In vitro Culture of Ovules and Embryos from some Incompatible Interspecific Crosses in the Genus *Arachis* L.

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Abstract

In vitro culture of ovules and embyros is commonly used to produce hybrids from incompatible crosses. In several interspecific crosses in Arachis, *ovule and/or embryo culture is necessary because the ovules do not develop fully in vivo.*

Ovule sizes from different crosses and different hormone treatments range from less than 0.3 mm to 5.0 mm, rarely 7.0 mm. Ovules smaller than 3.0 mm had to be cultured entire as the proembryos they contained were too small for dissection and culture. Ovules larger than 3.0 mm were dissected and their embryos cultured.

All the ovules were cultured on MS media with or without agar and with different concentrations of various hormones.

Depending on the hormones used and their concentrations, different responses were observed. Surface callus formation, greening, swelling, and browning were common in cultured ovules. Embryos emerged from some of the ovules and plantlets have been obtained. Cultured embryos showed similar responses, and some cultured embryos germinated, and developed into plantlets.

Résumé

Culture in vitro d'ovules et d'embryons issus de certains croisements interspécifiques incompatibles dans le genre Arachis L. : *La culture d'ovules et d'embryons in vitro est une méthode courante de production d'hybrides à partir de croisements incompatibles. La culture d'ovules et/ou d'embryons s'avère nécessaire dans plusieurs croisements interspécifiques dans le genre* Arachis *étant donné le développement incomplet des ovules in vitro.*

La taille des ovules provenant de différents croisements et ayant subi différents traitements hormonaux varie de moins de 0,3 mm à 5 mm, rarement 7,0 mm. Les ovules de taille inférieure à 3 mm doivent être cultivés entiers, les proembryons qu'ils contiennent étant trop petits pour la dissection et la culture. Les ovules de dimension supérieure à 3,0 mm sont disséqués et leurs embryons cultivés.

Tous les ovules sont cultivés sur des milieux MS gélosés ou non et à différentes concentrations de diverses hormones.

On observe des réponses différentes selon les hormones utilisées et leurs concentrations. Formation de cals en surface, verdissement, gonflement et brunissement sont courants chez les ovules cultivés. Des embryons se sont développés dans certains de ces ovules et de jeunes plants ont pu être obtenus. On constate les mêmes réactions chez les embryons cultivés : certains embryons cultivés ont germé et se sont développés en plants.

Introduction

Of the several methods available to overcome barriers to hybridization, in vitro culture of ovules and embryos, which would otherwise abort, has been the most commonly used, and hybrids have been produced in about 50 interspecific crosses by embryo culture (Raghavan 1977, Sastri 1984) and in more than ten interspecific crosses by ovule culture (Sastri 1984; Sastri et al. 1980, 1982,1983).

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Our earlier investigations on interspecific incompatibility in the genus *Arachis* indicated the need to culture ovules or embryos for hybrid production (Nalini and Sastri 1985). In the crosses between *Arachis hypogaea* × *Arachis* sp PI 276233, and other tetraploid accessions of the section *Rhizomatosae*, pegs were rarely formed. If pegs were formed, they degenerated before they entered the soil, and the seeds aborted. The ovules and the proembryos in these aerial pegs were very small. Our preliminary attempts to culture such immature ovules even from selfed pegs were not successful (Sastri et al. 1980). We have induced pegs and pods from some incompatible pollinations by hormone treatments (Nalini et al. 1983; Sastri and Moss 1982; Sastri et al. 1982, 1983) in sufficient numbers and maintained growth to a stage at which it was possible to dissect and culture ovules and embryos. The objective of the present investigation was to study the hormonal requirements in the medium for survival, sustaining growth, and promoting subsequent development of immature ovules or proembryos to produce hybrid plants from these crosses.

Methods

All pegs and pods induced from incompatible crosses (Nalini and Sastri 1985) were carefully removed from the soil about 30 to 40 days after the last hormone treatment. The soil was washed from the pods in running water, surface sterilized with Clorox for ten minutes, and washed thoroughly twice with sterile water. Washing in sterile water and subsequent operations were performed under aseptic conditions.

Ovules larger than 3.0 mm were generally dissected to extract the proembryos for examination and culture.

Ovules and embryos were cultured on Murashige and Skoog's (MS) medium (1962) with or without agar, with different concentrations of sucrose, and with hormones [kinetin (Kn), Benzylamino purine (BAP), Indole acetic acid (IAA), Naphthalene acetic acid (NAA)] at different concentrations.

When liquid media were used ovules were supported on filter paper bridges in 15 mm × 50 mm screw-topped glass vials such that the placenta was in direct contact with the filter paper. When agar media were used, the placenta or the placental areas of cultured ovules were kept in direct contact with the surface of the medium in (25 mm × 150 mm) rimless glass tubes. All the cultures were incubated at 25° C ± 3° with 10h photoperiod (fluorescent and incandescent illumination at about 4000 lux).

Results and Discussion

Ovule Cultures

Most ovules were between 1.0 and 3.0 mm, with some up to 5.0 mm, but rarely reaching 7.0 mm Since all the pods from hormone-aided pollinations on a plant were harvested on the same day their ages ranged from 50 to 70 days after pollination. There appeared to be no correlation between sizes of ovules and age after pollination.

The response of cultured ovules depended on their size at the time of culture, and the medium employed. The responses could be broadly categorized into the following:

a. increase in size of ovules with no change in color,
b. increase in size and greening of ovules,
c. increase in size and browning of ovules,
d. growth and emergence of embryos,
e. callus production from surface of ovules,
f. greening of ovules, and
g. browning (necrosis) and shrinkage of ovules.

The percentage of cultured ovules showing desirable responses on a few combinations of media are presented in Tables 1 and 2. Among the ovules from crosses of four cultivars with *Arachis* sp PI 276233 cultured on different media, cultivars MK 374 and TMV 2 were found to give better results on both liquid and semisolid media than those of other cultivars (Table 1, Plate 4b,c,d). On semisolid media the responses were slower than on liquid media. Ovules survived longer in both agar and liquid media containing BAP-NAA than when Kn-IAA combinations were used. But Kn-IAA appeared to be better than BAP-NAA at stimulating embryo growth, development and emergence from the ovules (Tables 1 and 2).

Martin (1970) observed a beneficial effect on ovule growth with kinetin and gibberellin; kinetin at 0.5-1.0 mg/l had the best effect. In our studies, even lower concentrations of kinetin, (0.1 or 0.2 mg/l), were better than BAP.

Table 1. Percentage ovules responding from different *Arachis hypogaea* cultivars crossed with *Arachis* sp PI 276233 on MS medium with different concentrations of kinetin and IAA

Concentrations of additives				Ovules responding[1] (%)			
				Cultivars			
Kn (mg/l)	IAA (mg/l)	Sucrose (%)	Agar (%)	Robut 33-1	MK 374	M 13	TMV 2
0 00	0 00	5	0	24 00			
0 10	0 10	5	0	22 50	20 08	16 00	85 36
0 10	0 20	5	0	41 17	29 00		75 00
0 10	0 50	5	0	0 00	5 71		
0 11	0 00	3	0	46 15	83 33		
0 22	0 00	3	0 7	9 52	66 66	28 57	92 85

1 Ovules which responded increased in size and became green

Ovules which had swollen were examined for embryo development Those which increased in size and remained green were generally found to possess poorly-developed embryos On the other hand, embryos with different degrees of growth were observed in many ovules which had increased in size and turned brown These embryos, when extracted and cultured on MS medium with agar, NAA (2 mg/l) and BAP (0 5 mg/l) produced callus and multiple shoots

Embryo Culture

Ovules, upto 3.0 mm long had a small globular proembryo measuring 0.1 or 0.2 mm. Those larger than 3.0 mm contained slightly larger embryos The small proembryos were likely to be affected by injury during dissection, and/or suffer desiccation during transfer to the culture vials Many such embryos have not responded to culture Therefore, the dissection of embryos from ovules smaller than 3 0 mm was discontinued Even in larger ovules, the size and stages of proembryos were found to vary greatly The embryos very rarely differentiated, typical stages of dicotyledonous embryo development were rarely observed They were mostly amorphous and globular although they had shown an increase in size (Fig 1a to d) Some showed both the cotyledonary initials, but a very poorly-developed embryo axis (Fig 2e,f), some had only one well-formed cotyledonary initial, while the other was rudimentary (Fig 2d) However, some of the embryos were successfully cultured in vitro (Fig 2b,c,d) (Table 3) The majority of the embryos were initially cultured on MS semisolid medium with 2 mg/l of NAA and 0 5 mg/l of BAP, a medium which was good for a range of tissue and organ cultures of *A hypogaea* and some wild species (Sastri et al 1982, 1983) This medium with some variations in concentrations of NAA was satisfactory for callus formation and shoot regeneration from some of the embryos cultured

Table 2. Percentage ovules responding from different *Arachis hypogaea* cultivars crossed with *Arachis* sp PI 276233 on MS medium with different concentrations of BAP and NAA.

Concentrations of additives				Ovules responding[1] (%)			
				Cultivars			
BAP (mg/l)	NAA (mg/l)	Sucrose (%)	Agar (%)	Robut 33-1	MK 374	M 13	TMV 2
0 50	2 00	2	0	0 00			
0 10	0 10	1	0	25 00			
0 05	2 00	3	0	33 33			
0 50	0 50	3	0 7	5 45			
0 50	0 75	3	0 7	0 00		85 71	
0 50	2 00	3	0 7	11 37	40 25	37 93	100 00
0 50	0 50	3	0		72 22		

1 Ovules which responded increased in size and became green

Figure 1. Poorly-differentiated embryos from *A. hypogaea* cv MK 374 × *Arachis* sp PI 276233

a. globular proembryo dissected from an ovule (1.5 mm) cultured on liquid medium (MS + Suc. 5% + Kn 0.1 ppm + IAA 0.1 ppm) for 20 days. (× 122)

b. swollen globular proembryo dissected from an ovule (2.5 mm) cultured on liquid medium (MS + Suc. 5% + BAP 0.5 ppm) for 74 days. (× 106)

c. heart-shaped, but elongated proembryo dissected from an ovule (3.0 mm) cultured on liquid medium (MS + Suc. 5% + Kn 0.25 ppm + IAA 1.0 ppm) for 46 days. (× 96)

d. overgrown late heart-shaped proembryo from an ovule (3.5 mm) cultured on liquid medium (MS + Suc. 5% + Kn 0.25 ppm + IAA 1.0 ppm) for 77 days. (× 54)

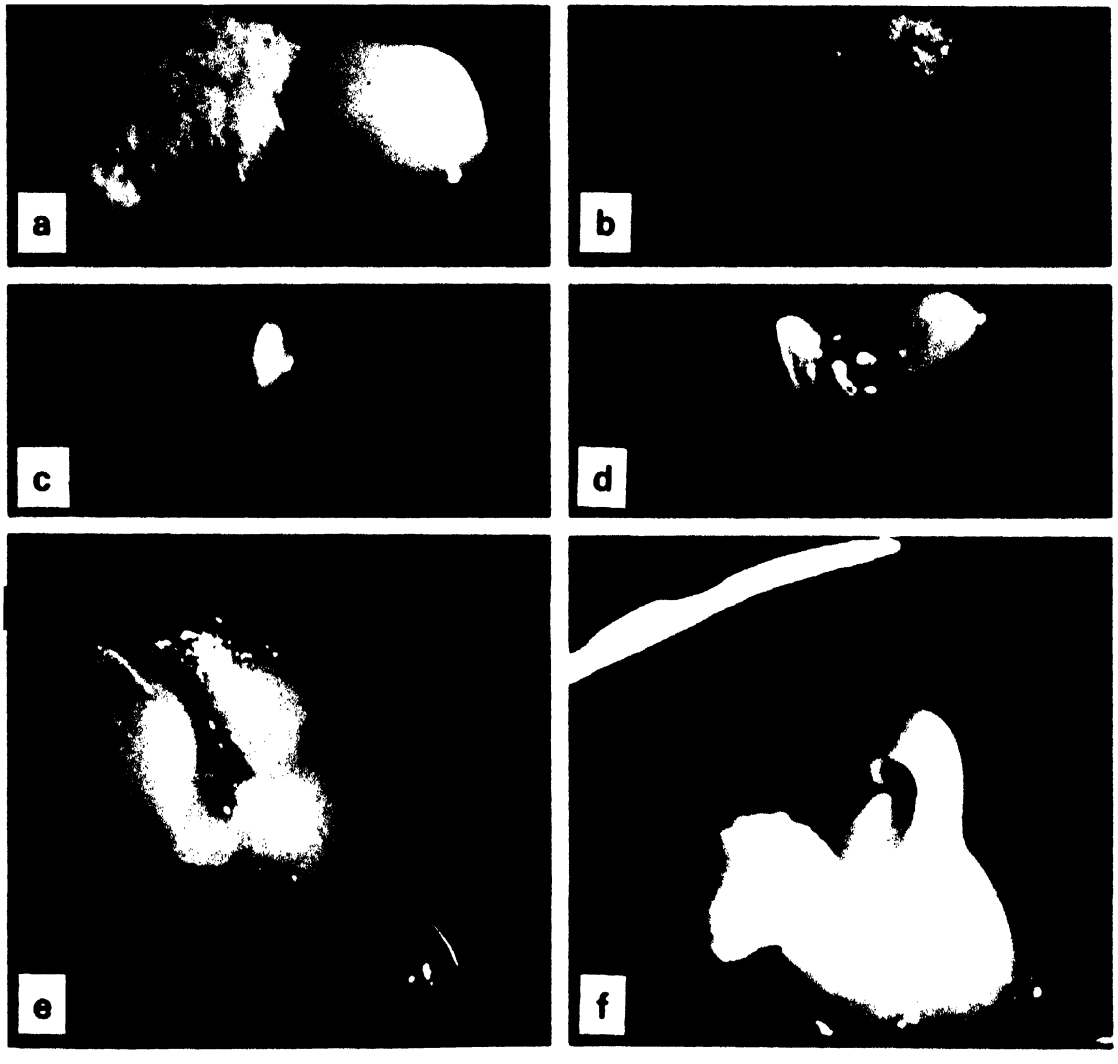

Figure 2 a. callusing ovule (0.5 mm) cultured on liquid medium (MS + Suc. 5% + Kn 0.1 ppm) for 41 days from *A. hypogaea* cv TMV 2 × *Arachis* sp Coll 9649. (× 11.7)

b. differentiating callus from a globular embryo dissected from a 3.0 mm ovule cultured on semisolid medium (MS + Suc. 3% + NAA 0.75 ppm + BAP 0.5 ppm) for 79 days from the cross *A. hypogaea* cv MK 374 × *A. glabrata*. (× 11.7)

c. late heart-shaped embryo dissected from an ovule (3.5 mm) cultured on liquid medium (MS + Suc. 5% + Kn 0.1 ppm) for 19 days from the cross *A. hypogaea* MK 374 × *Arachis* sp PI 276233. (× 11.4)

d. cotyledonary embryo with one well-formed cotyledon, dissected from an ovule (3.0 mm) cultured on liquid medium (MS + Suc. 5% + Kn 0.1 ppm) for 65 days from the cross *A. hypogaea* cv Chico × *Arachis* sp PI 276233. (× 13.3)

e. late cotyledonary embryo (note poorly-developed embryo axis) dissected from an ovule (2.0 mm) cultured on liquid medium (MS + Suc. 5% + Kn 0.1 ppm + IAA 0.2 ppm) for 47 days from the cross *Arachis hypogaea* cv Chico × *Arachis* sp Coll 9649. (× 12.2)

f. embryo similar to that shown in e, from the cross *A. hypogaea* cv MK 374 × *Arachis* sp PI 276233, 20 days after culture. (× 14.4)

Table 3. Percentage embryos responding from different *Arachis hypogaea* cultivars crossed with *Arachis* sp PI 276233 on MS medium.

Concentrations of additives						Embryos responding[1] (%)		
						Cultivars		
Kn (mg/l)	IAA (mg/l)	BAP (mg/l)	NAA (mg/l)	Sucrose (%)	Agar(%)	Robut 33-1	MK 374	TMV 2
0.10	0.1			5	0	6.66		
0.22	0			3	0.7			25.00
0	0	0.50	2.00	2		8.33		
0	0	0.50	0.50	3	0.7	3.57	26.11	
0	0	0.50	0.75	3	0.7	0.0	50.11	
0	0	0.50	2.00	3	0.7	21.42	20.00	27.27

1. Embryos which responded increased in size and became green

Conclusions

These studies on media and hormones have increased the survival, growth and development of hybrid ovules and embryos in culture. Changes in media necessary for continued survival, and better growth and development of ovules and embryos are being investigated, but there is now a method to produce hybrids in culture between species of *Arachis* which were previously incompatible. This is an important step towards transfer of desirable characters for the genetic improvement of *Arachis hypogaea*.

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