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Early Reproductive Ontogeny in Interspecific Crosses of *Arachis hypogaea* and Section *Arachis* Species

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Wild *Arachis* species have been recognized as sources of resistance to pests and pathogens that infect *A. hypogaea* L. and cause substantial yield losses. However, utilization of these genetic resources for crop improvement has been difficult. This study was conducted to (*a*) understand the processes of early embryo growth and development in four *Arachis* species, two *A. hypogaea* cultivars and their hybrids and (*b*) identify parental compatibilities in reciprocal crosses of *A. hypogaea*. The results indicated that delayed fertilization beyond 24 h, coupled with slow proembryo growth, leads to embryo abortion in many interspecific crosses. For example, in female *A. cardenasii* crosses, lack of or delayed fertilization leads to failure to obtain hybrids. When *A. batizocoi* was used as a female parent, delayed fertilization and the inability of quiescent proembryos to resume growth after soil penetration caused abortion. Embryos of *A. hypogaea* × *A. glandulifera* crosses developed normally during the first 21 d after fertilization, but then aborted at a later time. In this study, *A. hypogaea* was always a better female parent than the wild *Arachis* species. Increasing the number of pollinations per cross, using the cultivated species as the female parent, utilizing different *A. hypogaea* varieties, and embryo rescue techniques are suggested to improve the probability of obtaining interspecific hybrids in *Arachis*. © 1995 Annals of Botany Company

Key words: Peanut, interspecific hybrids, *Arachis*, wild species, incompatibilities.

INTRODUCTION

Wild species of *Arachis* are potential sources of resistance to the major diseases and insect pests of cultivated peanut. These genetic resources have encouraged peanut breeders to create interspecific hybrids to improve *A. hypogaea* L., and considerable progress has been made toward this goal with several species in section *Arachis* (see Stalker and Moss, 1987; Singh, Stalker and Moss, 1991; Stalker, 1992). In a crossing programme using two *A. hypogaea* cultivars and 22 section *Arachis* species accessions, in reciprocal, most species were successfully used to obtain hybrids (Stalker *et al.*, 1991). However, the degree of success varied significantly between the two cultivars and depended upon whether the cultivars or wild species were used as the female parent. Twenty-seven of 73 attempted crosses did not produce hybrids and all but 16 had less than 5% success. The low success rate between *A. hypogaea* and several section *Arachis* species was partially explained by Halward and Stalker (1987*a*, *b*) and Pattee and Stalker (1992*a*, *b*) who identified relative time sequences of embryo development and stages when abortion occurred. Stalker and Eweda (1988) and Ozias-Akins, Singsit and Branch (1992) also used embryo rescue techniques to recover interspecific hybrids with *A. hypogaea*.

The geocarpic feature of reproductive development in peanut is a complex process involving several physical, physiological, and genetic mechanisms. Two or 3 d after fertilization, a peg initiates a rapid growth phase at which time the embryos remain quiescent. The peg usually enters the soil within 7–10 d and then stops elongating. The tip swells to form a pod and the embryo resumes cell division and differentiation. Similar reproductive developmental patterns are believed to occur in both *Arachis* species and interspecific hybrids, but successful seed recovery is greatly reduced for crosses. Thus, a thorough understanding of the early reproductive events during the 2–3-week period following fertilization may provide clues about interspecific hybrid failures that are commonly observed in *Arachis*.

This study was conducted to (*a*) understand the processes of early embryo growth and development in four Arachis species, two *A. hypogaea* cultivars and their hybrids and (*b*) identify parental compatibilities in reciprocal crosses of *A. hypogaea*. For comparisons of reproductive development, the species used in this study were selected based on observations reported in Stalker *et al.* (1991) to represent taxa for which reciprocal hybrids can be obtained (*A. duranensis* Krapov. and W. C. Gregory), most reciprocals can be obtained but the wild species is the better female parent (*A. batizocoi* Krapov. and W. C. Gregory), the wild species is only successful as the male parent (*A. cardenasii* Krapov. and W. C. Gregory), and no mature hybrids have been obtained with *A. hypogaea* (*A. glandulifera* Stalker). The results will provide a more detailed understanding of critical stages leading to embryo abortion in peanut interspecific hybrids.

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MATERIALS AND METHODS

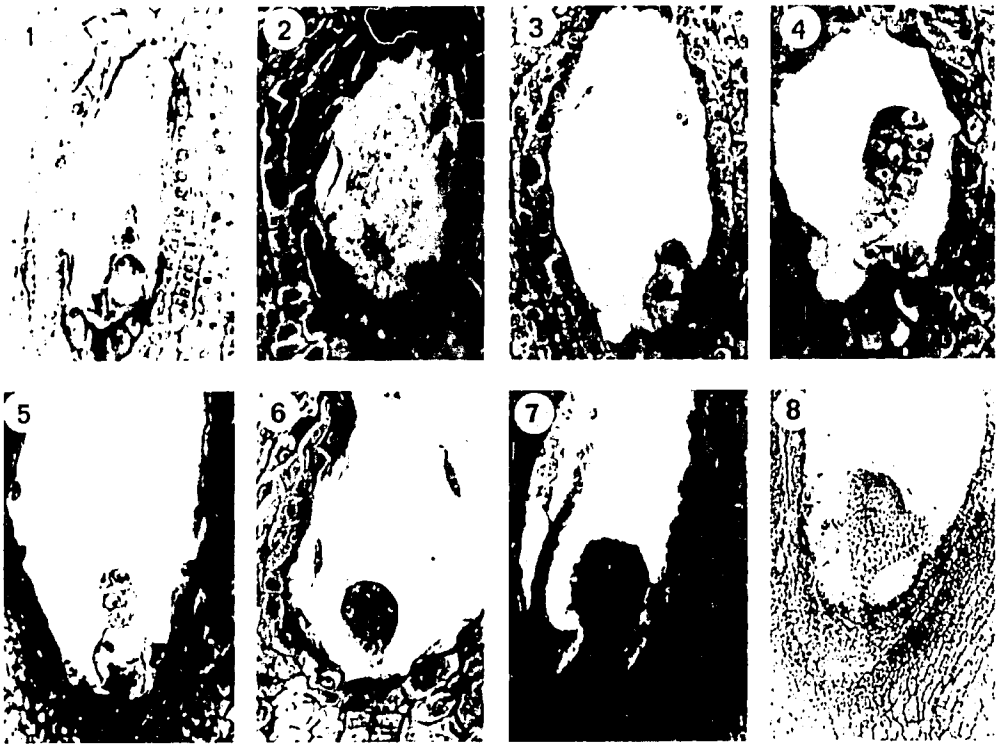
The plant material used in this study included two *A. hypogaea* ($2n = 4x = 40$) cultivars and four diploid ($2n = 2x = 20$) *Arachis* species. The cultivars were NC 6 (subsp. *hypogaea* var. hypogaea, a virginia market type) and Argentine (subsp. *fastigiata* var. vulgaris, a spanish market type). The diploids included two 'A' genome species, the annual *A. duranensis* (GKP 10038 1.1., PI 262133), and the perennial *A. cardenasii* (GKP 10017, PI 262141); one 'B' genome annual species, *A. batizocoi* (K 9484, PI 298639); and a 'D' genome annual species, *A. glandulifera* (GKSSC 30098, PI 468341). The plants were grown in wooden boxes filled with a 1:1:1 mixture of sand, soil, and a commercial potting mixture (Metromix-Grace Sierra Horticultural Products Co., 1001 Yosemite Dr., Milpitas, CA 95035, USA) in the greenhouse facilities at N. C. State University, Raleigh, North Carolina, USA. Calcium in the form of commercial limestone was applied to the soil mixture at the time of soil preparation. Plants were fertilized once a month with a soluble 15-30-15 commercial fertilizer.

TABLE 1. *Description of embryo growth standards (Pattee and Mohapatra, 1987)*

Embryo growth stage	Description
D2	Linear 2-tiered, 2-celled proembryo
D3	Linear 3-tiered, 3-celled proembryo
D4	Linear 4-tiered, 4-celled proembryo
D5	Linearly packed 8-celled proembryo
1-0	Enlargement of 2 basal tiers of cells of the proembryo initiating suspensor formation
1-1	Rapid division of the apical tiers of proembryo with differentiation of proembryo and suspensor
1-2	Embryo becomes a spherical mass of small isodiametric cells with a distinguishable suspensor
1-3	Early globular embryo
1-4	Early heart embryo

Crossing programmes were conducted in the greenhouse from Jun. to Sep. 1989, Jul. to Sep. 1990, and Jul. to Sep. 1991. Crosses between the two cultivars and four diploid species were made in reciprocal, resulting in a total of 16 combinations. On the day before anthesis the flower buds were emasculated between 1600 and 1900 h. Hand-pollination was completed the next morning between 0800 and 1000 h. Between 15 and 20 pollinated flowers were tagged with numbered metal bands for sampling at 1, 2, 3, 4, 5, 6, 7, 14, and 21 d after pollination. At least ten pollinated flowers were collected at each sampling stage, resulting in 90 samples per cross. A minimum of five self-pollinated flowers were collected for each genotype for the nine sampling times to use as controls. Most pegs had penetrated into the soil by 10 d after pollination, and pegs that were aerial at 14 or 21 d after pollination were considered to be aborted.

All harvested samples were fixed in FAA, dehydrated in



FIGS 1–8. Comparative embryo developmental stages in representative crosses of peanut as illustrated by *A. duranensis* and *A. hypogaea* hybrids. FIG. 1. A 1-d-old embryo sac of *A. duranensis* × NC 6 showing an egg cell with a basal vacuole indicating nonfertilization. ×64·4. FIG. 2. A starch-filled 1-d-old embryo sac of NC 6 × *A. duranensis* indicating nonfertilization. ×64·4. FIG. 3. A 3-tiered D3 stage proembryo at day 7 in *A. duranensis* × NC 6. ×64·4. FIG. 4. A 1-1 stage proembryo at day 7 in NC 6 × *A. duranensis*. ×64·4. FIG. 5. A 1-0 stage proembryo at day 14 in *A. duranensis* × NC 6. FIG. 6. A 1-2 stage embryo at day 14 in NC 6 × *A. duranensis*. ×64·4. FIG. 7. A 1-3 stage embryo at day 21 in *A. duranensis* × NC 6. ×32·2. FIG. 8. A 1-4 stage heart-shaped embryo at day 21 in NC 6 × *A. duranensis*. ×16·1.

TABLE 2. *Percentage of ovules and embryos in various reproductive developmental stages in the selfed tissues of wild* Arachis *species*

DAP*	Ovules: Number observed	Ovules: Unfertilized (%)	Ovules: Fertilized (%)	Proembryos (%): Developing	Proembryos (%): Quiescent	Proembryos (%): Aborted	Maximum embryo growth
A. batizocoi							
1	21	76·2	23·8	0	0	0	D1
2–3	33	6·1	6·1	75·8	0	12·0	D4
4–7	38	2·6	0	0	68·5	28·9	D4
14	18	0	0	0	77·8	22·2	D4
21	19	0	0	4·3	52·2	43·5	1-3
A. cardenasii							
1	26	100·0	0	0	0	0	—
2–3	22	91·0	0	9·0	0	0	D3
4–7	71	97·2	0	0	2·8	0	D3
14‡	—	—	—	—	—	—	—
21‡	—	—	—	—	—	—	—
A. duranensis							
1	16	100·0	0	0	0	0	—
2–3	31	51·6	3·2	45·2	0	0	D3
4–7	47	8·5	0	19·1	19·1	57·3	1-1
14	8	25·0	0	12·5	25·0	37·5	1-3
21	13	0	0	53·9	0	46·1	Heart
A. glandulifera							
1	27	92·6	7·4	0	0	0	D1
2–3	28	21·4	0	75·0	0	3·6	D4†
4–7	47	25·5	0	21·3	10·6	42·6	1-0
14	12	8·3	0	0	25·0	66·7	1-0
21	10	0	0	20·0	0	80·0	1-3

* DAP, Days after pollination.
† Starch observed in several embryo sacs.
‡ No reproductive tissues found.

an alcohol series, and embedded in paraffin. Serial sections of 10 μm thickness were made, mounted on slides, differentially stained with a safranin-O/fast green/orange G series, and observed under a light microscope as described by Pattee and Stalker (1992*a*). Specimens damaged during preparations were discarded. The embryo growth standards used were those of Pattee and Mohapatra (1987) (Table 1). Ovules were considered as unfertilized when a prominent egg cell with a basal vacuole was observed (Fig. 1) or starch grains were present in an embryo sac without an embryo (Fig. 2). Ovules containing one- or two-celled embryos were classified as fertilized, while those containing normally growing embryos were classified as developing. When embryos were at the same embryological growth stages over a relatively long period of time [usually between 4 and 14 d (pegs in soil) after pollination], they were considered as quiescent. The embryos were classified as aborted if they exhibited structural disorganization, disintegration of cells, or had consistently slower growth as compared to normally developing embryos collected at the same age.

A χ^2 test for independence of parental species effects *vs.* events of early reproductive ontogeny and embryo developmental stages was performed to support the conclusions. Data was pooled for days 2 and 3 and days 4–7 for this paper because no significant changes were observed in reproductive development within the two time frames. Yates correction factor was applied in the calculation of χ^2 values for all 2×2 contingency tables. χ^2 values were calculated using the data presented in Tables 2–7 based on the numeric data sets for each cross and compared to respective values at corresponding degrees of freedom to determine the statistical significance of the observed values.

RESULTS

The observations will be presented in two parts—those involving the six parental species which are considered as controls and those from the 16 crosses. The observations refer to early reproductive ontogeny on a time scale up to 21 d after pollination.

Early reproductive ontogeny in parental species

A low percentage of fertilized ovules, ranging from 0 to 7·4%, was observed up to 24 h after selfing for all genotypes except *A. batizocoi*, which contained 23·8% fertilized ovules (Tables 2 and 3). By day 3, embryo sacs with developed D3 or D4 stage proembryos were observed, indicating that fertilization must have occurred between 24 and 48 h after selfing in both the wild and cultivated species. Significant differences for unfertilized ovules and developing embryos were observed between NC 6 and Argentine ($P = 0·05$; $\chi^2 = 4·44$) where NC 6 contained more developing proembryos (75·6%) than Argentine (21·2%), indicating that reproductive development during the first 3 d occurred faster in

TABLE 3. *Percentage of ovules and embryos in various reproductive developmental stages in the selfed tissues of* A. hypogaea

DAP*	Ovules: Number observed	Ovules: Unfertilized (%)	Ovules: Fertilized (%)	Proembryos (%): Developing	Proembryos (%): Quiescent	Proembryos (%): Aborted	Maximum embryo growth
NC 6							
1	37	94·6	5·4	0	0	0	D2†
2–3	45	15·6	0	75·6	0	8·8	D4
4–7	85	8·2	0	37·7	15·3	38·8	1-0
14	19	0	0	63·2	0	36·8	Heart
21	16	0	0	62·5	0	37·5	Heart
Argentine							
1	35	97·1	0	2·9	0	0	D2†
2–3	33	45·5	30·3	21·2	0	3·0	D3
4–7	55	18·2	0	25·5	27·3	29·0	1-0
14	27	11·1	0	18·5	0	70·4	1-3
21	34	2·9	0	44·2	0	52·9	Heart

* DAP, Days after pollination.
† Starch observed in several embryo sacs.

TABLE 4. *Percentage of ovules and embryos in various reproductive developmental stages in the crosses between* A. duranensis *and* A. hypogaea

DAP*	Ovules: Number observed	Ovules: Unfertilized (%)	Ovules: Fertilized (%)	Proembryos (%): Developing	Proembryos (%): Quiescent	Proembryos (%): Aborted	Maximum embryo growth
A. duranensis × NC 6							
1	26	76·9	23·1	0	0	0	D1
2–3	36	58·3	11·1	30·6	0	0	D3
4–7	98	55·2	7·1	2·0	4·1	31·6	1-0
14	15	0	0	6·7	13·3	80·0	1-3
21	17	0	0	29·4	0	70·6	Heart
NC 6 × *A. duranensis*							
1	30	93·3	0	6·7	0	0	D2†
2–3	40	70·0	5·0	25·0	0	0	D2†
4–7	70	34·3	0	37·1	22·9	5·7	1-2
14	18	44·5	0	11·1	44·4	0	1-3
21	8	12·5	0	87·5	0	0	Heart
A. duranensis × Argentine							
1	16	87·5	12·5	0	0	0	D1
2–3	47	57·5	8·5	34·0	0	0	D2
4–7	72	38·8	1·4	2·8	13·9	43·1	1-0
14	6	0	0	0	0	100·0	D4‡
21	6	0	0	0	0	100·0	D4‡
Argentine × *A. duranensis*							
1	24	95·8	0	4·2	0	0	D2†
2–3	38	57·9	0	42·1	0	0	D4†
4–7	94	40·4	0	17·0	26·6	16·0	1-2
14	4	0	0	0	0	100.0	D4‡
21	10	0	0	0	0	100.0	1-0‡

* DAP, Days after pollination.
† Starch observed in several embryo sacs.
‡ Classified as aborted.

NC 6 (Table 3). Significant ($P = 0{\cdot}05$, $\chi^2 = 34{\cdot}05$) differences were observed for unfertilized ovules *vs.* developing proembryos among diploid species where a range from 9% for *A. cardenasii* to about 75% for *A. batizocoi* and *A. glandulifera* was observed (Table 2). Several embryo sacs of *A. glandulifera* also contained a few starch grains at day 3

Embryological observations between 4 and 7 d after selfing indicated different proembryo growth progressions among the *Arachis* species (Table 2). *Arachis cardenasii* had only 2·8% of its ovules fertilized whereas the percentage was at least 75% in the other species (Tables 2 and 3). Variation in maximum proembryo sizes was also observed by day 7, where *A. cardenasii* and *A. batizocoi* were at the D3 and D4 stages, respectively, but other species were at the

TABLE 5. *Percentage of ovules and embryos in various reproductive developmental stages in the crosses between* A. cardenasii *and* A. hypogaea

DAP*	Ovules: Number observed	Ovules: Unfertilized (%)	Ovules: Fertilized (%)	Proembryos (%): Developing	Proembryos (%): Quiescent	Proembryos (%): Aborted	Maximum embryo growth
A. cardenasii × NC 6							
1	16	93·8	6·2	0	0	0	D1
2–3	23	69·6	17·4	13·0	0	0	D3
4–7	57	73·7	7·0	1·8	7·0	10·5	D4
14	5	80·0	20·0	0	0	0	D1
21‡	—	—	—	—	—	—	—
NC 6 × *A. cardenasii*							
1	20	100·0	0	0	0	0	—
2–3	46	26·1	10·9	63·0	0	0	D4†
4–7	74	23·0	0	23·0	21·6	32·4	1-1
14	22	0	0	40·9	18·2	40·9	1-3
21	9	0	0	55·6	0	44·4	Heart
A. cardenasii × Argentine							
1	21	100·0	0	0	0	0	—
2–3	57	84·2	7·0	8·8	0	0	D3
4–7	63	90·5	0	3·2	0	6·3	D3
14	16	56·3	0	0	0	43·7	D3
21	5	40·0	0	0	0	60·0	D4
Argentine × *A. cardenasii*							
1	18	100·0	0	0	0	0	—
2–3	33	48·5	0	51·5	0	0	D3†
4–7	75	25·3	0	30·7	12·0	32·0	1-0
14	24	4·2	0	12·5	8·3	75·0	1-0
21	10	0	0	40·0	0	60·0	Heart

* DAP, Days after pollination.
† Starch observed in several embryo sacs.
‡ No reproductive tissues found.

1-0 to 1-1 stages. More than 25% of all proembryos aborted (except in *A. cardenasii* which did not have enough fertilized ovules to detect abortion) between days 4 and 7. Many of the non-aborted proembryos were now in a quiescent phase as pegs were rapidly elongating.

Pegs had penetrated the soil by 14 d for all species except *A. cardenasii*, for which no reproductive tissues were recovered. Both *A. hypogaea* cultivars had re-initiated growth, and significant differences for developing and aborted embryos were observed ($P = 0{\cdot}05$, $\chi^2 = 6{\cdot}31$). NC 6 contained a larger percentage of developing embryos (63·2%) than Argentine (18·5%). For diploid species, developing embryos were observed in *A. duranensis*, whereas in *A. batizocoi* and *A. glandulifera* they remained quiescent or were obviously aborting. The maximum embryo growth observed for Argentine and *A. duranensis* was the 1-3 stage, whereas several heart-shaped embryos were observed in NC 6.

At day 21, developing embryos were observed for all species (except *A. cardenasii*), although most embryos (52·2%) for *A. batizocoi* were still quiescent. This indicated that most pegs had ceased growth and that pods were forming. The pegs in *A. batizocoi* were somewhat longer than other species, which caused a delay in pod formation. Although half of the total number of embryos were aborted in *A. hypogaea* (Table 3), heart-shaped embryos were also observed in both cultivars. Similar results were seen in *A. duranensis*. Only early globular embryos were observed in *A. batizocoi* and *A. glandulifera*, and both species had a high frequency of abortion (Table 2).

Early reproductive ontogeny in interspecific crosses

'A' genome annual species: A. duranensis × A. hypogaea *and reciprocal.* Crosses involving *A. duranensis* as the female parent with either NC 6 or Argentine had 23 and 12% fertilized ovules, respectively, by 1 d after pollination. At day 3 there were 30–34% developing proembryos in both crosses (Table 4). Although the percentage of aborted proembryos was higher in *A. duranensis* × NC 6 than in selfed *A. duranensis*, a similar pattern of development was observed in hybrids and selfs (Tables 2 and 4; Figs 5 and 7). Maximum embryo development was to a heart-shape by day 21. The largest embryos observed for the cross *A. duranensis* × Argentine was the D4 stage, however, and all embryos appeared to abort by day 14. When *A. duranensis* was the female parent, it appeared that delayed fertilization, slow proembryo growth and early proembryo abortion (between 4 and 7 d) limited the numbers of hybrids (Figs 1 and 3), but for at least *A. duranensis* × NC 6, hybrids would have been expected if the seeds had remained in the soil until maturity.

In the reciprocal hybrid, NC 6 × *A. duranensis* showed normal embryo development and no abortion at either days

TABLE 6. *Percentage of ovules and embryos in various reproductive developmental stages in the crosses between* A. batizocoi *and* A. hypogaea

DAP*	Ovules: Number observed	Ovules: Unfertilized (%)	Ovules: Fertilized (%)	Proembryos (%): Developing	Proembryos (%): Quiescent	Proembryos (%): Aborted	Maximum embryo growth
A. batizocoi × NC 6							
1	16	100·0	0	0	0	0	
2–3	45	42·2	2·2	55·6	0	0	D3
4–7	84	32·1	1·2	4·8	27·4	34·5	D4
14	13	7·7	0	7·7	15·4	69·2	D5
21	15	13·3	0	20·0	0	66·7	1-3
NC 6 × *A. batizocoi*							
1	19	100·0	0	0	0	0	—
2–3	38	44·8	2·6	50·0	0	2·6	D3†
4–7	72	33·3	0	29·2	16·7	20·8	1-2
14	20	20·0	0	20·0	10·0	50·0	1-2
21	6	0	0	33·3	0	66·7	1-3
A. batizocoi × Argentine							
1	24	87·5	12·5	0	0	0	D1
2–3	32	78·1	3·1	18·8	0	0	D2
4–7	77	48·1	5·2	6·5	29·9	10·3	D4
14	22	13·6	0	4·6	40·9	40·9	D5
21	21	23·8	0	28·6	0	47·6	1-3
Argentine × *A. batizocoi*							
1	22	95·5	4·5	0	0	0	D1
2–3	42	16·7	0	83·3	0	0	D4†
4–7	70	22·9	0	37·1	24·3	15·7	1-1
14	15	0	0	20·0	20·0	60·0	1-2
21	13	23·1	0	15·4	0	61·5	1-3

* DAP, Days after pollination.
† Starch observed in several embryo sacs.

14 or 21 (Table 4; Figs 4, 6 and 8). In this cross, 87·5% of the embryos were in the late globular to heart shape stages at 21 d after pollination (Table 4; Fig. 8). Although Argentine × *A. duranensis* also contained early globular embryos by 7 d after pollination, they all aborted by day 14 (Table 4).

'*A*' *genome perennial species*: A. cardenasii × A. hypogaea *and reciprocal*. When *A. cardenasii* was the female parent, a high percentage of unfertilized ovules was observed throughout all sampling times (Table 5). Although 8·8% of the proembryos were developing in the cross *A. cardenasii* × Argentine and 13·0% in *A. cardenasii* × NC 6 by day 3, most of them aborted by day 7. The surviving proembryos continued very slow growth (maximum D4 stage in *A. cardenasii* × Argentine) at 21 d after pollination; this was considered as abnormal and they were classified as aborted.

The reciprocal crosses showed delayed fertilization beyond day 1 but, by day 3, NC 6 × *A. cardenasii* and Argentine × *A. cardenasii* had 63·0 and 51·5% developing proembryos, respectively. Between 4 and 7 d, both crosses showed a similar growth response with developing, quiescent and aborting proembryos (Table 5). A relatively large percentage of developed embryos were observed when either cultivar was used as a female parent, and heart-shaped embryos were observed at day 21.

'*B*' *genome annual species*: A. batizocoi × A. hypogaea *and reciprocal*. The crosses of *A. batizocoi* × NC 6 or Argentine contained few fertilized ovules 1 d after pollination (Table 6). The cross *A. batizocoi* × NC 6 showed significantly more (55·6%) developing embryos by day 3 than crosses with Argentine (18·8%). Many proembryos in both crosses were in a quiescent phase between days 4 and 7. At day 14, 40·9% of the embryos aborted in *A. batizocoi* × Argentine, whereas in *A. batizocoi* × NC 6 the percentage was higher at 69·2%. However, at least 20% of the embryos were at an early globular stage and apparently developing by day 21 in both crosses.

The cross Argentine × *A. batizocoi* had a higher fertilization percentage by day 3 and more developing embryos than NC 6 × *A. batizocoi*. Abortion initiated between 4 and 7 d in both crosses. By day 14, 50·0% of the embryos had aborted in NC 6 × *A. batizocoi* and 60·0% in Argentine × *A. batizocoi*. Both crosses had 20·0% developing embryos at day 14 and maximum growth to the late globular stage by day 21 (Table 6).

'*D*' *genome annual species*: A. glandulifera × A. hypogaea *and reciprocal*. Fertilization occurred earlier in the cross *A. glandulifera* × NC 6 than in the cross with Argentine (Table 7), but by day 3 these differences were not significant. Abortion initiated between 4 and 7 d, with 29·3% in *A. glandulifera* × Argentine, and 8·4% in *A. glandulifera* × NC 6 by day 7. By 21 d, about 50% of the embryos aborted, but a few also appeared to be developing in both crosses (Table 7). Growth was slower in *A. glandulifera* × Argentine than in *A. glandulifera* × NC 6, which had small globular embryos.

TABLE 7. *Percentage of ovules and embryos in various reproductive developmental stages in the crosses between* A. glandulifera *and* A. hypogaea

DAP*	Ovules: Number observed	Ovules: Unfertilized (%)	Ovules: Fertilized (%)	Proembryos (%): Developing	Proembryos (%): Quiescent	Proembryos (%): Aborted	Maximum embryo growth
A. glandulifera × NC 6							
1	23	86·9	13·1	0	0	0	D1†
2–3	37	51·4	2·7	45·9	0	0	D4
4–7	84	61·9	2·4	3·6	23·8	8·4	D4
14	18	33·3	0	33·3	16·7	16·7	1-0
21	28	32·1	0	14·3	3·6	50·0	1-3
NC 6 × *A. glandulifera*							
1	21	80·9	19·1	0	0	0	D1†
2–3	28	42·9	0	57·1	0	0	D4†
4–7	90	35·6	0	17·8	17·8	28·8	1-1
14	20	15·0	0	35·0	30·0	20·0	1-3
21	5	0	0	60·0	40·0	0	Heart
A. glandulifera × Argentine							
1	16	100·0	0	0	0	0	—
2–3	47	76·6	2·1	19·2	0	2·1	D3
4–7	82	47·5	0	9·8	13·4	29·3	D5
14	12	33·3	0	0	0	66·7	D4
21	14	50·0	0	7·1	0	42·9	1-2
Argentine × *A. glandulifera*							
1	25	96·0	0	4·0	0	0	D2†
2–3	38	10·5	7·9	76·3	0	5·3	D4†
4–7	72	15·3	0	23·6	16·7	44·4	1-0
14	8	0	0	12·5	0	87·5	1-2
21	13	7·7	0	23·1	38·5	30·7	1-3

DAP, Days after pollination.
† Starch observed in several embryo sacs.

The reciprocal cross Argentine × *A. glandulifera* had 4·0 % of the ovules with developing proembryos by day 1, whereas fertilization was delayed in the NC 6 crosses. However, by day 3, 57 % of the NC 6 females were fertilized (Table 7). A few embryo sacs contained starch in addition to developing proembryos up to 2 d after pollination in both crosses. Developing heart-shaped embryos were observed in both Argentine × *A. glandulifera* and in NC 6 × *A. glandulifera* at day 21 (Table 7).

Overall, crosses of *A. hypogaea* cultivars NC 6 and Argentine as female parents with the four diploid *Arachis* species showed no significant differences for fertilized ovules at day 1 ($P = 0{\cdot}05$, $\chi^2 = 0{\cdot}47$) or for developing embryos at day 7 ($P = 0{\cdot}05$, $\chi^2 = 1{\cdot}18$). However, significant differences were observed at days 14 ($P = 0{\cdot}05$, $\chi^2 = 9{\cdot}97$) and 21 ($P = 0{\cdot}05$, $\chi^2 = 9{\cdot}91$) for developing and aborted embryos. Hybrids involving *A. hypogaea* subsp. *fastigiata* cv. Argentine as the female parent contained more aborted embryos at days 14 and 21 than did *A. hypogaea* subsp. *hypogaea* cv. NC 6 (Tables 4–7).

DISCUSSION

All *Arachis* species produce underground pods, which makes monitoring reproductive development difficult in interspecific crosses where embryos often abort. Although pegs may appear healthy several weeks following fertilization, embryos of most interspecific crosses exhibit slow growth during this period which leads to abortion at a later stage. Smith (1954) reported that even in self-pollinated *A. hypogaea*, the pod/flower ratio is low, and for interspecific crosses the number of seeds obtained is further restricted because of several incompatibility barriers.

The results of this study indicated that a delay in fertilization beyond 24 h was common in both self- and cross-pollinated flowers. A similar observation was noted by Pattee and Stalker (1992*a*) in reciprocal crosses of cultivar NC 6 with *A. duranensis* and *A. stenosperma*. If the egg is usually fertilized 12–18 h after pollination, as reported by Smith (1956) for *A. hypogaea*, an extended delay in the process could disrupt normal developmental sequences. Conceivably, the delay in fertilization in this experiment *vs.* the report of Smith, could have resulted from different temperatures or other environmental conditions under which plants were grown; but tissues were collected over a long period during several summers and the cause of delayed fertilization is not clearly understood.

The observations indicated that detectable signs of abortion initiate in peanut crosses between days 4 and 7 after pollination. This supports the earlier report of Halward and Stalker (1987*b*) who observed that many interspecific hybrid embryos abort within 10 d after pollination.

Several processes were observed which may lead to failure to obtain hybrids from peanut crosses, including (*a*) no fertilization, (*b*) delayed fertilization, (*c*) inability of quiescent proembryos to resume growth after the peg enters the

soil, and (*d*) slow growth of proembryos. For example, in female *A. cardenasii* crosses, it appeared that lack of fertilization may be the main cause for hybridization failures. In female *A. batizocoi* crosses it appeared as though delayed fertilization coupled with the inability of quiescent embryos to resume growth after soil penetration were the causes for high rates of embryo abortion.

Several clues were provided on the applicability of different strategies to obtain peanut interspecific hybrids. First, NC 6 appeared to be a better female parent than Argentine. In this study, embryo sacs of crosses using NC 6 as female parent contained predominantly D3 or D4 stage embryos along with starch grains even up to 3 d after pollination; in contrast, the amount of starch granules was depleted in Argentine crosses. Pattee and Stalker (1991) hypothesized that starch granules in the embryo sac act as an energy source for the developing proembryo during the initial 3 d after pollination. Thus, sustained availability of more starch for a day longer in NC 6 *vs.* Argentine may partially be responsible for the better performance of NC 6 as female parent.

Secondly, *A. hypogaea* appears to be a better female than male parent for interspecific hybridization. Use of wild *Arachis* species as female parents resulted in high frequencies of unfertilized ovules or aborted embryos. Based on crosses reported with many other crop species, it was not unexpected that higher ploidy cultivated genotypes were superior to the diploid wild species (Hadley and Openshaw, 1980; Singh, Moss and Smartt, 1990). However, it may be worthwhile to produce lines with the nuclear genome of *A. hypogaea* in the cytoplasm of different *Arachis* species to exploit useful maternal traits.

Interspecific peanut hybrids are usually difficult to obtain even when *A. hypogaea* is used as the female parent because a large number of pollinations are necessary to obtain even a single hybrid (Stalker *et al.*, 1991). Reciprocal crosses are nearly impossible for many desirable crosses in section *Arachis* (Stalker, 1985; Stalker *et al.*, 1991). The limited number (20–25) of pollinations made for each sampling time in this study resulted in a few normally developing embryos and may partially explain why no developing embryos were observed at days 14 and 21 between Argentine and *A. duranensis* whereas the same cross was reported as successful by Stalker *et al.* (1991). Even though *A. glandulifera* embryos seemed to show normal growth until 21 d after pollination, no viable hybrids have been obtained (Stalker *et al.*, 1991). The conclusion is that these embryos abort relatively late in the reproductive cycle when embryo culture techniques could be successfully used to obtain mature plants.

In summary, the observations of this study, coupled with those of Pattee and Stalker (1991, 1992*a*, *b*), provide details of various factors affecting embryo growth and development of crosses in section *Arachis*. Increasing the number of pollinations per cross, using the cultivated species as the female parent, utilizing different *A. hypogaea* varieties, and embryo rescue techniques are suggested to improve the probability of obtaining interspecific hybrids in *Arachis*.

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