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Hybridization barriers among the species of *Arachis* L., namely of the sections *Arachis* (including the groundnut) and *Erectoides*

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Abstract

Some members of section *Erectoides* have been found resistant to early leaf spot disease, a serious constraint to groundnut productivity. These accessions do not cross with cultivated species. Crossing between one such diploid species, *A. paraguariensis* (ICGs 8130 and 8973) of section *Erectoides*, and the diploids, *A. batizocoi* and *A. duranensis*, and the tetraploid *A. hypogaea* (groundnut) of section *Arachis* has helped understand barriers to hybridization between sections. These crosses result in the development of normal pegs and pods, but with of ovule and embryo development restricted or non-existent. Such restricted growth and embryo abortion is found to be due to cessation of early endosperm development in *A. duranensis* (2x) × *A. paraguariensis* (2x), the non-development of endosperm beyond the coenocytic stage in *A. batizocoi* (2x) × *A. paraguariensis* (2x), and the overgrowth of nucellar tissue into the embryo sac in case of *A. hypogaea* (4x) × *A. paraguariensis* (2x).

The weak cross-compatibility between the species of two sections suggest relatively closer phylogenetic relationship between them, than with the other incompatible sections of the genus *Arachis*.

Introduction

Early leaf spot (ELS) disease caused by *Cercospora arachidocola* Hori, is a major yield-reducing factor in many groundnut-growing regions of the world. There have been many reports of sources of resistance to this disease in the cultivated groundnut (*Arachis hypogaea* L.), but the levels of resistance reported are not high or uniform across locations. Most of the resistant accessions have shown susceptibility when checked under high disease pressure (Singh et al., 1997). However, a few accessions of *A. paraguariensis* Chodat & Hassl. [ICGs 8130 (KFC 11462) and 8973 (GKP 10134)] of section *Erectoides* were found to be highly resistant to early leaf spot (Bock, personal communication 1985). This generated much interest in exploiting such exotic sources in breeding for resistance to ELS. However, it is also reported that the diploid species of section *Erectoides* are cross-incompatible with both the diploid and tetraploid (cultivated groundnut) species of section *Arachis* (Gregory & Gregory, 1979). In the study reported here an attempt was made to cross the two diploid accessions of *A. paraguariensis* (ICGs 8130 and 8973), representing the two subsp. *paraguariensis* and subsp. *capibarensis* Krapov. & W.C. Gregory (Krapovickas & Gregory, 1994) with accessions of two diploid species of section *Arachis*, *A. duranensis* Krapov. & W.C. Gregory and *A. batizocoi* Krapov. & W.C. Gregory (representing the A and B genomes respectively), and the tetraploid *A. hypogaea* (representing AABB genome). The initial pollinations produce a normal percentage of pegs and pods, like any other intrasectional crosses in genus *Arachis*, but only a few, under-developed seeds, indicating the presence of post-zygotic incompatibility. Therefore, in subsequent pollinations, the pods were harvested on different days after pollination to identify the reasons restricting ovule growth. This paper reports the results of these investigations and discusses the probable reasons restricting the growth of ovules and embryos, and the phylogenetic consequences. It further discusses the possible

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Table 1. List of *Arachis* accessions their identities, taxonomic affinity, and country of origin

ICRISAT (ICG)[a]	Species	Section	2n=	Origin
8138	*A. duranensis*	*Arachis*	20	Argentina
8956	*A. duranensis*	*Arachis*	20	Argentina
8958	*A. batizocoi*	*Arachis*	20	Bolivia
7633	*A. hypogaea*	*Arachis*	40	USA
13945	*A. hypogaea*	*Arachis*	40	Malawi
8130	*A. paraguariensis* ssp. *paraguariensis*	*Erectoides*	20	Paraguay
8973	*A. paraguariensis* ssp. *capibariensis*	*Erectoides*	20	Brazil

[a]ICRISAT groundnut number

Table 2. Crossability data between accessions of *A. duranensis*, *A. batizocoi*, and *A. hypogaea* of section *Arachis* and two accessions of *A. paraguariensis* of section *Erectoides*

Cross combinations	No. of pollinations	% pegs to pollination	% pods to pollination	% ovules to pollination	Average ovule size (mm)
A. duranensis (8183)					
×	56	54	43	2	2.7
A. para. ssp *capi.*[a] (8973)					
A. duranensis (8956)					
×	47	53	13	13	2.7
A. para. ssp *capi.*[a] (8973)					
A. batizocoi (8548)					
×	52	55	13	13	3.5
A. para. ssp *capi.*[a] (8973)					
A. hypogaea (7633)					
×	28	42	32	29	2.0
A. para. ssp *capi.*[a] (8973)					
A. duranensis (8183)					
×	43	28	19	16	2.5
A. para. ssp *para.*[b] (8130)					
A. duranensis (8956)					
×	86	46	7	2	2.5
A. para. ssp *para.*[b] (8130)					
A. batizocoi (8548)					
×	40	46	8	10	4.5
A. para. ssp *para.*[b] (8130)					
A. hypogaea (13945)					
×	12	42	25	33	2.0
A. para. ssp *para.*[b] (8130)					

[a]*A. para.* ssp *capi.* = *A. paraguariensis* subspecies *capibariensis*
[b]*A. para.* ssp *para.* = *A. paraguariensis* subspecies *paraguariensis*

in vivo and *in vitro* manipulations that could overcome such post-zygotic barriers in the establishment of hybrids.

Materials and methods

The accessions used are presented in Table 1, with their identities and taxonomic affinities.

All plants were grown in a greenhouse at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Asia Center, Patancheru (near Hyderabad), India, and first crosses involving species listed in Table 2 were made in 1990. They were repeated in the rainy season of 1991. In the crosses, species of the section *Arachis* were used as the female parent. Progenies from such diploid crosses can serve as bridge to facilitate the transfer of desirable traits to the cultivated tetraploid taxa, that is compatible with diploid species of section *Arachis*. In cross with tetraploid species, the cultivated tetraploid species would function as the recurrent parent to regain agronomic features.

The flower buds were emasculated between 1300–1500 h and pollinated the following day (0800–0900 h). Only one flower per node was pollinated. Nodes were tagged, and the success of fertilization measured by the number of pegs (gynophors). The pegs were harvested at intervals starting from 20 days to 50–60 days after pollination. Pods were removed from pegs, cleaned, and surface-sterilized. These pods were cut open checked for ovule formation. Any ovule that were found were placed on a filter paper bridges in tubes containing liquid Murashige and Skoog (MS) medium to sustain them for a week and allow them to grow. A set of ovules from such treatments were then cut open longitudinally to allow observations of the embryos' developmental stage. Thin longitudinal sections of the ovules were cut by hand and detailed anatomical observations made.

Results

Hybridization at diploid level

A. duranensis × *A. paraguariensis*

The percentage of pegs, pods, and ovules obtained from different pollinations in crosses involving two accessions of *A. duranensis* and two accessions of *A. paraguariensis* are summarized in Table 2. In these crosses, the developing ovules longitudinally cutting revealed hollow space in place of embryo sac with or without differentiated embryos. Probably the endosperm had either not been able to develop at all, or had dried up in an early growth stage, leaving the developing embryo without nutrition for further development (Figure 1a, Table 2).

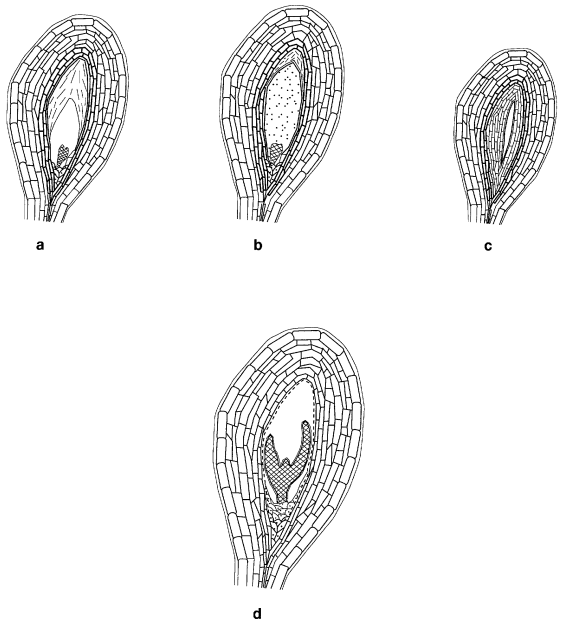

Figure 1. Schemical longitudinal section of ovule: a) *A. duranensis* × *A. paraguariensis*; b) *A. batizocoi* × *A. paraguariensis*; c) *A. hypogaea* × *A. paraguariensis* and d) self-fertilized ovule of *A. hypogaea* after 12 days of pollination.

A. batizocoi × *A. paraguariensis*

In this combination, the percentage of pegs, pods, and ovules obtained from 40 and 52 pollination made using two accessions of *A. paraguariensis* are listed in Table 2. The ovules in this combination were comparatively larger in size than those resulting from other two crosses. Most of these ovules contained a hollow cavity impregnated with fluid, with or without a differentiated or undifferentiated embryo. This indicates that the endosperm probably does not develop after a particular stage, and remains in the multinucleate coenocytic stage depriving the embryo access to nutrition due to physiological inability of endosperm (Figure 1b, Table 2).

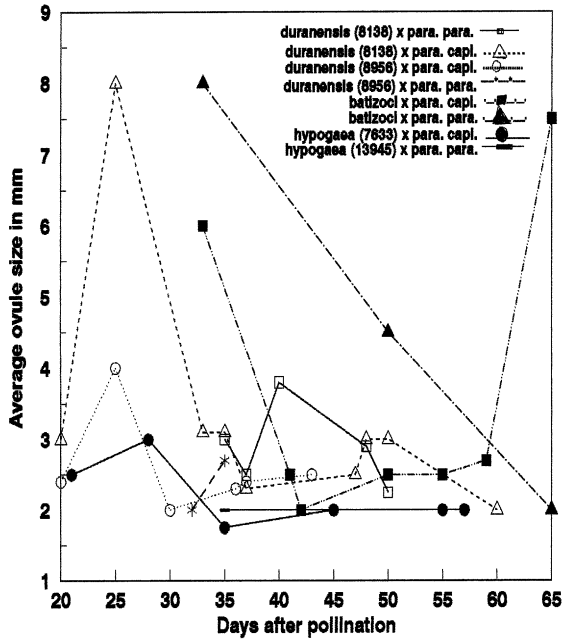

Figure 2. Ovule size after different days of pollination in various crosses studied. *A. para.* ssp. *capi.* = *A. paraguariensis* subspecies *capibariensis A. para.* ssp. *para.* = *A. paraguariensis* subspecies *paraguariensis*

Hybridization at tetraploid level

A. hypogaea (4x) × *A. paraguariensis* (2x)

The percentage of pegs, pods and ovules obtained from pollination between *A. hypogaea* and the two accessions of *A. paraguariensis* were similar to compatible crosses between diploid wild species of the section *Arachis* and *A. hypogaea*. They are summarized in Table 2. When these ovules were cut open to check the embryo development, only a line was observed in place of the embryo sac. Anatomical observations on hand-cut sections of these ovules showed the growth of nucellar tissue into the embryo sac, squeezing the embryo and restricting its development, resulting in a remnant line in place of the embryo sac, and a hardening of tissue due to its compression (Figure 1c, Table 2).

Plotting the size of ovules against days after pollination in the crosses studied indicated that in most crosses the ovule continued to grow until 25 to 32 days after pollination (Figure 2). Most of the ovules after this period were smaller than self-fertilized normally growing ovules.

Normally, following self-fertilization the embryo obtains nucellar and endospermic nourishment and thus grows vigorously for 10–12 days after pollination. It gradually occupies the total ovular space with two cotyledons, a young epicotyl and suspensor, leaving only a thin envelope of endosperm surrounding the embryo (Figure 2d).

Discussion

The successful pollination leading to fertilization, but impaired development of the resulting proembryos observed in these crosses between members of sections *Arachis* and *Erectoides* suggests a phylogenetic relationship between them relatively closer than that with the other six sections of genus *Arachis*. The members of six other sections do not even show such a degree of success on crossing (Gregory & Gregory, 1979). It may be recalled that members of section *Erectoides* and *Procumbentes* (Krapovickas & Gregory, 1994) were classified as series *Tetrafoliolatae* and *Procumbensae* of section *Erectoides* by Gregory et al. (1973). Subsequent crossability studies involving accessions from *Procumbensae* (Gregory & Gregory, 1979; Singh, 1989), and accessions of *Erectoides* (Tetrafoliolatae of Gregory et al. 1979) in the present investigation partially support the earlier taxonomic grouping because of similar crossability with section *Arachis* species.

Plotting the size of ovules against the days after pollinations in these crosses reported here indicated that in most crosses the ovule continued to grow up to 25 to 32 days after pollination, though slowly than normal self-fertilized ovules (Figure 2). However, it has to be pointed out, that some of such ovules do not develop or may even degenerate. The initial growth rate of ovules in diploid crosses was comparatively faster (though slower than normal) than in crosses with the cultivated tetraploid *A. hypogaea*. This is not unexpected because of the similar ploidy levels and greater genetic affinity between diploid wild species of the involved sections. In most cases, the ovules stops growing or even decrease in size, because they shrivel, turn brown, and degenerate. The average growth of ovules is not always linear as a function of days after pollination. In wide crosses particularly, growth is regulated by several factors that affect the embryo, endosperm, and maternal tissues. These could lead to embryo death and ovule collapse (seeds) (Raghvan, 1986). However, detailed observations on different crosses indicate a differential cross-compatibility reaction, though the final result is ovule collapse leading to the abortion of the developing embryo at an early stage. In

the crosses between diploid annual species of section *Arachis* and *A. paraguariensis*, post-zygotic incompatibility is because the endosperm that is essential to nourish the developing embryo does not develop. In crosses involving *A. duranensis*, development of the hybrid endosperm ceases at an early stage, leaving a hollow around the developing embryo and lack of nutrients. This was also observed in *Trifolium* crosses by Williams & White (1976). In crosses with *A. batizocoi*, the endosperm is not able to develop after a particular multinuclear stage, and remains fluid for a long time, probably because of low levels of cytokinin that hamper cell division in the developing endosperm, in the same way as Nesling and Morris (1979) observed in *Phaseolus* species crosses. In case of crosses between tetraploid *A. hypogaea* and *A. paraguariensis* accessions, the embryo is aborted due to the hypertrophic growth of nucellar tissue into embryo sac. This was observed by Cooper & Brink (1945) in *Lycopersicon* crosses, they called this process somatic plastic sterility. Hypertrophy of nucellar tissue crushes the embryo leaving only a vestiged remnant of the embryo sac.

These results indicate that in diploid crosses, the non-development of the endosperm, limits the development of embryos and ovules. Low levels of cytokinins in hybrid ovules have been implicated in this type of situation (Williams & White, 1976; Nesling & Morris, 1979). Therefore, as suggested by several authors (Sastri, 1984) an *in vivo* application of cytokinin at regular intervals 20 days after pollination may help to sustain the development of the endosperm and thereby the embryo. An exogenous supply of hormones to the developing embryos should result in the production of comparatively larger ovules that can either be cultured directly, or from which a fully differentiated embryo can be excised for *in vitro* rescue operation. Supplementing the hormones in culture media can further assist the development and differentiation of embryos, transforming them to plantlets, and overcoming post-zygotic incompatibility barriers. However, whether an exogenous application of cytokinin in these crosses can prevent degeneration of the embryos needs to be investigated. Nevertheless, the present investigation offers scope to create new hybrids and thus raises the possibility of introgressing desirable genes from non-crossable wild *Arachis* species into cultivated groundnut, either directly or through the hybrids established between diploid species of these sections, that could act as bridge. This process might involve several steps – such as making the hybrids fertile, and promoting meiotic recombinations through various cytogenetic manipulations to combine desirable features. These steps may or may not succeed. It is however, pertinent to pursue such techniques to produce hybrids in the combinations discussed here, so that desirable gene(s) can be transferred. Sastri and Moss (1982) and Singh et al. (1991) in the same way executed their transfer.

References

Cooper, D.C. & R.A. Brinks, 1945. Seed collapse following matings between diploid and tetraploid races in *Lycopersicon pimpinellifolium.* Genetics 30: 376–401.

Gregory, M.P. & W.C. Gregory, 1979. Exotic germplasm of *Arachis* L. interspecific hybrids. J. Heredity 70: 185–193.

Gregory, W.C., M.P. Gregory, A. Krapovickas, B.W. Smith & J.A. Yarbrough, 1973. Structure and genetic resources of peanuts. In: C.T. Wilson (Ed.), Peanut culture and uses, Amer. Peanut Res. and Educ. Asso. pp. 47–133. Stillwater, Oklahoma.

Krapovickas, A. & W.C. Gregory, 1994. Taxonomia del genera *Arachis (Leguminosae).* Bonplandia 8: 1–186.

Nelsing, F.A.V. & D.A. Morris, 1979. Cytokinin levels and embryo abortion in interspecific *Phaseolus crosses.* Z. pflanzenphysiol 91: 345–358.

Raghavan, V., 1986. Variability through wide crosses and embryo rescue. In: F. Constable & I.K. Vasil (Eds) Cell culture and somatic cell genetics of plants vol 3. Pages 613–653. Academic Press, In. San Diego, California.

Sastri, D.C., 1984. Incompatibility in Angiosperms: Significance in crop improvement. Adv. Applied Biology 10: 71–111.

Sastri, D.C. & J.P. Moss, 1982. Effect of growth regulators on incompatible crosses in genus *Arachis* L. J. Exp. Bot. 33: 1293–1301.

Singh, A.K., 1989. Exploitation of *Arachis* species for improvement of the cultivated groundnut. Part I. Transfer of genes from incompatible species of section *Erectoides* into *A. hypogaea.* ICRISAT, Patancheru 502 324, Andhra Pradesh India. (Progress report).

Singh, A.K., V.K. Mehan & S.N. Nigam, 1997. Sources of resistance to groundnut fungal and bacterial diseases: an update and appraisal. Information bulletin no 50 International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India. Pages 42.

Singh, A.K., H.T. Stalker & J.P. Moss, 1991. Cytogenetic and use of alien genetic variation in groundnut improvement. In: T. Tsuchiya & P.K. Gupta (Eds) Chromosome engineering in plants: genetic, breeding, evolution Part B. Developments in Plant Genetic and Breeding, 2B. Pages 65–77, Elsevier Science Publishers B.V. Amsterdam, The Netherlands.

Williams, E. & D.W.R. White, 1976. Early seed development after crossing of *Trifolium ambigum* and *T. repens.* N.Z.J. Bot. 14: 307–314.