In Vitro Reproductive Development of Peanut, *Arachis hypogaea* L., as Influenced by Plant Growth Regulators, Sucrose and pH[1]

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ABSTRACT

Research on *in vitro* embryo culture in *Arachis* has the objective of rescuing interspecific hybrid embryos which abort before they reach maturity. This study explored effects of the three exogenous plant growth regulators 1-naphthaleneacetic acid (NAA), gibberellic acid (GA_3), and 6-benzylaminopurine (6-BAP); sucrose; and medium pH on *in vitro* fruit and embryo development of *A. hypogaea* L. by culturing 10-d-old peg tips. Results indicated that medium containing 0.5 to 1.0 mg L^{-1} NAA was optimal for *in vitro* pod formation and embryo development. GA_3 did not have a significant influence and 6-BAP had negative effects on both *in vitro* fruit and embryo development. High concentrations of 6-BAP and NAA induced callus which inhibited ovary enlargement and embryo development. Sixty g L^{-1} sucrose was the best concentration for ovary enlargement and embryo development. Acidic medium was needed for *in vitro* reproductive development with pH 4.5-6.5 the most favorable. A pod formation frequency of 81%, a seed production rate of 90% (from pods recovered *in vitro*), and plant recovery of 33% were obtained for a medium containing 1.0 mg L^{-1} NAA and 0.5 mg L^{-1} GA_3 plus 60 g L^{-1} sucrose at pH 5.8. *In vitro*-recovered cotyledonary embryos between 4 and 10 mm long germinated precociously into seedlings at relatively higher frequencies than morphologically mature embryos which produced more vigorous plants.

Key Words: Embryo, ovule, pod, peg, *in vitro* culture, tissue culture.

Peanut is a valuable leguminous crop because seeds contain high percentages of protein and oil. Cultivated peanut is susceptible to many diseases, insects and nematodes, whereas many wild species of *Arachis* have high levels of resistances. However, introgression of wild germplasm to cultivated peanut is restricted because interspecific hybridization is difficult (5). Embryo abortion occuring soon after fertilization often is the cause for failure of interspecific hybridization (6,7,11,12), and *in vitro* culture of embryos is an approach to rescue hybrids. Studies by Bajaj *et al.* (1), Ozias-Akins (10), Sastri *et al.* (17), and Stalker and Eweda (20) indicated that rescuing heart-shaped embryos of interspecific hybrids is possible. However, since abortion often occurs at a stage prior to the heart shape (11,12), improved *in vitro* techniques are needed to rescue proembryos.

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Previous work has resulted in multicellular globular embryos derived from one- to two-celled proembryos of selfed *A. hypogaea* L. (8,13,14). Ziv and Sagar (23) achieved *in vitro* growth of *A. hypogaea* ovules and obtained viable young seedlings by a two-step process. Mature seeds were obtained from several-celled proembryos of *A. hypogaea* and *A. duranensis* Krapov. and W. C. Gregory by Feng *et al.* (2,3), but at a very low frequency. The objective of this research was to evaluate effects of plant growth regulators, sucrose, and medium pH on *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 summer of 1994. Selfed flowers were marked daily at anthesis with colored tags, and elongating aerial pegs were collected 10 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 then washed three times for 10 min each with sterile distilled water. Peg tips containing the ovary and peg meristem were excised 10 mm from the apex. Explants were cultured either in 250-mL flasks or 51 x 98-mm jars by inserting 2 to 3 mm of the cut end vertically into the medium with the apex end oriented upward. Each culture container included 50 mL of medium and one peg tip explant. 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 six or eight peg tips each were observed in different experiments.

The basal medium in all experiments contained the inorganic components of MS medium (9), the organic components of B5 medium (4), 0.3 g L^{-1} casein hydrolysate and 60 g L^{-1} sucrose except where specifically mentioned. All media were adjusted to pH 5.8, except when noted, and solidified with 6 g L^{-1} Difco Bacto agar. Deviations from the basal medium were made in experiments evaluating plant growth regulator, sucrose, and pH influences on reproductive development. An experiment was conducted to evaluate the effects of the exogenous plant growth regulators 1-naphthaleneacetic acid (NAA), gibberellic acid (GA_3), and 6-benzylaminopurine (6-BAP) (Table 1). In experiments studying the effects of sucrose and acidity of initial culture medium, the concentrations of NAA, GA_3, and 6-BAP were 1.0, 0.5, and 0.05 mg L^{-1}, respectively. In the sucrose experiment, concentrations of sucrose were increased from 0 to 150 g L^{-1} by increments of 15 g L^{-1}. In the experiment testing pH influences on reproductive development, the

Table 1. Effects of plant growth regulators on *in vitro* pod formation, embryo development, and plant regeneration of *A. hypogaea*.[a]

Conc. of growth regulator	Pod formation	Pod volume	Embryo development[b]			Seedling	Mature plant
			Prior to cotyledon	Immature seed	Mature seed		
mg L^{-1}	%	cm^3	%				
			NAA[c]				
0.00	12.4	6.2	8.4	4.2	8.4	8.3	0.0
0.50	53.8	6.0	12.6	20.8	16.6	29.2	12.5
1.00	61.9	5.1	12.6	20.8	29.2	25.0	12.5
2.00	20.5	1.0	20.8	0.0	4.2	0.0	0.0
			GA_3[d]				
0.00	38.1	4.2	20.8	4.2	20.8	4.2	0.0
0.05	30.0	3.7	16.6	12.6	4.2	16.7	0.0
0.50	61.9	5.1	12.6	20.8	29.2	25.0	12.5
1.00	39.5	3.8	16.6	12.6	12.6	12.5	8.3
			6-BAP[e]				
0.00	81.0	11.2	25.0	50.0	29.2	62.5	29.2
0.05	61.9	5.1	12.6	20.8	29.2	25.0	12.5
0.10	34.8	2.1	20.8	0.0	12.6	0.0	0.0
0.20	31.4	0.5	62.4	0.0	0.0	0.0	0.0
0.50	47.6	0.2	45.8	0.0	0.0	0.0	0.0
Control[f]	44.3	9.0	20.8	8.4	37.4	37.5	16.7
LSD (0.05)	36.1	3.6	32.6	17.4	25.6	22.5	15.2

[a]The data were averaged from three replicates of eight peg tips each.

[b]Mature seeds were those which contained fully developed cotyledons and thin seed coats; immature seeds were those which contained developing cotyledons which had not yet filled the capacity of the seeds; embryos prior to cotyledonary stage included all embryos at globular and heart-shaped stages.

[c]The concentrations of GA_3 and 6-BAP in the media were 0.5 and 0.05 mg L^{-1}, respectively.

[d]The concentrations of NAA and 6-BAP in the media were 1.0 and 0.05 mg L^{-1}, respectively.

[e]The concentrations of NAA and GA_3 in the media were 1.0 and 0.5 mg L^{-1}, respectively.

[f]The medium did not contain any exogenous growth regulator.

medium was adjusted with HCl and NaOH from pH 4.5 to 8.0 by 0.5-unit increments. pH values of media were measured before and after 90 d culture.

Pod number and size, ovule growth, embryo development, peg elongation, fresh weight of callus, and root number were recorded at the end of 90 d culture. 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 size was calculated by the formula: $3/4\ \pi\ L\ (W/2)^2$, where L and W were the length and width of pods or ovules, respectively. Only ovules and embryos in pods were scored for growth. Experimental data were analyzed using the Analysis of Variance Procedure and Correlation Analysis in the SAS system (16). Because no transformation was found which would stabalize the error variance (due to the large number of zeros in data sets), nontransformed data are presented.

After culturing for 90 d, selected explants were subcultured in an attempt to obtain plants. Previous experiments indicated that ovules smaller than 4 x 7 mm contained heart-shaped (or smaller) embryos, so these embryos were isolated from *in vitro*-developed pods and subcultured on MS media containing 60 g L^{-1} sucrose, 0.3 g L^{-1} casein hydrolysate, and 0.5 mg L^{-1} each of 6-BAP and GA_3 in the dark at 27 C. From the remaining pods, either cotyledonary embryos or seed were isolated and all cotyledonary embryos and two-thirds of the seeds were subcultured on the same medium, but in the light (50 $\mu E\ m^{-2}\ sec^{-1}$) for 12 hr duration to induce germination. After 1 mo, seedlings were transferred onto MS media with 0.5 mg L^{-1} indole-3-butyric acid (IBA), and seedlings were planted in soil in the greenhouse after roots were produced. One-third of the mature seeds were placed directly into the soil for germination.

Results

When GA_3 and 6-BAP concentrations were 0.5 and 0.05 mg L^{-1}, respectively, high percentages of pod formation were observed at both 0.5 and 1.0 mg L^{-1} NAA (Table 1). These pods had a relatively large volume. Seventy-five and 95% of the pods obtained at 0.5 and 1.0 mg L^{-1} NAA, respectively, contained cotyledonary embryos. Omitting NAA produced fewer pods, but they also were large and contained cotyledonary embryos (Table 1). When NAA was increased to 2.0 mg L^{-1}, the number of pods, pod size, and embryo development were significantly ($P \leq 0.05$) reduced (Table 1). Increasing NAA promoted callus formation significantly ($P \leq 0.05$), particularly at 2.0 mg L^{-1} (Table 2). Increasing the concentrations of NAA from 0 to 1.0 mg L^{-1} also enhanced root production.

In the GA_3 experiment, when NAA and 6-BAP concentrations were 1.0 and 0.05 mg L^{-1}, respectively, change of GA_3 levels did not significantly ($P > 0.05$) influence pod or embryo development. However, 0.5 mg L^{-1} GA_3 promoted a relatively higher frequency of pods, and appeared to produce more cotyledonary embryos than other GA_3 levels (Table 1). GA_3 had no significant effects on either callus or root formation (Table 2).

With 1.0 and 0.5 mg L^{-1} of NAA and GA_3, respectively, 6-BAP at 0 and 0.05 mg L^{-1} produced relatively more pods than the other concentrations tested (Table 1). Without 6-BAP or at the 0.05 mg L^{-1}, not only more pods were produced, but also larger pods and more developed embryos were observed. However, increasing 6-BAP from 0.1 to 0.5 mg L^{-1} significantly ($P \leq 0.05$) inhibited ovary enlargement, pod growth, and embryo development. At 0.2 and 0.5 mg L^{-1} 6-BAP, no cotyledonary embryos were recovered and most embryos were at the precotyledonary stage (Table 1). 6-BAP significantly ($P \leq 0.05$) induced callus formation and inhibited root production with increased concentrations (Table 2).

Basal medium, which did not contain growth regulators, gave 44.3% pods with an average size of 9 cm^3 (Table 1). Eleven seeds were recovered from 10 of these pods. These numbers were higher than those for most of growth regulator treatments except for the combination of 1.0 mg L^{-1} NAA and 0.5 mg L^{-1} GA_3, which produced the highest rate (81.0%) of pod formation, largest pods (11 cm^3), and the most seeds (19 seeds from 17 pods).

Correlations were estimated among the cultural responses for all treatments. Negative correlation coefficients were found between callus and pod number ($r = -0.14$, $P = 0.40$) and between callus and pod volume ($r = -0.81$, $P \leq 0.01$). Callus thus appeared unrelated to pod number, but increasing callus was significantly associated with decreasing pod size. Further, embryo development was positively related to pod development, and the correlation coefficients between pod volume and imma-

Table 2. Effects of NAA, GA_3, and 6-BAP on root formation, and callus production of peg tip explants of *A. hypogaea*.

Conc. of growth regulators	Root peg tip^{-1}	Callus peg tip^{-1}
mg L^{-1}	no.	g
	NAA[a]	
0.00	0.66	0.295
0.50	3.67	3.854
1.00	5.62	4.649
2.00	4.86	8.091
	GA_3[b]	
0.00	3.43	5.158
0.05	5.57	4.939
0.50	5.62	4.649
1.00	3.86	5.751
	6-BAP[c]	
0.00	7.48	0.461
0.05	5.62	4.649
0.10	4.33	8.061
0.20	2.19	9.533
0.50	1.33	11.122
Control[d]	4.91	0.144
LSD (0.05)	2.65	1.186

[a]The concentrations of GA_3 and 6-BAP in the media were 0.5 and 0.05 mg L^{-1}, respectively.

[b]The concentrations of NAA and 6-BAP in the media were 1.0 and 0.05 mg L^{-1}, respectively.

[c]The concentrations of NAA and GA_3 in the media were 1.0 and 0.5 mg L^{-1}, respectively.

[d]The medium did not contain any growth regulator.

ture seed, and pod volume and mature seed were 0.67 ($P \leq 0.01$) and 0.69 ($P \leq 0.01$), respectively. Positive correlation coefficients were observed between root number and both pod number ($r = 0.54$, $P \leq 0.01$) and pod volume ($r = 0.40$, $P \leq 0.01$). Most large pods had many roots which formed at the cut end of peg tip explants (Fig. 1).

A large range of media sucrose levels (30-135 g L^{-1}) was suitable for producing pods; however, a maximum number of pods was reached at 60 g L^{-1} sucrose (Table 3). Sucrose concentrations at 60-105 g L^{-1} resulted in larger pods than other concentrations (Table 3; Fig. 2). The most cotyledonary embryos were observed at 60-90 g L^{-1} sucrose. When sucrose levels were either at 0-30 g L^{-1} or at 135-150 g L^{-1}, pod formation (and especially size) and embryo development were dramatically suppressed (Table 3; Fig. 2). No ovary enlargement was observed when sucrose was deleted from the media.

Within the range of pH tested, pH 4.5-5.0 gave relatively more pods (Table 4) and pH 8.0 resulted in the fewest pods. However, pod size varied with the pH changes (Table 4; Fig. 3). As pH was raised from 4.5 to 8.0, pod size was gradually reduced. Neutral (pH 7.0) and basic (pH 7.5 and 8.0) media produced smaller pods than acidic (pH 4.5-6.5) media (Table 4; Fig. 3). Also embryo development was influenced by increased pH. At pH 4.5 and 5.0, more than 75% of the pods contained seeds whereas, for neutral pH media, only a few embryos

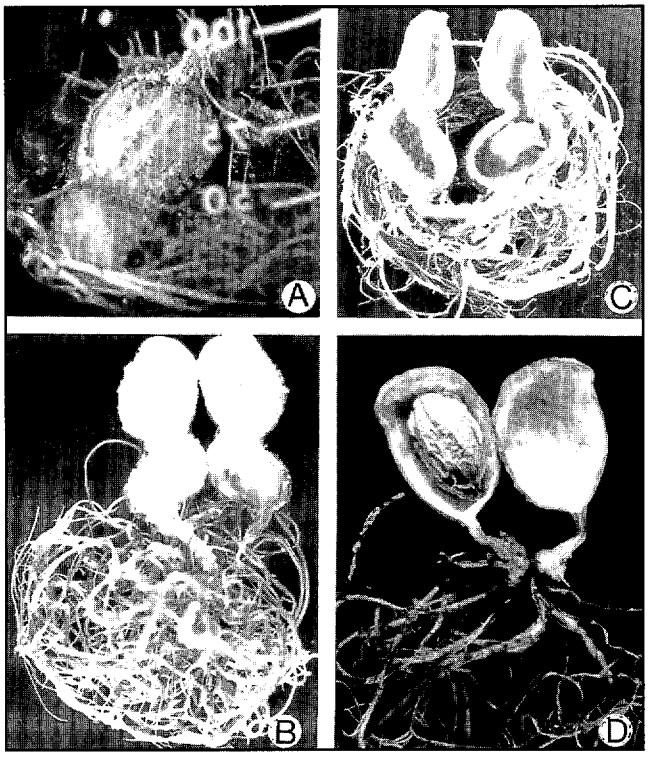

Fig. 1. (A) An *in vitro* double pod of *A. hypogaea* in the medium containing 1.0 mg L^{-1} NAA and 0.5 mg L^{-1} GA_3; (B) a double pod in which only the basal seed developed while the apical embryo degenerated; (C) a double pod which contained two small seeds; (D) a single pod *in vitro* recovered showing a mature seed inside the pod.

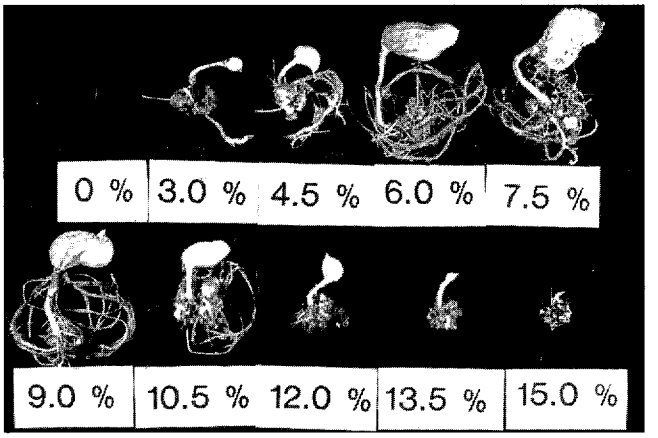

Fig. 2. Effect of sucrose concentrations on *in vitro* pod formation of *A. hypogaea*. The media contained 1.0 mg L^{-1} NAA, 0.5 mg L^{-1} GA_3, and 0.05 mg L^{-1} 6-BAP at pH 5.8.

Table 3. Effects of sucrose concentration on *in vitro* pod formation, embryo development, and plant regeneration of *A. hypogaea*.[a]

Conc. of sucrose	Pod formation	Pod volume	Embryo development[b]				Mature plant
			Prior to cotyledon	Immature seed	Mature seed	Seedling	
g L^{-1}	%	cm^3	%				
0	0.0	0.00	0.0	0.0	0.0	0.0	0.0
30	16.7	0.24	0.0	0.0	0.0	0.0	0.0
45	38.9	2.49	22.2	5.6	5.6	22.2	11.2
60	66.7	4.40	5.6	11.2	16.6	33.4	22.2
75	33.3	5.37	5.6	16.6	0.0	33.4	11.2
90	44.4	3.75	27.8	16.6	0.0	33.4	0.0
105	27.8	4.49	22.0	0.0	5.6	22.2	11.2
120	27.8	1.99	16.0	0.0	5.6	22.2	0.0
135	22.2	0.60	16.8	0.0	0.0	0.0	0.0
150	5.6	0.01	0.0	0.0	0.0	0.0	0.0
LSD (0.05)	17.3	1.88	27.6	7.2	12.4	39.6	21.8

[a]The media contained 1.0 mg L^{-1} NAA, 0.5 mg L^{-1} GA_3, and 0.05 mg L^{-1} 6-BAP. The data were averaged from three replicates of six peg tips each.
[b]The developmental stages of embryos were defined in Table 1.

Table 4. Effects of medium pH on *in vitro* pod formation, embryo development, and plant regeneration of *A. hypogaea*.[a]

Conc. of sucrose	Pod formation	Pod volume	Embryo development[b]			Seedling	Mature plant
			Prior to cotyledon	Immature seed	Mature seed		
g L^{-1}	%	cm^3	%				
4.5	66.7	5.80	27.8	22.2	33.4	33.3	16.7
5.0	72.2	5.41	11.2	16.6	44.4	55.6	22.8
5.5	38.9	4.93	11.2	0.0	27.8	22.2	16.7
6.0	50.0	4.21	16.6	16.6	16.6	22.2	5.6
6.5	44.5	2.13	16.6	5.6	5.6	5.6	0.0
7.0	55.6	0.81	22.2	0.0	5.6	5.6	5.6
7.5	38.9	0.25	27.8	0.0	0.0	0.0	0.0
8.0	16.7	0.08	33.4	0.0	0.0	0.0	0.0
LSD (0.05)	30.3	2.42	32.2	21.0	27.4	20.6	18.9

[a]The media contained 1.0 mg L^{-1} NAA, 0.5 mg L^{-1} GA_3, and 0.05 mg L^{-1} 6-BAP. The data were averaged from three replicates of six peg tips each.
[b]The developmental stages of embryos were defined in Table 1.

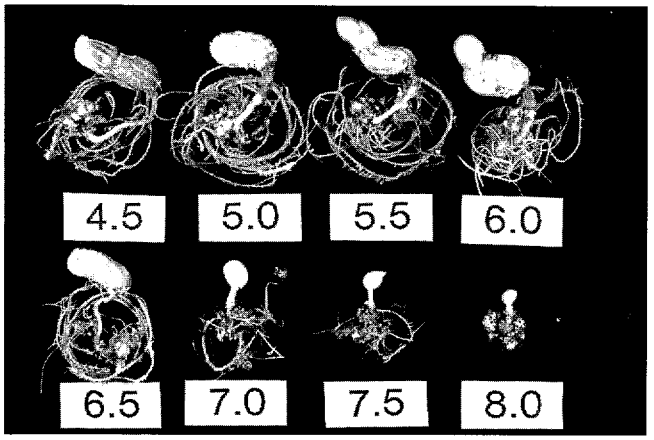

Fig. 3. Effect of medium pH on *in vitro* pod formation of *A. hypogaea*. The media contained 1.0 mg L^{-1} NAA, 0.5 mg L^{-1} GA_3, and 0.05 mg L^{-1} 6-BAP plus 60 g L^{-1} sucrose.

developed to the cotyledonary stage. For basic media, embryos did not develop beyond the early cotyledonary stage. After 90 d culture, the pH was remeasured and found to range between 4.21 and 4.51, regardless of the original values.

From the combined experiments to study effects of growth regulators, sucrose, and pH on reproductive development, a total of 231 pods were recovered from 576 peg tip explants, giving a 40.1% overall success rate. Eighty-one double pods were observed (Fig. 1A), and these contained either one large mature seed and an aborted embryo (Fig. 1B) or two small immature seeds (Fig. 1C). One hundred fifty single pods only had the basal ovary enlarged, and most of them contained one mature seed (Fig. 1D). From the 231 pods, 120 mature seeds (which contained fully developed cotyledons and thin seed coats) and immature seeds (whose developing cotyledons had not yet filled the capacity of the seed) were obtained. Thus, seeds were obtained from 21% of the total peg tip explants and from 52% of the pods.

Another 119 ovules were obtained which were smaller than 4 x 7 mm and presumably contained embryos prior to cotyledonary stage. After 2 mo subculture, 26.4% of the ovule explants had limited growth and the others died. Five cotyledonary embryos were recovered from the ovules cultured in the dark and they subsequently germinated after culture in the light.

Eighty-seven cotyledonary embryos were subcultured in the light to induce germination. The highest germination rate was observed when embryos were 4 to 10 mm long (Table 5), whereas the larger embryos had a relatively lower and slower germination rate. However, the seedlings originating from larger embryos grew faster and a larger number matured into plants than those from young embryos (Table 5). Another 33 seeds were placed in vermiculite to germinate, but only eight of them germinated. In total, 86 seedlings were recovered from 576 peg tip explants in the three experiments and 37 seedlings grew to maturity.

Discussion

Previous experiments using small culture tubes and 20 mL medium per peg tip explant indicated that high levels of NAA (4 mg L^{-1}) and 6-BAP (0.5 mg L^{-1}) suppressed *in vitro* development of fruits, ovules and embryos of *A. hypogaea* (3) and *A. duranensis* (2). The

Table 5. Relationship between size of *in vitro*-recovered *A. hypogaea* embryos and rates of embryo germination and plant recovery in MS media with 0.5 mg L^{-1} each of 6-BAP and GA_3 in the light.

Embryo length	Embryos		Germination	Mature plants
	Cultured	Germinated		
mm	no.		%	no.
<4	15	12	80.00	2
4-6	24	23	95.83	3
7-10	19	18	94.74	9
>10	29	18	62.07	16
Total/avg	87	71	81.61	30

results of this experiment using larger culture containers and 50 mL medium further indicated that optimal NAA levels were lower than 2 mg L^{-1}. NAA induced roots which may improve uptake of nutrients from media and produce endogenous hormones (19). GA_3 at the tested levels did not have significant effects on *in vitro* pod formation, embryo development, root or callus formation. Increased 6-BAP induced large amounts of callus which may have negatively affected *in vitro* reproductive development. Thus, addition of exogenous 6-BAP and GA_3 is not believed to be necessary for peanut peg tip culture of explants generated from selfed *A. hypogaea*.

Peg tip explants cultured on the basal medium also produced many pods and seeds when greater amounts of medium and culture space were provided as compared with previous experiments (3), where very few pods and seeds were obtained in basal medium when 2.5 x 15-cm culture tubes were used. Because peg tip explants consisted of embryos, ovules, ovary, meristem, and other tissues, they likely produced endogenous phytohormones. When an adequate nutrient supply was available, the levels of phytohormones already existing and synthesized *in situ* in peg tissues were apparently sufficient to induce *in vitro* growth and development along a parallel pattern as occurs *in vivo*.

Since the peg tip explants were cultured in the dark for the long period of 90 d, the only energy source for the tissues was sugars from the medium. In a previous experiment using small culture tubes, both pod and embryo development was observed, but the success rate for obtaining pods was relatively low at 10.2% (3). In this experiment using larger culture containers and more medium, a greater number (40.1% of pegs forming pods) and larger pods were observed, as well as enhanced embryo development. Thus, if variables other than container size did not affect the results between this and previous experiments, then a large quantity of nutrients and more culture space appears to be beneficial for *in vitro* reproductive development of peanut. In addition to container size, the only other known differences between previous experiments and this study were in the type of top used to seal the containers. Moss *et al.* (8) used parafilm around the edges of small petri dishes and Feng *et al.* (3) used an air permeable cap, whereas screw-on lids were used in this experiment. Significant differences in the gaseous atmosphere could have been present by the end of 90 d, and further testing is needed to discover the influence of gases on embryo development. Because pods and seeds developed at concentrations both above and below the 60 g L^{-1} optimum level, sucrose and additional factors likely affected *in vitro* reproductive of peanut.

This experiment also indicated that the reproductive responses were variable if the sucrose concentration was changed. Optimal levels of sucrose for *in vitro* ovary enlargement and embryo development of peanut were between 60 and 105 g L^{-1}. Young and Brown (21) found that osmotic potentials of *Phaseolus vulgaris* L. embryo sacs were between 3 to 10% sucrose-equivalent. If the same is true for peanut, then the adverse effects of high sucrose concentrations may be attributed to unfavorable osmotic potential.

In a previous experiment (3), 0.5 or 2.0 mg L^{-1} NAA produced many pods and greater embryo development, and a very low frequency of pod formation and ovule growth was observed in basal medium. In this experiment, however, 2.0 mg L^{-1} NAA was not beneficial for pod and embryo development, and the control medium produced many large pods and cotyledonary embryos. Thus, the changes in nutrient and environmental conditions within the culture container can greatly alter the need of growth regulators and the responses of tissues to growth regulators.

Zamotailov (22) reported an increase in basicity of the protoplasm in peanut embryo sacs after soil penetration by pegs. Shenk (18) assumed that a shift toward a higher oxidation state would favor fruit enlargement, thus an increased acidity would restrict growth. The results of the present experiment, however, indicated that increased acidity in the initial medium favored *in vitro* fruit enlargement and embryo development. Decreased pH is believed to benefit uptake of nitrogen and carbohydrates and transport across the cell plasma membranes (15).

Only eight out of 33 mature seeds germinated in vermiculite without addition of plant growth regulators. This suggests that *in vitro*-recovered seeds were dormant. Dormancy was broken by adding 0.5 mg L^{-1} each of 6-BAP and GA_3 to MS media for 13 of 17 seeds. Developing embryos at the cotyledonary stage had higher frequencies of germination than developed embryos, but only weak seedlings resulted after germination. It appears that embryos were becoming more dormant as they matured and, although dormancy makes *in vitro* experiments more complex, it may be beneficial for development and long-term growth.

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