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Review

THEORETICAL AND APPLIED GENETICS

In Vitro Induction of Haploid Plants from Unpollinated Ovaries and Ovules

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Summary. A recent advance in plant tissue culture and experimental embryology is the successful induction of haploid plants by the culture of unpollinated ovaries or ovules. It means that not only the microspore but also the megaspore or female gametophyte of angiosperms can be triggered in vitro to sporophytic development, thus opening a new way to genetical research and haploid breeding. Since data so far accumulated are attractive, though not so rich, this paper tends to offer a preliminary review on this problem, covering the historical description, culture experiments, embryological observations, cytological and genetical studies and a brief discussion on its perspectives.

Key words: Haploid induction – Ovary culture – Ovule culture – Gynogenesis

1 Historical Description and Present Status

Since the discovery of the first haploid in *Datura stramonium* in 1921, many attempts have been made to induce the parthenogenic development of the unfertilized egg or other embryo sac cells. Many methods have been tried and these can be classified roughly into two types. One is in vivo induction of haploidy by various physical, chemical or biological stimulants. Much experience has been gained and important achievements have been made in some cases in these (Lacadena 1974). However, none of them can be regarded as a widely applicable means. Beginning in the late 1950's, the Maheshwarian school of plant embryology pioneered another method of haploid induction via in vitro culture. Culture of unfertilized ovules or ovaries has been tried in many materials, e.g. *Cooperia* (Sachar and Kapoor 1958), *Zephyranthes* (Sachar and Kapoor 1959), *Althaea rosea* (Chopra 1958, 1962; see Maheshwari and Rangaswamy 1965), *Papaver somniferum* (Maheshwari et al. 1961), *Allium cepa* (Guha and Johri 1966), *Nicotiana tabacum* (Siddiqui 1964) etc. All experiments failed except in one particular case in which an obligate apomict, *Aerva tomentosa*, was dealt with (Murgai 1959; Puri 1963; see Maheshwari and Rangaswamy 1965). Summarizing the work done during this period, Maheshwari and Rangaswamy (1965) concluded: "Ovule culture may also prove useful in the artificial induction of parthenogenesis. A direct handling of the eggs of angiosperms is by no means easy because of the problems involved in removing them without injury. However, there is no such bar to the use of unfertilized ovules. While ovules excised at the zygote or 2- to 4-celled proembryo stage can be reared in vitro, unpollinated and unfertilized ovules have not proved amenable."

While ovule and ovary culture suffered setbacks, Guha and Maheshwari (1964) made a breakthrough in anther culture. This attracted the attention of researchers and as a result the culture of female tissues was neglected for about a decade. Yet from scattered reports during that period (e.g. Nishi and Mitsuoka 1969; Uchimiya et al. 1971; Mullins and Srinivasan

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Table 1. Methods and results of haploid induction by culture of unpollinated ovaries and ovules

Species	Stage of inoculation		Mode of inoculation	Culture media (Arrow means transfer to differentiating medium)
	Pollen	Embryo sac		
Hordeum vulgare	Binucleate to trinucleate	Nearly mature	Ovary culture	Miller macro- and microelements + Fujii vitamins + 2,4-D 2 ppm + Sucrose 10% → Same basic medium + Sucrose 2%
	–	1 – 2 days before anthesis	Vertical ovary culture	N6 + 2,4-D 0.5 ppm + NAA 1 ppm + KT 1 ppm + sucrose 3%
	Late uninucleate to trinucleate	Uninucleate to mature	Vertical flower culture	N6 + MCPA 1 – 2 ppm + NAA 1 ppm + KT 0.5 ppm + sucrose 3%
Triticum aestivum	Middle to late uninucleate	–	Ovary culture	N6 + NAA 0.5 ppm + KT 1 ppm + CH 500 ppm + sucrose 6% or N6 + 2,4-D 2 ppm + sucrose 8% → H + IAA 5 ppm + KT 2 ppm (or → N6 + IAA 0.2 ppm + KT 1 ppm)
Oryza sativa	Binucleate to trinucleate	Nearly mature	Ovary culture	Miller macro- and microelements + Morel vitamins + NAA 3 ppm + YE 2000 ppm + sucrose 6%
	Late uninucleate to binucleate	Uninucleate to mature	Float culture of unhusked flowers	N6 + MCPA 0.125 ppm + sucrose 3% → MS or N6 + IAA 0.5 ppm + KT 1 – 2 ppm + NAA 0.25 ppm + sucrose 3% (or → N6 + MCPA 0.033 ppm + sucrose 3%)
	Late uninucleate to early binucleate	Uninucleate to mature	Ovary culture	N6 + 2,4-D 2 ppm + CH 500 ppm + sucrose 4% → N6 + CH 500 ppm + KT 2 ppm + NAA 0.5 ppm + sucrose 3%
Nicotiana tabacum (in some cases *N. rustica* too)	Uninucleate	Embryo sac mother cell to megaspore tetrad	Ovary culture	H + IAA 0.5 – 1 ppm + KT 2 – 4 ppm + sucrose 2%
	–	–	Ovule culture	H or B5 + KT 2 ppm + IAA 0.5 ppm or H + IAA 0.5 ppm + charcoal 1%
	Late uninucleate to binucleate	uninucleate to mature	Ovary culture	H + inositol 150 ppm + Nicotinic acid 7.5 ppm + glycine 3 ppm + vit B1 4 ppm + vit B6 5.5 ppm + folic acid 1 ppm + IAA 0.5 ppm + KT 2 ppm + sucrose 2%
Gerbera jamesonii	–	Ovule bigger than half ovary cavity	Ovule culture	MS + IAA 0.5 ppm + BA 2 ppm + KT 2 ppm + sucrose 3 – 6% → modified MS + KT 2 ppm + BA 2 ppm + sucrose 4.5%

Abbreviations: 2,4-D (2,4-dichlorophenoxyacetic acid); NAA (naphthyleneacetic acid); KT (kinetin); MCPA (2-methyl-4-chlorophenoxyacetic acid); CH (casein hydrolysate); IAA (indole-3-acetic acid); YE (yeast extract); BA (6-benzylaminopurine); MS (Murashige-Skoog medium); B5 (Gramborg medium); H and N6 (specific media)

1976; Jensen et al. 1977; Pareek et al. 1980) some interesting results can be found. Uchimiya et al. (1971) cultured unpollinated ovaries of *Zea mays* and ovules of *Solanum melongena* and observed the division of haploid cells in their callus tissues. They believed that in vitro induction of haploid plants from angiosperm megagametophytes was possible. Jensen et al. (1977), dealing with cultured ovules of *Gossypium hirsutum,* found that the unfertilized polar nuclei could undergo free nuclear division and subsequent precocious cell formation; in addition, degeneration of one of the synergids took place just as it occurred after pollination.

So far as we know, the culture of unpollinated ovaries and ovules met with no success in haploid plant production until 1976 when San Noeum reported her first result in the ovary culture of *Hordeum vulgare.* Subsequently, Zhu and Wu (1979) obtained haploid plants from cultured ovaries of *Triticum aestivum* and *Nicotiana tabacum.* Yan et al. (1979) also raised a haploid albino plantlet in wheat ovary culture. Asselin de Beauville (1980) and Zhou and Yang (1980), using different methods, both succeeded in obtaining haploid plants from the ovary culture in *Oryza sativa.* In the meantime, ovule culture giving haploid plants was achieved by Cagnet-Sitbon (1980) in *Gerbera jamesonii* and by Ran (1980) in *Nicotiana tabacum.* Recently, haploid plants from ovules have been also produced in barley (Wang and Kuang 1981), rice (Kuo 1982), tobacco (Wu and Chen 1982), *Lilium daviddii* (Gu and Chen, unpub-

Table 1. (continued)

Culture conditions		Development pattern	Characteristics of regenerated plants	Author(s)
Temperature (°C)	Light (lux)			
27± 1	3000	Embryoid	Green haploids	San Noeum (1976, 1979)
25 – 28	1000	Direct shooting	Green haploids	Wang and Kuang (1981)
~25	Dark	Embryoid	–	Huang et al. (1982)
25 – 28	1500	Direct shooting or via callus	Green haploids	Zhu et al. (1979, 1981)
25 – 28	1000 – 3000	Direct shooting or via callus	Green haploids; some mixoploids	Asselin de Beauville (1980)
~25	Dark	Callus	Green haploids; some albinos and non-haploids	Zhou and Yang (1980, 1981)
24 – 28	2000	Callus or embryoids	Green haploids and diploids; Some albinos	Kuo (1981)
25 – 30	Light	Embryoid	Green haploids	Zhu et al. (1979, 1981)
25 – 28	Light	Direct shooting	Green haploids	Ran (1980)
25 – 28	1500	Embryoid; some via callus	Mainly green haploids	Wu and Chen (1982)
23	500 Lux or dark	callus	Green haploids; some diploids	Cagnet-Sitbon (1980)

lished) and *Zea mays* (Ao et al., unpublished). Success within such a short period indicate that the induction of haploids from female gametophytes via in vitro culture is not so inaccessible as had been thought before. Some data in this new field are shown in Table 1 for the convenience of reference.

2 Factors Affecting Culture Results

2.1 Genotype of Donor Plants

As in anther culture, a difference in response also exists among donor cultivars in ovary and ovule culture. For example, among 12 rice cultivars tested, 9 *japonica* and 2 of 3 *indica* cultivars could produce gynogenic calli with a percentage of ovaries ranging from 1.1% to 12%; 'Nang Ken No. 4', a *japonica* cultivar, showed the highest response in all experiments (Zhou and Yang 1982). The percentage of ovaries producing gynogenic calli in 4 wheat cultivars varied from 1.3% to 10.9% (Zhu et al. 1981). In *Nicotiana tabacum,* two cultivars had an induction frequency as high as 75% and 80%, but in another species, *N. rustica,* it was only 8% (Wu and Chen 1982). In ovule culture of 4 cultivars of *Gerbera jamesonii,* the percentage of ovules producing gynogenic calli ranged from 8% to 17% and the percentage of ovules regenerating plantlets was 0–5% (Cagnet-Sitbon 1980). These facts indicate that genotype plays an important role in culture.

2.2 Embryo Sac Stage

It is not easy to observe directly the embryo sacs at the time of inoculation: an indirect judgement by pollen stage is more feasible. To do this, it is better to deter-

mine the corresponding embryo sac stages later by paraffin sections. In barley and rice, some authors had good results only with late-staged ovaries, e.g. with nearly mature embryo sacs (San Noeum 1976, 1979; Asselin de Beauville 1980; Wang and Kuang 1981); others reported success with ovaries ranging from uninucleate to mature embryo sac stages (Zhou and Yang 1981 b, 1982; Kuo 1982; Huang et al. 1982). *Gerbera* ovules responded only when their size was larger than half that of the ovary cavity (Cagnet-Sitbon 1980). Tobacco ovaries inoculated at the uninucleate (Zhu et al. 1981; Wu and Chen 1982) or binucleate (Wu and Chen 1982) pollen stage resulted in haploid production. It seems that quite a wide range of embryo sac stages are responsive to gynogenic development, but in most cases the later stages give better results. This is unlike anther culture in which mature pollen usually can not be induced to androgenesis.

2.3 Cold-Treatment

Little data are available on this problem. Rice ovaries have been induced to gynogenesis when treated with low temperature (12–13 °C) for 6 days after inoculation (Zhou and Yang 1980); however, repeated experiments indicate that there is no merit in cold treatment, either on panicles before inoculation or on cultured ovaries after inoculation (unpublished). Tobacco flower buds could be pretreated at 0–4 °C for 12 h, but no comparative data were presented (Wu and Chen 1982). In *Gerbera,* cold pretreatment at 4 °C for 48 h did not increase the number of gynogenic calli (Cagnet-Sitbon 1980). It appears that low temperature is not likely to be as effective a treatment in ovary and ovule culture as it is in anther culture.

2.4 Culture Media

2.4.1 Basic Media

Most early work in the 1950s used Nitsch medium for ovule and ovary culture; however since the 70s, Miller, MS or N6 media have been used in successful experiments. Few experiments have been carried out to compare the effects of various basic media. In *Gerbera,* MS seems better than Knop and Heller (Cagnet-Sitbon 1980). An increase in the content of B group vitamins and glycine in H medium has been reported to have promoted induction-frequency in tobacco ovary culture (Wu and Chen 1982).

2.4.2 Exogenous Hormones

IAA (0.5–1 mg/l) and KT (2–4 mg/l) were supplemented in ovary culture of tobacco (Zhu and Wu 1979; Wu and Chen 1982). In *Gerbera* ovule culture, an auxin (IAA 0.5 mg/l) plus two cytokinins (BA and KT 2 mg/l each) proved most effective (Cagnet-Sitbon 1980). In graminaceous species, stronger auxins were adopted, e.g. 2,4-D 2 mg/l (San Noeum 1976) or 2,4-D 0.5 mg/l plus NAA 1 mg/l (Wang and Kuang 1981) for barley; NAA 3 mg/l (Asselin de Beauville 1980), MCPA 0.125 mg/l (Zhou and Yang 1980, 1981 a) or 2,4-D 2 mg/l (Kuo 1982) for rice; 2,4-D 2 mg/l for wheat (Zhu and Wu 1979). Comparative experiments in rice showed that when young flowers were cultured on liquid medium in the absence of exogenous hormone, there was a failure in the enlargement of the ovaries as well as in the production of gynogenic calli; an increase of MCPA concentration from 0.125 to 8 mg/l did favor ovary swelling, but high auxin levels (2 mg/l) stimulated callus formation from the ovary wall rather than from the embryo sac. Therefore, regulation of hormone level to enhance gynogenesis and inhibit proliferation of somatic tissue was considered to be a critical point in ovary culture (Zhou and Yang 1981 b, 1982).

2.4.3 Sucrose Concentration

Sucrose concentration used in ovary and ovule culture was 3–10% in barley, 8–14% in wheat, 3–6% in rice, 2% in tobacco and 3–6% in *Gerbera.* No exact comparative experiments were reported except in rice float culture, in which 1% and 9% sucrose proved unsuitable and 3–6% were recommended (Zhou and Yang 1981 b, 1982).

2.4.4 Solid and Liquid Media

Almost all experiments succeeded on solid media, the exception being rice, which has been induced to gynogenesis either on solid (Asselin de Beauville 1980) or liquid (Zhou and Yang 1980, 1981 a, b) media. A comparison showed the advantages of the latter over the former (Zhou and Yang 1982).

2.5 Modes of Inoculation

Usually ovaries were randomly orientated on the solid medium. However, San Noeum noticed that better results could be obtained when ovaries were inoculated so that their placentae side was facing downwards to the medium (personal communication). Wang and Kuang (1981) placed barley ovaries in a vertical situation with the cut surface in contact with the medium. Another question is which parts are involved in the inoculum. Generally saying, the term ovary culture is in its exact sense a pistil culture. Some researchers have used whole flower buds or even pieces of inflorescence (Murgai 1959; Puri 1963; see Maheshwari

and Rangaswamy 1965). Evidence has been given that in rice the best results of gynogenesis were obtained when an unhusked flower with pistil and stamens attached to the receptable was inoculated as a unit on liquid medium, the culture was less effective when the stamens were removed and was the worst when single pistils were used (Zhou and Yang 1981 b, 1982). In barley, whole flowers (with or without stamens) inoculated vertically on solid medium proved to be a better culture source than randomly placeo single pistils (Huang et al. 1982). It is of special interest that in *Gerbera* ovary culture failed whereas ovule culture succeeded (Cagnet-Sitbon 1980). It is unknown in this case whether the ovary wall has a harmful effect or whether it mechanically hinders the growth of gynogenic callus inside.

2.6 Culture Conditions

Ovaries or ovules have been incubated usually at 25–28 °C, either in darkness (Zhou and Yang 1980, 1981; Cagnet-Sitbon 1980) or with a 10–16 h photoperiod of 500 (Cagnet-Sitbon 1980), 1,000 (Wang and Kuang 1981), 1,500 (Wu and Chen 1982), 2,000 (Kuo 1982) or 3,000 (San Noeum 1976; Asselin de Beauville 1980) lux. No precise comparison has been reported.

3 Embryology of Gynogenesis

The induction of haploid plants from cultured ovaries or ovules indicates that sporophytic development of megaspores or megagametophytes occurs in the absence of amphimixis. However, direct embryological observation is necessary in order to know the origins of the embryos and the developmental process.

3.1 Starting Points of Gynogenesis

San Noeum (1979), trying to analyze the origin of gynogenic proembryos in barley ovaries, classified them into eight groups, i.e. from (a) egg, (b) egg and antipodals, (c) antipodals, (d) egg and one synergid, (e) egg and two synergids, (f) one or two synergids, (g) synergids and antipodals and (h) synergids, egg and antipodals. She also mentioned that the best results were obtained with proembryos from the egg or antipodal cells; synergids gave only a proliferation of the callus type. These conclusions were drawn from observations on dissected ovaries under a binocular microscope and thus are puzzling. Zhou and Yang (1981 a, c) carried out observations on paraffin sections of cultured rice ovaries. They saw that while inoculation occurred at the 1- to 4-nucleate embryo sac stages, gynogenesis was not initiated until the gametophytes matured during culture; the proembryos were mainly located at the micropylar end and were derived from the egg apparatus. These observations were basically confirmed by Kuo (1982) in rice and Huang et al. (1982) in barley. Recently, based on large scale observations on rice ovary culture, Tian, G. et al. (unpublished) were able to trace the detailed apogametic development from the very beginning of the synergids into proembryos and calli; they also observed the proliferation of the egg cell and antipodals. On the other hand, in tobacco, ovary culture gynogenesis was reported to be initiated either directly from megaspores (Zhu et al. 1981; Wu and Chen 1982) or mature egg cells (Wu and Chen 1982). It is interesting to note that unfertilized polar nuclei could divide into endosperm-like structures in cotton ovule culture (Jensen et al. 1977), and in rice (Zhou and Yang 1981 a, c) and barley (Huang et al. 1982) ovary culture. Nevertheless, these structures did not seem to serve as a nurse tissue for the gynogenic embryoids nor were they themselves regenerated into plantlets.

Usually there was only one gynogenic unit inside one embryo sac but several units inside one embryo sac have been found in barley (San Noeum 1979; Huang et al. 1982) as well as rice (Zhou and Yang 1981 a, c). Two possibilities can be supposed for the origin of such multiple units: polygenetic origin from different cells of the embryo sac, and monogenetic origin from a single cell, cleaving into several parts during subsequent development.

3.2 Characteristics of Gynogenic Development

As in pollen androgenesis, gynogenic plantlets may develop via embryoid or callus formation. To date in barley (San Noeum 1976, 1979; Huang et al. 1982) and tobacco (Zhu et al. 1981) only embryoids have been observed, but in rice (Asselin de Beauville 1980; Zhou and Yang 1981) and wheat (Zhu et al. 1981) both cases were identified. In barley ovary culture, the unfertilized eggs divided transversely into 2-celled proembryos, grew further into multicellular proembryos and then differentiated into embryoids more or less similar to in vivo zygotic embryos (Huang et al. 1982). In rice, under float culture conditions, proembryos were characterized by various morphological abnormalities, often leading to callus formation (Zhou and Yang 1981); detailed morphogenetic processes were further studied (Tiang et al., unpublished). Nagato (1979) has also observed various morphogenetic anomalies of rice embryos in caryopsis culture. It appears that in vitro culture may cause either zygotic or gynogenic embryos to behave quite differently from the in vivo developmental pattern. Thus, the problem of how to control gynogenic

development, shifting it to the pathway of normal embryogenesis, still remains to be solved.

3.3 Somatic Calli or Embryoids

A troublesome problem in ovary and ovule culture is the proliferation of somatic tissues which results in callus or embryoid formation. This not only makes it difficult to identify the gametophytic products from the sporophytic ones, but also may place inhibitory effects on the normal growth of the former. In fact, much previous work on ovary and ovule culture have merely induced somatic tissue proliferation (e.g. Maheshwari 1961; Mullins and Srinivasan 1976; Hsu and Steward 1976; Beasley 1977; Jensen et al. 1977; Pareek et al. 1980). Even in the cases of successful haploid induction, calli or embryoids might be simultaneously produced from such somatic tissues as ovary wall (Zhou and Yang 1982), funicle (Cagnet-Sitbon 1980), integument (Kuo 1982) or nucellus (Huang et al. 1982). Therefore, one should pay attention to the challenge of how to induce gynogenesis without the overabundant proliferation of somatic tissues. Hormonal regulation appears to play an important role in maintaining an optimal physiological balance (Zhou and Yang 1981 b, 1982).

4 Cytological and Genetical Characteristics of Regenerated Plants

4.1 Ploidy Level

Under conditions when ovary or ovule culture produced haploid plants, haploids were sometimes obtained exclusively (San Noeum 1976, 1979; Zhu and Wu 1979; Yan et al. 1979; Asselin de Beauville 1980; Wang and Kuang 1981; Zhu et al. 1981), but in other cases both haploids and non-haploids were observed. For instance, plantlets regenerated from gynogenic calli of rice embryo sacs were observed to have haploid as well as diploid and polyploid roots (Zhou and Yang 1980, 1981 a). A further large scale investigation of plantlets regenerated from 111 ovaries confirmed that 73.9% of them were haploid, 15.3% were mixoploid (Liu et al., unpublished). These non-haploids seemed to have originated more from a doubling during callus proliferation than directly from somatic tissues. Ovule culture of *Gerbera* induced 16 clones from gynogenic calli, of which 14 were haploid and 2 were diploid (Cagnet-Sitbon 1980). In tobacco, plants emerging directly from cultured ovaries were haploid, but those redifferentiating from callus were mostly diploid or mixoploid and a few were haploid (Wu and Chen 1982).

4.2 Albinism

Among species in which regeneration took place from cultured ovaries or ovules, *Hordeum vulgare, Nicotiana tabacum, N. rustica* and *Gerbera jamesonii* exclusively yielded green plants; *Oryza sativa* and *Triticum aestivum* produced both green plants and albinos. In wheat, plants regenerated from six ovaries were all green (Zhu et al. 1981). However, in another author's experiment, only one albino plantlet was reared from the same species (Yan et al. 1979). According to Asselin de Beauville (1980), rice plants regenerated from nine ovaries were all green. Dealing with rice ovary culture in three successive years, Zhou et al. found that green plants were dominant, although albinos did exist. In the first year, 5 ovaries were induced, among which 1 gave green plantlets and 4 gave albinos (Zhou and Yang 1980, 1981 a); in the second year, 24 of 30 ovaries gave green plantlets and another 6 gave albinos (Zhou and Yang 1981c); in the third year, from a total of 178 ovaries induced, 87.1% gave rise to green plantlets, 9.0% gave only albinos and the remaining 3.9% produced both green and albino plantlets (Liu et al., unpublished). Kuo (1982) also raised 12 green and 3 albino plants from rice ovary culture. It is evident that green plants are found in higher numbers in cereal ovary culture as compared with anther culture, in which albinism is a well known serious problem. For instance, San Noeum and Ahmadi (1980) have shown in barley cultivar 'Bérénicé' that 100% of the gynogenic plants were green; in contrast, 99% of the androgenic plants were albinos. Similarly, in rice cultivar 'Zao Geng No. 19', the percentage of green plantlets in ovary culture was 89.3%, but that in anther culture was only 36.4% (Liu et al., unpublished).

4.3 Other Characters

San Noeum and Ahmadi (1980) have compared two gynogenic and three androgenic doubled haploid lines with their original donor plants of the barley cultivar 'Bérénicé'. Block-trials in the field were made and the main agronomic characters were measured. Multivariate analysis showed that the groups of gynogenic doubled haploids were located close to the donor plants, while the androgenic ones were significantly distant from them. Analysis of progenies from selfing and reciprocal crosses confirmed the variability and showed important maternal and reciprocal effects, which led to the conclusion of new nucleocytoplasmic interactions.

5 Perspectives

The breakthrough of in vitro culture of unpollinated ovaries and ovules indicates that it may become an

alternative way to haploid breeding. Its special contribution may be found in the following cases: (a) in certain species where anther culture has not succeeded or has too low a response to be actually applied to breeding, ovary or ovule culture may offer a useful approach. (b) in male-sterile plants, the value of haploid induction from female parts is obvious and this possibility has really been proved by the experiments in tobacco (Zhu et al. 1980). (c) while albinism is a limiting factor to anther culture of some cereal crops, ovary culture can provide a relatively higher proportion of green plants, as mentioned above. (d) In such cases when pollen plants show some unwelcome variation in ploidy or other characters, perhaps the gynogenic offsprings may behave otherwise. In fact, it is yet too early to talk about the validity of these merits. A great deal of work has to be done before it will become a practical and fruitful technique in plant breeding.

Other prospects are in the theoretical stages. Almost all stages in the reproductive cycle of angiosperms can now be studied by in vitro culture. Culture experiments of microspore mother cell, anther and pollen, embryo, endosperm and pollinated ovary or ovule have significantly pushed and are pushing forward our knowledge about microsporogenesis, pollen androgenesis, fertilization, embryogenesis, endosperm development and fruit formation. The understanding of such processes has a relation to the genetics and breeding of plants. However, in vitro study of megasporogenesis and the female gametophyte appears to be a unique gap among them. Taking this into account, Heslop-Harrison (1980) has recently made an appeal for enhancing the genetical and physiological researches on the "forgotten generation" – the angiosperm gametophytes. It is understandable that the culture of immature ovaries and ovules may play an important role in filling this blank.

As reviewed by Raghavan (1976), the spontaneous origin of embryos from haploid components of the embryo sac has been recorded in about 100 species, but in the majority of cases it was merely a sporadic event and lacked cytologically substantiated accounts. As for induced parthenogenesis or apogamy, the existing methods are too laborious for routine work and the extremely low frequency of production of haploids has prevented more critical examination of their potential use. "Owing to such limitations, haploids originating from the embryo sac have had relatively little impact on contemporary studies and were regarded more as abnormalities than as basic tools for research. Technical considerations of isolating and culturing the delicate female gametophytes of angiosperms have thus far precluded attempts to study in vitro embryoid induction on them." Taking account of this, the significance of the culture of unpollinated ovaries or ovules is self-evident, not only due to its reproducible efficiency, but also because it can provide a basis for further approach to such important but yet unknown problems as why and how female gametophytic cells are directed to sporophytic development in the absence of fertilization.

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