GENETICA POLONICA
Vol. 28 1987 No. 3

INDUCTION OF GYNOGENESIS IN SELECTED PLANT SPECIES FROM THE FAMILY *PAPILIONACEAE*[1]

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Summary. Experiments concerning the induction of the development of haploid embryos from female gametophyte were conducted on plants from the family Papilionaceae. Ovules were isolated under sterile conditions and transferred onto agar media. Callusing ovules were observed in all 11 species under study in 50% of the inoculated ovules. Parthenogenetic embryos were found in the ovules of only two species — *Baptisia australis* and *Astragalus cicer*.

Studies on the obtaining of haploids by the induction of the embryo sac haploid cells are being undertaken more and more frequently. The use of that method has permitted to obtain haploid plants in *Hordeum vulgare* (Noeum 1976), *Nicotiana tabacum* (Zhu, Wu 1979), *Gerbera jamesonii* (Cagnet 1980, Sitbon 1981, Meynet 1985), *Oryza sativa* (Asselin de Beauville 1980, Chang, Hong-yan 1981), *Zea mays* (Troung-Andre, Demarly 1984) and *Beta vulgaris* (D'Halluin, Keimer 1985). In the culture of unpollinated ovules of *Lilium* (Prakash, Giles 1985) and *Cucurbita pepo* (Dumas de Valulx, Chambonnet 1985) diploid, aneuploid plants and haplo-diploid chimeras have been obtained.

In the present paper an attempt has been made to obtain haploids by gynogenesis in several species in the family *Papilionaceae*.

MATERIAL AND METHODS

The experiments concerning the induction of the development of haploid embryos from female gametophytes were conducted on the following plant species from the family *Papilionaceae*: *Astragalus cicer* ($2n=64$), *Astragalus danicus* ($2n=16$), *Astragalus falcatus* ($2n=16$), *Baptisia australis* ($2n=18$), *Coronilla coronata* ($2n=24$), *Genista tinctoria* ($2n=48$), *Laburnum anagyroides* ($2n=48$), *Lathyrus sativus* ($2n=14$), *Trifolium rubens* ($2n=28$), *Vicia unijuga* ($2n=12$), *Vicia variegata* ($2n=10$).

From plants growing in the Botanical Garden closed flowers with not yet pollinated anthers were taken. Pistils of various size were isolated and then fixed in

[1] Received for publication: August 1986.

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AA (3 : 1 absolute ethanol : icy acetic acid). The developmental stage of the embryo sacs was determined on microtome preparations made by the paraffin method (Gerlach 1972), stained with Heidenhain's iron hematoxylin with light green (Jensen 1962). Ovules at the stages of binucleate to mature embryo sacs were isolated from flowers under sterile conditions and transferred onto the basic medium acc. to Murashige and Skoog (1962) — MS in the following three combinations:

1) MS+0.2 mg/l zeatin
2) MS+1.0 mg/l 2,4-D
3) MS 6 2.0 mg/l KIN+0.5 mg/l BAP.

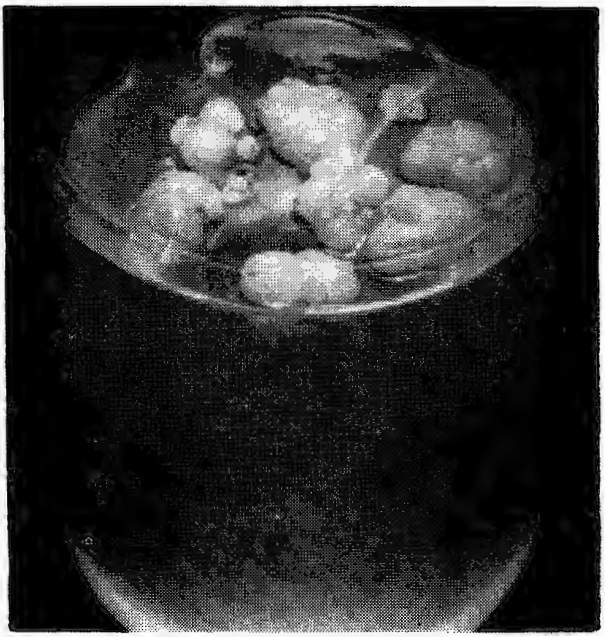
Before isolation of ovules the closed flowers were sterilized in a 0.2% of aqueous solution of $HgCl_2$ for 1 min., then rinsed with sterile water thrice. Totally about 7000 ovules were transferred onto the MS medium. The culture was performed at 22 - 25°C in darkness. Four weeks after callusing the ovules were transferred onto two kinds of media:

A) MS+0.2 mg/l 2,4-D+6% sucrose — to multiply callus
B) MS+1.0 mg/l BAP+1.0 mg/l IAA+6% sucrose — to induce organogenesis

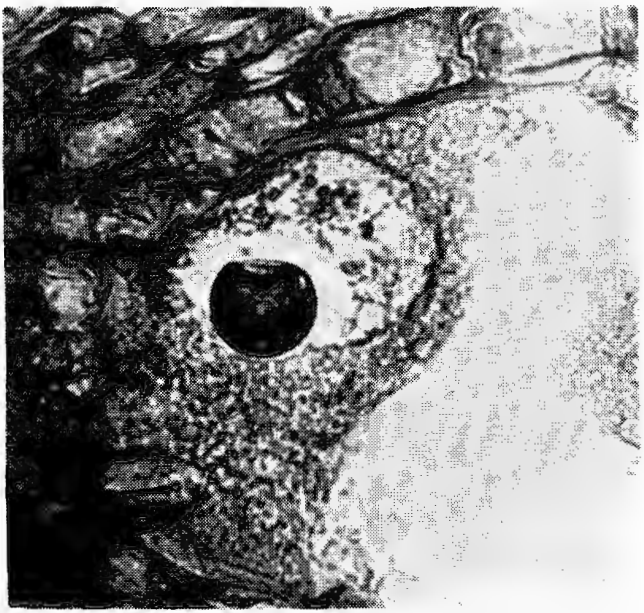
With the aim of performing a cytoembryological analysis 50 ovules from each species were fixed and cut after 3, 5, 7 and 11 days of culturing on the media. The preparations were made according to the method mentioned above in order to determine the developmental stage of the embryo sacs in the pistils before isolation of ovules for culture.

RESULTS

During the medium culture the ovules increased and dehisced, giving rise to a callus. Callusing ovules were observed in all the studied species in 50% of the inoculated ovules (Phot. 1). The largest number of callusing ovules was found in *Astragalus cicer* and *Astragalus falcatus*. It was observed that *Astragalus cicer*, beside the callus growing out of the ovule inside, has another source of callusing — at the place of excision of an isolated ovule. Generally, the ovules initiated callusing after about 2 - 4 weeks of culture, while the ovules of *Astragalus cicer* began to callus already after 9 days. After 4 weeks of culture the callusing ovules were transferred onto fresh media for the purpose of multiplying callus and inducing organogenesis. After callus multiplication on the A medium and its transferring onto the B medium regeneration of plants failed. It was found that only in the case of two species, *Astragalus cicer* and *Baptisia australis*, out of the 11 studied species, the microtome ovule preparations showed the development of parthenogenetic embryos. *Astragalus cicer* ovules 5 days after culture were observed to have embryo sacs, in which nearly all the nuclei, except the egg cell nucleus, were degenerated (Phot. 2). After 7 days of culture a 2-cellular embryo with visible traces of degenerated synergids beside it was noted several times (Phot. 3), whereas after 11 days



Phot. 1. Callusing ovules of *Astragalus cicer* after 3 weeks of culture on the MS medium containing vitamins acc. to Fuja, 3 mg/l 2,4-D and 6% sucrose

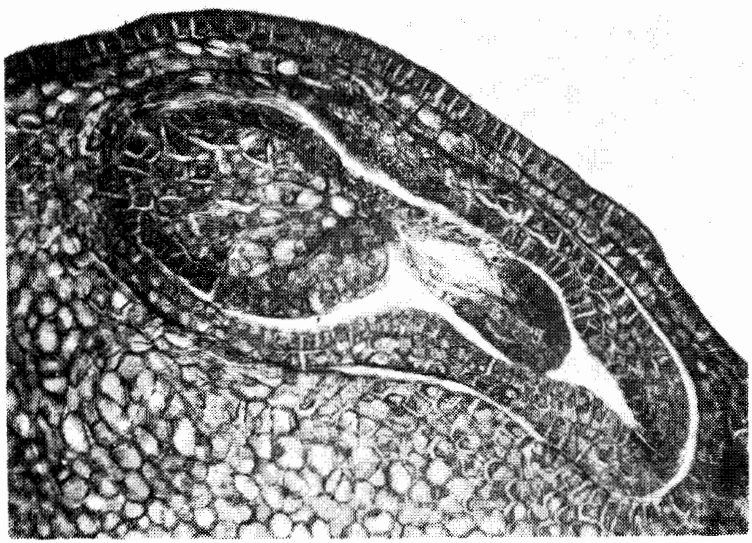


Phot. 2. The egg cell in the embryo sac of *Astragalus cicer* after 4 days of culture

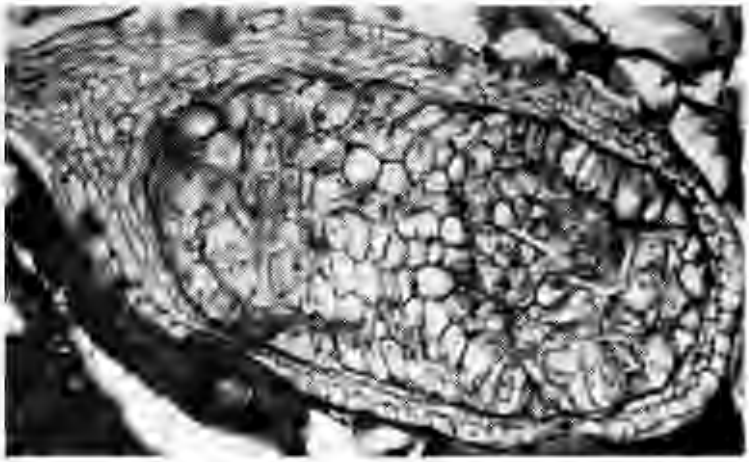
Phot. 3. A 2-cellular parthenogenetic embryo of *Astragalus cicer* after 7 days of culture

Phot. 4. A multicellular parthenogenetic embryo of *Astragalus cicer* after 11 days of culture

Phot. 5. Many nuclei in a dense cytoplasm in the place of the egg apparatus in *Astragalus* after 7 days of culture



Phot. 6. Somatic tissue growing into the cavity of the embryo sac of *Laburnum anagyroides*



Phot. 7. The cavity of *Vicia variegata* embryo sac completely grown over with the somatic tissue

only a single, large multicellular embryo was found (Phot. 4). Some embryo sacs of that species had multinuclear structures in the place of the egg apparatus (Phot. 5).

In *Baptisia australis*, spherical embryos consisting of several to a dozen or so cells were observed after 7 days of culture in four embryo sacs. All the embryos described in the both species, were always in the micropylar pole of the embryo sacs. Besides the pictures described above, some somatic tissue fragments growing into the embryo cavity (Phot. 6) and also completely grown over cavities of the embryo sacs were noted (Phot. 7).

DISCUSSION

The experiments here described are the first attempts to obtain haploids in the family *Papilionaceae* by gynogenesis. Studies were also conducted in that family on the obtaining of haploids via androgenesis, for instance in *Medicago sativa* (Robeva et al. 1984), and by crossing two tetra- and diploid forms of *Medicago sativa* (Bingham, Binek 1969).

The embryos described here were always in the place of the egg apparatus. Chang and Hong-Yan (1981) also observed embryos in *Oryza sativa* occurring only in the micropylar pole of the embryo sac, which developed from a single cell of the egg apparatus, whereas in *Hordeum vulgare* the embryos developed not only from the egg cell, but also from the antipods (San Noeum 1979). It may be assumed that the embryos of *Astragalus cicer* and *Baptisia australis* developed from the egg cell, which is supported by the observed by us 2-cellular embryos and degenerated synergids. The obtaining of parthenogenetic embryos has inclined us to extend studies on the obtaining of embryos and then, haploid plants in the family *Papilionaceae*. As follows from other studies concerning the obtaining of haploids by gynogenesis, the developmental stage of ovules transferred onto the medium seems to be of particular importance. San Noeum (1976, 1979) and Chang and Hong-Yan (1981) did not obtain haploids from the ovules inoculated onto the media and containing immature embryo sacs. The embryos described by the above authors, like those of *Astragalus* and *Baptisia*, developed only from a single cell of the mature embryo sac.

Acknowledgment. The authors are thankful to Prof. Dr. hab. Maciej Zenkteler for his valuable suggestions and help in preparing the manuscript.

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INDUKCJA GYNOGENEZY U WYBRANYCH GATUNKÓW ROŚLIN Z RODZINY *PAPILIONACEAE*

Streszczenie

Badano indukcję rozwoju zarodków haploidalnych z gametofitu żeńskiego roślin jedenastu gatunków z rodziny *Papilionaceae*. Zalążki izolowano w warunkach sterylnych, a następnie wykładano je na pożywki agarowe. Kalusujące zalążki obserwowano u wszystkich badanych gatunków w około 50% wyszczepionych zalążków. Analiza preparatów trwałych wykazała występowanie partenogenetycznych zarodków w zalążkach tylko dwóch gatunków, to jest *Baptisia australis* i *Astragalus cicer*.

ИНДУКЦИЯ ГИНОГЕНЕЗА У ВЫБРАННЫХ ВИДОВ РАСТЕНИЙ ИЗ СЕМЕЙСТВА *PAPILIONACEAE*

Резюме

Опыты относительно индукции развития гаплоидных зародышей из женского гаметофита проводились на растениях из семейства *Papilionaceae*. В стерильных условиях семяпочки выкладывались на агаровую среду. Каллусирующие семяпочки наблюдались у всех 11 исследуемых видов в 50% инокулированных семяпочек. Анализ гистологических срезов показал партеногенетическое развитие зародышей в семяпочках только двух видов, *Baptisia australis* и *Astragalus cicer*.