The effect of endo- and exogenous factors on growth and maturation of oilseed rape (*Brassica napus* cv. Jet Neuf) siliques cultured *in vitro*

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Abstract

The effect of developmental stage of siliques, growth regulators, agar and sucrose concentrations in the medium was investigated on the morphogenesis of the siliques and seed maturation. Regeneration of shoots, roots and callus occurred at the base of siliques during their growth. The developmental stage of the incubated siliques was the most important factor affecting the number of seeds, while the conditions of culture (agar and growth regulators) constituted a secondary factor. The reasons for the insignificant number of seeds produced could be the lack of fertilization of some of the ovules, limited ability of siliques to take up nutrients and the unspecific composition of the medium.

Key words: Brassica napus, silique growth in vitro, growth regulators

INTRODUCTION

The oilseed rape yield is determined by the number of siliques per plant, the number of seeds per silique and the weight of the seeds (I n a n a g a and K u m u r a 1987). The number of seeds per silique depends in turn on the number of ovules from which the seeds will develop (P e c h a n 1988). The average number of ovules per silique in cv. Jet Neuf is about 33 beside the number of developed seeds in self-pollinated plants — 17 and in cross-pollinated — 24 (Wojciechowski and Niedziela 1987). The factors affecting the number of seeds in silique are not well known. Nutrients and growth regulators are transported to developing siliques during the generative development of the plant. This limits the determination of the effects of various factors on the

development of seeds and the accumulation and synthesis in them of storage materials and other oilseed rape metabolites such as glucosinolates.

Recently, studies have been undertaken on *in vitro* cultures of ovaries, pistils, pods and siliques aimed at determining the influence of various factors on the growth of fruit and ripening of seeds in them. Excised organs are deprived of the growth correlations present in intact plants. *In vitro* cultures have been carried out of, among others, tomato ovaries (U r a l e t s 1980), soybean, alfalfa and pea pods (O b e n d o r f et al. 1983, W a n g et al. 1984, B a r r a t t 1986) and oilseed rape siliques (D r o z d o w s k a et al. 1989). This method has also been taken advantage of in *Brassica* genus to obtain interspecific hybrids (I n o m a t a 1978, B a j a j et al. 1986).

This paper is a continuation of studies on the morphogenesis of oilseed rape in *in vitro* cultures and on the metabolism of glucosinolates (R o g o z i ń s k a and D r o z d o w s k a 1981, 1988, D r o z d o w s k a et al. 1989). The effects of endo- and exogenous factors on the growth of oilseed rape siliques cultured *in vitro* as well as on the formation and maturation of seeds in them were studied.

MATERIAL AND METHODS

The siliques of winter oilseed rape (*Brassica napus* cv. Jet Neuf) were isolated from plants during seed setting and filling. Explants were sterilized in a 0.2% solutions of HgCl_2 and, after rinsing in sterile water several times, were placed in 100 cm³ Erlenmayer flasks containing 25 cm³ Murashige and Skoog medium (1962).

The medium was supplemented with vitamins according to G a m b o r g et al. (1968), casein hydrolysate (300 mg · dm⁻³) sucrose and growth regulators. The isolated siliques were cultured for a period of 6 weeks under conditions of a 16 h photoperiod at a light intensity of about 3000 lx and temp. $25 \pm 2^\circ\text{C}$.

The influence of the following endo- and exogenous factors on the growth of siliques and formation and maturation of seeds was studied:

- a. Developmental stage of the isolated silique (I — seed setting stage — initial length 2.0-2.5 cm, which corresponds to about six days after fertilization: II — seed filling stage — initial length 3.0-3.5 cm, about 8 days after fertilization: III — seed filling stage — initial length 4.0-4.5 cm, about 10 days after fertilization).
- b. Sucrose concentration — 2.0, 4.0, 6.0, 8.0%.
- c. Agar concentration — 0, 0.5, 0.8%.
- d. Kind and concentration of growth regulators — NAA (1-naphthaleneacetic acid), BAP (6-benzylaminopurine) and combinations of them.

For anatomical examinations the siliques were fixed in FAA, dehydrated in increasing concentrations of ethanol and embedded in paraffin. Sections 14 µm thick were stained with hematoxylin according to Heidenhein (B r o d a 1971).

The experiments were carried out in triplicate. The results were analyzed statistically by the Tukey test.

RESULTS AND DISCUSSION

Intense cell divisions occur during the first two weeks of oilseed rape embryogenesis. After they stop around 2-3 weeks following fertilization, the synthesis of lipids in seeds becomes more intense and precedes the synthesis of storage proteins. Various plant organs participate in the accumulation of storage materials in maturing oilseed rape seeds. An isotopic tracer method revealed that the leaves supply the most assimilates to the seeds (37%), followed by silique walls (32%) and stems (31%) (B r a r and T h i e s 1977).

Under field conditions, the growth of oilseed rape siliques and seed maturation are controlled by external and internal factors (I n a n a g a and K u m u r a 1987, B o u i l l e et al. 1989). *In vitro* silique culture may provide a better understanding of the processes related to their growth and the maturation of seeds, which is shown in this paper.

The age of the isolated silique, that is, the stage of development of the young embryo, is one of the factors which determines the growth of siliques and development of seeds in *in vitro* culture. More seeds matured in the siliques isolated during stage II and III, that is, around 8 and 10 days after fertilization, than did in those isolated during stage I, that is, in the youngest (Table 1). The dependence between the age of the isolated silique and its further growth was demonstrated earlier by B a j a j et al. (1986). They cultured fertilized ovaries *in vitro* in order to obtain hybrids between *Brassica napus* × *Brassica juncea* and found that the best period for isolation was 8-10 days after pollination.

Table 1

Effect of the developmental stage of isolated oilseed rape siliques, growth regulators and agar concentration in the medium on the number of matured seeds in siliques grown *in vitro* (growth period — 6 weeks)

Concentration of growth regulators (mg.dm ⁻³)*	Agar concentration (%)								
	0			0.5			0.8		
	Developmental stage of isolated silique								
	I	II	III	I	II	III	I	II	III
0	3	8	4	1	3	3	2	4	3
0.5 NAA	1	5	4	2	2	4	0	4	5
1.0 NAA	0	1	3	1	2	1	0	4	2
0.5 BAP	2	3	4	1	4	2	0	4	2
1.0 BAP	3	6	6	1	2	2	1	4	3
0.5 NAA+0.5 BAP	0	1	6	0	3	3	0	6	6
1.0 NAA+0.5 BAP	0	0	6	1	2	2	0	3	4
0.5 NAA+1.0 BAP	0	3	5	1	3	2	0	4	2
1.0 NAA+1.0 BAP	4	6	6	1	2	2	2	3	4
$\bar{x}$	1.44	3.85	4.89	0.78	2.59	2.33	0.78	3.96	3.44
Transf. LSD = 0.16 (P = 0.05)	1.26	1.89	2.26	1.08	1.69	1.67	1.04	2.06	1.96

* Each combination was comprised of 20 siliques.

Fig. 1. The effect to growth regulators and agar concentration on the morphogenesis of isolated oilseed rape siliques cultured *in vitro* (growth period — 4 weeks, A — liquid medium, B — 0.5% agar, C — 0.8% agar)

Fig. 2. The effect of growth regulators and sucrose on the morphogenesis of oilseed rape siliques cultured *in vitro*, after 4 weeks of growth (siliques were isolated in stage I of development): A — control; B — $1.0 \text{ mg} \cdot \text{dm}^{-3}$ NAA + $1.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP; C — 12-week-old plantlet differentiated at the base of the sillique

Analysis of the effects of growth regulators on the development of isolated oilseed rape siliques showed that growth regulators used individually as well as in combination affected the number of seeds and acted in conjunction with the other factors under study. This was confirmed by statistical analysis. NAA at a concentration of $0.5 \text{ mg} \cdot \text{dm}^{-3}$ was more effective in terms of the number of developed seeds than at a concentration of $1.0 \text{ mg} \cdot \text{dm}^{-3}$. BAP at a concentration of $1.0 \text{ mg} \cdot \text{dm}^{-3}$ also had a slight stimulatory effect on the maturation of seeds in siliques (stage II and III) cultured on a liquid medium. Better effects in terms of the number of seeds were obtained on medium with a combination of growth regulators. For siliques cultured on a liquid medium, that best concentration was $1.0 \text{ mg} \cdot \text{dm}^{-3}$ NAA + $1.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP, while on the agar medium, $0.5 \text{ mg} \cdot \text{dm}^{-3}$ NAA + $0.5 \text{ mg} \cdot \text{dm}^{-3}$ BAP (Table 1). The growth regulators also affected the morphogenesis of isolated siliques (isolated during stage II and III), while a combination of NAA and BAP — the formation of callus tissue (Fig. 1). Siliques isolated during earlier stages of development (I) and cultured on a medium containing $1.0 \text{ mg} \cdot \text{dm}^{-3}$ NAA + $1.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP showed a greater morphogenetic potential and, in addition, exhibited regeneration of shoots modified by the presence of sucrose (Fig. 2).

The structural components of the yield — the number of siliques, seeds and their weight depend not only on nutrients that are transported and accumulated, but also remain under the control of endogenous growth regulators. It was demonstrated that IAA synthesis occurs in maturing oilseed rape seeds and affects their filling, accumulation of photosynthates and the development of silique wall (Bouille et al. 1989). The number of seeds in the siliques of oilseed rape growing under filed conditions may be increased by the use of such exogenous auxins as 2,4-D (Morgan 1980) and IAA (Inanaga and Kumura 1987). The relatively slight stimulatory effect of NAA ($0.5 \text{ mg} \cdot \text{dm}^{-3}$) found in this study confirms the role of this group of regulators during seed development.

Cytokinins are also important for developing seeds. They may be synthesized in the seed itself or transported from other parts of the plant. Both the early stages of embryogenesis in rape and the development of siliques and their number depend on the presence of endogenous cytokinins (Bouille et al. 1989). The results obtained in this study indicate that seeds also matured in siliques which were incubated on a medium lacking growth regulators. It can be assumed then, that seeds contain endogenous phytohormones so their addition to the medium is not always necessary.

The other studied factors such as the presence of sucrose and degree of gelling of the medium by agar also affected the morphogenesis of siliques (Figs. 1, 2). This has to do with the osmotic potential of the medium which affects the availability of nutrients. This can explain the better growth in length and breadth of the oilseed rape siliques cultured on the less solidified media. Almost all of the seeds in siliques cultured on liquid media developed but did not attain ripeness.

As was shown by C o o k et al. (1988), reduction of the osmotic potential of the medium results in inhibition of the further stages of development of immature embryos and they begin to germinate precociously. A similar effects was observed in *in vitro* cultures of siliques of the studied variety of oilseed rape, cultured on agar medium (Fig. 3). The seeds which began to germinate in some of the siliques did not have a fully developed seed cover.

Fig. 3. The effect of growth regulators on the splitting of siliques and seed germination *in vitro*

Not very many seeds developed in the oilseed rape siliques cultured *in vitro*, their maximal number reached only 8 per silique. However, due to the synergistic effect all of the factors used in this experiment, the number of seeds was higher than that obtained in the experiment by B a j a j et al. (1986).

The anatomical studies were carried out in order to find out the reason for the small number of developed seeds. They showed that in the isolated siliques the ovules were present at various stages of development. Some of them were fertilized and were able to develop embryos and then seeds. Other ovules were shrivelled and dying (Fig. 4). Early seed abortion can be the result of fertilization of the egg cell only. In this case, no endosperm is formed which supplies the

Fig. 4. Cross section through siliques grown *in vitro* (siliques were isolated in stage III of development): A — control, before incubation; B — after 4 weeks of growth, visible cotyledons and embryo axis; C — degeneration of embryos

young embryo with nutrients. The degeneration of seeds can also be the result of their delayed development and resulting limited ability to compete with many normally developing seeds for available nutrients (Pechan 1988).

In the study presented here, it was found that the most significant of the tested factors affecting the growth of siliques and number of seeds in them was the stage of development of the isolated silique. The conditions of culture were of secondary importance, but did affect the further stages of siliques growth. It can be assumed that the limited number of seeds formed was the result of isolating siliques in which not all ovules had been fertilized, the limited ability of siliques to take up nutrients or of the composition of the medium which was not specifically worked out to the requirements of oilseed rape siliques. Determining the role of these factors will make it possible to use isolated oilseed rape siliques in studies on the further stages of growth and maturation of seeds. It may also contribute to the better understanding the processes involved in the synthesis and accumulation of storage materials and other metabolites.

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*Wpływ czynników endo- i egzogennych na wzrost i dojrzewanie
łuszczyń rzepaku ozimego (Brassica napus cv. Jet Neuf) w kulturach in vitro*

Streszczenie

Przebadano wpływ fazy rozwojowej łuszczyń, regulatorów wzrostu, stężenia agaru i sacharozy w pożywce na morfogenezę izolowanych łuszczyń rzepaku i dojrzewanie w nich nasion. W czasie wzrostu łuszczyń, u ich podstawy, następowało tworzenie pędów, korzeni i kalusa. Najistotniejszym czynnikiem wpływającym na liczbę nasion była faza rozwojowa inkubowanej łuszczyzny. Natomiast warunki kultury (agar i regulatory wzrostu) stanowiły czynnik drugorzędny. Przyczyną małej liczby wykształconych nasion może być niezapłodnienie wszystkich zalążków, ograniczona zdolność łuszczyń do pobierania substancji odżywczych i mało specyficzny dla rozwijających się nasion rzepaku skład pożywki.