Ovule and Embryo Culture of *Arachis hypogaea* and Interspecific Hybrids[1]

H. T. Stalker* and M. A. Eweda[2]

ABSTRACT

Interspecific hybridization in *Arachis* is difficult between species within sectional groups and nearly impossible among more distantly related species. Embryos usually abort early in the reproductive cycle; thus *in vitro* techniques are necessary to recover many desirable hybrid combinations in the genus. The objectives of this investigation were to develop techniques whereby mature plants could be recovered from otherwise aborting embryos. First, ovule culture was performed using eight genotypes, three levels of kinetin, and the two basal media Murashige and Skoog (MS) and N6. Two-tenths mg/L kinetin in media resulted in 24% of the ovules swelling to a size of 3-4 mm which could be used for excising embryos. Embryo culture was next performed on five genotypes. The transfer series (1) 0.2 mg/L kinetin for 21 days, (2) 0.5 mg/L 6-benzylamino-purine (BAP) for 14 days and, (3) MS without growth regulators resulted in 34.6% of ovules producing plants across genotypes; other transfer series either resulted in a lower percentage of plant recovery and/or tissues of some genotypes which did not survive to maturity. The BAP medium induced shoot growth, while root growth was induced on the MS without growth regulator medium. Approximately 90% of embryos transferred to a mist system after 7-9 weeks *in vitro* survived transplanting to soil. Two interspecific hybrids were recovered from incompatible hexaploid x diploid crosses, but only after roots were induced using a MS basal medium with 4 mg/L 1-naphthaleneacetic acid:2 mg/L indole-3-butyric acid in a fourth tissue transfer. The experiments illustrated the feasibility of rescuing embryos of *A. hypogaea* and interspecific peanut hybrids. The process is slow and will be most applicable to wide crosses which cannot be obtained by more conventional methods.

Key Words: Embryo culture, ovule culture, tissue culture, interspecific hybrids, *Arachis*.

A large number of *Arachis* species can potentially be utilized to improve the cultivated peanut *(A. hypogaea* L.). Several 40-chromosome populations have been derived after crossing *A. hypogaea* with other members of the genus (13,24), but hybrids have been restricted to members of section *Arachis*. Johansen and Smith (9) attributed failures to obtain *A. hypogaea* x *A. diogoi* Hoehne [not true *diogoi* vide Gregory and Gregory (5)] to slow growth and degeneration of the embryo accompanied by hypertrophy of integuments. In an *A. hypogaea* x *A. glabrata* Benth. cross, Murty *et al.* (15) found up to a 48-hr delay in fertilization and early embryo abortion. Sastri and Moss (20) observed large callose plugs along pollen tubes of *A. monticola* Krap. et Rig. pollinated with *A.* sp. coll. PI 276233 of section *Rhizomatosae,* but fertilization apparently took place in at least some of the crosses which subsequently aborted. Interspecific hybrids can abort as early as 6 days after fertilization (for example, in diploid x hexaploid crosses in section *Arachis*) or remain viable but undeveloped until the time of normal maturity (for example, hexaploid x diploid crosses in section *Arachis)* (6,7). Recovery of many interspecific hybrids in *Arachis* will involve recovery of small, but viable, embryos.

In vitro culture of ovules or embryos has been used as an aid to recovering interspecific hybrids in many genera (1,4,16,19). Numerous reviews have been published describing media requirements and technical aspects of tissue preparation (4,17,19,26,27). Embryo rescue techniques in *Arachis* were first reported in 1943 by Harvey and Schultz (8) and then by Nuchowiz (18) who used nearly mature embryos to recover plants. Martin (12) regenerated plants from 0.3 mm long peanut ovules, which probably corresponded to an embryo in the globular stage of development. However, in attempts to duplicate Martin's results, Sastri et al. (21) could only generate callus which later became necrotic. Johnson (10) concluded that *in vitro* culture of small embryos requires a two-step process where ovules can be cultured until they become large enough to excise embryos, and then embryos can be cultured after separation from maternal tissues to generate plants.

Embryo rescue techniques have been used to obtain interspecific hybrids between several *Arachis* species. Bajaj *et al.* (2) cultured 30-day-old F_1 embryos of a cross between *A. hypogaea* and *A. villosa* Benth. However, this is a hybrid combination which can be obtained without the aid of *in vitro* techniques. Mallakarjuna and Sastri (11) stimulated embryo expansion with growth regulators, and they subsequently observed shoot development. Several *A. monticola* x *A.* sp. coll. PI 276233 hybrids have been grown to maturity after growth regulators were applied to both flowers and pegs and *in vitro* techniques used to stimulate growth (20). Since the first successes with *A. monticola* hybrids, fewer than five intersectional hybrid plants have been recovered via embryo culture (25). Bajaj (1) reviewed the tissue culture literature and concluded that selfed and hybrid peanut embryos can be cultured *in vitro,* but application of techniques is yet to be realized. While reports have been made of tissue responses *in vitro* and a few plants have been recovered via ovule or embryo culture, uniform techniques to repeatably establish plants in the greenhouse are lacking.

The objective of this paper is to report results of *in vitro* experiments with ovules and embryos to recover plants from young embryonic tissues of peanut. Investigations were first conducted with *A. hypogaea* cultivars to test media and procedures for favorable plant growth. Methods were then applied to obtain plants from incompatible interspecific crosses in peanut.

Materials and Methods

Four *in vitro* experiments were conducted to test media and

[1]Paper no. 11761 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643. This research was partially funded by AID-Peanut CRSP Grant DAN-4048-G-SS-2065-00.

[2]Associate Professor of Crop Science, North Carolina State University, Raleigh, NC 27695-7629 and Assistant Professor, Department of Genetics, Cairo University, Giza, Egypt.

genotypic responses of peanut ovules and embryos. All plants were grown in 37 x 45 x 15-cm boxes in the greenhouse. Landplaster was added to the soil as a source of calcium, which is needed for embryo development. All tissues were placed on petri plates sealed with Parafilm and placed in a growth chamber with 28/21 C diurnal temperature with a 16-hr per day photoperiod and 33-75 μE per m^2 sec^{-1} of illumination.

Experiment 1

Flowers on plants of eight *A. hypogaea* cultivars were self-pollinated and pods collected 15 to 20 days after fertilization. Pods were rinsed in tap water, surface-sterilized in 2.6% sodium hypochlorite with 1 mL Tween 80 for 6 min and rinsed three times for 5 min each in distilled water. Ovules 2 mm or less in length were excised from pods and placed on filter paper bridges in bottles containing liquid N6 (3) media with 0.1 mg/L kinetin (kn) or Murashige and Skoog (MS) (14) media with 0.05, 0.1 or 0.2 mg/L kn. All media had pH = 5.8. Ovules were placed in a growth chamber and observed periodically until 60 days after culture when shoots, roots, greening and swelling responses were recorded.

Experiment 2

Pods were collected 21 to 25 days after fertilization, which corresponded to embryos in the heart to very early cotyledonary stage of development from *A. hypogaea* cultivars NC 4, NC 6 and Argentine and the breeding line NC Ac 18000. Embryos were aseptically dissected from pods and ovules and placed on a MS semi-solid basal medium supplemented with 50 g/L sucrose and one of the following four treatments: (a) no growth regulators, (b) 0.2 mg/L kn, (c) 0.2 mg/L 3-indoleacetic acid (IAA), or (d) 0.2 mg/L kn + 0.2 mg/L IAA. Growth responses were recorded after 21 days, at which time one-half of the embryos were transferred to a MS medium + 30 g/L sucrose + 0.5 mg/L 6-benzylaminopurine (BAP) and the other half to a MS medium + 30 g/L sucrose-growth regulators. Tissues remained on the second medium for an additional 30 days after which time shoot and root responses were recorded. Tissues with both shoots and roots were transferred to a sand medium in the greenhouse and misted with water for 10 sec every 15 min. After two additional weeks the young plants were transferred to soil, but left under the water mist system for 7 to 10 days and then transferred onto a shaded greenhouse bench. After another 5-7 days the plant were exposed to full sunlight.

Experiment 3

Embryos of *A. hypogaea* cultivars NC 4, Argentine and Spanhoma were harvested and sterilized as described in experiment 2. They were placed on a MS semi-solid medium supplemented with 50 g/L sucrose + combinations of four growth regulators (Table 3) in petri dishes for 21 days. Embryos were transferred to a MS medium + 30 g/L sucrose + 0.5 mg/L BAP medium for 14 days and subsequently transferred to a MS + 30 g/L sucrose medium without growth regulators. Growth responses were recorded, including shoots, roots and callus. Tissues with both shoots and roots were transferred to a soil medium in the greenhouse as previously described.

Experiment 4

One hundred ten plants of the interspecific hybrid 6*x*(*A. batizocoi* Krap. et Greg. (coll. K 9484) x NC Ac 18000) and 6*x*(NC Ac 18000 x *A. cardenasii* Krap. et Greg. *nom. nud.* (coll. GKP 10017) were grown in boxes in the greenhouse. They were used as female parents in a crossing program with the diploid (2n = 2*x* = 20) species *A. batizocoi* or *A. cardenasii*, thus totaling four hybrid combinations (Table 4). Flowers were hand-emasculated between 3 and 5 PM and pollinated and tagged between 8 and 10 AM the following morning. Thirty days after pollinations were made, the number of pegs and pods were recorded, tissues harvested and surface-sterilized as previously described. Ovules were aseptically removed from pods, placed on filter paper bridges in bottles with liquid MS medium + 0.2 mg/L kn, and placed in a growth chamber. Embryos were excised from ovules which swelled to a length of 4-5 mm and transferred to a semi-solid MS medium with 0.1 mg/L Kn for 14 days, transferred to a MS medium + 0.5 mg/L BAP and subsequently to a MS medium without growth regulators. Tissues which produced shoots but which failed to root were again transferred to a MS medium with 4 mg/L 1-naphthaleneacetic acid (NAA) + 2 mg/L indole-3-butyric acid (IBA). Seedlings with both shoots and roots were transferred to a misted sand bench and to a soil medium on a greenhouse bench as previously described. Vegetative cuttings were taken from suspected hybrid plants and rooted in a mist chamber. Root tips were collected, squashed and stained in aceto-orcein after which the chromosome number of plants was determined.

Results

Experiment 1

A total of 1040 ovules were cultured using eight *A. hypogaea* cultivars to investigate the effects of different growth regulators on ovule growth (Table 1). The 2-mm long ovules corresponded to embryos in the globular stage of development. After 60 days of culture, several reactions were observed including tissue enlargement, color changes from white to green and occasionally seedling germination. Although *ca.* 70% of the tissues turned green and swelled, growth was usually slow, resulting in only 11.9% reaching a size (4-5 mm) within 60 days which could easily be manipulated for dissection of embryos without injury to tissues. *In vitro* differences were observed among genotypes for swelling. For example, 17.7 and 25.8% of ovules from cultivars NC 4 and NC 6, respectively, enlarged from 2 to 4-5 mm in length when 0.1 mg/L kn was used in the medium; however, 0% enlargement was observed for cultivars Argentine and Pearl for the same medium. Genotypes also varied in physical appearance. Cultivars Pearl and Argentine usually remained white, whereas ovules of cultivars PI 275751, Robut 33-1, New Mexico Valencia, NC 4 and NC 6 usually turned green. Brown areas appeared on the surface of cultivar Spanhoma ovules, resembling areas of necrotic cells. The differences between MS and N6 basal media were statistically nonsignificant, and an arbitrary decision was made to use MS medium in future experiments.

Seedlings germinated in 60 days in nine ovules of cultivar NC 4 and two ovules of Argentine. All seedlings of Argentine were from tissues on MS medium with 0.1 mg/L kn, whereas four NC 4 seedlings originated from ovules cultured on a medium with 0.1 mg/L kn and the other five seedlings were cultured on a 0.2 mg/L kn medium. Of the 15 seedlings which germinated from ovules, only one had both shoots and roots, one NC 4 seedling had only roots, and the remaining 11 seedlings produced only shoots. The seedlings were transferred on a 3-week schedule for 12 months in attempts to induce both healthy shoots and roots. Only two seedlings were eventually transferred to the sand-mist system, but both died shortly after transfer to soil.

Experiment 2

Pods of cultivars NC 4, NC 6 and Argentine and the breeding line NC Ac 18000 were harvested 21-25 days after fertilization. Ovules 4-5 mm in length were dissected from the pods, which corresponded to embryos in the heart or very early cotyledonary stage of development. Preliminary experiments had shown that MS medium without growth regulators would support peanut explants and MS medium plus the growth regulator BAP would induce callus and shoots. Further, previously published reports (11) had indicated that kn and IAA probably induce embryo development in peanuts. Thus, genotypes were initially placed on media with the growth regulators IAA or kn and subsequently transferred to a MS medium without growth

Table 1. Ovules of *Arachis hypogaea* selfs cultured *in vitro* using two basal media and different kinetin levels.

Genotype	Subspecies/variety	MS media						N6	
		0.05 mg/L kn		0.1 mg/L kn		0.2 mg/L kn		0.1 mg/L kn	
		Cultures	Swelling[a]	Cultures	Swelling	Cultures	Swelling	Cultures	Swelling
		No.							
N.M. Valencia	fastigiata var. fastigiata			29	3			25	1
Pearl	fastigiata var. vulgaris			25	0			22	0
Spanhoma	fastigiata var. vulgaris			30	1			25	4
PI 275751	fastigiata var. vulgaris			17	4			30	1
Argentine	fastigiata var. vulgaris	121	0	36	0	58	13	89	4
NC 4	hypogaea var. hypogaea	58	0	113	20	42	8	16	0
NC 6	hypogaea var. hypogaea			97	25	139	37	8	0
Robut 33-1	hypogaea var. hypogaea			35	3			25	0
	TOTAL	179	0	382	56	239	58	240	10

[a]Swelling to 4-5 mm ovule which is size large enough to excise embryos.

regulators or + 0.5 mg/L BAP. Significant differences ($p < 0.01$) were observed among media and genotypes for shoot and root production and whole plant recovery. When IAA was added to the media, most embryos from the genotypes Argentine and NC Ac 18000 did not grow and remained a very pale white color. These two genotypes had abnormal shoot development and only a few transfers were made to the secondary MS media without growth regulators or + BAP (Table 2).

Table 2. *In vitro* culture of heart-shaped *Arachis hypogaea* embryos on a primary medium and secondary medium.

MS primary media	Genotype	No. primary cultures	Secondary media							
			MS - growth regulator				MS + 0.05 mg/L BAP			
			Transfers	Shoots	Roots	Plants	Transfers	Shoots	Roots	Plants
mg/L			No. cultured							
-Growth regulator	NC 4	28	12	0	5	0	12	2	5	0
	Argentine	61[a]	0	-	-	-	0	-	-	-
	NC Ac 18000	55[a]	0	-	-	-	0	-	-	-
	NC 6	68	28	18	21	18	25	11	7	2
	Total		40	18	26	18	37	13	12	2
+0.2 kn	NC 4	48	24	15	19	15	23	3	15	3
	Argentine	57	27	16	18	12	25	15	9	2
	NC Ac 18000	44	19	11	15	10	20	12	12	6
	NC 6	62	30	16	17	16	28	13	13	8
	Total		100	58	69	53	96	43	49	19
+0.2 IAA	NC 4	29	13	0	1	0	12	5	0	0
	Argentine	60[a]	0	-	-	-	0	-	-	-
	NC Ac 18000	58[a]	0	-	-	-	0	-	-	-
	NC 6	55	24	21	21	18	24	11	8	5
	Total		37	21	22	18	36	16	8	5
+0.2 kn and 0.2 IAA	NC 4	49	21	18	21	18	23	15	14	6
	Argentine	57	11	6	8	5	22	19	5	0
	NC Ac 18000	52[a]	0	-	-	-	0	-	-	-
	NC 6	42	19	9	10	9	21	12	15	2
	Total		51	43	39	32	66	46	34	8

[a]Tissues died before transfers.

Similar responses to both kn and IAA media were initially observed for cultivars NC 4 or NC 6 during the first 21 days in culture. However, significant differences ($p < 0.01$) for shoot and root growth were observed for tissues cultured on the kn and IAA primary media and transferred to secondary MS without growth regulators or with BAP in the media. For example, NC 4 plants were not recovered when 0.2 mg/L IAA was used initially, whereas 54 to 86% of the embryos grew into mature plants when the tissues were placed on the other three primary media and subsequently transferred to a MS without growth regulator medium. On the other hand, IAA did not adversely affect embryo growth of the cultivar NC 6. Overall, 0.2 mg/L kn was the best primary medium tested across genotypes because plants were recovered for all genotypes (Table 2).

When BAP was added to the secondary media, shoots were greener and larger than when MS without growth regulators were used. However, prolonged use of BAP resulted in excessive callusing. Roots produced on this medium generally grew slowly and were not branched.

Tissues transferred from the primary medium to a secondary medium without growth regulators resulted in shoots which were often small and deformed. However, healthy and branched roots were usually observed.

After both shoots and roots developed on a germinating seedling, tissues were transferred to a sand bench where seedlings with abnormal-looking leaves and shoots generally grew into 'normal-looking' plants. Roots also grew and branched into the sand. The best results were observed when the seedlings were placed in the sand for 3-4 weeks, transferred into a soil medium, but left in the mist system for an additional week, then put under an 80% shade on a greenhouse bench for 2 weeks and subsequently exposed to full sunlight. Approximately 90% of seedlings transferred to the sand-mist system grew to maturity. Plants recovered from *in vitro* culture were fertile and produced seeds.

Experiment 3

A second embryo rescue study was conducted to determine effects of growth regulators added to initial media on embryo growth and to determine whether a sequential transfer of embryos would enhance recovery of plants. Embryos of cultivars Argentine and NC 4 were first placed on one of seven media for 21 days (Table 3), transferred to MS + BAP media for 14 days, then to MS media without growth regulators until adequate responses were obtained for transfer to the greenhouse. Shoots or roots were rarely observed during the first 21 days when embryos were on the primary media. Shoots were generally initiated after transfer to MS +BAP media, but very few roots were produced. The second transfer to the MS media-growth regulators resulted in embryos producing many branched roots. Cultivar Argentine performed poorly in the experiment when the primary medium had 0.2 mg/L IAA, 0.2 mg/L kn + 0.2 mg/L NAA, or no growth regulators. When the primary medium contained 0.5 mg/L BAP, 43.1% of the Argentine embryos developed shoots and roots, as compared to 0 to 30% for other media tested (Table 3).

Table 3. *In vitro* responses of *Arachis hypogaea* embryos on three sequential media where second medium = MS + 0.5 ppm BAP and third medium = MS - growth regulator.

Primary media[a]				Genotype	No. cultures	No. transfers[b] to second medium	Rating after third medium[c]		
kn	IAA	BAP	NAA				Shoots[d]	Roots[d]	Plants[d]
mg/L							%		
0	0	0	0	NC 4	59	57	93	75	75
				Argentine	60	0	0	0	0
				Spanhoma	49	42	20	27	16
.2				NC 4	58	50	60	64	60
.2				Argentine	48	25	33	39	30
.2				Spanhoma	57	52	24	25	14
	.2			NC 4	24	22	84	76	76
	.2			Argentine	60	0	0	0	0
	.2			Spanhoma	38	37	18	37	16
		.5		NC 4	49	32	32	24	12
		.5		Argentine	48	47	63	51	43
.2			.2	NC 4	61	47	73	59	44
.2			.2	Argentine	47	0	0	0	0
	.2	.5		NC 4	55	41	53	64	33
	.2	.5		Argentine	51	49	52	71	29
		.5	.2	NC 4	44	36	25	55	25
		.5	.2	Argentine	48	30	9	36	0
LSD	.05						19	17	27
	.01						28	24	39

[a]Primary medium used for 21 days.

[b]Secondary medium used f 14 days.

[c]Third medium used for 14-21 days.

[d]% of original number of cultures.

Although plants were obtained from all media combinations when NC 4 embryos were used, success rates varied significantly ($p < 0.05$) between media. When 0.5 mg/L BAP was used in the initial medium, only 12% of the NC 4 embryos developed into mature plants, whereas 75 and 76% of the embryos produced plants when no growth regulators or IAA was used, respectively. Two-tenths mg/L kn in the primary medium resulted in the greatest overall percentage of recovered plants (Table 3).

Because Argentine had small seeds as compared to NC 4 and the Argentine cultivar performed poorly in experiments, embryos of a second variety *vulgaris* genotype (Spanhoma) were tested on three media combinations (Table 3). Embryo responses were more favorable for Spanhoma than for Argentine since plants were recovered on all three media sequences.

Experiment 4

A crossing program between hexaploid interspecific hybrids and diploid species of section *Arachis* resulted in approximately 11% pod set. The crossing program was designed to use different combinations of the A and B genomes to avoid sterility due to genomic incompatibilities. Pod formation ranged from 2.9% for 6*x*(*A. hypogaea* x *A. cardenasii*) x *A. batizocoi* crosses to 24.0% for 6*x* (*A. batizocoi* x *A. hypogaea*) x *A. cardenasii*) crosses (Table 4). All pods contained very small ovules.

From the 153 pods harvested, 124 ovules were cultured on filter paper bridges of which 63 expanded to a size where embryos could be dissected without injury. After embryos were cultured on the media sequence used in experiment 3, shoots were observed on 11 tissues of which nine came from the cross 6*x*(*A. batizocoi* x *A. hypogaea*) x *A. cardenasii*. This was one of the genomically balanced combinations where (B'B' x AABB) x A'A' would result in a AA'BB' tetraploid hybrid. However, roots were infrequently produced.

Embryos which produced shoots but not roots were transferred to MS media supplemented with 4 mg/L NAA + 2 mg/L IBA which has been reported to induce rooting in peanut (23). Four seedlings grew and were transferred to the greenhouse via a sand-mist system as previously described. The plants remained weak and spindly for several months before two of them died. The remaining two plants developed 'normal-looking' leaves and shoots approximately 5 months after transfers to a soil medium. Both plants flowered but averaged only 8.0 to 18.6% pollen viability as compared to the maternal plant in the original cross which had 79.0% fertility. Almost a year following the initial crosses, the plants remained small. One plant had a chromosome number of $2n = 40$ while the second one had $2n = 41$, thus confirming they were both hybrids.

Table 4. Interspecific hybridization between *Arachis* hexaploids and diploids and resulting plant recovery.

Cross	Genome	Pollinations	Pegs	Pods	Ovules cultured	Embryos	Shoot	Shoot + root	Plant
		No.							
6x(A. batizocoi x A. hypogaea) x A. cardenasii	(B'B'AABB) x A'A'	338	116	81	64	28	9	5	2
6x(A. batizocoi x A. hypogaea) x A. batizocoi	(B'B'AABB) x B'B'	281	57	22	20	15	1	0	0
6x(A. hypogaea x A. cardenasii) x A. cardensii	(AABBA'A') x A'A'	446	77	41	33	17	1	1	0
6x(A. hypogaea x A. cardenasii) x A. batizocoi	(AABBA'A') x B'B'	306	22	9	7	3	0	0	0
	Total	1371	272	153	124	63	11	6	2

Discussion

Ovule culture of *A. hypogaea* resulted in varying responses among genotypes ranging from tissue senescence to seedling germination. The most common response was swelling and greening of tissues. However, Mallinakara and Sastri (11) concluded that green expanding ovules are not always the best tissues to use for rescuing plants because embryos actually grew more in their experiments when swelling, but browning tissues were observed. A clear distinction needs to be made between tissues which swell, as reported in many articles of the literature, and ones which actually reach a size from which embryos can be excised. In this study, a genotypic response was observed because the large-seeded cultivars NC 4 and NC 6 generally had more tissue swelling than cultivars with smaller seeds even though all tissues were initially of a similar size.

Seedlings had been reported from peanut ovule culture by Martin (12), but there is difficulty determining whether he actually transferred tissues out of the culture medium into a soil mixture. Sastri *et al.* (21) were unable to repeat Martin's experiments and concluded that ovule culture for peanuts was not possible for directly obtaining hybrids. In this study, shoots and roots germinated from 2-mm *A. hypogaea* ovules which supports Martin's (12) conclusions concerning the possibil-

ity for obtaining plants from very small reproductive tissues. However, the percentage of ovules from eight cultivars (Table 1) which produced shoots or roots equalled less than 1.5% of cultured tissues. Only two seedlings had both shoots and roots, and neither seedling survived transplanting to soil. However, these techniques were employed to obtain sufficiently large interspecific hybrid embryos to culture from which two mature plants were obtained. Ovule culture techniques in *Arachis* are currently not feasible for recovery of large numbers of plants from very young tissues but are necessary when interspecific hybridization will not produce reproductive tissues large enough for directly using embryo rescue techniques.

Although Sastri *et al.* (22) indicated that embryo culture of *Arachis* is routine, preliminary work in this laboratory indicated that significant differences resulted for plant recovery among genotypes. Further, plant transfers from the culture tube into soil in the greenhouse for *Arachis* in general has had a very low success rate (25). Although techniques and various media combinations have been successfully used, methods described in the literature do not appear to be easily repeatable.

Most genotypes require growth regulators such as kn and BAP to support early embryonic development and shoot development. The embryo culture experiments showed that IAA does not induce embryo growth for at least some peanut genotypes. For a few cultivars such as NC 6, embryos can be successfully grown on a medium without growth regulators.

6-Benzylaminopurine induced shoot development on small peanut embryos cultured *in vitro*. When tissues were left on BAP medium for more than 2 weeks, tissues of most genotypes developed callus masses which generally inhibited further shoot or root development. Differences were observed among genotypes for response to BAP. For example, only 12% of the NC 4 embryos were recovered using this procedure. However, Argentine embryos had the highest frequency of plants recovered when BAP was used in both the primary and secondary media.

Transferring germinating seedlings that are derived from embryos of cultivated peanuts with a shoot plus even one small root to a sand-mist system appears to be an efficient method for establishing plants in the soil. After seedlings are placed in sand, shoots generally grow and roots proliferate. However, for the interspecific crosses in this study, a MS medium without growth regulators did not initiate roots. The addition of NAA and IBA, as suggested by Seitz *et al.* (23), initiated roots on several seedlings, but further testing will be needed to enhance the frequency of hybrid recovery.

Although genotype-media interactions were observed, the most satisfactory primary medium contained 0.2 mg/L kn. Presumably this growth regulator stimulated embryo development in addition to ovule enlargement. Tissue transfer to a MS + 0.5 mg/L BAP medium to induce shoot production and finally to a MS medium without growth regulators for root initiation gave satisfactory results in the experiments for *A. hypogaea*.

Although crossing hexaploid interspecific hybrids by diploid species should be an efficient method to quickly reduce the chromosome number from $2n = 60$ to $2n = 40$, embryos abort and thus restrict easy ploidy reduction. Application of embryo rescue techniques developed for *A. hypogaea* resulted in recovery of otherwise aborted hybrids, but at a low frequency. Although the two plants rescued *in vitro* continued to survive, the process thus far encompassed a year and plants are not yet nearly of a size to produce seeds. Further, pollen viability is low and several years may be required to restore fertility even though the explants should have balanced genomes. Nevertheless, this study demonstrated that incompatible species hybrids can be obtained by *in vitro* methods. Because of the problems encountered in terms of time and sterility in recovered plants, the techniques would be better reserved for the most distantly related interspecific cross combinations which cannot be obtained by other methods. Application of techniques reported in this paper should also be applicable to crosses among *A. hypogaea* and species of other sections of the genus, especially when functional roots can be more easily initiated on plants.

Literature Cited

1. Bajaj, Y. P. S. 1984. Peanut, pp. 193-225. *in* P. V. Ammirato, D. A. Evans, W. R. Sharp, and Y. Yamada (eds.), Handbook of Plant Cell Culture. Vol. 3. Macmillan Publ. Co., New York.
2. Bajaj, Y. P. S., P. Kumar, M. M. Singh, and K. S. Labana. 1982. Interspecific hybridization in the genus *Arachis* through embryo culture. Euphytica 31:365-370.
3. Chih-Ching, C. 1978. The N6 medium and its applications to anther culture of cereal crops, pp. 43-50. *in* Proc. Symp. on Plant Tissue Culture, May 25-30, 1978, Peking. Science Press, Peking.
4. Collins, G. B., N. L. Taylor, and J. W. De Verna. 1984. *In vitro* approaches to interspecific hybridization, pp. 323-383. *in* J. P. Gustafson (ed.), Gene Manipulation in Plant Improvement. Plenum Press, New York and London.
5. Gregory, M. P., and W. C. Gregory. 1979. Exotic germ plasm of *Arachis* L. interspecific hybrids. J. Hered. 70:185-193.
6. Halward, T. M., and H. T. Stalker. 1985. Embryo development in interspecific peanut hybrids. Agron. Abstr. 1985:56. (Abstr.).
7. Halward, T. M., and H. T. Stalker. 1986. Incompatibility mechanisms in interspecific peanut hybrids. Crop Sci. 27:456-460.
8. Harvey, R. H., and E. F. Schulz. 1943. Cultures of *Arachis* embryos. J. Amer. Soc. Agron. 35:631.
9. Johansen, E. L., and B. W. Smith. 1956. *Arachis hypogaea* x *Arachis diogoi*. Embryo and seed failure. Amer. J. Bot. 43:250-258.
10. Johnson, B. B. 1981. Embryo-ovule culture. Env. Exp. Bot. 21:439 (abstr.).
11. Mallikarjuna, N., and D. C. Sastri. 1985. *In vitro* culture of ovules and embryos from some incompatible interspecific crosses in the genus *Arachis* L., pp. 153-158. *in* Proc. Internat. Workshop on Cytogenetics of *Arachis*. ICRISAT, Patancheru, A.P., India.
12. Martin, J. P. 1970. Culture *in vitro* d'ovules d'arachide. Oleagineux 25:155-156.
13. Moss, J. P. 1980. Wild species in the improvement of groundnuts, pp. 525-533. *in* R. J. Summerfield and A. H. Bunting (eds.), Advances in Legume Science. Vol. 1. International Legume Conference, Royal Botanic Gardens, Kew.
14. Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
15. Murty, U. R., N. G. P. Rao, P. B. Kirti, and M. Bharathi. 1980. Cytogenetics and groundnut improvement. IARI Reg. Stn. Rep., Hyderabad, India. (cited from Mallikajuna and Sastri, 1985).

16. Narayanswami, S., and K. Norstog. 1964. Plant embryo culture. Bot. Rev. 30:587-628.
17. North, C. 1976. *In vitro* culture of plant material as an aid to hybridization. Acta Hort. 63:67-74.
18. Nuchowicz, A. 1955. Studies on the culturing of embryos and of embryo fragments of *Arachis hypogaea* L. Agricultura (Louvain) 3:3-37.
19. Raghavan, V. 1980. Embryo culture. Int. Rev. Cytol. Suppl. 11B: 209-240.
20. Sastri, D. C., and J. P. Moss. 1982. Effects of growth regulators on incompatible crosses in the genus *Arachis* L. J. Exp. Bot. 33:1293-1301.
21. Sastri, D. C., M. S. Nalini, and J. P. Moss. 1980. *In vitro* culture of *Arachis* ovaries and ovules, pp. 366-373. *in* P. S. Rao *et al. (eds.),* Proc. Symp. Plant Tissue Culture, Genetic Manipulation and Somatic Hybridization. Bhabha Atomic Research Center, Trombay, India.
22. Sastri, D. C., M. S. Nalini, and J. P. Moss. 1982. Tissue culture and prospects for improvement of *Arachis hypogaea* and other oil seed crops, pp. 42-57. *in* A. N. Rao (ed.), Proc. COSTED Symp. on Tissue Culture of Economically Important Plants. Singapore.
23. Seitz, M. H., H. T. Stalker, and P. Still. 1985. Regeneration of anther callus in *Arachis paraguariensis.* Proc. Amer. Peanut Res. Educ. Soc. 17:23 (abstr.).
24. Stalker, H. T. 1985. Cytotaxonomy of *Arachis,* pp. 65-79. *in* Proc. Internat. Workshop on Cytogenetics of *Arachis.* ICRISAT, Patancheru, A.P., India.
25. Stalker, H. T., and J. P. Moss. 1987. Speciation, cytogenetics and utilization of *Arachis* species. Ad. Agron. 41:1-40.
26. Williams, E. G., I. M. Verry, and W. M. Williams. 1982. Use of embryo culture in interspecific hybridization, pp. 119-128. *in* I. K. Vasil, W. R. Scowcroft, and K. J. Frey (eds.), Plant Improvement and Somatic Cell Genetics. Academic Press, New York.
27. Yeung, E. C., T. A. Thorpe, and C. J. Jensen. 1981. *In vitro* fertilization and embryo culture, pp. 253-271. *in* T. A. Thrope (ed.), Plant Tissue Culture: Methods and Applications in Agriculture. Academic Press, New York.

Accepted December 27, 1988