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# Cytology of Interspecific Hybrids in Section *Arachis* of Peanuts[1]

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## ABSTRACT

Interspecific hybrids between *Arachis correntina* (Burk.) Krap. *et* Greg. *nom. nud.* (coll. GKP 9530-31) and seven other diploid peanut species of section *Arachis nom. nud.* [syn. Axonomorphae (7)] were cytologically analyzed. Although hybrid plants were partially sterile, cytological barriers to gene exchange were nonexistent except for *A. batizocoi* Krap. *et* Greg. hybrids. *Arachis batizocoi* hybrids had between 0 and 4.67% pollen fertility, probably due to an average of 2.88 univalents per cell. Laggards and anaphase I bridges were observed in 85% of the hybrid cells. Because the cultigen, *A. hypogaea* L. (2n = 40), and the diploid wild species (2n = 20) are at different ploidy levels, hybridization results in sterile triploid plants. This is a major barrier to introgression from wild to cultivated varieties. In order to derive wild species of section *Arachis* at the same ploidy level as *A. hypogaea*, 572 three to six-day-old seedlings were colchicine-treated representing 34 interspecific *Arachis* section hybrid combinations. Eighty-one cytologically confirmed amphidiploids plus 49 probable ones based on plant morphology were isolated. After colchicine treatments, 26 autotetraploids were likewise produced from six species. The observations indicated that selection can occur at the diploid level in wild species for desired agronomic traits or for disease and insect resistances. Colchicine-treated selected hybrid seedlings would then serve as a pathway for overcoming the major sterility obstacle to introgressing germplasm into *A. hypogaea*.


Key Words: Amphidiploids.

The cultivated peanut, *Arachis hypogaea* L., is one of the world's most important legume crops. Recent trends have been toward monoculture with only four varieties accounting for 87% of the acreage and 90% of U. S. production during 1974 (8). New sources of germplasm resources are needed to improve yield, quality and resistances to plant pests. One source is the *ca.* 70 wild species of peanuts. Gregory *et al.* (7) divided the genus into seven botanical sections based on morphology and hybridization data. Intersectional hybridization is extremely difficult (5), while intrasectional crosses have been more successful. *Arachis hypogaea* (2n = 40) plus one other tetraploid and eight diploid species have been assigned to section *Arachis nom. nud.* [syn. *Axonomorphae* (7)]. Smartt and Gregory (16) hybridized seven diploid wild species plus *A. monticola* Krap. *et.* Rig. (2n = 40) with *A. hypogaea*. They obtained hexaploids after colchicine treatments or by natural chromosome doubling from the following hybrid combinations: *A. hypogaea* by *A. duranensis* Krap. *et* Greg. *nom. nud.* (K 7988), *A. villosa* Benth., *A. correntina* Burk. (Krap. *et* Greg.) *nom. nud.* (GKP 9530-31), and *A. cardenasii* Krap. *et* Greg. *nom. nud.* (GKP 10017). The authors were unable to restore fertility by doubling the chromosome numbers in other cultivated x wild species hybrid combinations by colchicine treatment of vegetative tissues. Application of colchicine to peanut stems of sterile hybrids has been more successful than seed applications (18).

Most peanut cultigens exhibit susceptibility in varying degrees to all major insect and disease pests (3, 4). Although high levels of resistance to many of these pests are found in wild species of the genus, only members of section *Arachis* have immediate potential value to cultivated varieties because species of other botanical sections do not readily hybridize with *A. hypogaea*. Pest resistances to the following insects and diseases have been found in section *Arachis:* moderate levels of resistance to the two-spotted spider mite, *Tetranychus urticae* Koch, in *A. correntina* (GKP 9548) (11), high levels of resistance to peanut rust, *Puccinia arachidis* Speg. in *A.* sp. HLK 408, PI 338279; *A.* sp. HLK 409, PI 337308; *A. batizocoi* Krap. *et* Greg., K 9484, PI 298639; *A. villosa* Benth., PI 210554; *A. correntina*, K 7830, PI 262137, and GKP 9548; *A. chacoense* Krap. *et* Greg. *nom. nud.*, K 7988, PI 219823 (Wynne and Stalker, unpublished); high levels of resistance to late leafspot, *Cercosporidium personatum* (Beck & Curtis) Deighton in *A. cardenasii*, GKP 10017, PI 262141 (1); and high levels of resistance to early leafspot, *Cercospora arachidicola* Hori, in *A. chacoense*, GKP 10602, PI 276235 (1) and *A.* sp. HLK 410 (Wynne and Stalker, unpublished). All the above-mentioned pests can cause severe yield losses, but the leafspots result in the most widespread defoliation and severe production limitations.

More efficient methods of combining the genomes of wild and cultivated species in section *Arachis* would greatly aid in the utilization of the diploid species. Selection at the diploid level for desired agronomic traits, especially in the area of pest resistance, may lead to more successful and rapid utilization of the germplasm resources. A prerequisite for utilization of selections at the diploid level is knowing species relationships and ancestors of cultivated peanuts. A rapid method for overcoming sterility barriers due to different ploidy levels must then be used for efficient introgression of germplasm from wild species into cultivated varieties.

The objectives of this investigation were to cyto-

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logically determine whether selection among *Arachis* section species at the diploid level and subsequent chromosome doubling are practical for introgressing germplasm from wild species to *A. hypogaea*. Chromosome pairing between the diploid species and *A. correntina* (coll. GKP 9530-31) will be used to cytologically determine species relationships and barriers to gene exchange. The results of seedling colchicine treatments will be presented as an indication of the potential for overcoming sterility of hybrids because of ploidy differences. The potential for using the wild species germplasm in a breeding program will then be discussed.

## Materials and Methods

Eight diploid members of section *Arachis* were used in this investigation (Table 1). Although many of the collections are as yet unnamed botanically, they are considered to be different species based on their unique plant morphologies (Gregory, personal communication). Reciprocal first-generation hybrids between *A. correntina* and the other diploid members of the section were cytologically observed. Morphological traits such as dominant yellow flowers, flower and leaf shape and pollen stainability were used to establish hybridity. Plants were grown in pots in the greenhouse and flower buds collected during the summer of 1978. Inflorescences were fixed and stored in Carnoy's solution. Anthers were squashed in aceto-carmine. Iron was avoided during all steps of the procedure because the cytoplasm turned black when it was added. Chromosome numbers and associations were confirmed by observing 25 late diakinesis or early metaphase stages in microsporocytes. As an estimate of male fertility, pollen stainability for all parents and hybrids was determined by the acetocarmine-glycine method (13). At least 300 pollen grains for each of two flowers were scored for stainability.

Three to five-day-old seedlings were treated with a 0.2% colchicine solution by inverting the seedling into the solution after cutting off part of the cotyledon (2). After 6 hours the seedlings were washed with water and planted in soil with the cotyledons exposed to light. Lateral branch cuttings were made and chromosome numbers confirmed by observing root tip chromosomes. The root tips were pretreated in a saturated solution of paradichlorobenzene for 1½ hours, hydrolyzed in 1 N HCl and stained with aceto-orcein.

## Results

Morphological and pollen stainability data confirmed hybrid origin of the plant materials. Yellow flower color is dominant to orange, and three hybrid combinations — *A. correntina* by *A.* sp. HLK 410, by *A. batizocoi*, and by *A.* sp. GKP 10038 — could be identified by the yellow flower color of $F_1$ hybrids since the male parents were yellow flowered. Flower and leaf size, leaf shape and pollen stainability were used for distinguishing hybrids of other crosses. Individual plants of a hybrid combination were usually variable for leaf and plant traits. The parental collections ranged between 89 and 99% pollen stainability, whereas interspecific hybrids ranged between 0 and 88.5%. Few differences were observed (Table 2) among reciprocal crosses except in hybrids involving *A.*

**Table 1. List of *Arachis* section species used and their collection sites.[a]**

| Collector[b] | Collector no. | PI no. | Species | Habit | Locality |
|---|---|---|---|---|---|
| K | -- | 210554 | *A. villosa* Benth. | Perennial | Colonia, Uruguay |
| HLK | 410 | 338280 | *A.* sp. | Annual | Porto Dom Pedro II, Parana, Brazil |
| K | 7988 | 219823 | *A. duranensis* Krap. et Greg. nom. nud. | Annual | Salta, Argentina |
| K | 9484 | 298639 | *A. batizocoi* Krap. et Greg. | Annual | Parapeti, Bolivia |
| GKP | 9530-31 | 262808-09 | *A. correntina* (Burk.) Krap. et Greg. nom. nud. | Perennial | Corrientes, Argentina |
| GKP | 10017 | 262141 | *A. cardenasii* Krap. et Greg. nom. nud. | Perennial | Robore, Bolivia |
| GKP | 10038 | 263133 | *A.* sp. | Annual | Salta, Argentina |
| GKP | 10602 | 276235 | *A. chacoense* Krap. et Greg. nom. nud. | Perennial | Puerto Casado, Paraguay |

[a]Table abstracted from Gregory et al. (7).

[b]Abbreviations of collectors' names: HL = Hammons-Langford, K = Krapovickas, GKP = Gregory-Krapovickas, Pietrarelli.

**Table 2. Pollen stainability and chromosome associations of first-generation *Arachis* section hybrids.**

| Hybrid | No. plants | Pollen stainability (%) | Avg chromosome associations/cell I | II |
|---|---|---|---|---|
| 8 parents (avg) | 16 | 96.4 | 0.01 | 9.99 |
| *A. correntina* x *A. villosa* | 2 | 87.1 | 0.20 | 9.90 |
| " x *A.* sp. HLK 410 | 3 | 66.8 | 0 | 10.00 |
| " x *A. duranensis* | 3 | 81.8 | 0 | 10.00 |
| " x *A. batizocoi* | 1 | 0 | 2.96 | 8.56 |
| " x *A.* sp. GKP 10038 | 1 | 62.6 | 0 | 10.00 |
| " x *A. chacoense* | 1 | 56.7 | 0 | 10.00 |
| *A. villosa* x *A. correntina* | 1 | 88.5 | 0.24 | 9.88 |
| *A.* sp. HLK 410 x " | 1 | 70.3 | 0.16 | 9.92 |
| *A. duranensis* x " | 1 | 78.0 | 0 | 10.00 |
| *A. batizocoi* x " | 1 | 4.67 | 2.80 | 8.60 |
| *A. cardenasii* x " | 2 | 63.0 | 0.04 | 9.98 |
| *A.* sp. GKP 10038 x " | 2 | 44.7 | 0.04 | 9.98 |
| *A. chacoense* x " | 1 | 59.2 | 0 | 10.00 |

sp. GKP 10038. A cytological explanation for low fertility levels in *A. batizocoi* hybrids will be presented in the following sections. The 87.1 to 88.5% pollen stainability of *A. villosa* by *A. correntina* hybrids (Table 2), along with very similar plant morphology, indicates a close relationship among the collections.

Cytological observation confirmed that the eight *Arachis* species have 20 chromosomes. No cytological abnormalities were observed in the collections and all plants had 10 bivalents except for 0.04 univalents per cell observed in *A. chacoense* and 0.08 univalents per cell in *A. villosa*. One species, *A. duranensis*, appeared to have a single bivalent which was much smaller at metaphase I than the other chromosomes of the *Arachis* section species. On the other hand, *A. batizocoi* had no visibly smaller bivalents than the other paired chromosomes in pollen mother cells (PMCs). Smartt *et al.* (17) observed root tip cells and found one distinctly smaller chromosome in all *Arachis* section species except *A. batizocoi*. This corresponds to the metaphase I observation in this study.

All hybrids with *A. correntina* had normal meiosis and usually 10 bivalents, except hybrids involving *A. batizocoi* which averaged 8.58 bivalents per cell (Table 2). No reciprocal differences were observed. Pollen mother cells in *A. batizocoi* hybrids had laggards or bridges in 127 of the 145 observed anaphase I cells. Although a few of the PMCs had 10 bivalents in these hybrids, most had 2-4 univalents with an upper range of 6 univalents observed in 6% of metaphase I cells. In addition, cytological analysis of *A. batizocoi* by *A. cardenasii* showed the same patterns of low fertility and 2-4 univalents per cell as the hybrids between *A. batizocoi* and *A. correntina*.

Five hundred seventy-two seedlings representing 34 hybrid combinations were treated with 0.2% solution of colchicine. Two hundred thirty-three of the treated seedlings survived the treatment and 81 amphidiploids representing 29 hybrid combinations were cytologically identified. An additional 49 hybrids are suspected of being polyploid based on morphological traits. Ninety seedlings of six species — *A.* sp. HLK 410, *A. duranensis*, *A. batizocoi*, *A. correntina*, *A.* sp. GKP 10038 and *A. chacoense* — were colchicine-treated and 26 autotetraploids were recovered. Complete chromosome doubling of all vegetative tissues was rare in colchicine-treated plants. Lateral cuttings were thus made to confirm chromosome numbers and to isolate tetraploid tissues. Amphidiploid and autotetraploid plants were usually less vigorous than their diploid counterparts with autotetraploids usually showing less vigor than amphidiploids. Several morphological traits such as gigas leaves and flowers, mottled or deformed leaves, and darker plant color were useful for identifying polyploid tissues. Pollen stainability of amphidiploid hybrid plants was variable and ranged between 10 and 92%. Utilization of germplasm of each hybrid combination will have to be considered on an individual basis.

## Discussion

Evidence from cytological investigations indicated that the species of section *Arachis* were closely related. The chromosomes of all observed species hybrids between *A. correntina* and seven diploid species, except those with *A. batizocoi*, paired normally. Gene exchange was likely among most interspecific hybrids in the group. The species were not fully compatible because fertility levels were low in many hybrid combinations (see Table 2) and several $F_1$ hybrid combinations produced few progeny. Resslar and Gregory (14) reported similar results for hybrids between *A. villosa, A. cardenasii, and A. duranensis. Arachis batizocoi* was cytologically the most distantly related species observed in the section, but even in this case an average of 8.58 bivalents per cell was observed. Cytological barriers to selection at the diploid level were thus absent in most observed interspecific hybrids.

Stebbins (19) concluded that A. hypogaea is a segmental alloploid because the species has one small chromosome pair in its genome (9, 10). Much speculation as to the genome donors has been presented in the literature. Varisai Muhammad (20) proposed *A. villosa* as one of the diploid progenitors, Seetharam *et al.* (15) proposed *A. duranensis* as a forerunner, Gregory and Gregory (6) indicated the most likely ancestors were *A. duranensis and A. cardenasii*, Smartt *et al.* (17) said that *A. batizocoi* and *A. cardenasii* were likely progenitors, and Krapovickas *et al.* (12) also indicated that *A. batizocoi* was a probable ancestor of *A. hypogaea*. Smartt *et al.* (17) proposed that two genomes are present in species of section *Arachis*, with *A. batizocoi* having the 'B' genome and the other known diploid species having the 'A' genome. However, based on the cytological survey of hybrids in our study, there appears to be only one genome present among the diploid species, with *A. batizocoi* having possibly two altered chromosomes in this genome. Cytological analyses of all possible crosses between *A. batizocoi* and other diploid *Arachis* section species, along with identifying the species karyotypes, will more clearly define the number of genomes in section *Arachis*. Although it is of great academic interest to discover the progenitors of a cultivated species, the underlying agronomic reason fur such investigations is to find the species which will hybridize with the cultigen, thus allowing germplasm introgression. Any of the observed species in section *Arachis* are potential gene donors to *A. hypogaea*. Recent collection trips to South America have expanded the germplasm resources in the genus. Many species

will probably be added to the current list, and perhaps the progenitor species to *A. hypogaea* will be discovered after a complete species collection is assembled.

The classical method for attempting to introgress germplasm from wild peanuts of section *Arachis* to the cultigen is by direct hybridization of the diploid and tetraploid species (16). Sterile hybrids are obtained from such crosses, and restoration of fertility at the hexaploid level after colchicine treatment is unpredictable. At best the process is long and tedious. When hexaploids have been obtained, they may remain at that ploidy level for many generations. For example, 6x (*A. hypogaea* x *A. batizocoi* ) hybrids have 30 bivalents and are stable at the hexaploid ploidy level (Stalker, unpublished). Other hybrid combinations, such as 6x (*A. hypogaea* x *A. cardenasii*) lose chromosomes and are now stable at 2n = 40. Either the wild species or cultivated plants would produce similar stabilization at 2n = 40 if used as a recurrent parent after several generations of backcrossing.

The results from these studies suggest that a more economical route to introgressing germplasm than producing triploid *A. hypogaea* -wild species hybrids may be to create autotetraploids or amphidiploids and subsequently cross the 4x plants with *A. hypogaea*. Although some sterility may still be encountered in first-generation plants, the problems of restoring fertility would be expected to be fewer than with triploid hybrids. Furthermore, selections could be made for desirable gene combinations in crosses at the diploid level before they are diluted by the *A. hypogaea* genome. In addition, many of these gene combinations may be difficult or impossible to obtain by crossing one diploid species at a time with *A. hypogaea*. Collections *A.* sp. HLK 410, *A. cardenasii* and *A. chacoense* each has high resistance to *Cercospora* leafspot pathogens. Second-generation hybrids among these species have been screened for leafspot resistance and selections made for hybridization with *A. hypogaea*. Colchicine-treated seedlings of one combination, *A.* sp. HLK 410 by *A. chacoense*, has 79 to 88% pollen stainability and hybridization with *A. hypogaea* would demonstrate the potential of these hybrids.

The incorporation of germplasm from wild into cultivated varieties of peanuts will require much effort. No one hybridization technique is expected to be the best method of introgressing germplasm from all species of the genus to the cultigen, but in many instances, making hybrids among wild diploids and selection at the diploid level may prove to be the most practical. The desired crosses can then be made with *A. hypogaea* at the 4x ploidy level. Where $F_2$ seeds cannot be obtained in some quantity because of sterility in diploid x diploid hybrids, this method will have severe limitations; in these cases diploid x tetraploid hybrids should be made and restoration of fertility at the hexaploid level will continue to be the predominant method of introgressing germplasm from wild to cultivated species.

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