EMBRYOGENESIS IN RECIPROCAL CROSSES OF ARACHIS HYPOGAEA cv NC 6 WITH A. DURANENSIS AND A. STENOSPERMA

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Improvement of agronomic and quality factors in Arachis hypogaea L. through interspecific hybridization with wild Arachis species is restricted because of reproductive barriers including genetic incompatibility. A description of embryogenesis and embryo abortion in reciprocal crosses between wild and cultivated Arachis species should clarify some of these reproductive barriers. This study documents embryogenesis in the diploids A. duranensis (K 7988) and A. stenosperma (HLK 410) in reciprocal crosses with A. hypogaea cv NC 6. A significant parental effect was observed among crosses. When NC 6 was used as the female parent in crosses with both diploid species, embryos developed at a near normal rate, while embryos in the reciprocal crosses showed retarded rates. Differences in embryo developmental morphology were not observed between the two wild species. When A. duranensis was used as a female parent, however, embryos aborted at a higher frequency. In contrast, A. stenosperma had delayed fertilization, but initial embryo development was much faster and by day 5 had attained the same level of development as A. duranensis. These observations illustrate that as attempts are made to utilize the genetic resources of Arachis, different approaches will be needed to overcome the multiplicity of reproductive barriers that restrict introgression of potentially desirable traits to cultivated peanuts.

Introduction

Reproductive barriers restrict interspecific hybridization and subsequent introgression of potentially useful genes from wild Arachis species to A. hypogaea L. Our understanding of the reproductive barriers in interspecific crosses of Arachis is limited. To overcome these barriers we need to define first embryogenesis in compatible, marginally compatible, and incompatible crosses between wild and cultivated Arachis species. Morphological and ontogenetic comparisons of ovary development between cultivated and wild species (Pattee et al. 1991; Pattee and Stalker, unpublished data) showed significant differences in ovary component structure, peg growth onset, peg growth rate, and starch grain presence in the embryo sac. Hypotheses have been developed concerning reproductive incompatibilities between Arachis species based on these observed differences, but further investigation is needed.

Embryology and associated anatomical changes in cultivated A. hypogaea have been investigated by Reed (1924), Banerji (1938), Smith (1950, 1956a, 1956b), Conagin (1957), Gerassimova-Navashina (1959), Periasamy and Sampoornam (1984), and Pattee and Mohapatra (1987). Embryo development in other Arachis species has been reported by Halward and Stalker (1987a) and Pattee et al. (1991), who found differences in growth rates between cultivated and wild taxa; but Bharathi and Murty (1984) found no significant differences. Limited information on embryogenesis and embryo abortion in reciprocal crosses between wild and cultivated Arachis species has not been published.

This study was undertaken to document embryogenesis and embryo abortion using A. duranensis Krap. et Greg. nom. nud. and A. stenosperma Greg. et Greg. nom. nud. in reciprocal crosses with A. hypogaea cv NC 6. A comparative basis is thus provided for (1) extending the knowledge on reproductive efficiency in marginally compatible crosses between Arachis species (Pattee and Stalker 1992), where both nonfertilization and embryo abortion contribute to low reproductive efficiency, and (2) comparing embryo-stage attainment in early developmental stages in the crosses with that in selfed ovaries.

Material and methods

Two diploid (2n = 2x = 20) species, Arachis duranensis (K 7988, PI 219823) and A. stenosperma (HLK 410, PI 338280), were used to make reciprocal crosses to the tetraploid (2n = 4x = 40) A. hypogaea cv NC 6, a large-seeded virginia type peanut. Arachis duranensis is an annual and was originally collected in northern Argentina. Arachis stenosperma is a perennial and was originally collected on the coast of Brazil. Both wild species have a similar A genome. Plants were grown in a greenhouse at North Carolina State University, Raleigh, North Carolina 27695-7625.
University from May through July, 1989, and April through July, 1990, using boxes filled with sand, commercial potting mixture, and top soil (1:1:1). The plants were fertilized regularly with a soluble nitrogen : phosphorus : potassium (20:20:20) fertilizer. Seventy-five grams of landplaster (U.S. Gypsum Co., Chicago, Ill. 60606) per box was applied as a source of calcium, which is necessary for embryo development.

The control flowers were allowed to self-pollinate and designated inflorescences were collected at anthesis and hand-pollinated between 8:00 AM and 8:30 AM the morning of anthesis. Thirteen flowers were presented by Pattee et al. (1991) on comparative embryo growth rates in Arachis duranensis and A. stenosperma, between A. stenosperma and NC 6, delayed syngamy occurred in A. stenosperma (figs. 2, 6; table 1). These data suggest that the onset of syngamy occurred just prior to D2. The typical proembryo observed at D2 and D2.5, however, is at the two-tier stage (fig. 6). Two possibilities are suggested by this inconsistency: (1) the sample size D1–1.5 was too small for the lower fertilization efficiency of A. stenosperma, and we failed to observe a fertilized ovule because of sampling error, or (2) A. stenosperma undergoes very rapid cell division following syngamy relative to A. duranensis. Both possibilities are realistic, based on the data presented by Pattee et al. (1991) on comparative embryo growth rates between A. stenosperma and A. duranensis, and indicated that A. stenosperma develops at a faster rate than does A. duranensis. Further, the observations by Pattee and Stalker (1992) on reproductive efficiency in crosses of A. duranensis with NC 6 show abortion to be a major factor in the failure to obtain seed from these crosses. Thus, abortion may also be a factor in the observed differences between these accessions of the two species. In making interpretations of these data one should remember that these species are marginally compatible and that there are no previous data with which to

<table>
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<th>Growth stage</th>
<th>A. duranensis NC 6</th>
<th>A. duranensis Self</th>
<th>A. stenosperma NC 6</th>
<th>A. stenosperma Self</th>
<th>A. hypogaea cv NC 6</th>
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**Results and discussion**

**Embryo sac comparison: anthesis vs. D1.5 (figs. 1–4)**

The differential in syngamy timing and proembryo development between the two wild species is illustrated by the comparative changes in the embryo sac of Arachis duranensis and A. stenosperma at stage D1.5 (figs. 1–4; table 1). With A. duranensis as the female parent, syngamy commences ca. 24 h after pollination and the typical proembryo attains the two-tier stage by D1.5 (fig. 1). In the reciprocal crosses the embryo in NC 6 was typically at the one-tier stage (fig. 3). Selfs of both parents (A. duranensis, NC 6) achieved syngamy prior to D2 (table 1); thus the finding of similar syngamy timing in the reciprocal crosses might be expected. The slight difference in the proembryo stage of development in the female NC 6 is thought to be the result of a slight delay in syngamy timing relative to A. duranensis. The D1 stage of *A. duranensis* had a few ovules that had just achieved syngamy while none of the ovules in NC 6 at D1 appeared to be in the process of syngamy.

In contrast to the similar timing for syngamy between *A. duranensis* and NC 6, delayed syngamy occurred in *A. stenosperma* (figs. 2, 6; table 1). These data suggest that the onset of syngamy occurred just prior to D2. The typical proembryo observed at D2 and D2.5, however, is at the two-tier stage (fig. 6). Two possibilities are suggested by this inconsistency: (1) the sample size D1–1.5 was too small for the lower fertilization efficiency of *A. stenosperma*, and we failed to observe a fertilized ovule because of sampling error, or (2) *A. stenosperma* undergoes very rapid cell division following syngamy relative to *A. duranensis*. Both possibilities are realistic, based on the data presented by Pattee et al. (1991) on comparative embryo growth rates between *A. stenosperma* and *A. duranensis*. Further, the observations by Pattee and Stalker (1992) on reproductive efficiency in crosses of *A. duranensis* with NC 6 show abortion to be a major factor in the failure to obtain seed from these crosses. Thus, abortion may also be a factor in the observed differences between these accessions of the two species. In making interpretations of these data one should remember that these species are marginally compatible and that there are no previous data with which to
make comparisons. Thus, the terms “normal” and “abnormal” can only be relative to observations from selfed parents. In these crosses it is apparent that significant differences occurred in the timing of the syngamy process and in the growth of the proembryo. An understanding of these differences is vital to our success in developing proembryo rescue techniques which overcome the incompatibilities associated with these crosses.

**Prequiescent Proembryo Development at D2.5–D5 (Figs. 5–15)**

Comparison of the hybrid proembryos (figs. 5–12) shows significant differences in development of the proembryo depending upon the female parent used in the crosses. Differences in stage of development are less apparent when comparisons are made between the D5 proembryo in the selfs (figs. 13, 14) with those of the female wild species interspecific crosses (figs. 9, 10). Comparison of proembryo development in female NC 6 crosses to selfs shows no differences (figs. 11, 12, 15).

Using the wild species as the female parent produced a proembryo that was retarded in developmental progress at stage D2.5 relative to the proembryo of the reciprocal crosses using NC 6 females (figs. 5 and 6 vs. figs. 7 and 8). By the D5 stage the difference becomes greater, at which time the female wild species proembryos are at the three-tier stage and the female NC 6 proem-
bryos are at the fully developed four-tier stage (figs. 9 and 10 vs. figs. 11 and 12). Comparison of D5 photomicrographs (figs. 9, 10) with those of Halward and Stalker (1987b) indicates attainment of a similar proembryo developmental stage at 6 d following pollination in crosses using *A. batizocoi* and *A. duranensis* females with hexaploid males. Their crosses had a similar embryo failure pattern as observed in interspecific crosses in this study. Six-day-old reciprocal crosses with the hexaploid as the female parent (Halward and Stalker 1987b) also indicated similar proembryo development to our D5 female NC 6 crosses. The retarded proembryo growth may be typical across many of the female wild species interspecific crosses in which abortion occurs prior to the heart stage of embryo development. When the wild species by hexaploid crosses were left on the plant to mature, several proembryos developed into tiny, shriveled, black seed, some of which could be successfully germinated in culture.

**COMPARATIVE EMBRYO DEVELOPMENT AT D10 (FIGS. 16–28)**

By D10, aerial and peg specimens that have penetrated the soil can be collected from reciprocal crosses. Comparative proembryo development in the aerial pegs from selfs and female wild species hybrids (figs. 16 and 17 vs. figs. 22 and 23) generally show the presence of more cells in selfed than in hybrid proembryos. This indicates a stronger, more rapidly developing proembryo...
in the selfed species than in the crosses when wild species were used as female parents at comparable days after pollination and stage of peg development. Comparisons of self (fig. 18) and female NC 6 (figs. 24, 25) aerial pegs show near equal development of the proembryo. This indicates a near-normal progression of proembryo development at this time for the female NC 6 hybrids as opposed to either retarded development or total abortion for wild species females.

Penetration into the soil by the peg tip is a major triggering event with respect to embryo development in cultivated species, but possibly not in many wild species. The *A. duranensis* and *A. stenosperma* wild species selfs did not show an onset of proembryo cell division following soil penetration (figs. 19, 20), whereas the NC 6 self proembryo had undergone development to the early globular stage (fig. 21). Female *A. stenosperma* peg tips that penetrated the soil surface had proembryos in a similar developmental stage as their selfed counterparts (figs. 26, 20). However, none of the female *A. duranensis* pegs that had penetrated the soil surface contained developing embryos. With NC 6 as the female parent with either *A. duranensis* (fig. 27) or *A. stenosperma* (fig. 28) both crosses showed normal embryo development to the globular stage; this is similar to NC 6 selfs after soil penetration at D10 (fig. 21).

**Observations on Embryo Abortion**

(Figs. 29–36)

The first stage of development at which embryo abortion could be detected varied among species. The earliest time of abortion was detected in *A. duranensis* crosses at D1.5 when the zygote was still in the one-tier stage (fig. 29). It would appear that abortion occurred because of a failure in cell division. Abortion in *A. stenosperma* crosses was also observed soon after syngamy, at stage D2.5, when the proembryo was at the two-tier stage (fig. 30). Although the lapsed time from pollination to abortion is different in the two crosses because of variation in syngamy timing, the physiological development stage is approximately the same for both species. *Arachis duranensis* is characterized by a slow cell division process following syngamy, whereas *A. stenosperma* has a rapid initial cell division to the two-tier stage. Thus, abortion initially occurs in both species at a physiological stage in which the reproductive process changes from one directed by the genetic information of the egg apparatus to a cell division process directed by the genetic information of the hybrid tissues. This can be considered the first critical physiological phase of development in hybrids. Developmental information in hybrids is apparently not properly synchronized for cell division.
to proceed in a normal progression, and abortion results.

The aborted proembryos observed at D5 (figs. 31, 32) in crosses with wild species females were all at a comparable stage of development with those observed for aborted proembryos at D1.5 in Arachis duranensis and at D2.5 in A. stenosperma. None of the aborting proembryos had progressed beyond the first critical physiological phase at D5, indicating that overcoming obstacles to early cell division may permit recovery of hybrid proembryos, or at least make it possible for hybrid tissues to continue cell divisions until the quiescent stage during peg elongation. The next critical physiological stage to be overcome has been proposed by Pattee and co-workers (Pattee and Mohapatra 1987; Pattee et al. 1988; Pattee et al. 1991) to be the resumption of cell division following the quiescent proembryo development period during peg elongation. The observed developmental stage of the aborted proembryos at D10, whether in aerial pegs or pegs that had penetrated the soil, supports their proposal. By D10 the tissues of aerial pegs may naturally begin to deteriorate and the observed aborted proembryos, at the three- or four-tier stage (figs. 33, 34) of D10 may have occurred because of this process. In pegs that had penetrated the soil, the cells of aborted embryos had undergone enlargement, but no cell division appeared to have taken place. Figures 35 and 36 represent the typical median serial sections of the examined D10 specimens from Arachis duranensis and A. stenosperma. In female A. duranensis crosses the failure to reinstate cell division upon soil penetration is a major reproductive barrier to interspecific hybridization because no developing embryos were observed beyond this stage.

Although embryo abortion is a factor in reducing reproductive efficiency in A. stenosperma crosses after soil penetration at D10, this phase is not an absolute barrier. With NC 6 as the female parent in crosses with wild species, embryo abortion was not a major factor affecting the reproductive efficiency of crosses up to D10 for pegs that penetrated the soil; aborted embryos were only observed during the early stages of development, and none was observed in any NC 6 D10 specimens. The combined results suggest that a maternal genetic effect exists controlling proembryo development.

Conclusions

Obtaining interspecific hybrids in Arachis is difficult because of a high frequency of embryo abortion. When A. hypogaea was used as the female parent, interspecific hybrids were generally easier to obtain than when it was the male parent.
Reciprocal crosses are desirable to meet several breeding objectives, however, especially when cytoplasmic effects are observed in the hybrids. Several approaches have been used to stimulate proembryos in *Arachis*, such as, nurse cultures (Moss et al. 1988; Pattee et al. 1988), ovule culture (Mallikarjuna and Sastri 1985a; Stalker and Edweda 1989), and in vivo growth regulators (Mallikarjuna and Sastri 1985b). To date, there has been limited success in achieving the goal of recovering many of the more desirable interspecific hybrids between *A. hypogaea* and distantly related *Arachis* species. Much of the problem for obtaining hybrid plants appears to be overcoming abortion at one or more of the several developmental phases that the proembryo goes through within the first few weeks after fertilization, i.e., (1) initiating cell division, (2) a quiescent phase while the peg elongates, and (3) reinitiation of growth after soil penetration. A final critical point is differentiation into a heart-shaped embryo, at which time it can be rescued in vitro (Stalker and Edweda 1989). This study has further defined the timing sequences of embryo abortion in peanut and indicates that several approaches to circumvent crossing barriers will need to be developed for individual crosses.

**Acknowledgments**

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**Literature cited**


