0 mg/l	38	345	34	0.90
200 mg/l	38	345	30	0.79
300 mg/l	38	352	31	0.82
c. gelling agents (26 cross-combinations)				
Difco-Bacto	50	536	23	0.46
Gelrite	50	536	45	0.90

3. Comparison of in-ovule embryo culture with ovary culture

The results of a comparison of ovary and ovule culture are given in Table 4.

Table 4. Efficiency of ovary culture compared to in-ovule embryo culture

	a. Ovary culture			b. Ovule culture		
	No. of buds pollinated (A)	No. of hybrids (B)	B/A	No. of buds pollinated (A)	No. of hybrids (B)	B/A
♀B.ol.xB.camp.	29	5	0.17	59	15	0.25
♀B.camp.xB.ol.	25	21	0.84	45	47	1.04
	Σ 54	**Σ 26**	**0.48**	**Σ 104**	**Σ 62**	**0.59**

DISCUSSION

The restricted gene pool of *B. napus* is an important limitation for todays rapeseed breeding. In contrast to this, a lot of genetic variation is available in *B. oleracea* and *B. campestris*. Efficient methods of interspecific hybridization enable the introgression of important genes from both species into *B. napus* by means of resynthesis and subsequent backcrossing to modern rapeseed cultivars.

Namai *et al.* (1980) reviewed the literature on *Brassica napus* resynthesis and an update of recent results is given in Table 5. The continously increasing sucess in interspecific hybridization reflects the enormous progress of *in vitro*-culture during the last decades. To date, embryo, ovary and in-ovule embryo culture are succesful in circumventing interspecific incompatibility in the genus *Brassica*.

Although crucifer-embryos were the object of the earliest investigation using *in vitro* culture of isolated embryos (Hannig 1904), it took more than 50 years for the first succesful application of embryo culture to resynthesize *B. napus* (Nishi *et al.* 1959).

The main disadvantage of embryo culture is the difficult and time-consuming preparation of *Brassica* embryos. The sucess in overcoming interspecific incompatibility is limited and the technique is only effective when the female parent is *B. oleracea* (Gland 1982; Chen 1989). Ovary culture proved to be much more efficient in this interspecific hybridization (see Table 5). Because the handling of *Brassica* ovaries requires much less effort and diligence, a lot of material can be accomplished in a shorter time frame. In contrast to embryo culture, hybrid plants were mostly obtained in the cross direction ♀ *B. campestris* x *B. oleracea* (Inomata 1978; Takeshita *et al.* 1980).

In-ovule embryo culture was first applied for the resynthesis of *B. napus* by Takeshita *et al.* (1980). Sacristan & Gerdemann (1986) and Diederichsen & Sacristan (1988) established this technique for the resynthesis of all *Brassica* amphidiploids and crosses between them.

Table 5. A survey of recent results on resynthesis of *Brassica napus*. through application of *in vitro* culture

Reference		*in vitro*-technique	No. buds pollinated (A)	No. hybrids (B)	B/A
a. ♀*B. campestris* x *B. oleracea*					
Matsuzawa	1978	ovary culture	400	155	0.39
Takeshita *et al.*	1980	embryo culture	30	1	0.03
Takeshita *et al.*	1980	ovary culture	154	30	0.19
Takeshita *et al.*	1980	ovule culture	157	46	0.29
Gland	1982	embryo culture	1170	0	0.00
Inomata	1984	ovary culture	88	24	0.27
Inomata	1985	ovary culture	568	381	0.67
Diederichsen *et al.*	1988	ovule culture	334	273	0.82
Plümper[1.]	1991	ovary culture	25	21	0.84
Plümper[1.]	1991	ovule culture	269	283	1.05
b. ♀*B. oleracea* x *B. campestris*					
Takeshita *et al.*	1980	embryo culture	93	20	0.22
Takeshita *et al.*	1980	ovary culture	81	0	0.00
Takeshita *et al.*	1980	ovule culture	105	43	0.41
Gland	1982	embryo culture	6.533	367	0.06
Diederichsen *et al.*	1988	ovule culture	671	491	0.73
Chen	1989	embryo culture	2.295	379	0.17
Plümper[1.]	1991	ovary culture	29	5	0.17
Plümper[1.]	1991	ovule culture	314	149	0.47

[1.] present investigation

The present investigation reports the resynthesis of 71 different *B. napus* lines from a wide range of its diploid ancestral species. The high frequency of sucessful interspecific hybridization in both cross directions is due to the application of in-ovule embryo culture. This *in vitro*-technique is easy to perform and not as

time-consuming as embryo culture. Compared to ovary culture, the sucess rate in receiving hybrid plants is higher, especially when *B. oleracea* was the female parent. Therefore in-ovule embryo culture is suggested to be the preferable method for *Brassica napus* resynthesis in research and breeding.

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