Custodio, A. R., C. E. Simpson & J. F. Montenegro Valls. 2021. Crossability between *Arachis gregoryi* (Fabaceae) and wild *Arachis* species with distinct genomes. Bonplandia 30(1): 91-106. http://dx.doi.org/10.30972/bon.3014669
Recibido 7 Febrero 2020. Aceptado 22 Abril 2020.
Publicado en línea: 23 de diciembre 2020. Publicado impreso: 15 de febrero 2021.
ISSN 0524-0476 impreso. ISSN 1853-8460 en línea.

Crossability between *Arachis gregoryi* (Fabaceae) and wild *Arachis* species with distinct genomes

Cruzabilidad entre *Arachis gregoryi* (Fabaceae) y especies silvestres de *Arachis* con distintos genomas

Adriana R. Custodio[1], Charles E. Simpson[2] and José F. Montenegro Valls[1]

Summary: Cross-compatibility studies in *Arachis* L. have brought to light possible new sources of genes for introgression in cultivated peanuts. A total of 32 different crosses were made, using accession V14957 of *A. gregoryi* C.E. Simpson, Krapov. & Valls as the female parent and *Arachis* species containing seven distinct genomes as male parents. The 3167 pollinations resulted in 153 confirmed inter and intraspecific hybrids. Viability estimates of paternal pollen by staining (PVS) varied from 46.75 to 99.17%, while the pollen count of the hybrids varied from 0.3 to 43.6%, this highest PVS resulting from an intraspecific combination, which suggests appreciable variability in accessions of *A. gregoryi*. Viability estimates of paternal pollen by germination (PVG) resulted in consistently lower values for all materials, ranging from 41.91% to 88.00%. *Arachis gregoryi* is important to expand the available diversity of species associated with the peanut B genome; it has a potential to be incorporated as a parent in crosses that aim to expand the genetic base of peanuts and may be useful for concentrating important genes in pre-breeding lines associated with the B genome.

Key words: Hybrids, interspecific crosses, peanut, pollen viability, wild relatives.

Resumen: Estudios de compatibilidad cruzada en *Arachis* L. han sacado a la luz posibles nuevas fuentes de genes para introgresión en el maní cultivado. Se hicieron un total de 32 cruzamientos distintos, utilizando *A. gregoryi* C.E. Simpson, Krapov. & Valls accesión V14957 como progenitor femenino y especies de *Arachis* que contienen siete genomas distintos como progenitores masculinos. Las 3167 polinizaciones resultaron en 153 híbridos inter e intraespecíficos confirmados. Estimaciones de viabilidad del polen paternal por tinción (PVS) variaron de 46.75 a 99.17%, mientras que el recuento de polen de los híbridos varió de 0.30 a 43.60 %, ese PVS más alto resultando de una combinación intraespecífica, lo que sugiere variabilidad apreciable entre accesiones de *A. gregoryi*. Las estimaciones de viabilidad del polen paterno por germinación (PVG) resultaron en valores consistentemente más bajos para todos los materiales, que van desde 41.91% a los 88.00%. *Arachis gregoryi* es importante para expandir la diversidad disponible de especies asociadas con el genoma B del maní; tiene el potencial de incorporarse como progenitor en cruzamientos que apuntan a la expansión de la base genética del maní y puede ser útil para concentrar genes importantes en líneas de pre-mejoramento asociadas al genoma B.

Palabras clave: Cruzamientos interespecíficos, híbridos, maní, parientes silvestres, viabilidad de polen.

[1] Embrapa Genetic Resources and Biotechnology, Parque Estação Biológica, PqEB, Av. W5 Norte (final). Caixa Postal 02372, Brasília, DF, Brazil, CEP 70770-917. E-mail: jose.valls@embrapa.br
[2] Texas A&M AgriLife Research, Stephenville, Texas, USA.

Introduction

The genus *Arachis* encompasses 82 species described based on the integration of varied criteria, such as morphological, cytogenetic, and molecular characterization, geographic distribution, and interspecific cross-compatibility (Gregory et Gregory, 1979; Krapovickas et Gregory, 1994; Valls et Simpson, 2005, 2017; Valls et al., 2013). It is typified by the common peanut, an allotetraploid cultigen with a genomic constitution AABB (Singh et Moss, 1984), that is well diploidized genetically (Bertioli et al., 2011).

Further to their relevance for biosystematic studies, interspecific crosses are a first step for gene introgression from wild relatives into the cultigen. However, different ploidy levels impose a barrier for direct gene transfer. While the common peanut has 2n=4x=40 chromosomes, most wild species of the taxonomic section *Arachis* are diploid, with 2n=2x=20 or 2n=2x=18 (Fernández et Krapovickas, 1994; Lavia et al., 2009). The section *Arachis* includes at least six distinct genomes, A (Robledo et al., 2009), D (Stalker, 1991; Robledo et Seijo, 2008), B, F, K (Robledo et Seijo, 2010), and G (Silvestri et al., 2015), considering the distribution patterns of both heterochromatic bands and rDNA loci.

Production of synthetic allotetraploids from sterile hybrids between diploid wild species related to the A and B peanut genomes often restores fertility, removing the reproductive barriers, and increasing the possibility of successful construction of pre-breeding lines derived from wild species and *A. hypogaea* (Simpson, 1991; Fávero et al., 2006; Foncéka et al., 2009). Therefore, knowledge on the crossing behavior of wild diploids most closely related to the peanut, those with the A and B genomes, will help to understand species relationships in the genus, and to produce amphidiploid lines of potentially successful use in peanut breeding programs.

Arachis gregoryi is part of a small group of species genetically associated to *A. ipaënsis* (Burow et al., 2009; Robledo et Seijo, 2010), the source of the B genome of *A. hypogaea* (Kochert et al., 1996; Seijo et al., 2004, 2007; Fávero et al., 2006; Bertioli et al., 2016).

In the framework of peanut breeding based on the incorporation of related wild species germplasm, research on crossability of wild *Arachis* species with *A. hypogaea* has been concentrated for decades on species that share the A genome with the crop, and with assorted species then thought to be associated to the B genome (Simpson et Starr, 2001; Singh et Moss, 1984). A review of the genomic characteristics of species in the taxonomic sect. *Arachis* (Robledo et Seijo, 2010) established a group of true B genome species, gathering *A. ipaënsis* and *A. williamsii*, exclusive to Bolivia, and each represented in genebanks by a single available accession, as well as three species occurring in Brazil, *A. magna* (also occurring in Bolivia), *A. gregoryi*, and *A. valida*. The restricted diversity of *A. ipaënsis* demands further exploration of the closely related species sharing the B genome, for further progress in peanut breeding.

This paper analyses crossing relationships of *A. gregoryi* with members of section *Arachis*, including representatives of the A, B, D, F, G, and K genomes, as well as the AB configuration, and with *A. paraguariensis*, of the taxonomic section *Erectoides*.

Material and Methods

Crosses were made during two consecutive spring/summer periods, under screenhouse conditions, from October 2006 to late May 2007, and from September 2007 to April 2008, at Embrapa Genetic Resources and Biotechnology, located in Brasilia, Distrito Federal, Brazil.

The female parent in all crosses was *A. gregoryi*, accession number VS 14957 (Table1).

It was collected in the wild, in 2004, at the same original site as VSGr 6389, this one collected in 1981, and is a member of the same natural population already represented in crossing experiments (Valls et Simpson, 2005; Fávero et al., 2015a, b).

Male parents represent the genomic and cytological variation so far detected in the taxonomic section *Arachis* and one species

of section *Erectoides* (Table 1). Single plants of the female parent were cultivated in rectangular pots, each receiving pollen from a single and specific male donor.

Emasculation of the *A. gregoryi* 14957 flower buds was conducted each day, from 4 to 7 PM. The flower keel was carefully taken out with tweezers, for full anther exposure and removal, and the standard petal was folded again over the exposed stigma, for protection. Pollination of the emasculated flowers was conducted from 7 to 11 AM, the next morning. Pollen grains were transferred from the specific donor with tweezers, which were washed in alcohol between crosses. Emasculated flowers were misted with a fine water spray to facilitate pollen grain adherence to the stigma. Crosses were made during the flowering period of each pair of parents.

Pegs were visible approximately 15 days after pollination and were individually monitored until ripening, or to the end of the life cycle of the individual female parent. Spontaneous seedlings were carefully transferred to smaller, individual plant pots for further development. Harvested fruit segments were dried at room temperature and shelled seeds were later put to germinate under laboratory conditions.

Pollen viability of the parents was estimated both by staining and *in vitro* germination. Pollen of F_1 hybrids was analyzed by staining only. Eight flower buds were collected from each parent at weekly intervals during the crossing period. Pollen grains were set on a slide, stained with 2% aceto-carmine:glycerine, covered with a cover slip, and allowed to stain for 30 minutes in a moist chamber. Counting of well-formed and ill-formed grains was made under the microscope, including five samples of 100 grains each per slide. The analysis by *in vitro* germination followed the protocol of Niles et Quesenberry (1992). A basic solution of Medium 11 (10 mg H_3BO_3, 30 mg de Ca $(NO_3)_4$ H_2O, 20 mg $MgSO_4$ + 7 H_2O, 10 ml KNO_3, make up to 100 ml of water), with subsequent addition of 1.5 g sucrose to each 10 ml of basic solution at the time of preparation of the slides. The slides with the pollen grains plus a drop of basic solution are stored in humid chamber for 2 h. The number of pollen tubes larger than the pollen grain was counted. Average counts per flower bud were calculated, and the compiled results were expressed in percentage. The percentage of successful pollinations was calculated by division of the number of hybrids obtained by the number of pollinations performed. These results were multiplied by 100 (Table 2).

Results

Pollen viability estimates

Pollen viability estimates by staining, of the parental plants, varied from 46.75% for *A. helodes* to 99.17% for *A. magna* accession 30097 (Table 2). Values for all additional accessions were over 61.91%, with an average of 87.78%. Pollen viability estimates by germination varied from 41.70% for *A. helodes* to 88.00% for *A. diogoi*. Other accessions were over 41.91%, with an average of 75.33%.

Of hybrids that produced flowers (Table 2), the highest estimate of 43.6% was obtained for the intraspecific hybrid *A. gregoryi* 14957 × *A. gregoryi* 14753. Estimates for other flowering hybrids varied from 0.3% for *A. gregoryi* × *A. glandulifera* to 12% for *A. gregoryi* × *A. hoehnei* 30006.

Hybridization

A number of 153 confirmed hybrids resulted from the total of 3167 hand pollinations (Table 2). Of those, 1368 were made from October 2006 to late May 2007, and 1799 from September 2007 to April 2008. The number of pollinations in the distinct crossing combinations varied according to the number and quality of flowers produced by the respective female and male parents (Table 2). Some crossing combinations were repeated in the second season, due to low flower production, lack of flower synchrony between parents, or temporary low pollen estimates of the male parent in the first season.

Several seedlings voluntarily produced in the pots of their female parent died at early stages, so that it was impossible to determine whether they were hybrids or just maternal offspring.

Table 1. List of the *Arachis* species studied, accession codes, accession collectors and numbers, origin and geographic coordinates.
Tabla 1. Lista de especies de *Arachis* estudiadas, código de accesión, colector y número de accesión, origen y coordenadas geográficas.

Arachis species [1]	BRA-Code [2]	Accession [3,4,5]	Origin [6]	Municipality	Lat (S)	Long (W)	Alt (m)
A. batizocoi Krapov. & W. C.Greg.	00064972-3	K 9484	BOL/SC	Parapeti	20°05'	63°14'	700
A. benensis Krapov., W. C. Greg. & C. E.Simpson	00065872-4	KGSPSc 35005	BOL/BE	Trinidad	14°47'	64°55'	155
A. cruziana Krapov., W. C. Greg. & C. E. Simpson	00065840-1	WiSVg 1302-2	BOL/SC	San José de Chiquitos	18°50'	60°53'	285
A. diogoi Hoehne	00065993-8	Vp 5000	BRA/MS	Corumbá	17°50'	57°33'	92
A. duranensis Krapov. & W. C. Greg.	00065723-9	VNvEv 14167	ARG/SA	Salta	24°50'	65°27'	1206
A. glandulifera Stalker	00065512-6	VSPmSv 13738	BRA/MT	Porto Esperidião	16°13'	59°07'	160
A. gregoryi C. E. Simpson, Krapov. & Valls	00065978-9	VOfSv 14753	BRA/MT	Pontes e Lacerda	15°59'	59°33'	275
	00065982-1	VOfSv 14767	BRA/MT	Vila Bela da S. Trindade	16°05'	59°58'	247
	00066017-5	VS 14957	BRA/MT	Vila Bela da S. Trindade	15°22'	60°14'	230
	00066020-9	VS 14962	BRA/MT	Vila Bela da S. Trindade	15°23'	60°13'	220
A. helodes Mart. ex Krapov. & Rigoni	00064934-3	VSGr 6325	BRA/MT	Santo Antônio do Leverger	15°52'	56°04'	150
A. hoehnei Krapov. & W. C. Greg.	00065832-8	KG 30006	BRA/MS	Corumbá	18°15'	57°28'	100
	00065620-7	VMPzW 13985	BRA/MS	Corumbá	19°31'	57°25'	85
A. hypogaea L. "Xingu" type	00063839-5	VGaRoSv 12549	BRA/MT	São José do Xingu	10°49'	50°41'	345
A. hypogaea subsp. *hypogaea* var. *hypogaea*	00063838-7	VGaRoSv 12548	BRA/MT	São José do Xingu	10°49'	50°41'	345
A. hypogaea subsp. *hypogaea* var. *hirsuta* H. A. Köhler	00063836-1	Mf 1538 [ex ARG]	ECU/PI	Quito	0°02'	78°26'	2560
A. hypogaea subsp. *fastigiata* Waldron var. *fastigiata*	00064542-4	IAC 'Tatu'	BRA/SP	Campinas	22°53'	47°03'	665
A. hypogaea subsp. *fastigiata* var. *peruviana* Krapov. & W. C. Greg.	00063833-8	Mf 1560 [ex ARG]	ECU/ES	Quinindé	0°07'	79°25'	260
A. hypogaea subsp. *fastigiata* var. *vulgaris* Harz	00223807-9	IAC 'Tatuí'	BRA/SP	Campinas	22°53'	47°03'	665
A. hypogaea subsp. *fastigiata* var. *aequatoriana* Krapov. & W. C. Greg.	00063831-2	Mf 1678 [ex ARG]	ECU/SU	Shushufindi	0°22'	76°39'	390
A. ipaënsis Krapov. & W. C. Greg.	00065831-0	KGBPScS 30076	BOL/TA	Ipa	21°00'	63°25'	650
A. kempff-mercadoi Krapov., W. C. Greg. & C. E. Simpson	00064968-1	V 13250	BOL/SC	Santa Cruz de la Sierra	17°41'	63°08'	420
A. krapovickasii C. E. Simpson, D. E. Williams, Valls & I. G. Vargas	00065839-3	WiSVg 1291	BOL/SC	San José de Chiquitos	18°14'	60°51'	314

Arachis species [1]	BRA-Code [2]	Accession [3,4,5]	Origin [6]	Municipality	Lat (S)	Long (W)	Alt (m)
A. kuhlmannii Krapov. & W. C. Greg.	00065544-9	VSPmSv 13779	BRA/MT	Cáceres	16°13'	57°23'	190
A. magna Krapov., W. C. Greg. & C. E. Simpson	00065836-9	KGSSc 30097-o	BOL/SC	San Ignácio de Velasco	16°22'	60°58'	370
	00065540-7	VSPmSv 13761	BRA/MT	Vila Bela da S. Trindade	15°21'	60°04'	210
	00065671-0	VSPmSv 13765	BRA/MT	Glória d'Oeste	15°48'	58°23'	150
	00065516-7	VOfSv 14724	BRA/MT	Vila Bela da S. Trindade	15°19'	60°03'	204
A. microsperma Krapov., W. C. Greg. & Valls	00065646-2	VMPzW 14042	BRA/MS	Porto Murtinho	22°05'	57°34'	102
A. monticola Krapov. & Rigoni	00219752-3	SeSnHoCh 2775	ARG/JU	Lozano	24°04'	65°24'	1546
	00065721-3	VOa 14165	ARG/JU	Yala	24°07'	65°23'	1436
A. palustris Krapov., W. C. Greg. & Valls	00065019-2	VPmSv 13023	BRA/TO	Filadélfia	07°25'	47°37'	192
A. paraguariensis Chodat & Hassl. subsp. *paraguariensis*	00065159-6	VRGeSv 7677	BRA/MS	Bela Vista	22°08'	56°44'	168
A. schininii Krapov., Valls & C. E. Simpson	00065028-3	VSPmSv 9923	PRY/AM	Bella Vista	22°20'	56°19'	246
A. simpsonii Krapov. & W. C. Greg.	00065536-5	VSPmSv 13728	BOL/SC	San Matías	16°19'	58°26'	120
A. stenosperma Krapov. & W. C. Greg.	00064932-7	VSv 10309	BRA/MT	Rondonópolis	16°28'	54°39'	235
A. valida Krapov. & W. C. Greg.	00065464-0	VPzRcSgSv 13514	BRA/MS	Corumbá	19°07'	57°32'	90
A. vallsii Krapov. & W. C. Greg.	00065027-5	VRGeSv 7635	BRA/MS	Miranda	20°07'	56°42'	100
A. villosa Benth.	00065862-5	VMiIrLbGvAn 14309	BRA/RS	Uruguaiana	29°47'	57°13'	50
A. williamsii Krapov. & W. C. Greg.	00065838-5	WiDc 1118	BOL/BE	Trinidad	14°48'	64°53'	150

[1] Species additionally cited in the text: *A. linearifolia* Valls, Krapov. & C. E. Simpson.
[2] Germplasm accession code in the Embrapa Alelo Portal.
[3] Collector's abbreviations: An= A. Carneiro; B= D. J. Banks; Ch= J. Chalian; Dc= D. Claure; Ev= A. V. Etcheverry; G= W. C. Gregory; Ga= M. L. Galgaro; Ge= M. A. N. Gerin; Gr= A. Gripp; Gv= F. R. Galvani; Ir=B. E. Irgang; Ho= D. Hojsgaard; K= A. Krapovickas; Lb= L. R. M.Baptista; M= J. P. Moss; Mi= S. T. S. Miotto; Nv= L. J. Novara; Oa= O. Ahumada; Of= F. O. Freitas; P= J. R. Pietrarelli; Pm= R. N. Pittman; Pz= E. A. Pizarro; R= V. R. Rao; Rc= R. C. Oliveira; Ro= D. M. S. Rocha; S= C. E. Simpson; Sc= A. Schinini; Se= J. G. Seijo; Sg= A. K. Singh; Sn= V. G. Solís Neffa; Sv= G. P. Silva; V= J. F. M.Valls; Vg= I. G. Vargas; Vp= V. J. Pott; W= W. L. Werneck; Wi= D. E. Williams.
[4] Mf= Estación Experimental de Manfredi, Córdoba, Argentina. Accession numbers assigned for the 1997-1998 regeneration season (All three accessions originally collected in Ecuador).
[5] IAC= Instituto Agronômico de Campinas, São Paulo, Brazil. IAC cultivars.
[6] Countries/Departments, provinces or states: ARG= Argentina/JU= Jujuy; SA= Salta; BOL= Bolivia/BE= Beni; SC= Santa Cruz; TA=Tarija; BRA= Brazil/MT= Mato Grosso; MS= Mato Grosso do Sul; TO= Tocantins; RS= Rio Grande do Sul; SP= São Paulo; ECU= Ecuador/ES= Esmeraldas; PI= Pichincha; SU= Sucumbíos; PRY= Paraguay/AM= Amambay.

According to the percent of successful pollinations, crossing results were classified into four heterogeneous groups (Table 2). Six crossing combinations, which failed or did not perform well in the first crossing season, for reasons beyond our control, were repeated in the second season, this time showing positive results. Such combinations conducted in both seasons are reported individually within the framework of the four groups, and the results of the second attempt obviously better reflect their crossing potential.

The first group encompasses crossing combinations that produced the highest percent of hybrids per pollination events (17.1 to 11.9%), but most of the hybrids produced did not flower. Group 1 involves *A. gregoryi* × *A. hoehnei* 13985 (17.1%), *A. gregoryi* × *A. kuhlmannii* (15.18%), *A. gregoryi* × *A. williamsii* (14.3%), *A. gregoryi* × *A. kempff-mercadoi* (14.0%), *A. gregoryi* × *A. schininii* (13.3%), *A. gregoryi* × *A. microsperma* (12.5%), and *A. gregoryi* × *A. stenosperma*, as well as *A. gregoryi* × *A. vallsii* (both 11.9%). Five of the male parents are diploid species associated to the A peanut genome, one to the B genome. The genomes of *A. hoehnei* 13985 and of *A. vallsii* are yet to be determined (Table 2).

Concerning male parents with the A genome, the cross of *A. gregoryi* with *A. kuhlmannii* produced 24 fruit segments, which generated 12 hybrid plants with pollen viability estimates of 4.8%, in average. Hybrids of *A. gregoryi* × *A. kempff-mercadoi* produced quite normal plants, with a typical main axis and ascending lateral branches, but no flowers. Hybrids with *A. microsperma* also were quite normal plants, but did not flower. Showing excellent survival, hybrids with *A. schininii* produced highly proliferated lateral branches, but an abnormally short main axis, and no flowers. Hybrids of *A. gregoryi* × *A. stenosperma* showed slow growth and early loss of the main axis, although the lateral branches remained alive for several months.

Hybrids with the B genome species *A. williamsii*, that stood out as a good result obtained in the first season, showed very intensive vegetative growth, with lateral branches extending longer than 2.5 meters, intensive flowering, and average pollen viability estimate of 2.6%, but no peg formation.

The hybrid of *A. gregoryi* × *A. hoehnei* 13985 showed a fast growing rate, erect habit, and intensive lateral branching. However, the plants did not flower, so that pollen viability estimates are not available.

Crosses of *A. gregoryi* × *A. vallsii* produced 22 fruit segments, which resulted in ten seedlings. Although their hybrid nature could not be confirmed, due to very slow growth and no flowers, the leaflets of the hybrids showed the paternal parent trait, narrow and lanceolate, not of maternal origin.

The second group of crossing combinations gathers four crosses with a percent of success from 9.6 to 5.4%, involving *A. gregoryi* × *A. valida* (9.6%), *A. gregoryi* × *A. magna* 30097 (9.5%), *A. gregoryi* × *A. villosa* (8.2%) and *A. gregoryi* × *A. diogoi* (5.4%), the first two male parents associated to the peanut B genome, and the last two to the A genome.

In hybrids of *A. gregoryi* × *A. valida*, lateral branches were well developed and over 2.5 m long. Flowering was intensive, with an average pollen viability estimate of 5.45%. In spite of this low figure, one peg was produced, showing a fruit segment that, although well formed, did not complete its development and deteriorated in the soil.

The hybrid of *A. gregoryi* × *A. magna* 30097 presented average pollen viability estimate of 7.12%.

With a rate of success of 8.2%, crosses of *A. gregoryi* × *A. villosa* produced four plants, three of which survived well, and were additionally multiplied by cuttings, but never flowered. The hybrid status of the seedlings was easily confirmed by the dense pubescence on the adaxial leaflet surface, a paternal feature.

Hybridization of *A. gregoryi* × *A. diogoi* produced three small, but vigorous plants, which did not flower.

The third group of hybrids, with a low rate of success between 3.6 and 0.6%, encompasses 15 crossing combinations, involving *A. gregoryi* × *A. hypogaea* subsp. *fastigiata* var. *aequatoriana* (3.6%), *A. gregoryi* × *A. krapovickasii* (3.5%), *A. gregoryi* × *A. ipaënsis* (2.8%), *A. gregoryi*

× *A. helodes* (2.7%), *A. gregoryi* × *A. schininii* (2.7%), *A. gregoryi* × *A. simpsonii* (2.5%), *A. gregoryi* × *A. cruziana* (2.5%), *A. gregoryi* × *A. magna* 14724 (2.2%), *A. gregoryi* × *A. hypogaea* subsp. *hypogaea* var. *hirsuta* (2.0%), *A. gregoryi* 14957 × *A. gregoryi* 14753 (1.7%), *A. gregoryi* × *A. glandulifera* (1.7%), *A. gregoryi* × *A. benensis* (1.4%), *A. gregoryi* × *A. hypogaea* subsp. *fastigiata* var. *fastigiata* (1.4%), *A. gregoryi* × *A. batizocoi* (1.2%), *A. gregoryi* × *A. duranensis* (1.1%), and *A. gregoryi* × *A. hoehnei* 30006 (0.6%). Male parents in this group include representatives of three botanical varieties of *A. hypogaea*, as well as species either associated to the A or B genomes, besides representatives of the D, F and K genomes, additionally described for species of the *Arachis* section (Stalker, 1991; Robledo et Seijo, 2008, 2010).

In the intraspecific cross *A. gregoryi* 14957 × 14753, 24 fruit segments were formed, but only two hybrid plants survived. Pollen viability estimates varied from 36-55.6%. Although the anthers did not open properly at anthesis, and, consequently, release of pollen-grain was restricted, the two hybrid plants produced many pegs with average thickness of 0.68mm, about half the peg thickness measured on the female parent. This F_1 plant behaved as typically annual, dying out at the end of the reproductive period. Seeds germinated voluntarily in the pot, generating ten F_2 plants.

Crosses involving *A. gregoryi* with *A. ipaënsis* produced 39 fruit segments, that had no spontaneous germination, with the hybrid pollen viability estimate of 5.4%.

Seedlings resulting from two crossing combinations, involving *A. gregoryi* × *A. batizocoi*, showed abnormal growth from the first stages. Fruit segments produced in the respective female plant pots were harvested, some already starting germination. Seedlings and seeds were grown *in vitro*. The 82 pollinations made in the cross of *A. gregoryi* × *A. batizocoi* resulted in the production of 61 fruit segments, which would imply a very high rate of success, if they developed normal seeds and then plants. However, 46 seeds germinated spontaneously producing abnormal seedlings, lacking the aerial organs above the cotyledons. So, the seeds from the 15 remaining fruit segments were set to germinate *in vitro*. Only a single hybrid plant developed in a greenhouse, but it did not survive, when later transferred to screenhouse conditions. Embryos rescued *in vitro* still persist but need to be acclimatized.

The crossing of *A. gregoryi* × *A. cruziana* resulted in 36 fruit segments. Seedlings were vigorous, forming adult plants with lateral branches longer than 2.5 m, and intensive flowering. The average pollen viability estimate was 5.22%.

Concerning *A. gregoryi* × *A. krapovickasii*, 19 fruit segments were formed. The seeds germinated spontaneously, generating vigorous seedlings, but did not continue the development after showing the first leaves. Only one plant survived and flowered, allowing the pollen viability estimate of 0.4%.

The crossing combination *A. gregoryi* × *A. glandulifera*, representing the B and D genomes, produced 27 well-formed fruit segments, but 25 had aborted embryos, and one seed did not reach full maturity. The single well-formed seed produced a normal, flowering plant, with an estimated pollen viability of 0.3%. The hybrid status was easily confirmed by the presence of glands in the abaxial leaflet surface, a typical paternal feature.

In a cross involving *A. gregoryi* × *A. benensis*, which represents the F genome (Robledo et Seijo, 2010), 25 fruit segments were formed, with spontaneous germination of five seeds. Pollen viability estimates were quite low, 0.5% in average.

In a second crossing combination that required *in vitro* support, involving *A. gregoryi* × *A. hoehnei* 30006, 59 seeds were produced, of which 43 germinated spontaneously. Although under intensive attack of mites, just like the male parent, seedlings started in good shape, but soon died. The remaining 16 seeds were set to germinate *in vitro*. Only a single plant survived in the greenhouse, and later, under screenhouse conditions. It had profuse lateral branching, with ascending-erect habit, and it

Table 2. Classification of male parent *Arachis* species and accessions used, with respective genome. Female parent always *Arachis gregoryi* V 14957.

Tabla 2. Clasificación de las especies de *Arachis* parentales masculinas y accesiones utilizadas, con el respectivo genoma. Parental femenino siempre *Arachis gregoryi* V 14957.

Group	Male parents	Accession	Genome[3]	%SH[4]	%PVS[5a]	%PVG[5b]	NFS[6]	NH[7]	%PVH[8]	HB/BH[9]
1	*A. hoehnei*[2]	V 13985	?	17.1	93.85	82.1	34	19	NF	GSD
	A. kuhlmannii[2]	V 13779	Apn	15.1	74.97	67.25	24	12	4.8	GSD
	A. williamsii[1]	Wi 1118	B	14.3	97.65	79.35	28	10	2.6	GSD
	A. kempff-mercadoi[2]	V 13250	Ach	14,0	61.92	41.9	17	7	NF	GSD
	A. schininii[2]	V 9923	Apl	13.3	92.25	77.9	14	11	NF	GSD
	A. microsperma[2]	V 14042	A	12.5	95.4	86.1	8	8	NF	GSD
	A. stenosperma[2]	V 10309	Apn	11.9	84.22	78.9	27	16	NF	DES
	A. vallsii[2]	V 7635	?	11.9	94.3	81.15	22	10	NF	GSD
2	*A. valida*[1]	V 13514	B	9.6	71.92	60.05	36	11	5.45	GSD
	A. magna[1]	K 30097	B	9.5	99.17	82.45	11	4	7.12	GSD
	A. villosa[1]	V 14309	A	8.2	93.97	84.2	4	4	NF	GSD
	A. diogoi[2]	Vp 5000	Apn	5.4	95.45	88.00	20	3	NF	GSD
3	*A. hypogaea* var. *aequatoriana*[2]	Mf 1678	AB	3.6	76.82	69.25	46	4	NF	AE/DES
	A. krapovickasii[1]	Wi 1291	K	3.5	93.7	86.15	19	2	0.4	DES
	A. ipaënsis[2]	K 30076	B	2.8	94.37	83.75	39	3	5.4	GSD
	A. helodes[2]	V 6325	Apn	2.7	46.75	41.7	22	3	NF	GSD
	A. schininii[1]	V 9923	Apl	2.7	97.17	87.45	5	1	NF	GSD
	A. simpsonii[2]	V 13728	Apn	2.5	81.42	66.95	18	2	NF	GSD
	A. cruziana[1]	Wi 1302-2	K	2.5	98.37	87.4	36	3	5.22	GSD
	A. magna[1]	V 14724	B	2.2	98.62	72.15	4	2	10.45	GSD
	A. hypogaea var. *hirsuta*[2]	Mf 1538	AB	2.0	90.95	72.85	13	1	NF	AE/DES
	A. gregoryi[1]	V 14753	B	1.7	93.9	75.75	24	2	43.6	DES
	A. glandulifera[1]	V 13738	D	1.7	95.85	60.65	27	1	0.3	AE/GSD
	A. benensis[2]	K 35005	F	1.4	93.8	86.55	25	1	0.6	GSD
	A. hypogaea var. *fastigiata*[2]	IAC 'Tatu'	AB	1.4	69.57	57.75	31	1	NF	AE/DES
	A. batizocoi[1]	K 9484	K	1.2	89.6	86.2	61	1	NF	IV/DES
	A. duranensis[2]	V 14167	Apelo	1.1	91.55	82.35	32	1	NF	DES
	A. hoehnei[1]	K 30006	?	0.6	90.2	78.85	59	1	12	IV/DES
4	*A. diogoi*[1]	Vp 5000	Apn	0.0	90.27	68.5	4	0	-	LS/DES
	A. duranensis[1]	V 14167	Apl	0.0	87.95	82.2	0	0	-	LS/LQF/DES
	A. stenosperma[1]	V 10309	Apn	0.0	92.75	84.95	0	0	-	LS/LQF/DES
	A. ipaënsis[1]	K 30076	B	0.0	94.55	78.95	10	0	-	DES

Group	Male parents	Accession	Genome[3]	% SH[4]	% PVS[5a]	% PVG[5b]	NFS[6]	NH[7]	% PVH[8]	HB/BH[9]
	A. gregoryi[1]	V 14767	B	0.0	95.25	81.3	30	0	-	DES
	A. gregoryi[1]	V 14962	B	0.0	84.57	73.8	6	0	-	LS/DES
	A. magna[1]	V 13761	B	0.0	94.17	84.1	0	0	-	LS/LQF
	A. magna[1]	V 13765	B	0.0	96.62	72.15	8	0	-	DES
	A. vallsii[1]	V 7635	?	0.0	88.6	73.1	0	0	-	LS/LQF
	A. hypogaea "Xingu type"[2]	V 12549	AB	0.0	62.8	50.25	3	0	-	LS
	A. hypogaea var. *hypogaea*[2]	V 12548	AB	0.0	83.27	72.65	11	0	-	AE/DES
	A. hypogaea var. *peruviana*[2]	Mf 1560	AB	0.0	88.12	74.95	1	0	-	LS/LQF/ DES
	A. hypogaea var. *vulgaris*[2]	IAC 'Tatuí'	AB	0.0	88.15	72.25	39	0	-	AE/DES
	A. monticola[2]	Sj 2775	AB	0.0	90.9	82.3	10	0	-	AE/DES
	A. monticola[2]	V 14165	AB	0.0	81.1	78.3	8	0	-	AE/DES
	A. palustris[2]	V 13023	G	0.0	86.1	75.85	18	0	-	DES
	A. paraguariensis[2]	V 7677	E	0.0	87.5	79.45	8	0	-	AE/DES

[1] First cross season 2006/2007.
[2] Second cross season 2007/2008.
[3] A genome subgroups (Robledo et al. 2009): Ach= Chiquitano; Apn= Pantanal; Apl=La Plata River Basin.
[4] Percentage of success for obtaining hybrids (% SH)
[5] a, b Percentage of viability of the pollen-grain of the parents by staining (% PVS) and by germination (% PVG)
[6] Number of fruit segments produced by crossing combination (NFS)
[7] Number of confirmed hybrids (NH)
[8] Percentage of estimated viability of pollen-grain of hybrids by staining (% VPH)
[9] Hybrids behavoir/bariers for hybridization (HB/BH): good seedling development (GSD), death at the early stages of seedling development (DES), abortion of the embryo (AE), material cultivated in vitro (IV), low synchrony of flowering between parents (LS), low quantity of flowers of the parents (LQF), no flowering (NF).

flowered. The pollen viability estimate was 12%, with extremes of 9% and 18.2%.

The fourth group involves crosses in which the resulting seeds did not become established as plants, so that the hybrid character could not be confirmed (Table 2). Additionally, the crosses *A. gregoryi* × *A. diogoi*, *A. gregoryi* × *A. duranensis*, *A. gregoryi* × *A. stenosperma*, *A. gregoryi* × *A. ipaënsis*, and *A. gregoryi* × *A. vallsii*, as performed in the first season, as well as *A. gregoryi* × *A. gregoryi* 14767, *A. gregoryi* × *A. gregoryi* 14962, *A. gregoryi* × *A. magna* 13761, *A. gregoryi* × *A. magna* 13765, *A. gregoryi* × *A. vallsii*, *A. gregoryi* × *A. hypogaea* "Xingu" type, *A. gregoryi* × *A. hypogaea* subsp. *fastigiata* var. *peruviana*, and *A. gregoryi* × *A. palustris* were not possible because of low flowering synchrony between parents (LS), or due to low quantity of flowers produced by the parents (LQF), or potential hybrid offspring that died at early stages of seedling development (DES).

In crosses of *A. gregoryi* × *A. hypogaea* subsp. *hypogaea* var. *hypogaea*, *A. gregoryi* × *A. hypogaea* subsp. *fastigiata* var. *vulgaris*, *A. gregoryi* × *A. monticola* 2775, *A. gregoryi* × *A. monticola* 14165, *A. gregoryi* × *A. paraguariensis*, embryos aborted (AE) and/or seedlings died at early stages of development (DES).

Factors like low flowering synchrony and low quantity of flowers produced by the male parents, and, most of the time, low vigor of flowers after manipulation for hybridization were significant in the lack of success in the production of hybrid plants.

Discussion

Pollen viability estimates

No method for estimation of pollen viability is fully satisfactory, as the stains used in chemical tests react with chemical constituents or structures of the grains, and may not reflect the real germinating capacity (Stanley et Linskens, 1974). In spite of this, estimates of pollen viability are important to ensure that the plant materials involved present, at least in theory, the ability to trigger the complex reproductive process that involves morphological, physiological and genetic aspects.

Comparing the pollen staining and germination estimates, the difference of 10 to 20% is considered a common fact, most possibly due to overestimation with the staining technique and underestimation with germination, as concerns the viable pollen grains (Galetta, 1983). Even though some authors do not consider pollen staining as a viable estimate of plant fertility, the present authors are not as skeptical as to the positive aspects of this technique. Pollen staining has been reported in the evaluation of *Arachis* crossing programs by several authors (Gregory et Gregory, 1979; Simpson, 1991; Krapovickas et Gregory, 1994; Wondracek-Lüdke et al., 2015; Fávero et al., 2015a, b) and is generally accepted by *Arachis* researchers as a useful estimate of fertility.

Although a few accessions have shown lower pollen estimates for some time along the crossing season, all others consistently presented high estimates, so that all male parents were considered fit to fertilize the flowers of the female parent. It is not uncommon for a species such as *A. helodes* to have a low pollen count when some strenuous environmental conditions occur. If daytime temperature exceeds 42ºC, fertile plants of many *Arachis* species may have a pollen stain of < 50% for two or three consecutive days (Simpson CE, unpublished results).

Hybridization

The female parent *A. gregoryi* 14957, from the same site of 6389, has shown a broad crossing plasticity, as it was successfully pollinated and developed fruit segments in most crossing combinations, involving distinct genomes, ploidy levels, and taxonomic sections. Although these two accessions were assigned distinct collection numbers and germoplasm codes (BRA), due to the long time elapsed between field collections, previous data and the results of the present research confirm the potential value of this species and of the wild population represented by both the 6389 and 14957 accessions for hybridization studies.

The possibility of polyploidization of the hybrid *A. gregoryi* 6389 × *A. linearifolia*, producing an amphidiploid, which was then successfully crossed with *A. hypogaea*, has been demonstrated (Fávero et al., 2015a). So, it is estimated that additional accessions of *A. gregoryi*, with a B genome somewhat similar to that of *A. ipaënsis*, may be used as efficient bridge species for the introgression of genes from wild *Arachis* species into the cultivated peanut.

Group 1

Crossing combinations in this group were the most successful, although they showed differentiated potential. The five hybrid combinations involving B × A genome holders (*A. gregoryi* × *A. kempff-mercadoi*, *A. kuhlmannii*, *A. microsperma*, *A. schininii*, or *A. stenosperma*, the last two with worse results in the first season), all showing low pollen counts, are good candidates for production of colchicine induced highly fertile amphidiploids. Diploid hybrids of *A. gregoryi* with species showing the A chromosome pair are of special interest to peanut breeding, once their chromosome doubling will produce lines with the AABB genome constitution, for direct crossing with *A. hypogaea*. Furthermore, several of the A genome species of which hybrids where obtained show variable degrees of resistance/tolerance to foliar diseases caused by *Cercospora arachidicola* Hori (early leaf spot), *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton (late leaf spot), and *Puccinia arachidis* Speg. (rust) (Fávero et al., 2009, 2015a).

It must be noted that hybrids of *A. gregoryi* × *A. stenosperma* have already been reported,

but they involve different accessions of each parental species (Fávero, 2015b; Valls et Simpson, 2005; Leal-Bertioli et al., 2017). These previous hybrids flowered in both experiments cited, showing low pollen viability estimates, from 0.4 to 4.9%. Besides peculiarities of the distinct accessions involved, environmental differences at the distinct experimental facilities may not be ruled out as responsible for the lack of flowering in the present study.

The easy B × B crossing of *A. gregoryi* × *A. williamsii*, associated to the very low pollen viability estimates of the hybrid produced, suggests a potential for successful restoration of fertility through chromosome doubling. Such tetraploid line, gathering characteristics of two B genome species, may be useful for gene pyramidation in the B side.

The easiest crossing, that of *A. gregoryi* × *A. hoehnei* 13985, the most prolific male parent in this crossing program, may be of high interest for peanut breeding, but further study of the *A. hoehnei* genome relationships is still on demand.

Seven accessions of *A. hoehnei* are available in the Embrapa Wild *Arachis* Genebank, all from the state of Mato Grosso do Sul, Brazil. But they are not uniform, showing differences in their reproductive cycle and crossing behavior, and doubts still persist about the presence or absence of the A chromosome pair in distinct accessions of the species (Fernández et Krapovickas, 1994; Custodio et al., 2013).

Easy crossing of *A. gregoryi* × *A. vallsii*, attained in the second season, but by no means in the first, is not only of genetic, but also of taxonomic importance. *Arachis vallsii* was originally described as a member of the taxonomic section *Procumbentes* (Krapovickas et Gregory, 1994). Cytogenetic evidence has confirmed its closer relationship to the *Arachis* section, although it does not show the A chromosome pair (Lavia et al., 2009) nor a typical B genome configuration (Robledo et Seijo, 2010). Confirmation of the crossability of *A. gregoryi* × *A. vallsii* suggests a possibility of incorporating some peculiar features of the latter into peanut breeding programs, especially those related to the ecological preferences of *A. vallsii*, which thrives in periodically flooded areas, producing its underground fruits in muddy soils (Simpson et al., 2018).

Group 2

As well as *A. gregoryi*, associated to the B genome (Robledo et Seijo, 2010), *A. valida* has a potential value for peanut breeding, as it shows resistance to leaf spots (Fávero, 2009), and possibly also to flooding, considering the area of occurrence of its natural populations (Krapovickas et Gregory, 1994). In a parallel study (Wondracek-Lüdke et al., 2015), *A. valida* has shown a similar potential to that of *A. gregoryi* for producing hybrids with all representatives of the B genome.

Concerning the hybrid of *A. gregoryi* × *A. magna* 30097, similar low values for pollen viability estimates were obtained in Texas (Simpson et Faries, 2001), involving the same male parent and the 6389 accession of *A. gregoryi*, already mentioned as a member of the same natural population of our female parent. This means that, although *A. gregoryi* and *A. magna* seem to be closely related species, even showing individual plants somewhat difficult to discriminate on morphological grounds, and have several sympatric populations, they must have developed genetic barriers, which maintain their reproductive isolation and allow for their persistence as distinct species.

As to the *A. gregoryi* × *A. villosa* hybridization, the latter, collected in Brazil on the edge of the Uruguay river, is cytogenetically, as well as geographically considered a member of the La Plata River Basin subgroup of A genome species, therefore narrowly related to *A. duranensis* (Robledo et al., 2009). Due to this close cytological affinity to the A genome donor of *A. hypogaea* (Seijo et al., 2004), this AB diploid hybrid is of utmost interest for chromosome duplication and incorporation to peanut breeding programs.

Another *A. gregoryi* × *A. villosa* hybrid, obtained by using, respectively, the *A. gregoryi* accession 6389 and the Uruguayan accession of *A. villosa* VGoMrOv 12812, not tested here, had a much lower percent of success (0.92%), but flowered, with estimated pollen viability of 7.67% (Fávero, 2015b).

The *A. gregoryi* × *A. diogoi* diploid hybrid is another interesting line for chromosome

duplication, once the paternal species, then represented by the distinct Paraguayan accession GKP 10602, has been used to produce the amphidiploid line that resulted in the release of the cultivar 'COAN' (Simpson et Starr, 2001), which has near immunity to the root-knot nematode species *Meloidogyne arenaria* (Neal) Chitwood and *M. javanica* (Treub) Chitwood. Natural populations of *A. diogoi* occur along the Paraguay river basin and its tributaries, in areas subject to periodic flooding (Krapovickas et Gregory, 1994), and have shown resistance to early and late leaf spots, thrips, and jassids (Fávero, 2009).

Group 3

In spite of the distinct ploidy levels involved, crosses of *A. gregoryi* × *A. hypogaea* did not require special techniques. However, embryo abortion at early stages of seed formation was frequent. Mature seeds were produced in crosses with three varieties (*aequatoriana*, *hirsuta*, and *fastigiata*). None has shown dormancy, and they generally germinated, but most seedlings died at early stages. All four surviving plants of *A. gregoryi* × *A. hypogaea* subsp. *fastigiata* var. *aequatoriana* were triploid (2n=3x=30) and highly sterile, while the single surviving plant of *A. gregoryi* × *A. hypogaea* subsp. *hypogaea* var. *hirsuta* was too weak, and roots were not adequate for chromosome counting, but its morphology, quite different from the female parent, led to the assumption of the hybrid state. Also, a single slow growing, confirmed cytologically as a triploid plant was rescued from the combination *A. gregoryi* × *A. hypogaea* subsp. *fastigiata* var. *fastigiata*, which, although healthy, never flowered.

Manipulation of triploid interspecific hybrids of *Arachis* to produce useful breeding lines is laborious. It is first necessary to duplicate chromosomes to produce a fertile hexaploid line, which is then backcrossed to *A. hypogaea*, until it stabilizes at 2n=40, due to chromosome loss (Simpson, 1991, 2001), but with no guarantee of a balanced AABB genome structure. In spite of the more complicated pathway, triploid hybrids produced in this program have a potential use in peanut breeding. Different behavior presented by crossing combinations of *A. gregoryi* with these three botanical varieties of *A. hypogaea* would recommend additional trials, involving the remaining varieties, as well as accessions of the "Xingu" type, morphologically divergent, so far unclassified at the subspecific level, and more closely related to *A. monticola* (Nascimento et al., 2020), of which no hybrids were obtained in this study.

Considering B × B hybrids, it is noteworthy that only one intraspecific combination (*A. gregoryi* 14957 × 14753) has been successful, out of three attempted. The accessions tested, 14957 and 14753, are from adjacent counties in Mato Grosso State, but were collected in nature 99 km apart. Although pollen viability estimates of this hybrid reached 43.6%, the highest figure for all hybrids obtained in the present study, it is far below estimates of over 90% mentioned for other intraspecific and even some interspecific crosses involving other *Arachis* species (Krapovickas et Gregory 1994, Simpson et Faries 2001; Fávero et al., 2015 a, b; Wondracek-Lüdke et al., 2015). So, crossing data obtained in this study for the specific accession used as the female parent should be taken carefully as concerns other *A. gregoryi* materials, except in comparisons with the 6389 accession, recognized as a member of the same natural population.

Contrary to the general trend, seeds resulting from the *A. gregoryi* × *A. ipaënsis* crossing did not present spontaneous germination. Lack of spontaneous germination is a useful trait, allowing for timely use of the hybrid seed in breeding programs. Due to the relevance of *A. ipaënsis* in the origin of *A. hypogaea* (Kochert et al., 1996; Seijo et al., 2004, 2007; Fávero et al., 2006; Bertioli et al., 2016), and the fact that there is only one accession of the former species available in genebanks, hybrids with *A. ipaënsis* are important for potentially expanding the possibilities of introgression of favorable characteristics associated to the B genome, for the improvement of peanut cultivars.

Similar to the hybrids with *A. ipaënsis*, and contrary to the behavior of the *A. gregoryi* × *A. magna* 30097, seeds of *A. gregoryi* × *A. magna* 14724 did not show spontaneous germination. This emphasizes the diversity

of the available *A. magna* accessions, already put in evidence by molecular cytogenetics (Custodio et al., 2013) and molecular marker analyses (Moretzsohn et al., 2013).

Although at lower rates of success, once again B × A genome crosses produced interesting candidates for induced chromosome doubling for direct crossing with *A. hypogaea* at the tetraploid level, including the one involving *A. duranensis*, the A genome donor of *A. hypogaea* (Kochert et al., 1996; Seijo et al., 2004, 2007; Fávero et al., 2006; Bertioli et al., 2016), besides *A. helodes*, *A. schininii*, and *A. simpsonii*.

Crosses of the B genome *A. gregoryi* with representatives of the D, F and K genomes, though important to the comprehension of genetic and evolutionary aspects of the genus, are not likely to produce amphidiploids as close to *A. hypogaea*, but that does not exclude their interest for peanut breeding, once such species eventually present favorable attributes, related to biotic and/or abiotic constraints. For instance, the K genome *A. batizocoi* has been used in the construction of TxAG-6, the breeding line that gave rise to the cultivar 'COAN' (Simpson et Starr, 2001). While, in the present study, hybrid seedlings of *A. gregoryi* × *A. batizocoi* produced mostly abnormal seedlings, lacking the aerial organs above the cotyledons, a reverse hybrid involving the same accession of *A. batizocoi* and *A. gregoryi* 6389 has shown normal development and flowering, with a pollen viability estimate of 4.5% (Simpson et Faries, 2001).

The low pollen viability estimate of the *A. gregoryi* × *A. glandulifera* hybrid possibly reflects a wide genetic distance between the parents involved. Intraspecific hybrids involving distinct accessions of *A. glandulifera*, all from Bolivia, and interspecific hybrids of *A. glandulifera* × *A. batizocoi* and *A. duranensis*, have been obtained elsewhere (Stalker, 1991). However, despite 835 pollinations in both ways, involving *A. glandulifera* and two cultivars of *A. hypogaea* representing subspecies *hypogaea* and *fastigiata*, not a single triploid hybrid was obtained (Stalker, 1991). Successful crosses of *A. gregoryi* × *A. glandulifera* may open a pathway to bringing desirable genes of the highly prolific *A. glandulifera* into pre-breeding lines similar to the peanut B genome, eventually conditioned to chromosome duplication.

It is interesting to notice the distinct results shown by the crossing combination *A. gregoryi* × *A. hoehnei*. The most successful crossing in this study has been obtained using *A. hoehnei* 13985 as the male parent, while that with *A. hoehnei* 30006 lies in the third group. The founder seed of the male parent *A. hoehnei* 30006 has been collected in the field at the same natural site of the type collection (Krapovickas et Gregory, 1994), so this accession is the most typical of *A. hoehnei*, while other accessions listed at the moment under the same name, such as 13985, may belong to a distinct taxon, yet to be described (Custodio et al., 2013).

Group 4

Although this group encompasses unsuccessful crosses or crosses in which the hybrid nature of seed produced could not be confirmed, this cannot be taken as a definitive indication of incompatibility. For example, the unsuccesfull hybrization of *A. gregoryi* with *A. diogoi*, *A. duranensis*, and *A. stenosperma* (three A genome species) and *A. vallsii* (undetermined genome) in the first season was superseded by successful crosses in the second season. Such differences, which we cannot easily account for, may be under the influence of several factors, such as environmental factors, vigor of plants, attack of pests, and even the manipulation of the flowers of each parent during emasculation. But it must be considered that they did not affect crosses in the first season so intensely, when involving *A. williamsii*, *A. valida* and *A. magna* 30097 (B genome species), and *A. villosa* (A genome).

The genetic diversity of accessions of a same species that show different crossability results needs to be evaluated. This refers to the distinct varieties and off-types of *A. hypogaea*, as well as to species such as *A. gregoryi*, *A. magna*, and *A. hoehnei*.

In summary, it has been shown that *A. gregoryi* is a promising diploid wild species to be incorporated into peanut breeding programs. It has shown plasticity for crosses with species of several genome types present in the taxonomic section *Arachis*. Its inclusion in breeding

programs will expand the diversity of species and breeding lines associated to the B genome, with special attention to the actual availability of a single accession of *A. ipaënsis*, involved in the origin of *A. hypogaea*.

Similarly positive crossing results with distinct botanical varieties of *A. hypogaea* indicate that the use of *A. gregoryi* in pre-breeding efforts is a possibility, also through the more laborious triploid/hexaploid/backcrossing strategy.

Finally, crossability of *A. gregoryi* with several representatives of the A genome group, and the general sterility of the AB hybrids, suggests a good potential use of *A. gregoryi* in pre-breeding efforts through the amphidiploid strategy, which has already produced relevant results in the genetic breeding of the cultivated peanut.

Acknowledgements

This work is part of the doctoral thesis of the first author, at the Federal University of Santa Catarina, Graduate Program in Plant Genetic Resources, and a Post-Doctoral Scholarship CAPES/CNPq/Protax. The authors would like to thank the support of Nacional Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES) and Embrapa Genetic Resources and Biotechnology, for the scholarships and grants received (310026/2018-0, 312215/2013-4, 313763/2013-5, 401939/2013-8, 483860/2012-3, 561768/2010-2, 311488/2006-4, and 001/2011/Projeto 43).

Bibliography

Bertioli, D. J., Cannon, S. B., Froenicke, L., Huang, G., Farmer, A. D., Cannon, E. K., Liu, X., Gao, D., Clevenger, J., Dash, S., Ren, L., Moretzsohn, M. C., Shirasawa, K., Shirasawa, K., Huang, W., Vidigal, B., Abernathy, B., Chu, Y., Niederhuth, C. E., Umale, P., Araújo, A. C., Kozik. A., Kim, K. D., Burow, M. D., Varshney, R. K., Wang, X., Zhang, X., Barkley, N., Guimarães, P. M., Isobe, S., Guo, B., Liao, B., Stalker, H. T., Schmitz, R. J., Scheffler, B. E., Leal-Bertoli, S. C., Xun, X., Jackson, S. A., Michelmore, R. & Ozias-Akins, P. (2016). The genome sequence of *Arachis duranensis* and *Arachis ipaënsis*, the diploid ancestors of cultivated peanut. Nature Genetics 48: 438-446. https://doi.org/10.1038/ng.3517

Bertioli, D. J., Seijo, G., Freitas, F. O., Valls, J. F. M., Leal-Bertioli, S. C. M., Moretzsohn, M. C. (2011). An overview of peanut and its wild relatives. Plant Genetic Resources 9: 134-149. https://doi.org/10.1017/S1479262110000444

Burow, M. D., Simpson, C. E., Faries, M. W., Starr, J. L. & Paterson, A. H. (2009). Molecular biogeographic study of recently described B- and A- genome *Arachis* species, also providing new insights into the origins of cultivated peanut. Genome 52: 107-119. https://doi.org/ 10.1139/g08-094

Custodio, A. R., Seijo, G. & Valls, J. F. M. (2013). Characterization of Brazilian accessions of wild *Arachis* species of section *Arachis* (Fabaceae) using heterochromatin detection and fluorescence *in situ* hybridization (FISH). Genetics and Molecular Biology 36: 364-370. http://dx.doi.org/10.1590/S1415-47572013000300011

Fávero, A. P., Simpson, C. E., Valls, J. F. M. & Vello, N. (2006). Study of the evolution of cultivated peanut through crossability studies among *Arachis ipaënsis*, *A. duranensis* and *A. hypogaea*. Crop Science 46: 1546-1552. https://doi.org/ 10.2135/cropsci2005.09-0331

Fávero, A. P., Moraes, S. A., Garcia, A. A. F., Valls, J. F. M. & Vello, N. (2009). Characterization of rust, early and late spot resistance in wild and cultivated peanut germoplasm. Scientia Agricola 66: 110-117. http://dx.doi.org/10.1590/S0103-90162009000100015

Fávero, A. P., Santos, R. F., Simpson, C. E., Valls, J. F. M. & Vello, N. (2015a). Successful crosses between fungal-resistant wild species of *Arachis* (section *Arachis*) and *Arachis hypogaea*. Genetics and Molecular Biology 38: 353-365. http://dx.doi.org/10.1590/S1415-475738320140376

Fávero, A. P., Pádua, J. G., Costa, T. S., Gimenes, M. A., Godoy, I. J., Moretzsohn, M. C. & Michelotto, M. D. (2015b). New hybrids from peanut (*Arachis hypogaea* L.) and synthetic amphidiploid crosses show promise in increasing pest and disease tolerance. Genetics and Molecular Research 14: 16694-16703. http://dx.doi.org/10.4238/2015.December.11.17

Fernández, A. & Krapovickas, A. (1994). Cromosomas y evolución en *Arachis* (Leguminosae). Bonplandia 8: 187-220. http://dx.doi.org/10.30972/bon.81-41499

Foncéka, D., Hodo-Abalo, T., Rivallan, R., Faye, I., Sall, M. N., Ndoye, O., Fávero, A. P., Bertoli, D. J., Glaszmann, J. C., Courtois, B. & Rami, J. F. (2009). Genetic mapping of wild introgressions into cultivated peanut: a way toward enlarging the genetic basis of a recent allotetraploid. BMC Plant Biology 9: 103. http://dx.doi.org/ 10.1186/1471-2229-9-103

Galetta, G. J. (1983). Pollen and seed management. En Moore, J. N. & Janik, J. (ed.), Methods in Fruit Breeding, pp. 23-47. Purdue University Press, Indiana.

Gregory, M. P. & Gregory, W. C. (1979). Exotic germ plasm of *Arachis* L. interspecific hybrids. The Journal of Heredity 70: 185-193. https://doi.org/10.1093/oxfordjournals.jhered.a109231

krapovickas, A. & Gregory, W. C. (1994). Taxonomía del género *Arachis* (Leguminosae). Bonplandia 8: 1-186. https://dx.doi.org/10.30972/bon.81-43559

Kochert, G., Stalker, H. T., Gimenes, M., Galgaro, L., Lopes, C. R. & Moore, K. (1996). RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (Leguminosae). American Journal of Botany 83: 1282-1291. https://doi.org/10.1002/j.1537-2197.1996.tb13912.x

Lavia, G. I., Ortiz, A. M. & Fernández, A. (2009). Karyotypic studies in wild germoplasm of *Arachis* (Leguminosae). Genetic Resources and Crop Evolution 56: 755-764. https://doi.org/10.1007/s10722-008-9399-6

Leal-Bertioli, S. C. M., Moretzsohn. M. C., Santos, S. P., Brasileiro, A. C. M., Guimarães, P. M., Bertioli, D. J. & Araujo, A. C. (2017). Phenotypic effects of allotetraploidization of wild *Arachis* and their implications for peanut domestication. American Journal of Botany 104: 379-388. https://doi.org/10.3732/ajb.1600402

Moretzsohn, M. C., Gouvea, E. G., Inglis, P.W., Leal-Bertioli, S. C. M., Valls, J. F. M. & Bertioli, D. J. (2013). A study of the relationships of cultivated peanut (*Arachis hypogaea*) and its most closely related wild species using intron sequences and microsatellite markers. Annals of Botany 111: 113-126. https://doi.org/10.1093/aob/mcs237

Nascimento, E. F. M. B., Leal-Bertioli, S. C. M., Bertioli, D. J., Chavarro, C., Freitas, F. O., Moretzsohn, M. C., Guimarães, P. M., Valls, J. F. M. & Araujo, A. C. G. (2020). Brazilian Kayabi Indian accessions of peanut, *Arachis hypogaea* (Fabales, Fabaceae): origin, diversity and evolution. Genetics and Molecular Biology 43: e20190418. https://doi.org/10.1590/1678-4685-GMB-2019-0418

Niles, W. L. & Quesenberry, K. H. (1992). Pollen Germination of Rhizoma Peanut cv. Florigraze. Peanut Science 19: 105–107. https://doi.org/10.3146/i0095-3679-19-2-11

Robledo, G. & Seijo, G. (2008). Characterization of the *Arachis* (Leguminosae) D genome using fluorescent *in situ* hybridization (FISH) chromosome markers and total genome DNA hybridization. Genetics and Molecular Biology 31: 717-724. http://dx.doi.org/10.1590/S1415-47572008000400019

Robledo, G. & Seijo, G. (2010). Species relationships among the wild B genome of *Arachis* species (section *Arachis*) based on FISH mapping of rDNA loci and heterochromatin detection: a new proposal for genome arrangement. Theoretical and Applied Genetics 121: 1033-1046. http://dx.doi.org/10.1007/s00122-010-1369-7

Robledo, G., Lavia, G. I. & Seijo, G. (2009). Species relations among wild *Arachis* species with the A genome as revealed by FISH mapping of rDNA loci and heterochromatin detection. Theoretical and Applied Genetics 118: 1295-1307. http://dx.doi.org/10.1007/s00122-009-0981-x

Seijo, J. G., Lavia, G. I., Fernández, A., Krapovickas, A., Ducasse, D. A. & Moscone, E. A. (2004). Physical mapping of 5S and 18-25S rRNA genes evidence that *Arachis duranensis* and *A. ipaënsis* are the wild diploid species involved in the origin of *A. hypogaea* (Leguminosae). American Journal of Botany 91: 1294-1303. http://dx.doi.org/10.3732/ajb.91.9.1294

Seijo, J. G., Lavia, G. I., Fernández, A., Krapovickas, A., Ducasse, D. A., Bertioli, D. J. & Moscone, E. A. (2007). Genetic relationships between the cultivated peanut (*Arachis hypogaea,* Leguminosae) and its close relatives revealed by double GISH. American Journal of Botany 94: 1963-1971. http://dx.doi.org/ 10.3732/ajb.94.12.1963

Silvestri, M. C., Ortiz, A. M. & Lavia, G. I. (2015). rDNA loci and heterochromatin positions support a distinct genome type for `x=9 species´ of section *Arachis* (*Arachis* Leguminosae) Plant Systematic and Evolution 301: 555-562. https://doi.org/10.1007/s00606-014-1092-y

Simpson, C. E. (1991). Pathways for introgression of pest resistance into *Arachis hypogaea* L. *Peanut Science* 18: 22-26. https://doi.org/10.3146/i0095-3679-18-1-8

Simpson, C. E. (2001). Use of wild *Arachis* species/ introgression of genes into *A. hypogaea*. Peanut Science 28: 114-116. https://doi.org/10.3146/i0095-3679-28-2-12

Simpson, C. E. & Faries, M. J. (2001). Advances in the characterization of diversity in section *Arachis*: archeological evidence, crossing results and their relationship in understanding the origins of *Arachis hypogaea* L. III Simpósio de Recursos Genéticos para América Latina e Caribe. Resumos. Londrina: IAPAR, p. 103.

Simpson, C. E. & Starr, J. L. (2001). Registration of "COAN" peanut. Crop Science 41: 918. https://doi.org/10.2135/cropsci2001.413918x

Simpson, C. E., Custodio, A. R., Rodrigues, L. S., Penaloza, A. P., Valls, J. F. M. & Cason, J. M. (2018). Using *Arachis Vallsii* Krapov. & W.C. Greg. as a bridge species for introgression in *Arachis*. American Peanut Research and Education Society Annual Meeting Proceedings, 50, 2018, Williamsburg, VA. https://apresinc.com/meetings/annual-meeting/abstracts.

Singh, A. K. & Moss, J. P. (1984). Utilisation of wild relatives in the genetic improvement of *Arachis hypogaea* L.5. Genome analysis in section *Arachis* and its implications in gene transfer Theoretical and Applied Genetics 68: 355-364. https://doi.org/10.1007/BF00267889

Stalker, H. T. (1991). A new species in section *Arachis* of peanuts with a D genome. American Journal of Botany 78: 630-637. https://doi.org/10.1002/j.1537-2197.1991.tb12587.x

Stanley, R. G. & Linskens, H. F. (1974). Pollen Biology, Biochemistry and Management. Springer-Verlag Berlin Heidelberg. https://trove.nla.gov.au/version/20162284

Valls, J. F. M. & Simpson, C. E. (2005). New species of *Arachis* (Leguminosae) from Brazil, Paraguay and Bolivia. Bonplandia 14: 35-64. http://dx.doi.org/10.30972/bon.141-21387

Valls, J. F. M. & Simpson, C. E. (2017). A new species of *Arachis* (Fabaceae) from Mato Grosso, Brazil, related to *Arachis matiensis*. Bonplandia 26: 143-149. http://dx.doi.org/10.30972/bon.2622575

Valls, J. F. M., Costa, L. & Custodio, A. R. (2013). A novel trifoliolate species of *Arachis* (Fabaceae) and further comments on the taxonomic section *Trierectoides*. Bonplandia 22: 91-97. http://dx.doi.org/10.30972/bon.2211257

Wondracek-Lüdke, D. C., Custodio, A. R., Simpson, C. E. & Valls, J. F. M. (2015). Crossability of *Arachis valida* and B genome *Arachis* species. Genetics and Molecular Research 14: 17574-1758. https://doi.org 10.4238/2015