PRODUCTION OF HAPLOIDS IN OILSEED RAPE (BRASSICA NAPUS L.) BY IMPROVED MICROSPORE CULTURE

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SUMMARY

Induction of haploid embryoids of oilseed rape (Brassica napus L.) from isolated microspores cultured according to LICHTER (1982) is strongly determined by the donor plant genotype. This genotype dependence is largely overcome by using the following modifications of the original method:

- omission of the cold-pretreatment of buds
- selection of buds with petals half as long as the anthers
- culture of microspores in a 32/25°C temperature regime
- use of a two-layer culture medium with activated charcoal.

Regeneration of plants was induced by culturing the haploid embryoids on a solid MURASHIGE and SKOOG (1962) medium supplemented with 0.2 mg/l 6-BAP. Other hormone combinations were tested for their shoot regeneration abilities.

INTRODUCTION

Culture of isolated microspores as a method to produce haploids in oilseed rape was first described by LICHTER (1982). By several modifications of the original method CHUONG and BEVERSDORF (1985) could evidently increase the yield of haploid embryoids. In the present investigation a further modified technique is described that was successfully applied to produce haploids of six winter rape genotypes.

MATERIALS and METHODS

The genotypes used in this study were six winter rape F_1-hybrids kindly supplied by the Norddeutsche Pflanzenzucht, Hans-Georg Lembke KG, Hohenlieth (Table 1). Twelve seeds of each F_1 were shown in a compost: peat mix in the greenhouse in autumn 1985. The young plants were vernalized at 2 - 4°C for eight weeks. In February 1986 they are transfered to the greenhouse in a 20-25/15°C day/night temperature regime and a 16 h photoperiod. The plants were fertilized three times a week with a 0.2% solution of a 15 N : 11 P_2O_5 : 15 K_2O fertilizer. Buds of about 3 mm length were selected sequentially before opening of the first flower. Selected buds were surface-sterilized in a 0.5% (w/v) solution of calcium-hypochlorite supplemented with 0.1% sodium-dodecylsulfate for 5 min and then rinsed three times with sterile deionized water. Buds with petals approximately half as long as anthers were gently

squashed in a 250 µm sieve with the sieve surface covered with liquid culture medium according to LICHTER (1982). The bud suspension was passed through a 40 µm sieve and microspores collected by centrifugation at low speed. After one wash with culture medium the microspores were suspended in culture medium 0.25 ml for each bud initially present. The microspore suspension was incubated in 1.5 ml portions in 35 mm sterile petri dishes coated with a thin layer of culture medium, solidified with 1.0% agar and supplemented with 0.5% activated charcoal, according to JOHANSSON (1986). The microspores were incubated for two days at 32°C in the dark, afterwards at 25°C in red light. Embryoids were transferred to solid hormone free MS medium (MURASHIGE and SKOOG 1962) supplemented with 5% sucrose. Shoots were induced on MS-medium with 0.2 mg/l 6-BAP and 1% sucrose. After root induction the plantlets were transplanted to soil and placed in the greenhouse.

RESULTS

About three weeks after starting the microspore culture macroscopically visible embryoids were observed. Their number was strongly genotype-dependent (Table 1). In total, 3.35 embryoids per cultivated bud and 0.56 embryos per anther, respectively, were obtained. Data varied between 0.90 and 13.56 embryoids per bud or 0.15 and 2.26 embryoids per anther.

Table 1: Number of embryoids and plantlets formed in microspore culture experiments with six winter rape F_1 hybrids, realized in Göttingen in spring 1986

F_1 hybrid	Cultured buds	Anthers	Embryoids	Embryoids/ anther	Subcultured embryoids	Plantlet regeneration (%)
Jupiter x Mo 1197/85/3	232	1392	353	0.25	240	20.4
Jupiter x Mo 1939/85/2	236	1416	212	0.15	200	9.5
Jet Neuf x Mo 1939/85/2	236	1416	475	0.34	240	18.3
Bienvenu x Mo 1197/85/2	215	1290	2915	2.26	240	25.4
Bienvenu x Mo 1939/85/2	199	1194	413	0.35	240	37.9
Mikado x Mo 1939/85/2	229	1374	138	0.10	120	17.5
Total	1347	8082	4506	0.56	1280	22.3

The most vigorous embryoids were subcultured on solid MS-medium. Seven months after start of the embryo culture, 22.3% of the initially present embryoids had developed into plantlets that could be transferred to soil (Table 1). Plantlet formation varied between 9.5 and 37.9% for the different genotypes investigated. All plantlets resulted from secondary shoot regeneration of the primary embryoids.

DISCUSSION

In the present investigation an improved microspore culture technique was applied to the production of haploids in winter oilseed rape. From all used genotypes microspore-derived embryoids were obtained. The embryoid frequency varied between 0.15 and 2.26 embryoids per anther, with an average of 0.56. Previous authors reported on embryoid frequencies of 0.04 - 0.58, most of them obtained from anther culture (Table 2). The rate of plantlet regeneration from the embryoids depends on the genotype and the used culture medium. In the present investigation 22.3% of all cultured embryoids regenerated plants from secondary

Table 2: Number of microspore-derived embryoids per anther and plant regeneration frequency in Brassica napus realized by different authors

Author	Donor plant type (1)	Method (2)	Embryoids per cultured anther	Plantlet regeneration (%)
Keller and Armstrong (1978)	s	a	0.16*	17.3 - 28.9*
Renard and Dosba (1980	w	a	0.04*	52.1
Lichter (1981)	s	a	0.27*	33.0
Lichter (1982)	s	m	0.34*	-
Dunwell et al. (1983)	s	a	0.14*	-
Naleczynska and Cegielska (1984)	s,w	a	0.08*	54.2*
Chuong and Beversdorf (1985)	s	m	-	5.8*
Dunwell and Thurling (1985)	w	a	0.58*	-
Dunwell et al. (1985)	w	a	0.25*	-
Present investigation	w	m	0.56	22.3

(1): w = winter rape, s = spring rape
(2): a = anther culture, m = microspore culture
* : Value calculated from the published data

shoots. The regeneration frequency in previous experiments varied between 5.8 and 54.2% (Table 2). The most important factors that influence the regeneration are the development stage of the initial embryo and the composition of the culture medium. Abnormal or small embryoids fail to develop further (CHUONG and BEVERSDORF 1985). By other authors irregular embryo development on subculture media is reported (KELLER and ARMSTRONG 1978, LICHTER 1981, NALECZYNSKA and CEGIELSKA 1984). The increase of plantlet regeneration by improving the embryoid culture conditions is subject of present experiments, results of which will be published later.

This work was sponsored by BMFT/AIF, Bonn, and the Norddeutsche Pflanzenzucht, Hohenlieth. Stimulation and support by Prof.Dr. G. Röbbelen and Dr. M. Frauen are also gratefully acknowledged.

REFERENCES

CHUONG, P.V., and W.D. BEVERSDORF, 1985: High frequency of embryogenesis through isolated microspore culture in Brassica napus L. and B. carinata Braun. Plant Science 39, 219-226

DUNWELL, J.M., M. CORNISH, A.G.L. de COURCEL and J.E. MIDDLEFELL-WILLIAMS, 1983: Induction and growth of 'microspore-derived' embryos of Brassica napus ssp. oleifera. Exp.Bot. 34, 1768-1778

- , - and - , 1985: Influence of genotype, plant growth temperature and anther incubation temperature on microspore embryo production in Brassica napus ssp. oleifera. Exp.Bot. 36, 679-689

- , and N. THURLING, 1985: Role of sucrose in microspore embryo production in Brassica napus ssp. oleifera. Exp.Bot. 36, 1478-1491

JOHANSSON, L.B., 1986: Effects of activated charcoal, cold treatment and elevated CO_2-concentrations on embryogenesis in anther cultures. In: HORN, W., C.J. JENSEN, W. ODENBACH and O. SCHIEDER (eds.): Genetic Manipulation in Plant Breeding. W. de Gruyter, Berlin-New York, 1986, pp. 257-264

KELLER, W.A., and K.G. ARMSTRONG, 1978: High frequency of microspore-derived plants from Brassica napus anther cultures. Z.Pflanzenzüchtg. 80, 100-108

LICHTER, R., 1981: Anther culture in Brassica napus in a liquid culture medium. Z.Pflanzenphysiol. 103, 229-237

- , 1982: Induction of haploid plants from isolated pollen of Brassica napus. Z.Pflanzenphysiol. 105, 427-434

MURASHIGE, T., and F. SKOOG, 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol.Plant. 15, 473-497

NALECZYNSKA, A., and T. CEGIELSKA, 1984: Dihaploid production in Brassica napus L. by in vitro androgenesis. Genet.Polon. 25, 271-276

RENARD, M., and Francoise DOSBA, 1980: Etude de l'haploidie chez le colza (Brassica napus L. var. oleifera Metzger). Ann. Amélior. Plant. 30, 191-210