Arachis hypogaea Plant Recovery Through *In Vitro* Culture of Peg Tips[1]

Q. L. Feng, H. T. Stalker, H. E. Pattee*, and T. G. Isleib[2]

ABSTRACT

In vitro culture of embryos in *Arachis* is necessary to recover interspecific hybrids which otherwise abort soon after fertilization. The objective of this research was to develop *in vitro* techniques to promote proembryo development so that plants can be recovered. Aerial peg tips consisting of embryos, ovules, and peg meristem of *Arachis hypogaea* L. cv. NC 6, were collected 7, 10, and 14 d after self-pollination. Peg tips were cultured in the dark on combined MS and B5 media with NAA, GA_3 and 6-BAP for 90 d. The effects of plant growth regulators on *in vitro* reproductive traits, including peg elongation, callus and root production, pod formation, ovule and embryo development were variable. Results indicated that 10-d-old peg tips, which contained eight-celled proembryos, had more embryo development and pod formation than 7- and 14-d-old peg tips. Medium with 4 mg L^{-1} NAA and 0.5 mg L^{-1} 6-BAP suppressed *in vitro* development of pods, ovules and embryos and induced large amounts of callus. Media with lower concentrations of NAA, GA_3, and 6-BAP caused development of more and larger pods and ovules. The development of young embryos from proembryos was observed and mature seeds were obtained by an *in vitro* one-step process. Peanut plants were obtained both from *in vitro*-recovered embryos and from mature seeds.

Key Words: Embryo, ovule, pod, tissue culture, growth regulators, peanut.

Embryo development within the genus *Arachis* is characterized by aerial fertilization and early embryo development, followed by peg elongation and an accompanying quiescent phase of the embryo. Pod formation occurs after the peg enters the soil. At this time embryo growth is resumed, differentiation occurs, and a seed is produced. Embryo development is temporally and spatially interrelated with peg elongation, which is controlled by meristematic activity proximal to the base of the ovary. Failure of embryo growth, peg elongation, pod development or resumption of embryo development following quiescence can cause abortion. The complex reproductive development in *Arachis* is believed to cause many interspecific hybrid failures in the genus (6,7,9,17,19).

One approach to rescue hybrids is *in vitro* culture of embryos. Although Harvey and Schultz (8) first cultured *Arachis* embryos in 1943, plants were not obtained until Nuchowicz (15) cultured mature tissues using *in vitro* techniques. Martin (11) reported formation of a few plants from *A. hypogaea* L. ovules which were only 0.3-mm long, but his methodology has not yet been reproduced. Bajaj *et al.* (1), Feng and Pan (3), and Ziv and Zamski (24) cultured pegs and observed ovary enlargement, but no seeds or highly developed ovules. After using *in vivo* plant growth regulator treatments followed by *in vitro* culture, Mallikarjuna and Sastri (10) obtained plantlets from an *A. hypogaea* x *A.* sp. (section *Rhizomatosae*) cross.

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Moss and Stalker (12) cultured 4-, 10-, and 20-d-old peg tips of *A. hypogaea* selfs and hybrids with (4 mm long) or without (1 mm long) meristem. Their results indicated that several selfed and hybrid embryos grew to the globular stage after 21 d in culture. Moss *et al.* (13), Pattee *et al.* (19), and Rau *et al.* (20) later found similar results with 1- to 4-d-old selfed peg tips of *A. hypogaea*. Ziv and Sagar (23) cultured 4- to 5-cm pegs of *A. hypogaea* and observed swelling after 14 d. They removed the embryos, subcultured them in the dark, and reported seedling development. Bajaj *et al.* (1), Stalker and Eweda (22), Ziv and Sagar (23), and Ozias-Akins *et al.* (16) were successful in culturing heart-shaped or early cotyledonary embryos of several *A. hypogaea* genotypes and interspecific hybrids to obtain plants. Feng *et al.* (4) obtained mature seeds from several-celled proembryos of *A. duranensis* Krapov. and W.C. Gregory by culturing 7- to 14-d-old pegs. Thus, it appears feasible to recover peanut plants from differentiated embryos. However, application of *in vitro* techniques to rescuing hybrids has been mostly unsuccessful because of the high frequency of abortion within the first few days after pollination (7,17,18).

The objective of this research was to use the *in vitro* techniques of peg tip culture to reproducibly recover peanut plants from proembryos of *A. hypogaea*. Peg tips were used as nurse tissue, and a series of plant growth regulators were evaluated for *in vitro* reproductive growth and development in *A. hypogaea*.

Materials and Methods

Plants of *A. hypogaea* cv. NC 6 were grown in 15 x 15-cm pots in a greenhouse at North Carolina State University, Raleigh, NC during the summers of 1992 and 1993. Selfed flowers were identified daily with colored tags at anthesis, and elongating aerial pegs were excised 7, 10, or 14 d later. Pegs were rinsed in running tap water for 5 min, surface-sterilized in 70% ethanol for 10 sec, 0.1% mercuric chloride for 8 min, and washed three times for 10 min each with sterile distilled water. Peg tips were excised 10 mm from the apex to obtain the ovary and meristem. They were placed in 2.5 x 15-cm glass culture tubes by inserting 2 to 3 mm of the cut end vertically into the medium. The cultures were placed in the light (50 $\mu E\ m^{-2}\ sec^{-1}$) for 1 d and then kept in the dark at 27±1 C for 90 d in growth chambers except for brief periodic observations. Three replications of 10 peg tips each were observed.

An additional 10 pegs were excised at each 7, 10 or 14 d after pollination. These were fixed in FAA (70% ethanol:glacial acetic acid:formalin, 18:1:1) for 72 hr and stored in 70% ethanol until dissection to determine the embryonic stage at the initiation of culturing. Specimens were dehydrated in an alcohol series, paraffin embedded according to Berlyn and Miksche (2), sectioned at 7 µm thickness, stained with safranin-fast green, and prepared for observation by light microscopy.

The basal medium contained the inorganic components of MS medium (14), the organic components of B5 medium (5), 0.3 g L^{-1} casein hydrolysate and 60 g L^{-1} sucrose. All media were adjusted to pH 5.8 and solidified with 6 g L^{-1} agar. Treatments consisted of three plant growth regulators with four concentrations as follows: 1-naphthaleneacetic acid (NAA) (0.5, 1.0, 2.0, 4.0 mg L^{-1}), gibberellic acid (GA_3) (0.05, 0.10, 0.50, 1.00 mg L^{-1}), and 6-benzylaminopurine (6-BAP) (0.05, 0.10, 0.20, 0.50 mg L^{-1}). Only 16 of the 64 possible treatment combinations were used, and the 16 treatments (Table 1) were arranged so each growth regulator was combined once with each concentration of the other two compounds. The control was the basal medium without any growth regulator.

At the end of 90 d culture, tip swelling, pod formation, ovule growth, peg elongation, fresh weight of callus, and root number were recorded. Pods were distinguished by being at least 3 mm long and 2 mm wide and having netted veins on the surface of the pericarp. Pod and ovule volume was calculated by the formula: $3/4\ \pi\ L(W/2)^2$, where L and W were the length and width, respectively, of pods or ovules. Only ovules in pods were scored for growth. Callus at the surface of the cut end and along the peg length was weighed. The data were analyzed by performing response surface regression (RSREG) procedure in the SAS system (21). The RSREG procedure was used to determine the contribution of each growth regulator and their interactions to observed responses in the experiment. A best fit model was developed to generate predicted values which were used to plot a response surface. The shape of the response surface was analyzed to determine the optimal range of growth regulator levels or the directions in which subsequent growth regulator concentration experiments should be conducted.

Ovules isolated from *in vitro*-developed pods were sequentially subcultured for 30 d each on MS media containing 60 g L^{-1} sucrose, 0.3 g L^{-1} casein hydrolysate, and the following series of selected concentrations of plant growth regulators: 0.2 mg L^{-1} 6-BAP + 0.05 mg L^{-1} GA_3 in the first month; 0.2 mg L^{-1} 6-BAP + 0.05 mg L^{-1} NAA in the second month; and 0.1 mg L^{-1} IBA (indole-3-butyric acid) in the third month. The cultures were placed in a growth chamber at 27±1 C using a 12-hr photoperiod. Several ovules from each treatment were fixed in FAA to histologically determine the stage of embryo development.

Seeds were aseptically removed from pods and germinated either on MS medium with 0.2 mg L^{-1} each of 6-BAP and GA_3 or in vermiculite in a growth chamber at 27±1 C in the light. If seeds did not germinate within 20 d, they were removed, dried for 7 to 10 d at room temperature, and floated in a solution of 5 mg L^{-1} each of GA_3 and 6-BAP at pH 6 for 5 hr. The seeds were then placed on filter paper with 10 mL of the above solution in 9.5x7.5-cm petri dishes and sealed with parafilm. Ethylene gas was injected into the container to a final concentration of 100 ppm. At 4-d intervals, the container was opened, resealed, and reinjected with ethylene gas at the same concentration. After germination, the seedlings were placed in potted soil in a culture room for 7 to 10 d and then transferred to the greenhouse.

Results

Aerial peg tips of NC 6 collected at 7 d after self-pollination had four- to eight-celled and four-tiered proembryos (Fig. 1A, D) within green ovules smaller than 0.3 mm in length. Ten-d-old peg tips contained 8- to 16-celled, 5- to 6-tiered proembryos (Fig. 1B, E). Eight to 32 cells were observed in the proembryos of 14-d-old peg tips (Fig. 1C, F).

Peg tip explants had several responses to different media during culture, including peg elongation, callus

Table 1. *In vitro* reproductive traits of *A. hypogaea* peg tips excised 7, 10, and 14 d after pollination in different treatments of plant growth regulators for 90 d culture.

Tr.	NAA	GA_3	6-BAP	Peg tip age	Obs.	Length	Callus	Root	Pod formation[a]		Ovule growth				Max. embryo growth[b]	
											Apical		Basal		Apical	Basal
no.	---- mg L^{-1} -----			DAP	no.	cm	g	no.	%	mm^3	%	mm^3	%	mm^3		
1	0.5	0.05	0.50	7	23	3.84	0.663	0.0	4.4	179.9	4.4	1.2	4.4	21.2	NG	C
	"	"	"	10	19	2.76	1.071	0.1	5.3	564.4	5.3	7.1	5.3	31.7	G	C
	"	"	"	14	29	2.97	1.412	0.0	6.9	124.9	10.5	0.4	10.5	6.3	G	HS
2	0.5	0.10	0.20	7	28	5.17	0.879	0.2	10.7	245.5	7.1	1.2	10.7	18.4	NG	S
	"	"	"	10	30	3.34	1.235	0.3	30.0	333.1	20.0	1.9	30.0	20.3	G	S
	"	"	"	14	30	2.81	1.253	0.5	26.7	73.1	20.0	0.5	23.3	4.2	G	G
3	0.5	0.50	0.05	7	28	6.17	0.406	0.4	21.4	854.3	21.4	4.2	21.4	118.2	G	S
	"	"	"	10	30	3.78	0.913	0.4	40.0	352.7	36.7	1.1	40.0	252.0	G	S
	"	"	"	14	29	2.99	1.306	0.4	24.1	136.4	24.1	0.3	24.1	41.8	G	S
4	0.5	1.00	0.10	7	24	4.50	0.576	0.2	4.2	169.0	4.2	0.9	4.2	11.0	NG	NG
	"	"	"	10	30	3.48	0.830	0.4	6.7	195.6	3.3	7.1	3.3	20.7	NG	HS
	"	"	"	14	26	3.03	1.341	0.1	15.4	105.9	7.7	0.9	7.7	10.4	G	G
5	1.0	0.05	0.05	7	25	4.22	0.529	0.2	20.0	32.5	12.0	0.1	20.0	0.9	G	G
	"	"	"	10	29	3.35	0.878	1.3	17.2	57.6	10.3	0.2	17.9	2.2	G	HS
	"	"	"	14	28	3.28	1.293	2.3	17.9	30.7	14.3	0.2	17.9	1.1	G	G
6	1.0	0.10	0.10	7	28	5.21	0.555	0.7	7.1	41.8	3.6	0.9	7.1	1.1	NG	G
	"	"	"	10	28	3.24	1.116	0.4	10.7	24.4	10.7	0.1	10.7	0.4	NG	G
	"	"	"	14	28	2.72	1.304	0.6	10.7	72.8	7.1	0.5	10.7	1.3	G	HS
7	1.0	0.50	0.50	7	26	3.73	0.659	0.0	0.0	—	0.0	—	0.0	—	NG	NG
	"	"	"	10	22	2.78	1.873	0.0	4.6	65.8	4.6	0.2	4.6	2.6	G	G
	"	"	"	14	29	2.47	1.923	0.0	3.5	26.5	0.0	—	0.0	—	NG	NG
8	1.0	1.00	0.20	7	24	5.31	1.236	0.3	8.3	39.6	4.2	0.6	4.2	10.6	NG	HS
	"	"	"	10	28	3.27	1.060	0.3	7.1	89.3	7.1	0.5	7.1	0.6	NG	G
	"	"	"	14	29	3.16	1.738	0.6	6.9	67.9	6.9	0.9	6.9	2.8	G	G
9	2.0	0.05	0.20	7	27	4.37	1.338	0.0	3.7	26.5	0.0	—	3.7	0.1	NG	NG
	"	"	"	10	28	2.90	1.652	0.0	7.1	26.5	7.1	0.1	7.1	0.1	NG	G
	"	"	"	14	29	3.11	2.478	0.5	3.5	56.4	3.5	0.2	3.5	1.2	G	G
10	2.0	0.10	0.50	7	25	2.77	1.373	0.0	0.0	—	0.0	—	0.0	—	NG	NG
	"	"	"	10	26	2.65	1.560	0.0	3.9	34.4	3.9	0.2	3.9	2.6	NG	G
	"	"	"	14	28	2.70	2.883	0.0	3.6	43.2	3.6	0.2	3.6	1.2	G	NG
11	2.0	0.50	0.10	7	25	3.76	0.405	0.0	16.0	97.0	12.0	0.3	16.0	6.2	NG	HS
	"	"	"	10	26	3.57	1.076	0.6	23.1	107.8	19.2	1.0	19.2	30.2	G	S
	"	"	"	14	30	3.42	1.991	1.4	16.7	259.0	13.3	2.0	16.7	28.7	G	S
12	2.0	1.00	0.05	7	22	7.14	1.031	1.2	27.3	468.6	22.7	1.2	27.3	82.8	NG	S
	"	"	"	10	30	3.57	1.043	0.8	36.7	402.4	30.0	0.8	36.7	126.0	G	S
	"	"	"	14	29	3.49	1.547	1.0	20.7	249.7	17.2	2.0	20.7	43.4	G	S
13	4.0	0.05	0.10	7	21	3.31	1.604	0.0	4.8	179.9	4.8	0.6	4.8	3.9	NG	NG
	"	"	"	10	26	2.72	1.657	1.5	3.9	186.7	3.9	1.2	3.9	21.2	NG	G
	"	"	"	14	28	2.62	2.401	2.2	3.6	36.0	3.6	0.2	3.6	1.2	NG	G
14	4.0	0.10	0.05	7	25	5.76	1.093	1.2	0.0	—	0.0	—	0.0	—	NG	NG
	"	"	"	10	29	2.88	1.207	0.6	3.5	26.5	0.0	—	3.5	0.2	NG	NG
	"	"	"	14	29	2.99	2.121	2.5	3.5	57.6	3.5	0.2	3.5	2.6	NG	G
15	4.0	0.50	0.20	7	21	3.87	1.258	0.0	0.0	—	0.0	—	0.0	—	NG	NG
	"	"	"	10	23	2.72	2.239	0.0	4.4	26.5	4.4	0.2	4.4	0.9	NG	G
	"	"	"	14	27	3.23	2.611	0.2	0.0	—	0.0	—	0.0	—	NG	NG
16	4.0	1.00	0.50	7	26	3.59	1.536	0.0	0.0	—	0.0	—	0.0	—	NG	NG
	"	"	"	10	25	2.37	2.744	0.0	4.0	31.7	4.0	0.1	4.0	0.9	NG	NG
	"	"	"	14	29	2.42	2.744	0.0	0.0	—	0.0	—	0.0	—	NG	NG
17	0.0	0.00	0.00	7	24	5.92	0.061	1.5	4.2	35.8	4.2	0.2	4.2	1.2	NG	G
	"	"	"	10	27	3.12	0.049	0.5	3.7	0.9	3.7	0.2	3.7	1.2	G	G
	"	"	"	14	30	2.75	0.105	1.8	0.0	—	0.0	—	0.0	—	NG	NG

[a]Pod formation was distinguished from tip swelling by significantly swelling to at least 3.0 x 2.0 mm (length x width) and having netted veins formed on the pericarp surface. The volume of pod and ovule was calculated based on the formula $3/4\,\pi\, L(W/2)^2$, where L and W were the length and width, respectively.

[b]NG = no growth or not observed, G = globular stage, HS = heart-shaped stage, C = cotyledonary stage, S = seed.

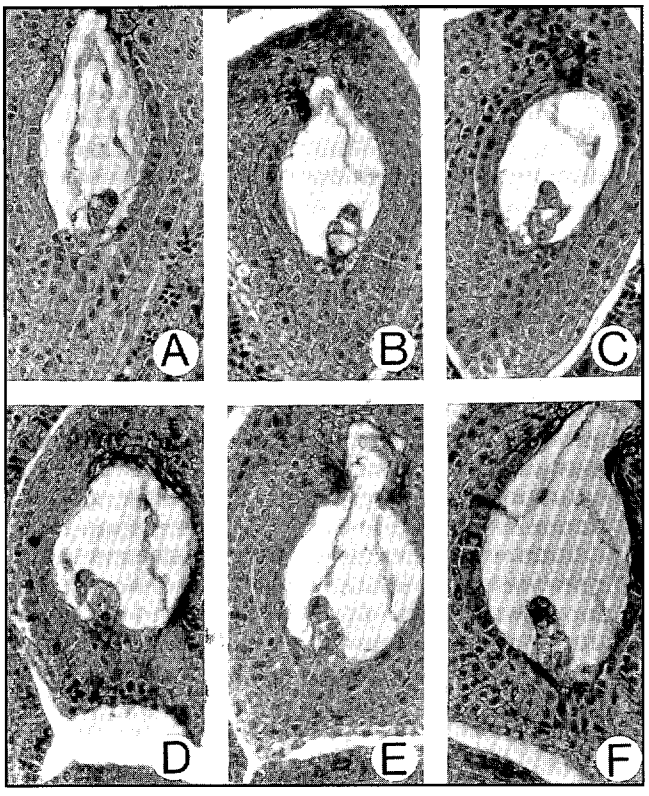

Fig. 1. Longitudinal sections of the embryo sac and proembryo of *A. hypogaea*: (A) a 7-d-old apical embryo at four-tier stage, x600; (B) a 10-d-old apical embryo at four-tier stage, x600; (C) a 14-d-old apical embryo at five-tier stage, x600; (D) a 7-d-old basal embryo at four-tier stage, x600; (E) a 10-d-old basal embryo at five-tier stage, x600; (F) a 14-d-old basal embryo at six-tier stage, x600.

production, root formation, ovary enlargement, and ovule and embryo development (Table 1). Because of the incomplete factorial design of the experiment, the effects of interaction among the three growth regulators were confounded with the main effects, so a simple comparison of main effects of the growth regulators on the responses could not be made. Thus, a response surface analysis using the SAS RSREG procedure (21) was performed to estimate main and interaction effects. In the analysis of peg elongation, NAA had no significant effect and no significant interactions were observed between NAA and the other two growth regulators. However, a significant interaction effect was observed between GA_3 and 6-BAP. At relatively low concentrations (0.05-0.2 mg L^{-1}) of 6-BAP, GA_3 promoted peg elongation at the higher concentrations. The promoting effect of GA_3 was suppressed at the higher concentration (0.5 mg L^{-1}) of 6-BAP. In general, 7-d-old peg tips elongated more than 10- and 14-d-old peg tips (Table 1).

Callus formed either at the cut surface or along the length of the peg tip explants. The RSREG analysis indicated that there was no significant interaction between GA_3 and either of the other two growth regulators for callus production. An increase in GA_3 concentration did not change the amount of callus. Both NAA and 6-BAP stimulated callus production with increased concentrations, and they had a positive interaction effect on callus production. Older peg tips produced more calli than younger tissues (Table 1).

Roots were produced either directly from explant tissues or from callus at the cut surface. Because few roots were produced in the experiment, no significant effects of plant growth regulators on root production were found. However, when 6-BAP was at a low concentration (for example, treatments 3, 5, 12, and 14), increased levels of NAA promoted root formation. When 6-BAP was at a high level, the promoting effect of NAA was suppressed (for example, treatments 1, 7, 10, and 16) (Table 1).

From the 1365 cultured peg tips, 139 pods (10.2%) were obtained across all treatments and stages. Some *in vitro* pods formed aerially above the medium, but in most cases pods formed below the medium surface (Fig. 2). Pod formation varied from 0 to 40% in different treatments depending on different growth regulators used and on peg tip age (Table 1). When NAA was at 4 mg L^{-1} (treatments 13-16), a low frequency of pod formation was observed regardless the concentrations of the other

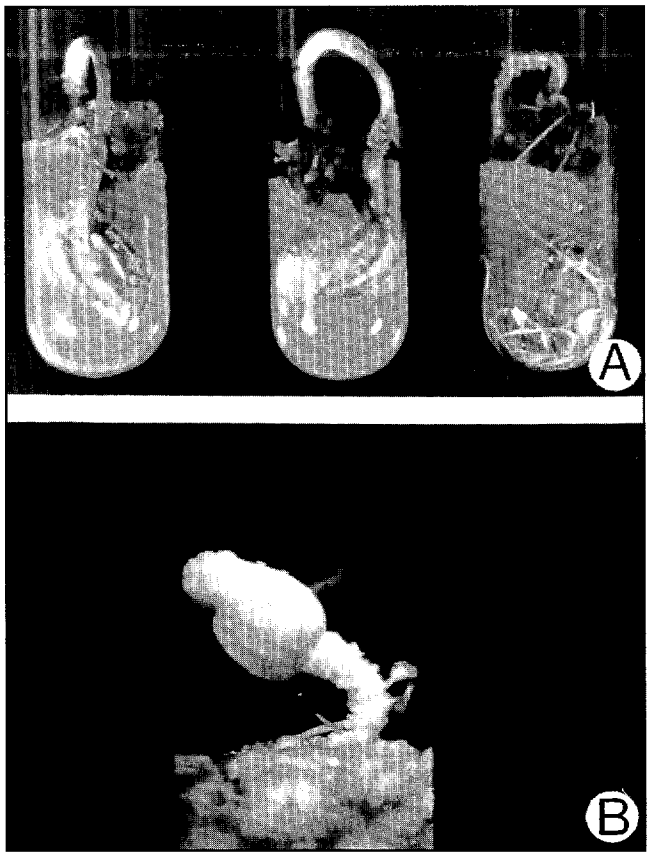

Fig. 2. (A) Mature pods from 7- (left) and 10-d-old (middle and right) peg tips of *A. hypogaea* after 90-d culture on the media with 0.5 mg L^{-1} NAA + 0.5 mg L^{-1} GA_3 + 0.05 mg L^{-1} 6-BAP (left) and 2.0 mg L^{-1} NAA + 1.0 mg L^{-1}. GA_3 + 0.05 mg L^{-1} 6-BAP (middle and right). (B) An *in vitro* aerially formed pod of *A. hypogaea* after 90-d culture on the medium containing 2.0 mg L^{-1} NAA + 1.0 mg L^{-1} GA_3 and 0.05 mg L^{-1} 6-BAP.

two regulators. In addition, when the 6-BAP concentration was 0.5 mg L^{-1} (treatments 1, 7, 10, and 16), pod formation was reduced.

The RSREG analysis indicated that there were no significant interactions between 6-BAP and either of the other two growth regulators for pod formation. However, a significant ($P \leq 0.01$) interaction between NAA and GA_3 was observed (Fig. 3). At the lowest concentration (0.5 mg L^{-1}) of NAA, changing GA_3 concentrations did not significantly affect the frequency of pod formation. At higher concentrations of NAA (for example, 2.0 mg L^{-1}), higher rates of GA_3 produced more pods. At low concentrations of GA_3, corresponding low concentrations of NAA were necessary to obtain a high frequency of pod formation; and at higher concentrations of GA_3, a greater amount of NAA was needed to produce pods. Differences in pod and ovule size were observed between the low and high concentrations of GA_3 and NAA. For example, at 0.5 mg L^{-1} NAA, the average pod, apical ovule and basal ovule sizes were 288, 2, and 46 mm^3, respectively; whereas at 2.0 mg L^{-1} NAA, the pod, apical and basal ovules had volume of 148, 0.7, and 27 mm^3, respectively.

No significant interactions were found between peg tip age and the three growth regulators NAA, GA_3 or 6-BAP for pod formation. Differences in pod formation were observed, however, among three tissue ages. Ten-d-old peg tips had the highest rate of pod development, with the largest frequencies observed in treatments 3 (40.0%) and 12 (36.7%).

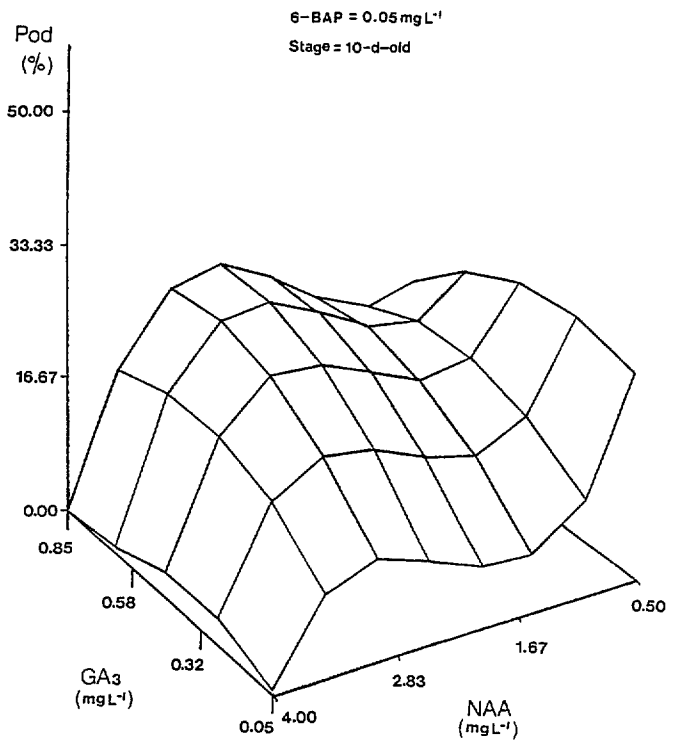

Fig. 3. Response surface of pod formation in 10-d-old peg tips of *A. hypogaea* to different concentrations of NAA and GA_3.

Twenty-six seeds and 218 ovules (that enlarged to at least 0.5 x 0.5 mm) were obtained from 139 pods. These ovules contained embryos at different stages from globular to cotyledonary (Fig. 4). Within the pods, basal embryos almost always developed farther than apical embryos. For example, in treatments 3 and 12, when basal embryos were at a cotyledonary stage, the apical embryos usually developed only to a globular stage (Table

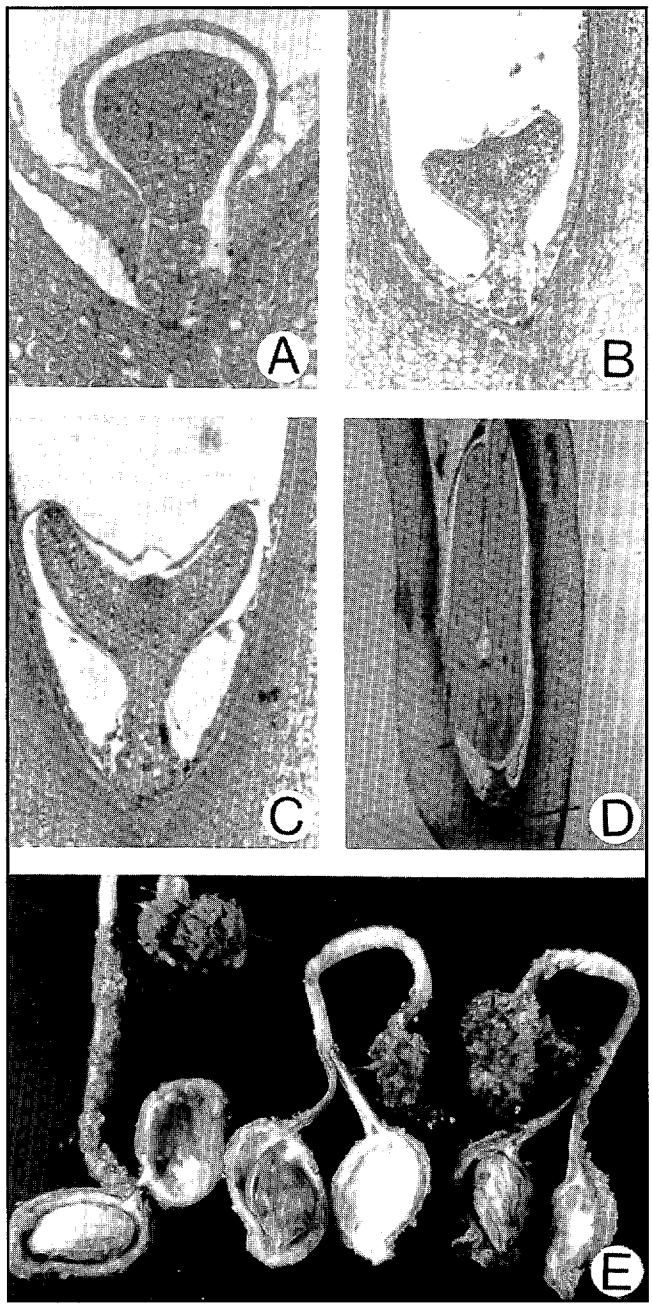

Fig. 4. Longitudinal sections of the embryo sac and embryos of *A. hypogaea* showing the different stages of *in vitro* developed embryos observed after 90-d culture: (A) at globular stage, x200; (B) at heart-shaped stage, x100; (C) at early cotyledonary stage, x80; (D) at late cotyledonary stage, x20; (E) mature seeds recovered from 7- (left) and 10-d-old (middle and right) proembryos through peg tip culture for 90 d.

1). In general, treatments that favored pod formation also increased ovule growth and embryo development.

One hundred twenty-two ovules were subcultured on MS media with 0.2 mg L^{-1} 6-BAP and 0.05 mg L^{-1} GA_3, but 60% of them died within 2 wk. Another 21% of the ovules, which were less than 3 mm long, turned green but did not grow and finally died. The ovules (14.9% of total) larger than 3 mm in length initiated growth, but never produced shoots. Five embryos germinated during subculturing on the MS media with 0.2 mg L^{-1} 6-BAP and 0.05 mg L^{-1} GA_3 into seedlings which were subsequently transferred to the greenhouse and mature plants were obtained.

Twenty-six seeds were obtained from basal ovules in treatments 2, 3, 11, and 12 (Table 2). These seeds had thin, brown seed coats and two fully developed cotyledons (Fig. 4). Twelve relatively small seeds were placed on MS media with 0.2 mg L^{-1} of each 6-BAP and GA_3. Three seeds germinated within 30 d, one germinated in 90 d, and eight died after remaining dormant for 120 d. In addition, 14 larger seeds were placed in vermiculite for 14 d. Two of these seeds germinated, two died, and 10 remained dormant. Two weeks later the dormant seeds were removed from the vermiculite, dried at room temperature, and treated with 100 ppm ethylene gas plus 0.5 mg L^{-1} of each 6-BAP and GA_3. Eight of these seeds germinated. A total of 15 mature plants were recovered from all ovules and seeds obtained *in vitro*.

Table 2. Number of ovules, seeds, seedlings, and plants recovered from the *in vitro* culture of peg tips of *A. hypogaea* in the five best treatments of plant growth regulators.

Tr.	Cultured explants	Ovules enlarged		Seeds obtained		Seedlings obtained		Plants recovered	
no.	no.	no.	%	no.	%	no.	%	no.	%
2	88	33	37.5	4	4.6	1	1.1	1	1.1
3	87	49	56.3	8	9.2	6	6.9	5	5.8
5	82	25	30.5	0	0.0	1	1.2	1	1.2
11	81	26	32.1	4	4.5	2	2.5	1	1.2
12	81	42	51.9	10	12.4	9	11.1	7	8.6
Total	419	175		26		19		15	
Avg			41.8		6.2		4.5		3.6

Discussion

Embryo development from the globular to the heart-shaped stage is a critical step for which *in vitro* techniques need to be developed (13, 19, 20). In this research, large embryos and mature seeds were recovered from proembryos through a one-step *in vitro* procedure. This demonstrates that the entire developmental processes from ovaries to fruits and from proembryos to mature seeds in peanut can be achieved *in vitro*.

Culture method, selection of explants, and effects of plant growth regulators were studied. Previous attempts to culture peanut pegs have been primarily with short peg tips without meristems (12,13,19,20). Although the removal of meristems from pegs can eliminate inhibitory effects of elongation on embryo development, the cut surface is so close to the ovary that *in vitro* growth of ovules and embryos is probably influenced by wounding effects. Cutting the pegs close to the ovules may be unfavorable for uptake of nutrients from the medium because the developed vascular tissues in pegs may have served a critical growth function by translocating nutrients to the developing embryos through the peg tissues. Further, the peg tip must swell to form a pod to allow room for the embryo to grow. The culture of longer peg tips may overcome these disadvantages and allow embryos to develop along a more normal developmental pattern. The conclusion is that culturing longer peg tips with the meristem is a more effective procedure than using short peg tips to achieve *in vitro* reproductive development of peanut.

Ten-d-old peg tips had more reproductive development *in vitro* than 7- or 14-d-old pegs. The reason was probably associated with 7-d-old peg tips having more elongation and 14-d-old peg tips having more calli than 10-d-old tissues. Excess elongation and calli could have suppressed ovary enlargement and embryo development.

The highest concentration of NAA (4 mg L^{-1}) and 6-BAP (0.5 mg L^{-1}) tested suppressed *in vitro* development of fruits, ovules, and embryos. NAA at 0.5 mg L^{-1} appeared to induce more pod formation and larger pods and ovules. Two mg L^{-1} NAA also induced pod formation and ovule growth, but the sizes of pods and ovules were relatively smaller. This was due to a suppressing effect of the calli induced by relatively high levels of NAA.

Because the peg tip explants were placed in the dark for the long period of 90 d, the only energy source for the tissues was sugars from the medium. Although pod and embryo development was observed and a maximum of 40% pod formation per treatment was obtained, the overall success rate of 10.2% was relatively low. Further, most pods contained only one developed embryo. An increased nutrient supply may be beneficial to *in vitro* reproductive development, which could be obtained by using culture containers larger than the 2.5 x 15 cm ones in this experiment.

A repeatable method to obtain plants from proembryos of *A. hypogaea* is now available for peanut. The *in vitro* technique can be used to study the developmental process of peanut pods and embryos and should be applicable for attempting to overcome embryo abortion in interspecific hybrid crosses.

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