Embryo Rescue in Wide Crosses in *Arachis*. 2. Embryo Development in Cultured Peg Tips of *Arachis hypogaea*

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ABSTRACT

Embryo rescue techniques in *Arachis* are potentially important for recovering interspecific hybrids which have the propensity to abort. Pegs are commonly produced in interspecific crosses, but either they fail to reach the soil because growth is arrested, or pods are produced but embryo development is never re-initiated. Peg tips, with the ovule and embryo, of *A. hypogaea* L. cv. 'NC 6', were used to determine whether peg tips can be used as nurse tissue for *in vitro* culture of embryos. Tissues were collected 1, 2, 3 and 4 d after self-pollination, after which peg meristems were removed from half the pegs, and cultured on five media combinations. Continued reproductive development was observed for embryos cultured at all four collection days; however, the highest frequency of growth was observed in 1-d-old tissues. Evidence is presented that meristematic activity may restrict embryo growth in the 2- to 4-d-old embryos and, once the sequence of events is initiated to slow embryo growth, it is not easily reversed *in vitro*. Achievements of embryo growth to multicellular, globular stages (stages 1-1 or 1-2) encourage the development of methods to recover very young embryos through tissue-culture techniques.

Key words: Embryo culture, morphology, interspecific hybridization, *Arachis hypogaea*, comparative light and scanning electron microscopy, peanuts, groundnuts.

INTRODUCTION

Arachis (Leguminosae) contains a large number of species in South America. Although taxonomic description of all *Arachis* species is not complete, the genus includes 22 described and more than 40 undescribed species (Gregory, Krapovickas and Gregory, 1980). *Arachis hypogaea* L. is widely cultivated and the most economically important species of the genus. Its seeds have a high percentage of oil and protein, and many human consumable products are made from them. Several wild species of *Arachis* are superior to *A. hypogaea* for disease and insect resistance, and introgression of these traits into *A. hypogaea* would be highly beneficial. However, because incompatibility barriers exist between *A. hypogaea* and most *Arachis* species and differences in ploidy levels are also present, interspecific hybridization is difficult for many desirable hybrid combinations (Gregory and Gregory, 1979; Singh, Sastri and Moss, 1980). Incompatibility barriers commonly occur before the peg reaches the soil surface and result in embryo abortion (Johansen and Smith, 1956; Halward and Stalker, 1987).

Embryo rescue techniques in *Arachis* are potentially important for recovering interspecific hybrids which have the propensity to abort. Abortion may occur as either a pre- or post-fertilization event in *Arachis*, but post-zygotic abortion is believed to be most common. In *Arachis*, an intercalary meristem exists 1·5–3·0 mm from the peg tip and is responsible for peg elongation (Jacobs, 1947). The meristem becomes active about the second day after anthesis and commences a rapid growth phase at about day 4 (Pattee and Mohapatra, 1986). Synchronous with the onset of rapid peg elongation the embryo becomes quiescent in growth and does not resume cell division until after the peg has entered the soil (Pattee and Mohapatra, 1987). Pegs are commonly produced in interspecific

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...ecause growth is arrested or pods are produced but embryo development is never re-initiated (Johansen and Smith, 1956; Sastri and Moss, 1982; Halward and Stalker, 1987). Developing the capability of culturing embryos, which exist in these normally abortive circumstances, is thus important for recovering many interspecific hybrids. To meet this objective, Martin (1970) cultured ovules which were 7–10 d old and only 0·3 mm in length. Although he recovered plants, Sastri, Nalini and Moss (1980) could only produce callus in attempts to duplicate Martin's experiments. Mallikarjuna and Sastri (1985) reported enlargement and greening of 1 to 3 mm ovules with interspecific hybrid embryos and variation among genotypes in culture. Ziv and co-workers (Ziv and Zamski, 1975; Zamski and Ziv, 1976; Ziv, 1981; Ziv and Sagar, 1984; Thompson, Ziv and Deitzer, 1985) studied physiological mechanisms governing peg, pod, ovule and embryo development using pegs both excised from and attached to the plant. They were successful in culturing excised ovules with an average volume of 3·0 mm^3, which corresponds to the heart-stage of embryo development (Ziv and Sagar, 1984; Thompson *et al.*, 1985). Ziv and Zamski (1975) and Ziv (1981) excised pegs which then elongated and initiated pod, ovule and embryo development under various culture conditions. However, the size and amount of growth of the embryo and ovule were not reported. Embryos not developed to the heart stage have not been repeatedly cultured to plant maturity *in vitro*.

Alternative techniques to those reported in the literature for *Arachis* will be needed to repeatedly rescue young peanut embryos. Nurse cultures have been used to culture young embryos from interspecific crosses of other legume species (Williams and deLautour, 1980). Culturing peg tips, with enclosed embryos, is a means of culturing the embryo without detaching it from maternal tissues. Moss, Stalker and Pattee (1985, 1987) showed that when peg tips are grown *in vitro*, ovules may continue to enlarge after 14 d in culture. Further, when the peg meristem remains attached to the peg, ovule growth is suppressed. The objective of this investigation was to determine whether peg tips can be used as nurse tissue for *in vitro* culture of embryos. This paper reports embryo growth in 1- to 4-d-old peg tips of *A. hypogaea* which have the meristem either left intact or excised.

MATERIALS AND METHODS

Arachis hypogaea cultivar 'NC 6' was grown in the field at the Central Crops Research Station, Clayton, North Carolina during the summer of 1985. Selfed flowers were tagged on the day of anthesis and nodes were excised 1, 2, 3 or 4 d later. The pegs, with a portion of the nodal tissues attached, were separated in the laboratory from bracts, stipules and other flowers at the node.

The peg and attached nodal tissue were sterilized in 600 ml of 20% Clorox (sodium hypochlorite 5·25% solution) with 3 ml Tween 80 for 15 min and washed three times for 15 min each in sterile distilled water. Peg length was measured under a dissecting microscope fitted with an ocular micrometer, after which peg tips were excised. Peg tips with meristems were cut from the basal nodal tissue, and peg tips without meristems were also cut transversely 1 mm from the tip. The 1 mm peg tips were then assumed not to have meristematic tissues, as previously shown (Moss *et al.*, 1987).

Peg tips were cultured *in vitro* using 40 age × media × meristem combinations. The excised peg tip was placed partly in the culture medium in a 60 × 15 mm plastic disposable petri plate sealed with parafilm. Each plate contained 9 ml of medium based on Murashige and Skoog's (1962) major and minor nutrients, with the addition of myoinositol at 100 mg l^{-1}, nicotinic acid at 1 mg l^{-1}, thiamine HCl at 10 mg l^{-1}, pyroxidine at 1 mg l^{-1} and glycine at 2 mg l^{-1}. Five media were used in the study, and they were adjusted to pH = 5·7 and solidified with agar, 7 g l^{-1}. The five media differed in concentration of sucrose, indoleacetic acid (IAA) and kinetin (Kn) (Table 1). Five hundred and sixteen cultures were incubated at 27 °C in darkness and scored 21 d after anthesis, which corresponded with the stage of growth at which ovule and embryo development would normally have been in a rapid growth phase if tissues had not been removed from the plant (Pattee and Mohapatra, 1987). Pegs were then scored for growth response in culture, including ovule enlargement, callus growth and peg elongation (Table 2). Selected samples from each scoring classification were harvested and fixed in

TABLE 1. *Concentrations of three constituents of the five MS media used to culture 1- to 4-d-old peg tips of* Arachis hypogaea in vitro

Medium	Sucrose (g l^{-1})	IAA (mg l^{-1})	Kn (mg l^{-1})
1	125	0·2	0·1
2	30	0·4	0·1
3	30	1·0	0·1
4	30	1·0	0·0
5	30	0·0	0·1

.e 2. Growth response of 1- to 4-d-old Arachis hypogaea *peg tips of selected ages for the five growth media cultured for 21 d*

Growth response	Day 1		Day 2		Day 3		Day 4		
	−M*	+M	−M	+M	−M	+M	−M	+M	Total
Ovule outgrowth									
(1) Basal ovule only	9	6	8	3	13	1	6	1	47
(2) Basal ovule + peg callus	10	3	5	2	6	0	10	2	38
(3) Basal ovule + peg split	6	3	2	0	2	1	2	0	16
(4) Basal ovule + peg callus + split	1	1	0	0	0	0	0	0	2
(5) Apical ovule only	0	0	0	1	0	0	0	1	2
Total	26	13	15	6	21	2	18	4	105
Callus development									
(6) At cut end	17	8	26	19	30	31	24	24	179
(7) Covering peg	2	4	1	5	0	0	0	0	12
(8) At mid-peg only	0	0	1	1	0	6	0	0	8
(9) Callus + peg elongation	0	0	1	2	0	4	0	11	18
Total	19	12	29	27	30	41	24	35	217
Peg elongation									
(10) Elongate only	0	7	3	2	2	2	2	9	27
No response	19	34	20	24	19	15	22	14	167
Total	64	66	67	59	72	60	66	62	516

* M, meristem.

FAA (9 parts 70% EtOH:0·5 parts glacial acetic acid:0·5 parts formalin) for 48 h and then stored in 70% EtOH until processed for light microscopy (LM) and scanning electron microscopy (SEM). Dehydration and paraffin embedding of specimens were accomplished according to Berlyn and Miksche (1976). Paraffin-embedded tissues were sectioned at 7 μm thickness and prepared for correlative LM and SEM as described by Pattee, Mohapatra and Agnello (1983, 1985). Staining for LM was with safranin-fast green. SEM was conducted at 15 kV with the aid of a JEOL T200 microscope and photomicrographs were made with Polaroid P/N type 55 film.

Classification of embryo development was accomplished by visual and measurement comparison to the typical embryogenesis data of Pattee and Mohapatra (1987) for selected growth stages. Stages D-1 to D-5 are samples collected respectively 1–5 d post-anthesis at 0900 h. Stages 1-0, 1-1 and 1-2 are defined as: 1-0, peg just penetrated soil surface; 1-1, slight swelling of peg tip; and 1-2 peg tip beginning to turn during early pod development.

RESULTS

Based on observed tissue responses, peg tips cultured *in vitro* for 21 d were divided into four primary categories and 10 sub-response divisions (Table 2). Across all ages (Table 2), 68% of the cultured peg tips had ovule enlargement, callus growth or peg elongation, while 32% of the pegs showed no visible response. The effects of meristem removal and age on growth response can be seen by comparing the percentage change in growth responses across age for plus or minus meristem. When the meristem was removed, 70 ± 3% of the pegs had either callus or ovule growth regardless of age. However, the presence of the meristematic tissue zone initially suppressed growth response for day 1 tisues, where only 48% responded. Growth-response levels increased to 59, 64 and 77%, respectively, for 2-, 3- and 4-d-old pegs with meristems.

The observed external morphological changes in the cultured peg tips suggested the need for examination of changes in internal morphology, i.e. embryo development, across the four major response categories of ovule outgrowth, callus growth, peg elongation and no response. To determine the degree or relative age advancement of the embryo after 21 d in culture, pictorial embryo development standards (Fig. 1A–H) were assembled, based on the work of Pattee and Mohapatra (1987) on embryogenesis. Comparison of embryo growth within cultured pegs with the standards showed that embryo growth continuance ranged from 17 to 47% (Table 3). The highest

FIG. 1. LM of longitudinal sections of embryos at selected stages. Samples were field-grown, cv. NC 6. × 32. A, Day 1 stage. B, Day 2 stage. C, Day 3 stage. D, Day 4 stage. E, Day 5 stage. F, 1-0 stage. G, 1-1 stage. H, 1-2 stage. PtN, pollen tube nucleus.

3. Percentage distribution of embryo development among cultured peanut pegs of selected developmental stages

Initial development stage	ovule	Cultured embryo growth stages attained							Abnormal or no growth	Total observations
		D-2	D-3	D-4	D-5	1-0	1-1	1-2		
D-1	Apical	12	12	3	6	8	4	2	53	67
	Basal	8	3	2	4	4	4	3	72	
D-2	Apical	—	10	10	0	5	5	0	70	21
	Basal	—	5	5	5	10	0	0	75	
D-3	Apical	—	—	6	0	0	16	0	78	18
	Basal	—	—	0	11	6	0	0	83	
D-4	Apical	—	—	—	8	16	0	0	76	38
	Basal	—	—	—	5	5	8	0	82	
										144

TABLE 4. *Summary of morphological observations (%) from selected growth responses of peanut peg tip cultures*

Growth responses	Total observations	Apical ovule*				Basal ovule			
		Normal				Normal			
		Total	With embryo	Without embryo	Abnormal	Total	With embryo	Without embryo	Abnormal
Ovule outgrowth	73	89	63	26	8	32	20	12	63
Callus growth	26	84	65	19	8	70	58	12	12
Peg elongation	6	84	67	17	16	83	33	50	17
No response	38	90	74	16	10	87	69	18	13
	144								

* Unfertilized samples were not included in table, so totals do not add up to 100%

frequency of continued embryo growth occurred within pegs harvested at the D-1 stage (47%). The most advanced stage of embryo growth (stage 1-2) was also achieved with stage D-1 peg material. Selected examples of typical embryo growth after *in vitro* culture to stages D-5 to 1-2 are illustrated in Fig. 2A–D (compare Fig. 1E–H). The frequency of abnormal or no embryo growth was also lowest in stage D-1 apical ovules (53%). Other peg stages and ovule positions showed a closely grouped response frequency for abnormal or no embryo growth (71–83%) (Table 3). The high frequency of abnormal or no embryo growth responses is not believed to be related to the failure of stage D-2 to D-4 peg cultures to achieve the 1-2 embryo growth stage, since stage D-1 basal achieved a 3% growth to stage 1-2, while having a 72% abnormal or no embryo growth response.

The removal of the meristem resulted in significantly more basal ovules growing out of peg tissues (Table 2), and a higher frequency of abnormal basal ovule growth than when meristems were not removed (Table 4). Even when normal basal ovule growth occurred, there was a significantly lower frequency (20%) of ovules with the embryo present. The peg-elongation growth-response values for with and without embryos in the basal ovule probably do not represent expected response values, because of the rapid growth of ovule cells which compressed the embryo sac, thus causing abortion or abnormal growth of the embryo. On the other hand, apical ovule development did not appear to be affected by basal ovule outgrowth, callus growth or peg elongation. The uniform 83–89% normal embryo growth for apical embryos strongly suggests a standard-condition response level against which future studies could be evaluated.

Correlation LM and SEM of the same microtomed section (Pattee *et al.*, 1985) was used to

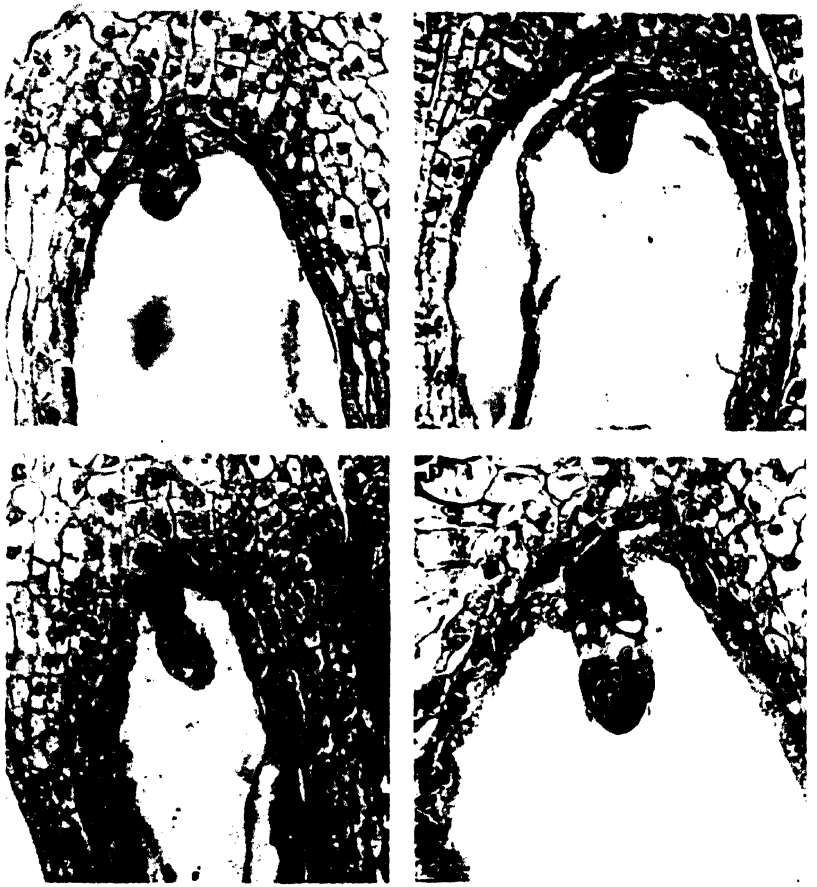

FIG. 2. LM of longitudinal sections of cultured embryos. × 325. A, Day 5, B, 1-0 stage. C, 1-1 stage. D, 1-2 stage.

investigate and illustrate embryo development in the cultured peg tips. All presented illustrations are representative of median longitudinal sections of the ovule and embryo. Typical ovule and embryo development for NC 6 peanuts at stages D-1 to D-5 and stages 1-0, 1-1 and 1-2 (Fig. 1A–H) show that the D-1 stage may encompass syngamy of the egg nucleus and the pollen tube nucleus. Following successful fertilization, normal development into an early globular embryo (stage 1-2) can occur. Figure 1A also shows the pollen-tube entrance route into the egg cell and the pollen tube with enclosed nucleus in juxtaposition to the egg nucleus. Embryo development in cultured peg tips was not considered significant unless it had progressed beyond the last peg-collection stage, D-4. Comparison of longitudinal sections of the significant embryo development stages D-5, 1-0, 1-1 and 1-2 from typical growth (Fig. 1E–H) and cultured peg tips (Fig. 2A–D) by LM shows excellent agreement in the cell structure of the embryo and the ovule. The SEM photomicrographs (Figs 3A–D and 4A–D) present complementary evidence that cell structure and

FIG. 3. SEM of longitudinal sections of field-grown embryos at selected stage. Using same sections as shown in Fig. 1 E–H. × 1000. A, Day 5 stage. B, 1-0 stage. C, 1-1 stage. D, 1-2 stage. Fse, fracture line between suspensor and

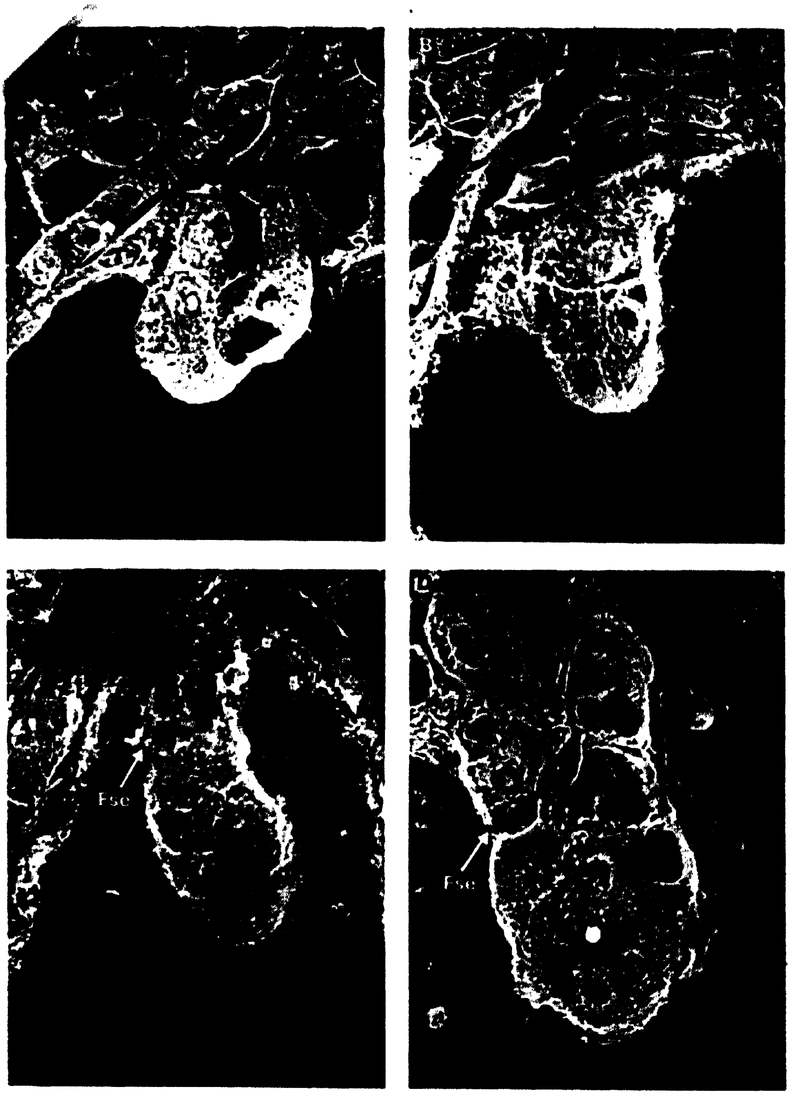

FIG. 4. SEM of longitudinal sections of cultured embryos as seen in Fig. 2. $\times 1000$. A, Day 5 stage. B, 1-0 stage. C, 1-1 stage. D, 1-2 stage. Fse, fracture line between suspensor and embryo.

opment progressed normally for cultured embryo and associated ovule tissues. Overall visual comparisons between reproductive tissues which developed on the plant and in cultured tissues is in close agreement. The characteristic structural weakness between the suspensor and embryo cells at their junction point, observed as fracturing, is evident in both tissue groups (Figs 3C–D and 4C–D). Morphometric measurements of the cell nucleus under both LM and SEM conditions also produced complementary results. The diameter of nuclei from normal and cultured peg-tip embryos under LM averaged 2·7 and 3·0 μm, respectively, while the same nuclei under SEM averaged 3·0 and 2·9 μm, respectively. The agreement between the morphometric measurements under LM and SEM of the nucleus minimizes the probability of artifact occurrence between the two procedures.

DISCUSSION

Plant recovery after *in vitro* embryo culture in *Arachis* has only been possible for reproductive tissues which have reached the heart-stage of development. This corresponds to an age ranging from 20 to 30 d after fertilization, depending upon the species and whether embryos developed after self- or cross-fertilization. However, many highly desirable interspecific hybrids abort before embryos reach the heart stage. For example, Johansen and Smith (1956) reported embryo abortion in crosses between *A. hypogaea* and *A. diogoi* Hoehne (not true *diogoi*, see Gregory and Gregory, 1979) at 10–12 d after fertilization. Early embryo abortion also occurred after crosses between *A. hypogaea* and *A. glabrata* Benth were made (Murty *et al.*, 1980). Halward and Stalker (1987) reported even earlier embryo abortion in diploid × hexaploid interspecific crosses. Techniques thus need to be developed for rescuing pro-embryos or immature embryos, which will only develop to the globular embryo stage when left to mature on the plant.

Martin (1970) reported the only successful recovery of a very immature peanut embryo when he obtained a plant following ovule culture. However, the procedure has proved unrepeatable (Sastri *et al.*, 1980). Johnson (1981) proposed a two-step procedure, where ovules are initially cultured to stimulate growth and then embryos cultured to generate plants. Although a relatively high frequency of ovules will turn green and swell (Mallikarjuna and Sastri, 1985), few reached a size where the embryo can be dissected without tissue damage (Stalker, 1986). The continued embryo growth of 1- to 4-d-old embryos in this investigation indicates the potential for utilizing peg tips as a nurse tissue for embryo rescue.

A comparison of morphological changes in peg tissues with embryo development indicates that selecting specific visual responses will not indicate which embryos will grow *in vitro*. For example, peg tips which had no visible change after 21 d in culture had as many embryos which continued growth as those pegs which elongated or produced callus. However, if excessive ovule growth occurs, a high probability exists for the embryo to be crushed. Because outgrowth of the basal ovule was more frequent in pegs which had the meristem removed, the outgrowth is believed to result from absence of a physical barrier preventing ovule enlargement. Apical embryos in the same peg tips are unaffected by growth of basal ovular tissues.

Although continued reproductive development was observed for embryos cultured at all four collection days, the highest frequency of growth was observed in 1-d-old tissues. This is believed to be related to peg meristematic activity, or lack of activity, in 1-d-old pegs. During normal reproductive development in *Arachis*, the embryo ceases cell division as the peg meristem becomes active. Since the peg meristem initiates activity the second day after fertilization, it is expected to suppress embryo growth in the 2- to 4-d-old embryos. Comparison of apical embryo growth among days (which eliminates bias due to abnormal embryo growth in basal ovule tissues) indicates that 47% of 1-d-old embryos grew, while only 22–29% of the older embryos continued growth. This gives further evidence that meristematic activity in the peg may restrict embryo growth and, once the sequence of events is initiated to slow embryo growth, it is not easily reversed *in vitro*.

Growth of embryos to multicellular stages (stages 1-1 or 1-2 in Table 3) encourage the development of methods to recover very young embryos through tissue-culture techniques. Future work will be needed in longer-range experiments to subculture peg tips, to determine if embryo development will continue to a stage where mature plants can be recovered.

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., S. K., 1977. A quantitative approach to source potentials and sink capacity among reciprocal grafts ... Crop Science 17, 559–62.

Iozyo, Y., 1970. Growth and development of tuberous

Sisju-Charran, G., 1978. Enzyme genesis in sweet potato tuber tissue. Ph.D. Thesis, University of the West Indies, Trinidad.

Togari, Y., 1950. A study of tuberous root formation in

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LITERATURE CITED

Berlyn, G. P. and Miksche, J. P., 1976. *Botanical Microtechnique and Cytochemistry*, 326 pp. The Iowa State University Press, Ames, Iowa.

Gregory, M. P. and Gregory, W. C., 1979. Exotic germplasm of *Arachis* L. interspecific hybrids. *Journal of Heredity* **70**, 185–93.

Gregory, W. C., Krapovickas, A. and Gregory, M. P., 1980. Structure, variation, evolution and clasification in *Arachis*, pp. 469–81. In *Advances in Legume Science*, eds J. R. Summerfield and A. H. Bunting, 1049 pp. Royal Botanic Gardens, Kew.

Halward, T. M. and Stalker, H. T., 1987. Incompatibility mechanisms in interspecific peanut hybrids. *Crop Science* **27**, 456–60.

Jacobs, W. P., 1947. The development of gynophore of the peanut plant, *Arachis hypogeae* L. I. The distribution of mitoses, the region of greatest elongation, and the maintenance of vascular continuity in the intercalary meristem. *American Journal of Botany* **34**, 361–70.

Johansen, E. L. and Smith, B. W., 1956. *Arachis hypogaea* × *Arachis diogoi*. Embryo and seed failure. *American Journal of Botany* **43**, 250–8.

Johnson, B. B., 1981. Embryo–ovule culture. *Environmental and Experimental Botany* **21**, 439.

Mallikarjuna, N. and Sastri, D. C., 1985. *In vitro* culture of ovules and embryos from some incompatible interspecific crosses in the genus *Arachis* L., pp. 153–8. In *Proceedings of the International Workshop on Cytogenetics in* Arachis, ed. J. P. Moss, 191 pp. ICRISAT, Patancheru, A.P., India.

Martin, J. P., 1970. Culture *in vitro* d'ovules d'arachie. *Oléagineux* **25**, 155–6.

Moss, J. P., Stalker, H. T. and Pattee, H. E., 1985. *In vitro* culture of *A. hypogaea* peg tips. *Proceedings of the American Peanut Research and Education Society* **17**, 22.

—— —— —— 1988. Embryo rescue in wide crosses in *Arachis*. I. Culture of ovules in peg tips of *Arachis hypogaea*. *Annals of Botany* **61**, 1–7.

Murashige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473–97.

Murty, U. R., Rao, N. G. P., Kirti, P. B. and Bharathi, M., 1980. *Cytogenetics and Groundnut Improvements*. IARI Regional Station Report Hyderabad, India.

Pattee, H. E. and Mohapatra, S. C., 1986. Hypanthium and style senescence in relation to ovary development in *Arachis hypogaea* L. *Botanical Gazette* **147**, 302–11.

—— —— 1987. Anatomical changes during ontogeny of the peanut (*Arachis hypogaea* L.) fruit. Mature megagametophyte thru heart-shaped embryo. *Botanical Gazette* **148**, 156–64.

—— —— and Agnello, E. K., 1983. Specimen preparation techniques for scanning electron microscopy of developing peanut pegs. *Peanut Science* **10**, 93–7.

—— —— —— 1985. A technique for comparative light and scanning electron microscopy of the same deparaffinized microtomed section. *Journal of Microscopy* **137**, 111–14.

Sastri, D. C. and Moss, J. P., 1982. Effects of growth regulators on incompatible cross in the genus *Arachis* L. *Journal of Experimental Botany* **33**, 1293–301.

—— Nalini, M. S. and Moss, J. P., 1980. *In vitro* culture of *Arachis* ovaries and ovules, pp. 366–73. In *Proceedings of the Symposium on Plant Tissue Culture, Genetic Manipulation and Somatic Hybridization*, eds P. S. Rao, M. R. Heble and M. S. Chadha, 436 pp. Bhadha Atomic Research Centre, Trombay, India.

Singh, A. K., Sastri, D. C. and Moss, J. P., 1980. Utilization of wild *Arachis* species at ICRISAT, pp. 82–90. In *Proceedings of the International Workshop on Groundnuts*, ed. R. W. Gibbons, 325 pp. ICRISAT, Pantancheru, A.P., India.

Stalker, H. T., 1986. Rescue of *Arachis hypogaea* L. embryos by *in vitro* culture. *Proceedings of the American Peanut Research and Education Society* **18**, 27.

Thompson, L. K., Ziv, M. and Deitzer, G. F., 1985. Photocontrol of peanut (*Arachis hypogaea* L.) embryo and ovule development *in vitro*. *Plant Physiology* **78**, 370–3.

Williams, E. G. and Delautour, G., 1980. The use of embryo transplanted nurse endosperm for the production of interspecific hybrids in pasture legumes. *Botanical Gazette* **41**, 252–7.

Zamski, E. and Ziv, M., 1976. Pod formation and its geotropic orientation in peanut, *Arachis hypogaea* L., in relation to light and mechanical stimulus. *Annals of Botany* **40**, 631–6.

Ziv, M., 1981. Photomorphogenesis of the gynophore, pod and embryo in peanut, *Arachis hypogaea* L. *Annals of Botany* **48**, 353–9.

—— and Sagar, J. C., 1984. The influence of light quality on peanut (*Arachis hypogaea* L.) gynophore pod embryo development *in vitro*. *Plant Science Letters* **34**, 211–8.

Ziv, M. and Zamski, E., 1975. Geotropic responses and pod development in gynophore explants of peanut (*Arachis hypogaea* L.) cultured *in vitro*. *Annals of Botany* **39**, 579–83.

., S. K., 1977. A quantitative approach to source potentials and sink capacity among reciprocal grafts of sweet potato varieties. *Crop Science* 17, 559–62.

HOZYO, Y., 1970. Growth and development of tuberous roots in sweet potato. In *Proceedings of the International Symposium on Tropical Root Crops* 2, 22–3.

KAYE, S. J., MAGNUSON, C. E. and FARRIS, Y., 1982. Assimilation patterns of carbon in developing sweet potatoes using ^{11}C and ^{14}C, pp. 95–118. In *Sweet Potato: Proceedings of the First International Symposium*. Asian Vegetable Research and Development Center, Taiwan.

LOWE, S. B. and WILSON, L. A., 1974. Comparative analysis of tuber development in six sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars. 2. Inter-relationships between tuber shape and yield. *Annals of Botany* 38, 319–26.

SEIJU-CHARRAN, G., 1978. Enzyme genesis in sweet potato tuber tissue. Ph.D. Thesis, University of the West Indies, Trinidad.

TOGARI, Y., 1950. A study of tuberous root formation in sweet potato. *Bulletin of National Agriculture Experimental Station, Tokyo* 68, 1–96.

TSUNO, Y. and FUJISE, K., 1965. Studies on dry matter production of sweet potato. *Bulletin of the National Institute for Agricultural Science* Series D, no. 13.

WILSON, L. A., 1982. Tuberization in sweet potato (*Ipomoea batatas* (L.) Lam.), pp. 79–94. In *Sweet Potato: Proceedings of the First International Symposium*, Asian Vegetable Research and Development Center, Taiwan.

WILSON, L. A. and LOWE, S. B., 1973. The anatomy of the root system in West Indian sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars. *Annals of Botany* 37, 633–43.