Embryo Rescue in Wide Crosses in *Arachis*. 1. Culture of Ovules in Peg Tips of *Arachis hypogaea*

J. P. MOSS*, H. T. STALKER†‡ and H. E. PATTEE§

* *International Crops Research Institute of the Semi-Arid Tropics, Andhra Pradesh 502 324, India and* † *Department of Crop Science,* § *USDA Agricultural Research Service and Department of Botany, North Carolina State University, Raleigh, NC 27695, USA*

Accepted: 24 April 1987

ABSTRACT

Interspecific hybridization in *Arachis* is restricted by early embryo abortion for many cross-combinations. Rescue of young embryos *in vitro* within a week after fertilization is necessary to recover these embryos before they abort. Peg tips, with the ovule and embryo tissues, of *A. hypogaea* L. cv. 'NC 6', were cultured to compare ovule growth, callus production and peg elongation. 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. One-day-old pegs had significantly ($P = 0{\cdot}01$) more ovule growth than older tissues. Presence of the meristem had a greater inhibition to ovule growth for 2- to 4-d pegs than for 1-d-old pegs. Significantly more callus was produced on 4-d pegs than younger tissues, and kinetin had the greatest stimulatory effect on callus. Elongation of pegs with the meristem attached was observed most often in media with high sucrose levels. The observations indicate that very young ovules can be grown *in vitro*, and techniques may be applicable to rescue of young embryonic tissues of *Arachis*.

Key words: Ovule culture, interspecific hybridization, *Arachis hypogaea*, peanuts.

INTRODUCTION

All species in the genus *Arachis* produce underground fruits. After fertilization, a gynophore or peg is produced, which elongates and grows into the soil. This elongation is the result of meristematic activity proximal to the ovary (Jacobs, 1947). Pegs initiate growth about 2 d after fertilization and have maximal elongation 6–8 d later (Smith, 1956; Pattee and Mohapatra, 1986). Gibberellic acid has a significant stimulatory effect on peg elongation (Amir, 1969; Sastri and Moss, 1982), while auxin inhibits peg elongation and is associated with fruit enlargement (Jacobs, 1951). Ziv (1981) also reported that light is necessary for peg elongation. When pegs fail to reach the soil, they remain viable for several days and then wither. Although peg elongation follows a sigmoidal growth pattern, the embryo initially grows slowly, and only initiates a rapid growth phase after peg elongation has ceased and pod development has been initiated (Smith, 1956; Halward and Stalker, 1987; Pattee and Mohapatra, 1987).

‡ For correspondence.

Embryo rescue techniques in peanuts are potentially important for recovering aborted interspecific hybrids. Abortion may occur either as a pre- or postfertilization event in *Arachis*, but postzygotic abortion is believed to be most common in the group. In *Arachis*, an intercalary meristem exists 1·5–3·0 mm below the ovarian cavity and is responsible for peg elongation (Jacobs, 1947). Since the meristem initiates activity between the first and third day after fertilization, the effect of the meristem on ovule growth should be detectable. Pegs are commonly produced in interspecific crosses but fail to reach the soil either because growth is arrested or because embryo development never initiates the rapid growth phase (Johansen and Smith, 1956; Sastri and Moss, 1982; Halward and Stalker, 1985). The capability of culturing very young embryos is thus important to recovering hybrids. To meet this objective, Martin (1970) regenerated plants from ovules which were 7–10 d old and only 0·3 mm in length. However, Sastri, Nalini and Moss (1980) could only produce callus in attempts to duplicate Martin's experiments. Mallikarjuna and Sastri

0305-7364/88/010001+07 $03.00/0

(1985) reported enlargement and greening of 1 to 3 mm interspecific hybrid ovules and variations among genotypes in culture. Ziv and Zamski (1975), Ziv (1981), and Ziv and Sagar (1984) observed callus and recovered mature seeds from pegs allowed to grow on the plant and into media until ovules were 3 mm^3 (corresponding to embryos in the heart stage) and then cultured *in vitro*. Ziv and Sagar (1984) further found that embryos cultured at the post-globular or early heart stage would develop into young seedlings under red, blue or far-red subsaturated flux densities, but pod formation was inhibited. However, attempts to culture very young embryos have thus far been unsuccessful, and media and techniques must be further developed to rescue aborting embryos.

Nurse cultures have been used to culture young embryos from interspecific crosses (Williams and deLautour, 1980). Culturing peg tips, which have the fertilized embryos, is a means of culturing the embryo without detaching it from maternal tissues. Preliminary experiments (Moss, Stalker and Pattee, 1985) have shown that ovules can continue to grow in cultured peg tips and that the peg meristem affects ovule and callus growth. The objective of this investigation was to determine whether peg tips could be used as nurse tissue for *in vitro* culture of ovules. This paper reports ovule growth in 1- to 4-d-old peg tips of *A. hypogaea* L. 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 with 3 ml Tween 80 for 15 min and washed three times for 15 min each in sterile distilled water. Peg length was measured and the colour of the peg tip was recorded, after which peg tips were excised under a dissecting microscope fitted with an ocular micrometer. Peg tips with meristems were cut from the basal nodal tissue, but peg tips without meristems were also cut transversely 1 mm from the tip. Based on previous results (Moss, Stalker and Pattee, 1985), the 1 mm peg tips were then assumed not to have meristematic tissues. Variation in position of the ovules was observed, evidenced by variation in the distance of the basal ovule from the peg tips, so a record was maintained as to whether the basal ovule had been cut at the time of dissection. Also, records were maintained for the basal ovule being easily visible, partly visible or totally obscured by peg tissue when the cut surface was observed.

A total of 709 peg tips were cultued *in vitro* using 40 age × media × meristem combinations. The excised peg tip was placed partially 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 (mg l^{-1}): myoinositol (100), nicotinic acid (1), thiamine HCl (10), pyroxidine (1) and glycine (2). The pH was 5·7 and 7 g l^{-1} agar was used. The five media differed in concentration of sucrose, indoleacetic acid (IAA) and kinetin (Kn) (Table 1). Cultures were incubated at 27 °C in the dark and scored 14 d after anthesis.

The data were analysed using the general linear models procedure of the statistical analysis system (SAS). Covariate analysis was performed using peg-tip colour, peg length and ovule position as covariates to determine their effects on observed reactions.

RESULTS

After 14 d incubation in the dark, 11 different reactions were recorded on the peg tissues (Table 2). Of the 709 cultured peg tips, 43·5% had either ovule growth, callus or elongation. The most frequent reaction was callus, in 26·2% of the tissues. Callus was most frequently observed at the cut end, but also in the middle of the peg and at times covering the entire cultured tissue. Ovules expanded and emerged out of the peg tissue in 18·3% of all cultures (Fig. 1). Significantly, in 74·6% of all pegs which had ovule growth no callus was observed on the surrounding peg tissues. Elongation was observed in only 4·1% of the cultured peg tips. The 11 reactions were then combined into three larger groups including ovule growth, callus production and peg elongation for analyses of reactions in culture.

TABLE 1. *Concentrations of three constituents of the five MS media used to culture 1- to 4-year-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

TABLE 2. *Reactions of peg-tip cultures on four culture dates*

Reaction	Responding cultures								Total
	Day 1		Day 2		Day 3		Day 4		
	−M*	+M	−M	+M	−M	+M	−M	+M	
Ovule growth									
(1) Basal ovule only	25	2	18	0	26	0	14	0	85
(2) Basal ovule + peg callus	10	1	7	1	3	0	10	0	32
(3) Basal ovule + peg split	0	5	6	0	1	0	0	0	12
(4) Basal ovule + peg callus + split	0	1	0	0	0	0	0	0	1
(5) Apical ovule	0	0	0	1	0	0	0	0	1
Callus growth									
(6) At cut end	6	4	12	16	15	26	23	39	141
(7) Covering peg	3	0	0	0	0	0	0	0	3
(8) At mid-peg only	1	1	1	1	1	1	0	0	6
(9) Callus + peg elongation	0	0	0	2	0	1	0	0	3
Elongation									
(10) Elongate only	0	9	0	2	1	5	0	8	25
No reaction	43	56	44	52	53	62	45	45	400
Total	88	79	88	75	100	95	92	92	709

* +M, pegs with meristem; −M, pegs without meristem.

When pegs were collected for culture, morphological variation was observed among tissues collected over days, for example, elongation with increased time and a change in colour of the peg tip. Initially, 94% of the pegs were green, but after 4 d 84% were light to dark purple. Peg tip colour was used as a covariate to test significance as an indicator of ovule growth but was found not to have influenced culture reactions. Peg length was similarly tested and the trait had a significant ($P = 0{\cdot}01$) effect on ovule growth where less ovule growth was observed as the peg elongated. Peg length had no effect on callus or peg elongation and had significant interactions with day of collection ($P = 0{\cdot}01$) and with presence or absence of the meristem ($P = 0{\cdot}05$). For 1-d-old pegs which did not have the meristem removed, an average of 12·8% ovule growth was observed after 14 d in culture, whereas none of the similarly prepared pegs collected at 4 d had ovule growth.

In the overall experiment, ovule growth was most influenced by the presence or absence of the peg meristem, where significantly ($P = 0{\cdot}01$) more ovules grew when the meristem was removed (Table 3). The effectiveness of removing the meristem by cutting 1 mm from the tip was assessed by observing the cut surface at the time of first culture. No peg tissue was observed between the cut surface and the ovule in 72·9% of the peg tips (Table 4). In the remaining pegs the ovule was not visible after cutting the peg. The ovule position was used as a covariate with culture reaction and was non-significant for ovule growth, callus production or peg elongation.

Ovule growth for 1-d-old peg tips ranged between 4 and 20% of the tissues when the meristem was left on the peg. However, no ovule growth was observed in similar 3- or 4-d-old tissues. For all pegs cultured at 1 d, 25·1% had ovule growth whereas they grew in only 11·8–14·0% of the 2- to 4-d-old pegs.

An analysis of variance showed that the media had a non-significant effect on ovule growth in the overall experiment. This was largely due to different responses of peg tips over collection days for the different media. However, significant differences were observed for media when they were compared within specific collection days (Table 3). Media which contained kinetin were generally superior than the media without kinetin for 1- to 3-d-old tissues. Indoleacetic acid appeared to have a neutral effect on ovule growth during the first few days of collection. However, by day 4 the 1 mg l^{-1} IAA:0 mg l^{-1} Kn medium was among the highest group for ovule development.

For callus production both age of tissue and media had significant ($P = 0{\cdot}01$) effects on growth. As older peg tissues were cultured, a greater

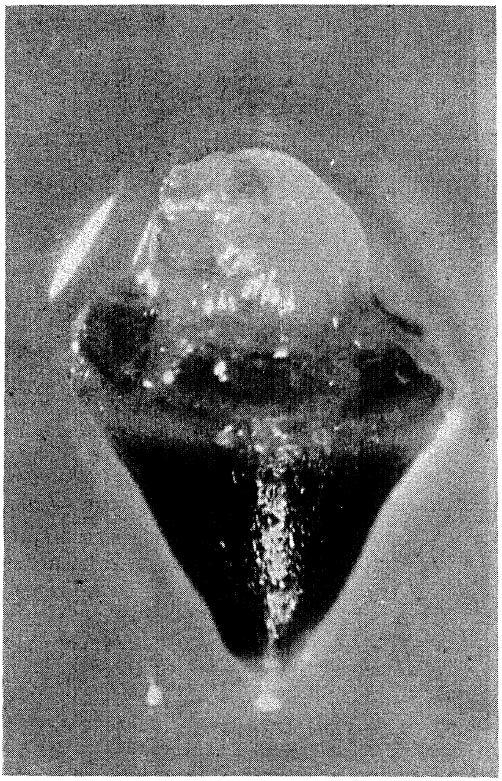

FIG. 1. Ovule growing out of 4-d-old peg tip after 21 d in culture.

percentage of the cultured pegs produced callus, with a maximum of 70·0% for 4-d-old pegs when the meristem remained attached and a 0 mg l^{-1} IAA:0·1 mg l^{-1} Kn ratio was used in the media. Overall, 26·5% of the 1-d-old pegs produced callus, whereas 35·5–41·9% of the 2- to 4-d-old pegs produced callus. For the overall experiment, the 0 mg l^{-1} IAA:0·1 mg l^{-1} Kn produced significantly ($P = 0·05$) more callus, averaging 48·8% of the pegs with callus, than the other media. The 0·2 mg l^{-1} IAA:0·1 mg l^{-1} Kn (plus a high sucrose level of 12·5%) produced significantly less callus, averaging callus on only 10·5% of the pegs. The remaining three media were intermediate and statistically the same. Kinetin thus had the greatest stimulatory effect on callus, and high sucrose levels apparently restrict callus growth.

Significant age × meristem interactions ($P = 0·05$) existed for callus initiation. Younger pegs produced more callus ($P = 0·01$) when the meristem was removed, whereas 2- and 3-d-old peg tissues with and without meristems had nearly the same frequencies of callus. Four-day-old pegs produced significantly ($P = 0·01$) more callus on pegs with a meristem. The increase in numbers of pegs producing callus for older tissues was due to increased frequencies of callus on tissues with the meristem attached rather than a decrease on pegs with the meristem removed, as compared to 1-d-old pegs.

Significant media × meristem ($P = 0·05$) interactions were also observed for callus production. Media with 1·0 mg l^{-1} IAA:0·1 mg l^{-1} Kn and 0 mg l^{-1} IAA:0·1 mg l^{-1} Kn had higher frequencies of callus on pegs with a meristem at day 1, but by 4 d the frequencies were reversed for pegs with and without a meristem. When the 1·0 mg l^{-1} IAA:0 mg l^{-1} Kn medium was used, the frequency of callus was always significantly greater when the meristem was left on the peg. The media consisting of 0·2 mg l^{-1} IAA:0·1 mg l^{-1} Kn and 0·4 mg l^{-1} IAA:0·1 mg l^{-1} Kn had nearly the same frequencies across all collection days. The mixed reactions of specific media for pegs with and without a meristem thus resulted in significant interactions.

Peg elongation was significantly greater ($P = 0·01$) when the meristem was left on the peg. The few exceptions – elongation when the peg was cut 1 mm from the tip – were probably due to residual meristematic tissues remaining on the peg. Media effects were also significant ($P = 0·05$) over all media for the experiment.

DISCUSSION

Nurse cultures have been used in several legume genera to recover interspecific hybrid embryos (Williams and deLautour, 1980). With peg-tip cultures the embryo is cultured within the ovule, and the placenta and other ovule tissue are undamaged, as is the funiculus attaching the ovule to the peg tissue. This may be a disadvantage in hybrids if there is an unfavourable reaction between the embryo and maternal or endosperm tissues. However, in the present study, where all tissues were selfs, growth of very young ovules was observed in 16·1% of all cultured peg tips. The growth occurred relatively fast, with significant differences being observed after 14 d in culture.

Embryo rescue and growth to plant maturity in *Arachis* is possible for tissues which survive until the heart stage of development (Sastri, Nalini and Moss, 1981). Younger tissues must go through a two-step process, where ovules can be cultured until the embryo reaches a size where it can be excised from maternal tissues (Johnson, 1981). Mallikarjuna and Sastri (1985) reported that ovules which were at least 3·0 mm in length could be cultured. Embryo rescue has thus been limited to tissues which survive for at least 15 d after

TABLE 3. *Summary of observed reactions of peg tips collected 1–4 d after pollination and cultured with and without meristems on five different media*

Age (d)	Media (mg l^{-1})			Meristem	No. of pegs cultured	No. of cultures responding (%)		
	Sucrose	IAA	Kn			Ovule	Callus	Elongation
1	125	0·2	0·1	N	31	40·6	9·4	0·0
	125	0·2	0·1	Y	23	4·0	0·0	32·0
	30	0·4	0·1	N	14	40·0	6·7	0·0
	30	0·4	0·1	Y	13	6·7	6·7	0·0
	30	1·0	0·1	N	15	40·0	46·7	0·0
	30	1·0	0·1	Y	14	26·7	13·3	6·7
	30	1·0	0·0	N	13	26·7	6·7	0·0
	30	1·0	0·0	Y	15	6·7	13·3	0·0
	30	0·0	0·1	N	15	40·0	53·3	0·0
	30	0·0	0·1	Y	15	20·0	13·3	0·0
2	125	0·2	0·1	N	31	12·1	3·0	0·0
	125	0·2	0·1	Y	24	3·9	3·9	11·6
	30	0·4	0·1	N	15	20·0	33·0	0·0
	30	0·4	0·1	Y	14	0·0	20·0	0·0
	30	1·0	0·1	N	14	53·3	26·7	0·0
	30	1·0	0·1	Y	14	6·7	26·7	0·0
	30	1·0	0·0	N	14	6·7	26·7	0·0
	30	1·0	0·0	Y	13	0·0	35·7	7·1
	30	0·0	0·1	N	13	20·0	46·7	0·0
	30	0·0	0·1	Y	10	6·7	46·7	6·7
3	125	0·2	0·1	N	42	31·0	2·4	2·4
	125	0·2	0·1	Y	40	0·0	5·0	12·5
	30	0·4	0·1	N	14	40·0	26·7	0·0
	30	0·4	0·1	Y	14	0·0	26·7	0·0
	30	1·0	0·1	N	15	20·0	20·0	0·0
	30	1·0	0·1	Y	14	0·0	26·7	0·0
	30	1·0	0·0	N	14	20·0	20·0	6·7
	30	1·0	0·0	Y	15	0·0	73·3	6·7
	30	0·0	0·1	N	15	33·3	46·7	0·0
	30	0·0	0·1	Y	12	0·0	46·7	0·0
4	125	0·2	0·1	N	43	34·9	37·2	0·0
	125	0·2	0·1	Y	43	0·0	22·7	11·4
	30	0·4	0·1	N	14	6·7	40·0	0·0
	30	0·4	0·1	Y	14	0·0	46·7	6·7
	30	1·0	0·1	N	15	6·7	26·7	0·0
	30	1·0	0·1	Y	14	0·0	60·0	0·0
	30	1·0	0·0	N	11	36·4	9·1	0·0
	30	1·0	0·0	Y	11	0·0	54·5	18·2
	30	0·0	0·1	N	9	33·3	6·7	0·0
	30	0·0	0·1	Y	10	0·0	70·0	0·0
±s.e.						1·64	1·71	0·75

pollination. However, many hybrid combinations in the genus abort within the first week after pollination, and development of earlier-stage rescue techniques must also be achieved before desired hybrids can be obtained. The investigation into culture of peg tips, with ovules inside, demonstrates a method to potentially recover hybrids by culturing very young tissues *in vitro*.

In *Arachis*, the embryo and ovule must remain small until the peg penetrates the soil and pods are initiated or else the peg would split, resulting in seed abortion. Meristem activity initiates in *A. hypogaea* between the first and third day after fertilization (Smith, 1956; Pattee and Mohapatra, 1986). Meristematic activity is thus believed to inhibit ovule growth in peg-tip cultures. This is in contrast to the reports of Badami (1935) and Jacobs (1951), who indicated that the embryo

TABLE 4. *Position of ovule, relative to a cut at 1 mm from* Arachis hypogaea *peg tip, at 1 to 4 d after anthesis*

Age (d)	Number	Frequency of ovules (%)			
		Cut through	Visible	Partially visible	Not visible
1	92	11	61	21	7
2	93	8	56	34	2
3	102	7	62	31	0
4	93	4	68	28	0
Total	380	$\bar{x}$... 7	62	29	2

controls elongation by secretion of hormones which stimulates tissue at the basal side of the ovules.

While peg elongation *per se* is not expected to influence ovule growth when hybrids are to be recovered *in vitro*, callus production is undesirable because it can compete directly with the ovule for nutrients and in some cases completely cover the ovule. The lower percentage for very young pegs may have been a reflection of non-growing pegs having compact meristematic tissues which were not as amenable to callus formation as rapidly growing tissues. The high sucrose level of 12·5%, vs 3%, appeared to suppress callus for at least the first few days of peg development.

One-day-old pegs had significantly more ovule growth than did 2- to 4-d-old tissues. This was possibly due to an inactive meristem in 1-d-old pegs. Further, the data indicated that ovules will develop on a range of media, but highest levels of growth may be media-dependent on specific days of culture. For pegs collected 1 d after pollination there is difficulty in separating fertilized reproductive tissues from non-fertilized pegs. All pegs at this age were small and green. By the second day after pollination the peg tip begins to turn purple and by 4 d fertilized peg tissues are easily identified. Because only obviously growing pegs were collected from days 2–4, which accounted for approx. 70% of the potential pegs which could have been cultured if all pegs were put on to media, all ovules should have developing embryos. However, even though a higher frequency of 1 d ovules grew, an expected 30% of the pegs are probably without embryos. Additional histological and culture work will be necessary to actually discern the feasibility of culturing peg tips to recover plants for very young tissues. Culturing of peg tips before meristematic activity is initiated would appear to be desirable. The results indicate that culturing peg tips has potential for rescuing very young reproductive tissues of peanuts. The techniques have application for recovering zygotes of hybrids which either do not express peg elongation or abort within the first week after fertilization.

ACKNOWLEDGEMENTS

Submitted as paper no. 603 of the journal series of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and paper no. 10403 of the journal series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601. Research was conducted by the senior author while on sabbatical study leave at N.C. State University. Use of trade names of specific materials does not constitute endorsement by the United States Department of Agriculture or the North Carolina Agricultural Research Service to the exclusion of others which also may be available. This investigation was supported in part by USAID Peanut CRSP grant no. DAN-4048-G-SS-2065-00.

LITERATURE CITED

AMIR, J., 1969. A study on the reproductive stage of the groundnut, *Arachis hypogaea* L. A survey. *Oléagineux* **32**, 225–9.

BADAMI, V. K., 1935. Botany of groundnut. *Journal of Mysore Agricultural & Experimental Union (India)*. Part I: **14**, 188–94; part II: **15**, 58–70.

HALWARD, T. M. and STALKER, H. T., 1985. Embryo development in interspecific peanut hybrids. *Agronomy Abstracts* **1985**, 56.

—— —— 1987. Comparison of embryo development in wild and cultivated *Arachis* species. *Annals of Botany* **59**, 9–14.

JACOBS, W. P., 1947. The development of the gynophore of the peanut plant, *Arachis hypogaea* 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.

—— 1951. Auxin relationships in an intercalary meri-

stem: further studies on the gynophore of *Arachis hypogaea* L. *American Journal of Botany* **38**, 307–10.

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 (abstr.).

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, International Workshop on Cytogenetics of* Arachis, ed. J. P. Moss, 191 pp. ICRISAT, Patancheru, A.P., India.

MARTIN, J. P., 1970. Culture *in vitro* d'ovules d'arachide. *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 & Education Society* **17**, 22 (abstr.).

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

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

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

SASTRI, D. C. and MOSS, J. P., 1982. Effects of growth regulators on incompatible crosses 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, Symposium on Plant Tissue Culture, Genetic Manipulations and Somatic Hybridization*, eds P. S. Rao, M. R. Heble and M. S. Chadha, 436 pp. Bhabha Atomic Research Centre, Trombay, India.

—— —— —— 1981. Tissue culture and prospects for improvement of *Arachis hypogaea* and other oil seed crops, pp. 42–57. In *Proceedings, COSTED Symposium on Tissue Culture of Economically Important Plants*, ed. A. N. Rao. Singapore.

SMITH, B. W., 1956. *Arachis hypogaea*. Embryogeny and the effect of peg elongation upon embryo and endosperm growth. *American Journal of Botany* **43**, 233–40.

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**, 252–57.

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 and embryo development *in vitro*. *Plant Science Letters* **34**, 211–18.

—— 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.