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Embryo sac development during the culture of placenta attached ovules of *Melandrium album*

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Abstract

Ovules of *Melandrium album*, attached to the placentas and containing immature embryo sacs, were cultured *in vitro*. The embryo sacs developed according to the *Polygonum* type but about 70 % of them degenerated during the culture. Gametophytes of a non-typical structure were found in a few ovules, *i.e.* there appeared more nuclei or cells as in the *Polygonum* type and the arrangement of nuclei or cells was not true to type. Several-celled structures observed in some embryo sacs were recognized as gynogenetic embryoids.

Introduction

Although the experiments on gynogenesis in higher plants have been carried on intensely since the early eighties, the development of embryo sacs in young ovaries or ovules cultured *in vitro* has been studied in a few species only. Under conditions favouring the development of gynogenetic embryos, the female gametophytes of *Hordeum vulgare* and *Oryza sativa* developed as *in vivo* (Huang *et al.* 1982, Zhou *et al.* 1986). However, detailed studies showed that apogamic embryos of rice were often formed in the embryo sacs differing somewhat from the *Polygonum* type (Li and Yang 1986).

The former studies on the placenta culture in *Melandrium album* revealed that gynogenetic plants regenerated from parthenogenetic embryos (Mól 1992). The embryo sacs of *M. album* develop *in vivo* according to the *Polygonum* type (Breslawetz 1929). It is worthy to ascertain if the embryo sac development diverges *in vitro* from the norm, especially in relation to the induction of gynogenesis in this species.

Material and methods

Placentas with the attached ovules were excised from young floral buds of *Melandrium album* (Mill.) Garcke and cultured, as described before (Mól 1992),

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on Miller-Fujii medium (Miller 1963, Clapham 1971) supplemented with 2.0 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 6 % sucrose. Development of embryo sacs was studied in the explants of two stages designated as I and II (Fig. 1). In all, 100 explants were analysed after 6 and 12 h, and 1 to 5 d of culture. The *in vivo* development of embryo sacs was also examined.

The material was prepared by paraffin method as mentioned earlier (Mól 1992). The embryo sacs with a damaged structure indicating their degeneration were left out of account as the percentages and the mean numbers of embryo sacs were calculated for particular periods of culture. A Student *t*-test or a Cochran and Cox *C*-test were used to calculate the levels of significant differences in the mean number of embryo sacs per explant (Fig. 2). Drawings were made from the slides observed in a microscope *Biorom T* (*IOR* Bucharest) with a drawing attachment.

Results

The *in vitro* development of embryo sacs of *Melandrium album* is presented in Fig. 1. In the ovules containing megasporocytes (stage I), meiosis completed on the first day of culture.

	Percentage of embryo sacs at different developmental stages								Time of culture	Number of embryo sacs analysed
explants at stage I								99.7	5 d	383
					0.1	3.4	0.6	0.6	95.0	4 d	868
					1.5	9.9	0.8	20.8	66.9	3 d	948
				0.4	7.6	13.5	0.8	18.4	54.7	2 d	777
		0.1	0.6	59.8	30.7	5.2	0.6	1.0	0.8	1 d	918
	2.1	6.3	13.4	54.8	19.2	3.0	0.4	0.4		12 h	666
	2.4	3.0	21.6	55.9	17.1					6 h	719
	2.4	14.5	6.6	64.5	12.0					excision	959
	megasporogenesis										
explants at stage II				13.4	37.4	27.8	1.2	17.7	2.3	excision	1286
				14.7	30.8	29.4	1.2	19.3	4.5	6 h	1006
				5.5	19.6	22.4	1.7	18.1	31.8	12 h	762
				3.9	13.2	10.8	0.7	20.4	50.8	1 d	825
					0.7	0.8	0.4	5.2	91.7	2 d	943
						0.2			98.5	3 d	826
					0.3	0.7	0.3		98.6	4 d	742
									100.0	5 d	456

Fig. 1. Development of embryo sacs in the placenta attached ovules of *Melandrium album* cultured *in vitro*. In the explants at stage I, the ovules at megasporogenesis were also taken into account.

At stage I, the gametophytes developed rapidly from 1- or 2-nucleate into nearly or fully mature ones on the second day of culture. In the explants excised at stage II, 2- to 4-nucleate embryo sacs became mature or nearly mature during 12 - 24 h. From the fourth day of culture at stage I and from the second day at stage II, over 90 % non-degenerated gametophytes were mature. Synergids began to degenerate in many embryo sacs at that time. The synergids of *M. album* became damaged also in unfertilized embryo sacs *in vivo* after 6 d of anthesis

During the flower development in *M. album* (*in vivo*), some new ovules still appeared on the placentas, so the mean number of embryo sacs per ovary increased from 143 ± 42 in the flower buds at stage I to 261 ± 53 in the opening flowers. The formation of ovule primordia and meiosis occurred also *in vitro* at stage I, thus the slight increase in the mean number of embryo sacs per explant was noticed on the first day of culture (Fig. 2). A statistically significant decrease in this number was observed when 1- to 4-nucleate gametophytes developed into 7-celled or mature ones; *i.e.* during the second day of culture at stage I and after 12 h at stage II (Figs. 1, 2). Thereafter, this number did not decline markedly till the fifth day of culture although over 90 % of non-damaged gametophytes became mature in the explants at stage II as early as on the second day. After 5 d of culture, the explants still contained about 30 % of embryo sacs in which no visible damages to the cell structure were distighuished. When *in vivo* pollination did not occur, 23 % and 8 % of embryo sacs remained non-damaged after 6 and 12 d, respectively.

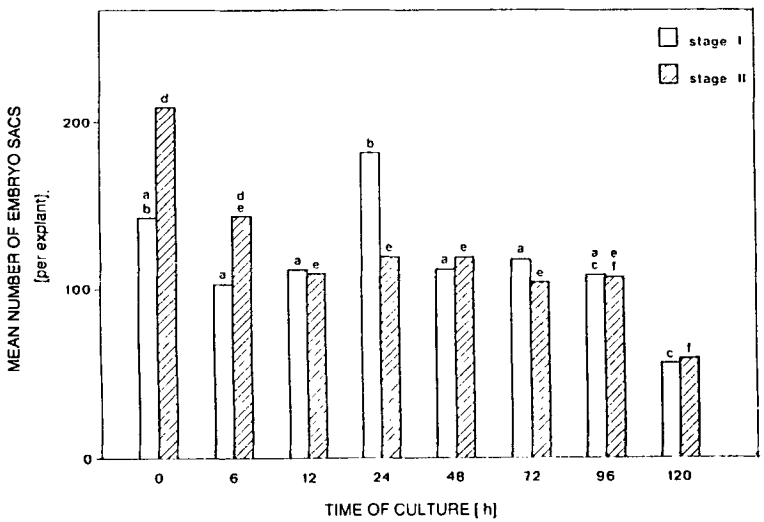

Fig. 2. Alterations of the mean number of embryo sacs per explant (ES/expl) during the culture of placenta attached ovules of *Melandrium album*.
At stage I, the ovules containing archesporial cells, megasporocytes, and megaspore tetrads were included in calculations. Columns with the same letter are not significantly different at a 0.05 confidence level.

Almost all female gametophytes of *M. album* represented *in vitro* developmental stages characteristic of the *Polygonum* type. However, thirty (0.3 %) non-typical embryo sacs were found. They often contained more than 8 nuclei, free or separated by the cell walls (Fig. 3 A - D). In 4- to 8-nucleate embryo sacs, the non-typical arrangements of nuclei were observed; *i.e.* 3 + 1, 1 + 2 + 1, 4 + 1, 4 + 2, 2 + 4, 5 + 3, and 3 + 3 + 2. At the micropylar end of some 8-nucleate gametophytes, 2- or 3-nucleate cells formed instead of a typical egg apparatus, and only two antipodal cells developed at the chalazal end (Fig. 3 E, F). Moreover, five embryo sacs contained two egg cells. Several-celled structures appeared in nine embryo sacs at the micropylar end (Fig. 3 G, H) and in one embryo sac at the chalazal end (Fig. 3 I). Those structures, which could be regarded as gynogenetic embryoids, usually consisted of vacuolated and relatively large cells. In control experiments *in vivo*, no shift from the *Polygonum* type was detected.

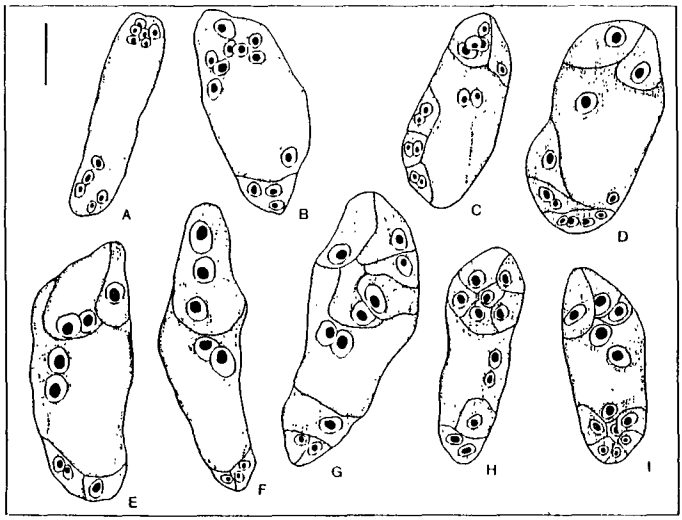

Fig. 3. Examples of the non-typical embryo sacs of *Melandrium album* observed in the ovules from placenta culture. *A, F* - 12 h of culture, explant at stage II; *B, I* - 1 day, stage I; *C,D,G* - 2 days, stage I; *E* - 6 h, stage II; *H* - 3 days, stage II. Micropylar end upwards. Bar = 20 μm.

Discussion

The *Polygonum* type of development was dominant in the ovules of *Melandrium album* attached to the placentas and cultured *in vitro*, and non-typical embryo sacs formed sporadically only. Li and Yang (1986) found that *in vitro* apogamy occurring in rice was related to the non-typical development of gametophytes. However, the other study of gynogenesis in *Oryza sativa* revealed that the embryo sacs were following the *Polygonum* type (Zhou *et al.* 1986). In tobacco also, the embryo sacs

matured after meiosis in young ovaries cultured *in vitro*; however, at high concentrations of phytohormones, the gametogenesis was delayed and a percentage of abnormal gametophytes increased (Lobanova and Enaleeva 1988). In the involucres of *Cenchrus ciliaris* cultured on a hormon-free medium, sexual and aposporic embryo sacs developed in the same manner as *in vivo*; *i.e.* according to the *Polygonum* and *Panicum* type, respectively (De Groote and Sherwood 1984). The results of above-mentioned studies indicate a considerable uniformity of the embryo sac development *in vitro* and *in vivo*.

Throughout the culture of placentas with ovules, many gametophytes of *M. album* degenerated during the maturation rather than after that. Also in cultured involucres of *C. ciliaris* (De Groote and Sherwood 1984) and ovaries of *Nicotiana tabacum* and *N. rustica* (Zhu *et al.* 1981., Lobanova and Enaleeva 1988), most embryo sacs died *in vitro* after about one week.

Development of parthenogenetic embryos in the placenta culture of *M. album* has been described earlier (Mól 1992). The results presented here suggest another origin of gynogenetic embryos in this species. Some non-typical embryo sacs contained several-celled embryoids as early as after 1 or 2 days of culture. In the explants at stage I, the egg apparatus just began to form at that time. Thus, at least at stage I, those embryoids could not arise from mature egg cells. The non-typical embryo sacs of *M. album* contained often more than 8 nuclei. Then, the unusual arrangement of nuclei caused probably disturbances of cellularization at the ends of embryo sac and induced the formation of more than three cells or few-nucleate cells. The proces of cell wall formation in the embryo sac is relatively fast, and the arrangement of nuclei at micropylar or chalazal end affects the spatial orientation of expanding cell walls (Kapil and Bhatnagar 1981, Cass *et al.* 1985, 1986, Bhandari and Chitralekha 1989, Folsom and Cass 1990).

The studies on *in vitro* gynogenesis in various species revealed that gynogenetic embryos originated from the egg cells, synergids or antipodal cells. The formation of embryoids directly from megaspores was observed in tobacco and lily only (Wu and Cheng 1982, Gu and Cheng 1983). The present results concerning *M. album* indicate for the first time that gynogenetic embryoids may also be formed in multi-nucleate embryo sacs most likely due to non-typical organization of the cells.

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