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The genome donors of the groundnut/peanut (*Arachis hypogaea* L.) revisited *

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Abstract

Arachis hypogaea, the cultivated groundnut is a tetraploid with an AABB genomic constitution. The available literature on the origin of groundnut reveals that there is general agreement that the cultivated groundnut has evolved from the wild tetraploid species *A. monticola*, with which it crosses freely to produce fertile hybrids. However, the issue of actual diploid ancestors of *A. monticola* is still unresolved. Both cytogenetic and molecular evidences support *A. duranensis* being the most probable progenitor and donor of the A genome to *A. hypogaea*. For the B genome, the cytogenetic evidence suggests *A. batizocoi* to be the most probable progenitor, but the RFLP banding pattern indicates that *A. batizocoi* is more distantly related to *A. hypogaea* than other species of section *Arachis*. RFLP banding pattern indicates *A. ipaensis* to be one of the closest species to *A. hypogaea* and the possible donor of the B genome. The present article critically analyzes the available data, which suggests that until an amphidiploid is produced synthetically between *A. duranensis* × *A. ipaensis* and crossed successfully with *A. hypogaea* to produce a fertile hybrid, this issue would remain unresolved. *A. batizocoi* would remain the most probable donor of the B genome because of its directly demonstrable cytogenetic affinity.

Introduction

The genus *Arachis*, established on morphological and cross-compatibility relationships between its species has been divided taxonomically into nine sections (Krapovickas & Gregory, 1994). Generally species within sections (irrespective of ploidy level), are cross-compatible, species of different sections are cross-incompatible or only weakly cross-compatible (Gregory & Gregory, 1979). Weak cross- compatibility means that species have sufficient prezygotic compatibility to produce pegs and pods but do not produce any seeds. The groundnut (*A. hypogaea* L.) is a tetraploid with 2n = 40. It is included in the section *Arachis* with another tetraploid species, *A. monticola* Krap. et. Rig., and several wild diploid species. It is widely accepted that two diploid *Arachis* species, belonging to the section *Arachis* have produced *A. monticola*, which on domestication gave rise to the cultigen *A. hypogaea* (Gregory & Gregory, 1976). However, the issue of the actual diploid progenitors of *A. monticola* is still unresolved. Some investigators who have recently identified possible progenitors of *A. monticola* based on molecular evidence, have tended to ignore previous inferences based on cytogenetic evidence. This situation is quite understandable, since it is often difficult to reconstruct evolutionary history. One is dependent on indirect evidence to provide insight into what might have happened in the evolution of a new species. Greater weight at present tends to be given to evidence collected through use of recently developed technologies, which are presumed (often without supporting evidence) to have higher powers of resolution. Nevertheless, whenever possible experimental verification is sought, through attempted recapitulation of the evolutionary process artificially. One cannot reproduce exactly, either the path of evolution or the product, because of the very

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long time-gap and can only approximate the process and the product. In the present discussion, various hypotheses regarding possible ancestral wild diploid species that could have contributed to the evolution of *A. monticola/A. hypogaea*, are considered in the light of the evidence collected through use of phytogeographical, cytogenetical, biochemical, and molecular marker techniques. These hypotheses are also critically analyzed in respect of the artificial production of a synthetic amphidiploid similar to, and relatively cross-compatible (making allowance for the time-interval), with today's *A. monticola/A. hypogaea*. It is hoped that discussion could pinpoint the weakness and strengths of different hypotheses, help reach a tentative conclusion and review the status of the inferences which have been drawn on the ancestry of *A. hypogaea* to the present time.

Genomic constitution of the section Arachis

Based on karyomorphology and cross-compatibility relationships, three different genomes have been proposed for section *Arachis* (Smartt, 1964; Smartt et al., 1978; Singh & Moss, 1982, 1984a, b; Stalker, 1991). Most diploid species are thought to have the 'A' genome, which is characterized by the presence of a small pair of chromosomes. These species are freely cross-compatible, and produce fertile hybrids with near normal chromosome pairing and various levels of pollen and pod fertility (Smartt & Gregory, 1967; Stalker & Wynne, 1979; Gregory & Gregory, 1979). A second genome the 'B' has been identified in *A. batizocoi* Krapov. & W.C. Gregory, characterized by the presence of a pair of chromosomes with a secondary constriction proximal to the centromere. This is freely cross-compatible with other species of section *Arachis*, producing sterile hybrids with incomplete chromosome pairing in meiosis, with little or no pollen or seed fertility (Smartt et al., 1978; Singh & Moss, 1982, 1984a). Further, based on cross-compatibility and chromosome pairing studies in hybrids between these species and *A. hypogaea*, the evidence suggested that the tetraploid *A. hypogaea* has an AABB genomic constitution. Stalker (1991) identified a third genome 'D' in *A. glandulifera* Stalker, characterized by an asymmetrical karyotype and freely cross-compatible with diploid species carrying either the A or B genomes to produce sterile hybrids, (like those involving *A. batizocoi* Krapov. et W.C. Gregory), but cross-incompatible with *A. hypgaea* unlike the A and B genome species. Cross-compatibility relationships may be influenced by genetic factors and may not always be very accurate indicators of genomic relationships, and therefore require use of a large number of accessions for conclusions; however chromosome pairing in hybrids is mostly reflective of genomic homology.

Proposed hypotheses

A review of the literature indicates that there is still considerable uncertainty and speculation regarding the putative ancestors of *A. hypogaea*. It is generally believed that *A. hypogaea* originated from the wild allotetraploid species, *A. monticola*. *Arachis monticola* has a very circumscribed range and is naturally found only in north western Argentina. This region is also thought to be the centre of origin of the cultivated groundnut. *Arachis monticola* is the only other wild tetraploid species in the section *Arachis*, and is freely cross-compatible with *A. hypogaea* (Krapovickas & Rigoni, 1957; Singh & Moss, 1984a), making it most probably the direct progenitor of *A. hypogaea* (Gregory & Gregory, 1976). This has been confirmed by the production of fully fertile hybrids between these two species with normal chromosomal pairing and high pollen and pod fertility and release of advance generation/varietal material in the US (Hammons, 1970). There is, however, controversy regarding the original diploid ancestors that gave rise to the allotetraploid *A. monticola* (groundnut progenitor). *Arachis monticola* and *A. hypogaea* are both considered to be segmental allopolyploids. The ultimate test for the identification of their putative genome donors requires the production of synthetic amphidiploids from the putative ancestral diploid wild species, in various combinations, followed by the crossing of these amphidiploids with selected tetraploid cultivars. The cross between such synthetic amphidiploids and the natural amphidiploid species that produces the most fertile hybrid, would confirm a close ancestral relationship between these diploid species and *A. hypogaea*, as well as offering the possibility of transfer of useful genes from diploid species to the cultivated groundnut.

Studies of hybridization between *A. hypogaea* and the diploid species of section *Arachis* started in the early fifties and a number of species, *A. villosa* (Krapovickas & Rigoni, 1951), *A. villosa* Benth., *A. cardenasii, A. helodes* Mart. et Krapov. et Rig. *A. duranensis* and three more (Smartt & Gregory, 1967), *A. correntina* (Birk.) Krapov. et Rig. (Kumar et al., 1957), *A. villosa* and *A. duranensis* and their hybrid (Raman, 1960; Raman & Kesavan, 1965) were suc-

cessfully crossed. All these pointed to closer genetic affinity of these taxa with *A. hypogaea*. Among these, except *A. duranensis*, the rest were geographically dispersed widely. However, Gregory and Gregory (1976) were the first to suggest *A. duranensis* Krapov. et W.C. Gregory (annual) and *A. cardenasii* Krapov. et W.C. Gregory (perennial) as possible cultigen progenitors based on their long experience with wild *Arachis* species in South America, on morphological similarities and interspecific cross-compatibilities. It was also based on the presumption that a perennial and an annual species of *Arachis* were involved in the evolution of *A. hypogaea*.

Smartt et al. (1978) advanced the possibility of species like *A. batizocoi* and *A. cardenasii*, which have many characters that might be expected to occur in genome donors to *A. hypogaea* are possible ancestors. Their candidacy could also be supported on morphological and cytogenetical grounds, and most importantly by the fact that *A. batizocoi* was then the only species known with the B genome and that it was crossable with *A. hypogaea*, while there were many with the A genome, and that hybrids involving *A. batizocoi* with any other diploid species of section *Arachis* were sterile. Such sterile F1 hybrids can produce the most fertile and stable hybrids on amphidiploidization, (cf *A. monticola*).

Singh (1986b, 1988) further advanced the Smartt (1978) suggestion and inferred a segmental amphidiploid origin of *A. hypogaea* with *A. duranensis* and *A. batizocoi* as the most probable donors of the A and B genome respectively. This was based on karyomorphological similarities (Singh & Moss, 1982) and detailed genome analysis. Cross-compatibility, chromosome pairing, and the pod fertility in hybrids between the species of section *Arachis* (irrespective of ploidy level) (Singh & Moss, 1984a) were studied in genome analysis. In addition an extensive evaluation of chromosome pairing and pollen fertility in hybrids between cultivated tetraploid *A. hypogaea* on the one hand and synthetic autotetraploids (Singh, 1986a), and amphidiploids (Singh, 1986b) involving species of section *Arachis* on the other. Higher mean bivalent associations in hybrids between *A. hypogaea* × AABB synthetic amphidiploids involving *A. batizocoi*, compared with those in hybrids of *A. hypogaea* × AAAA synthetic amphidiploids thus support the theory of an amphidiploid origin of *A. hypogaea* from species genetically close but differentiated in chromosome structure. Furthermore, in crosses between *A. hypogaea* and AABB amphidiploids, the amphidiploid involving *A. batizocoi* and *A. duranensis* produced the highest bivalent associations, and pollen and pod fertility. This gave a clear indication that this amphidiploid's genomic constitution is the closest yet produced artificially to that of *A. hypogaea*. However, chromosome pairing, pollen and pod fertility were not completely normal. This is understandable since we have no means of knowing when *A. monticola* actually arose. Both diploid ancestors and cultivated tetraploid species may well have undergone evolutionary divergence, becoming significantly different genetically from the original forms. These conclusions are supported by general morphological similarities, sympatric phytogeographical distribution, some phytochemical features, protein profile studies of these species, their autotetraploids, and derived amphidiploids (Singh et al., 1991).

Recently, Kochert et al. (1991), based on results of RFLP studies, suggested that *A. ipaensis* Krapov. et W.C. Gregory, *A. duranensis* and *A. spegazzinii* (= *A. duranensis*) are the closest diploid relatives of *A. hypogaea*, and that *A. batizocoi* did not appear to be closely related to *A. hypogaea*. Reconstruction of the tetraploid RFLP banding patterns in various combinations suggested that *A. ipaensis* combined with either *A. duranensis* or *A. spegazzinii* was the combination that came closest to the tetraploid *A. hypogaea*. Since then there have been further studies (Paik-Ro et al., 1992) using molecular marker techniques supporting a more distant relationship of *A. hypogaea* with *A. batizocoi* than with any other proposed diploid ancestor, suggesting that *A. batizocoi* cannot be the donor of the 'B' genome to *A. hypogaea*. However all these studies did support close genetic affinity of *A. duranensis* with *A. hypogaea*. They now suggest that *A. ipaensis* and *A. duranensis* are the donors of the 'B' and 'A' genomes respectively. This contention is supported by the karyotype studies of Fernandez and Krapovickas (1994) who has found in *A. ipaensis* a karyotype which lacks the characteristic 'A' chromosome pair. From this it has been inferred that it is a 'B' genome species and thus an alternative to *A. batizocoi* as the 'B' genome donor to *A. monticola/A. hypogaea*.

Congruency of various hypotheses with results

Though both *A. duranensis* and *A. cardenasii* proposed as putative ancestors of *A. hypogaea* by Gregory and Gregory (1976) cross with *A. hypogaea*, the possibility of hybridization between such a perennial and annual species, both with A genomes, evolving an amphidiploid like *A. monticola* is remote. This is

because crossing between these diploid species results in the production of a fully fertile diploid F_1 hybrid (Stalker & Wynne, 1979; Singh & Moss, 1984a) that can propagate without any necessity for doubling of chromosomes to regain fertility. Secondly *A. cardenasii* has been found immunologically distant from *A. hypogaea* (Klozová et al., 1983).

Similarly, the hypothesis of Smartt et al. (1978) that *A. batizocoi* and *H. cardenasii* were Band A genome donors also presents some difficulty. Synthetic amphidiploids from these two species have not yet been produced, unlike other combinations of A and B genome species (Singh, 1986b). So it is not possible to test experimentally an artificial amphidiploid in crosses with the cultigen, also *A. cardenasii* has been shown to be rather distant from *A. hypogaea* on immunological grounds (Klozová et al., 1983). This may be related to the failure to produce an amphidiploid.

Concerning *A. ipaensis*, one of the putative parents suggested by Kochert et al. (1991), to date all the evidence produced in favor is indirect and circumstantial, particularly that based on homology of some DNA sequences revealed through RFLP. Though these two species are sympatric, the proposers of *A. ipaensis* as one of the progenitors have yet to produce an amphidiploid from *A. duranensis* × *A. ipaensis* and cross it successfully with either *A. monticola* or *A. hypogaea*. This would provide the necessary confirmatory evidence in support of their hypothesis. The fact that one of the authors of this hypothesis in an earlier publication (Stalker et al., 1991) reports, that 81 and 76 pollinations respectively using *A. ipaensis* as male on *A. duranensis* and *A. hypogaea* as female did not result in any hybrid could indicate genetic isolation of *A. ipaensis* from both *A. duranensis* and *A. hypogaea*, raising doubts that *A. ipaensis* is a progenitor of *A. monticola* or *A. hypogaea*. The crossability between species sometimes may be affected by a few genetic factors. Nevertheless, crossability between species and particularly high levels of chromosome pairing in their hybrids provide direct and stronger evidence on genomic homology than the homology established based on rather few probes/enzyme combinations, which reflect homology of only a few of the thousands of sequences coded in the genome. Moreover, in such studies some probe/enzyme combinations may implicate certain diploid species, while the same species may be ruled out by other probe/enzyme combinations. Therefore, unless a very large number of probe/enzyme combinations is considered which provide full coverage of the genomes in question it is premature to draw such conclusions. Additionally, support from detailed cross-compatibility experiments involving a number of accessions and cytological behavior of their hybrids is essential. Until then, these inferences based on limited studies of few probes/clones/primers will remain questionable.

A. batizocoi and *A. duranensis*, the progenitors proposed on the basis of cytogenetical evidence, are parapatric and are distributed in the same area of northern Argentina and southern Bolivia, that has been considered the region of origin of *A. hypogaea*. In addition to the close cytogenetic affinity established through karyomorphological studies and genome analysis, synthetic amphidiploids involving *A. batizocoi*, the B genome species and many other A genome species including *A. duranensis* have been established (Singh, 1986a, b). Further, it was observed that AABB amphidiploids were more fertile than AAAA amphidiploids; AABB amphidiploids crossed more easily with *A. hypogaea* than AAAA amphidiploids, and the amphidiploid between *A. batizocoi* × *A. duranensis*, when crossed with *A. hypogaea* produced fertile hybrids showing an average more than 16 bivalents. This higher chromosome pairing resulted in the formation of fertile gametes (as indicated by pollen fertility), which on fertilization produced mature seeds (Singh, 1986b, 1988). In this way direct evidence that this combination of genomes is very close to that of those species that gave rise to the original wild *A. monticola* was provided. These observations have been supported by the experience of those involved in the transfer of genes from wild *Arachis* species into *A. hypogaea*. Krapovickas et al. (1974) were able to produce a hybrid between *A. hypogaea* × *A. batizocoi*, which stabilized at the tetraploid level to produce a hybrid called by them *A. batizogogea* (still maintained at some places). Singh and Moss (1984a, b) and several others recorded production of hybrid between *A. hypogaea* and *A. batizocoi* to be as easy as with any other species of section *Arachis* and that the triploid hybrid produced from this cross has a very similar level of chromosome pairing. This triploid hybrid was also one of the first to produce fertile pegs and pods through unreduced and hypermonoploid (n + 1) gametes (Singh & Moss, 1984b). Based on these observations, Singh (1986b) suggested that the amphidiploid route involving *A. batizocoi* (or another diploid species) to be the most effective route for introgression of genes from wild *Arachis* species into the cultivated groundnut to produce fertile progeny. This view has been strengthened by the experience of Singh and associates, and that of sever-

al others worldwide involved in bringing about gene introgression (Simpson et al., 1991; Starr et al., 1995). Subsequent studies with seed protein profiles (that are the product of the greater part of the genome rather than just a few DNA sequences) also supported these inferences, where construction of protein profiles combining the seed protein of *A. batizocoi* and *A. duranensis* resulted in a profile with 67% homology with that of *A. hypogaea* (Singh et al., 1991).

Conclusions

Hence, based on cytogenetic evidence, *A. batizocoi* and *A. duranensis* still appear to be the most probable progenitors of *A. hypogaea*. The proposition that *A. ipaensis* is one of the progenitors will not be acceptable until an amphidiploid is produced synthetically between *A. duranensis* × *A. ipaensis* and successfully crossed with *A. hypogaea* to produce a fertile hybrid. This would establish the genetic homology of this synthetic amphidiploid and *A. hypogaea*. Till then this issue still stands unresolved.

The status of *A. ipaensis*, whether it has a B genome or another also needs further investigation. The apparent absence of a small pair of chromosome (which is often difficult to confirm and used perhaps too loosely to designate the A genome) is not necessarily valid cytogenetic evidence (Fernandez et al., 1994). It should be investigated meiotically for the absence of a small bivalent (which is more consistent) and be crossed with wide range of A and B genome species/accessions to investigate crossability relationships and chromosome pairing pattern in hybrids to establish genomic relationships. As of today the results indicate that it neither has A genome, as it did not cross with *A. duranensis*, nor has it the B genome, as it produced sterile hybrids with *A. batizocoi* with an average of 7.79 bivalent association and 0.8% pollen fertility (Stalker et al., 1991). The use of protoplast fusion to produce an (*A. ipaensis* × *A. duranensis*) hybrid could be attempted if reciprocal conventional crosses using as wide a range of accessions as possible failed. This could provide material on which the critical test of crossing with *A. monticola/A. hypogaea* could be carried out.

References

Fernandez, A. & A. Krapovickas, 1994. Chromosomas y evolucion en *Arachis* (*Leguminosae*). Bonplandia 8: 188–220.

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, 1976. Groundnut. In: N.W. Simmonds (Ed.), Evolution of Crop Plants: 151–154. London: Longman Group Ltd.

Hammons, R.O., 1970. Spancross – A new peanut variety, Reg. no. 3. Crop Sci. 10: 459.

Klozová, E., V. Turková, J. Smartt, K. Pitterová & J. Svachulová, 1983. Immunological characterization of seed protein of some species of genus *Arachis* L. Biol. Plant 25: 201–208.

Kochert, G., T. Halward, W.D. Branch & C.E. Simpson, 1991. RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor. Appl. Genet. 81: 565–570.

Krapovickas, A. & V.A. Rigoni, 1951. Estudios citologicos en el genero *Arachis*. Rev. Invest. Agric. 5: 289–294.

Krapovickas, A. & V.A. Rigoni, 1957. Nuevas especies de *Arachis* vinculadas al problema del origen del mani. Darwiniana 11: 431–455.

Krapovickas, A., A. Fernandez & P. Seeligman, 1974. Recuperacion de la fertilidad en un hibrido interspecifico esteril de *Arachis* (*Leguminosae*). Bonplandia 3: 129–142.

Krapovickas, A. & W.C. Gregory, 1994. Taxonomia del genero *Arachis* (*Leguminosae*). Bonplandia 8: 1–187.

Kumar, L.S.S., R. D'Cruz & J.G. Oke, 1957. A synthetic allohexaploid in *Arachis*. Current Sci. 26: 121–122.

Paik Ro, O.G., R.L. Smith & D.A. Knauft, 1992. Restriction fragment length polymorphism evaluation of six peanut species within the *Arachis* section. Theor. Appl. Genet. 84: 201–208.

Raman, V.S., 1960. Studies in the genus *Arachis*. IX. A fertile synthetic tetraploid groundnut from the interspecific backcross *A. hypogaea* × *A. villosa*. Indian Oilseeds J. 4: 90–92.

Raman, V.S. & P.C. Kesavan, 1965. A tetraploid hybrid in *Arachis* and its bearing on differentiation between diploid species. Indian J. Genet. Plant Breeding 25: 80–84.

Simpson, C.E., 1991. Pathways for introgression of pest resistance into *Arachis hypogaea* L. Peanut Sci. 18: 22–26.

Singh, A.K., 1986a. Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. Part 7. Autotetraploid production and prospects in interspecific breeding. Theor. Appl. Genet. 72: 164–169.

Singh, A.K., 1986b. Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. Part 8. Synthetic amphidiploids and their importance in interspecific breeding. Theor. Appl. Genet. 72: 433–439.

Singh, A.K., 1988. Putative genome donors of *Arachis hypogaea* (Fabaceae), evidence from crosses with synthetic amphidiploid. Plant Syst. & Evolution 160: 143–151.

Singh, A.K. & J.P. Moss, 1982. Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. 2. Chromosome complements of species of section *Arachis*. Theor. Appl. Genet. 61: 305–314.

Singh, A.K. & J.P. Moss, 1984a. Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. 5. Genome analysis in section *Arachis* and its implications in gene transfer. Theor. Appl. Genet. 68: 355–364.

Singh, A.K. & J.P. Moss, 1984b. Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. 6. Fertility in triploids. Cytological basis and breeding implications. Peanut Sci. 11: 17–21.

Smartt, J., 1964. Cross compatibility relationships between the cultivated peanut *Arachis hypogaea* L. and other species of the genus *Arachis*. Ph.D. thesis. North Carolina State University, Raleigh.

Smartt, J. & W.C. Gregory, 1967. Interspecific cross compatibility relationships between the cultivated peanut *Arachis hypogaea* L. and other species of the genus *Arachis*. Oléaginuex 22: 455–459.

Smartt, J., W.C. Gregory & P.M. Gregory, 1978. The genomes of *A. hypogaea* L. Cytogenetic studies of putative genome donors. Euphytica 27: 665–675.

Stalker, H.T. & J.C. Wynne, 1979. Cytology of interspecific hybrids in section *Arachis* of Peanuts. Peanut Sci. 6: 110–114.

Stalker, H.T., 1991. A new species – section *Arachis* of peanuts with D genome. Amer. J. Bot. 78: 630–637.

Stalker, H.T., J.S. Dhesi, D.C. Parry & J.H. Hohn, 1991. Cytological and interfertility relationships of *Arachis* section *Arachis*. Amer. J. Bot. 78: 238–246.

Starr, J.L., C.E. Simpson & T.A. Lee, 1995. Resistance to *Meloidogyne arenaria* in advanced generation breeding lines of peanut. Peanut Sci. 22: 59–61.