Genomic affinity in hybrids of B-genome *Arachis* species: new genetic resources toward peanut improvement

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Abstract: *Peanut (Arachis hypogaea L.) is an important oilseed crop and food legume that is highly susceptible to biotic and abiotic factors. Wild Arachis species represent useful genetic resources because of their resistance to pests and diseases. In particular, understanding the affinity between B-genome species is important for broadening the genetic base of A. hypogaea (AABB genome), since only one population of A. ipaënsis is known, and relatively few other species have been described. Therefore, we aimed to determine the genomic affinity between A. ipaënsis and A. williamsii by developing hybrids. Microsatellite markers verified the hybrid status, and chromosome pairing and pollen viability results revealed that the species share high genomic affinity. Additionally, the presence of dyads detected in sporad and pollen analyses indicated the production of 2n gametes. These results will be useful for performing evolutionary studies and developing strategies for introgression programs to improve the B genome of A. hypogaea.*

Keywords: *Peanut, wild relatives, meiotic analysis, pollen viability*

INTRODUCTION

Peanut (*Arachis hypogaea* L., Fabaceae) is one of the most cultivated legumes in the world, as it is a valuable source of oil and protein (Kamdar et al. 2020). It is an allotetraploid (2n=4x=40; AABB) originated by hybridization between *A. duranensis* Krapov. & W.C. Greg. (2n=2x=20; AA) and *A. ipaënsis* Krapov. & W.C. Greg. (2n=2x=20; BB), and subsequent chromosome duplication (Seijo et al. 2007, Bertioli et al. 2016), likely via sexual polyploidization (García et al. 2020).

Crop yield can be negatively affected by diseases and pests. However, wild relatives can be exploited to enhance peanut performance because they constitute natural sources of resistance (Stalker 2017). Currently, the only commercially acceptable method for improving cultivated peanut is traditional gene introgression (Cason et al. 2019), which is restricted to species of the section Arachis, as *A. hypogaea* belongs to this group. The introgression of resistance genes from wild species into *A. hypogaea* can be performed by obtaining induced hybrids from crosses between wild diploid species, duplicating chromosomes of the hybrids using colchicine, and crossing with the peanut (Simpson 1991). Since closely related species are more likely to produce fertile progenies, selecting

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parents is a main factor in the success of crossings. Therefore, understanding the relationships between species is crucial for the introgression and pyramiding of resistance genes in *A. hypogaea* and justifies more intensive studies.

Arachis ipaënsis and *A. williamsii* Krapov. & W.C. Greg. (2n=2x=20; BB) are resistant to several pests, and recently conducted evaluations have shown that neither species shows symptoms of smut infection (Blas et al. 2019). Peanut smut is a soil-borne disease caused by the fungus *Thecaphora frezii* Carranza & Lindquist and is currently the main economically important peanut disease in Argentina (Rago et al. 2017). Thus, the possibility of transferring some of the resistance genes from both species into the B genome of peanut is of great interest. Although several crosses between B genome species have been conducted, there is only one report of *A. ipaënsis* × *A. williamsii* (Simpson and Faries 2001), and no meiotic studies have been performed to analyze their genomic homology. Meiotic studies provide relevant information on the fertility of hybrids, and therefore can accelerate the success of breeding programs (Krycki et al. 2020). Furthermore, the ability of hybrids to produce viable unreduced gametes, such as those reported in [*A. duranensis* × *A. ipaënsis*]^{2x} hybrids (García et al. 2020), may increase with interspecific crosses and environmental factors (Kreiner et al. 2017). Understanding this ability of *Arachis* hybrids is useful for inducing polyploidy in breeding programs.

Thus, we proposed the development of hybrid materials by performing reciprocal crosses between *A. ipaënsis* and *A. williamsii*, to certify hybridization using microsatellite markers, and analyze the meiotic behavior and determine the production of unreduced gametes in the F_1 hybrid progeny. We aimed to establish the relationships between these two B-genome *Arachis* species, considered of interest for introgression programs of resistance to key diseases in elite peanut genotypes.

MATERIAL AND METHODS

Plant material

Arachis ipaënsis, from Krapovickas et al. 30076 collection, and *A. williamsii* from Williams and Claire 1118 collection of the Instituto de Botánica del Nordeste (IBONE), Corrientes, Argentina, were used. The species were collected in the Tarija and Beni Departments of Bolivia, respectively, and are 2n=2x=20 with B genome. The hybrids obtained [*A. ipaënsis* × *A. williamsii*]^{2x} and [*A. williamsii* × *A. ipaënsis*]^{2x} are referred to here as IpaWil and Willpa, respectively.

Crosses and hybrid identification

Reciprocal crosses were performed between October 2015 and April 2016 in a greenhouse, according to the methods of Favero et al. (2006). During June and July 2016, the fruits were harvested and stored in the Germplasm Bank of the IBONE, and in September, the seeds were placed in individual germinators.

To identify true hybrid individuals, the seedlings were analyzed using microsatellite markers. The markers were developed by different authors, but the primer sequences, references, and other information were compiled on PeanutBase (https://peanutbase.org/search/marker). Total DNA was extracted according to the method described by Inglis et al. (2018). DNA amplification reactions were performed by multiplex PCR using until three fluorescences (6-FAM, HEX, and NED) by reaction and similar annealing temperatures. The PCR of microsatellite loci was performed as described by Moretzsohn et al. (2013). For allele sizing, the electropherograms were analyzed using the GeneMapper® software (Applied Biosystems).

Meiotic and pollen analysis

Pollen mother cells (PMCs) from young flower buds were stained in 2% lactopropionic orcein, and the frequency of all PMCs with normal and abnormal behavior was recorded from prophase I to telophase II. In addition, sporads colored with carmine:glycerin (1:1) were analyzed. A minimum of 300 sporads per flower bud was counted for each hybrid combination. The meiotic index was calculated as follows:

% *MI*=[(*number of normal tetrads* ×100)/*total of post meiotic products counted*] (Love 1951). Sporads with four microspores of the same size were considered normal tetrads, and any deviation was considered abnormal. The presence of dyads, triads, and monads was recorded as evidence of the production of 2n and 4n gametes.

Pollen viability was estimated in three flowers from each individual hybrid using the same technique as described for sporads. Regularly shaped and darkly stained pollen grains were considered viable and counted using ImageJ software (Rasband 1997). *Pollen viability = (stained pollen grains ÷ total pollen grains)×100*. Viable pollen grains from progenitors were measured with ImageJ software, using the Em index (Van Campo 1957). The production of unreduced pollen in the hybrids was detected following the Darlington (1937) criteria: pollen grains 1.25 times larger than the average size of each individual hybrid were considered as large 2n, whereas pollen grains 0.25 times smaller than the average size were described as small (García et al. 2020). Accordingly, viable pollen grains were classified as normal, small, or large 2n. Pollen size data were not normally distributed; therefore, we ran a one-way non-parametric ANOVA (Kruskal-Wallis test) (α level = 0.05) to evaluate the existence of significant differences among pollen sizes from *A. ipaënsis*, *A. williamsii*, IpaWil, and WilIpa. Multiple *a posteriori* comparisons were calculated between the mean ranks of each taxon using Dunn's test. Statistical analyses were performed using R software v 3.5.3 (R Core Team 2019).

RESULTS AND DISCUSSION

Crosses and hybrid identification

Only one accession of *A. ipaënsis* is known. Thus, understanding the crossability and relationships between the B-genome species is necessary to broaden the genetic base of *A. hypogaea* in breeding programs. In this study, *A. ipaënsis* was used as the female parent in 158 pollinations (44.89%), with peg development in 48.73%, whereas *A. williamsii* was the female parent in 194 crosses (55.11%), forming pegs in 25.26% of cases. After harvest, 66 seeds were obtained from *A. ipaënsis* × *A. williamsii* crosses and 44 seeds were collected from the reciprocal crosses. Although the formation of pegs and seeds does not guarantee hybrid development, values were high in both crosses, with greater returns when *A. ipaënsis* was the female parent. Custodio et al. (2021) obtained greater fruit numbers and success rates for *A. gregoryi* × *A. williamsii* crosses than for *A. gregoryi* × *A. ipaensis*. Similarly, Wondracek-Lüdke et al. (2015) obtained higher values of these parameters for *A. valida* × *A. williamsii* than for *A. valida* × *A. ipaënsis.* Considering the results of our crosses, B genome species with many collected accessions, such as *A. gregoryi*, *A. magna,* and *A. valida*, could use *A. williamsii* as a bridge species with the B genome of *A. hypogaea*, which has 99.96% homology with the *A. ipaënsis* genome (Bertioli et al. 2016). However, hybrids obtained in the studies mentioned above had low or null fertility, suggesting that more relationship studies between B genome species are required to evaluate this possibility.

Only seedlings obtained from seed germination were used in the analyses; the remaining seeds were stored in the *Arachis* Germplasm Bank of IBONE. A total of 47 microsatellite markers were screened for polymorphism against both parents and 18 were informative (PM36, PM45, PM73, PM170, PM210, pPGPSeq2H11, pPGSseq14H6, pPGSseq16C6, RN8C09, RN20C10, RN23A07, AC2B05, TC3E05, TC4G02, TC7E04, IPHAM108, GM1989, and Ad_FI308518). The 18 polymorphic markers confirmed that four of the eight WilIpa seedlings were true F_1 hybrids, three were self-pollinated, and one was the result of pollination by an unknown male parent. Of the four IpaWil seedlings, three were true F_1 hybrids and one was self-pollinated. Microsatellite markers were chosen because of their codominant nature, and the ability to identify the seedlings as truly hybrid, self-pollinated, or undesirably cross-pollinated. Selfed and contaminated seedlings were discarded, and seven true hybrids were analyzed.

Meiotic analysis

In *Arachis*, crossability studies have been useful at the genus level for grouping species into nine taxonomic sections (Krapovickas and Gregory 1994), and chromosome pairing in F_1 hybrids has been used to trace affinities and group species (Singh and Moss 1984). We analyzed chromosomal pairing in metaphase I and meiotic behavior from prophase I to telophase II.

Chromosome pairing

The analysis of 175 and 133 PMCs of IpaWil and WilIpa hybrids, respectively, revealed five meiotic configurations, including univalent (I), bivalent (II), trivalent (III), and quadrivalent (IV) associations. The most frequent association was bivalent, averaging 9.63 in IpaWil and 9.44 in WilIpa, while univalents ranged from zero to four per PMC, with means of 0.32 and 0.54, respectively. Multivalents were observed at very low frequencies in the two hybrid combinations. The

meiotic configuration 10II (Figure 1A) was the most common, with values of 83.43% in IpaWil and 76.69% in Willpa, while 9II+2I (Figure 1B) represented 9.14% and 15.04% of the PMC analyzed in the respective hybrids, and the remaining three configurations (8II+4I, 8II+1III+1I, and 8II+1IV; Figures 1C and D) accounted for less than 8% of the cells.

In cytological studies performed on *Arachis* intragenomic hybrids between A-genome species, the mean bivalent association ranged from 9.1 to 9.9, while the number of univalents ranged from zero to four per PMC with means from 0.05 to 1.35 (Singh and Moss 1984, Stalker et al. 1991). Tallury et al. (2005) reported mean values of 0.3 univalents and 9.9 bivalents in hybrids between K genome species. Although there are no reports of meiotic behavior in hybrids between B genome species, the hybrids analyzed in this study presented high frequencies of bivalents, with low or null presence of univalents. Closely related species are expected to have a high number of bivalents (Stalker et al. 1991),

Table 1. Meiotic abnormalities and sporads in hybrids between *A. ipaënsis* and *A. williamsii*

Phases	Meiotic abnormalities	IpaWil			Willpa		
		N	n	%	N	n	%
PI	Normal	141	136	96.45	583	306	52.49
	Cytomixis		5	3.55		227	47.51
MI	Normal	749	546	72.90	395	259	65.57
	Out of plate chromosomes		188	25.10		103	26.08
	Cytomixis		15	2.00		33	8.35
AI	Normal	37	37	100	-	-	-
TI	Normal	279	199	71.33	120	120	100
	Cytomixis		52	18.64		-	-
	Out of nucleus chromosomes		28	10.03		-	-
PII	Normal	219	136	62.10	114	47	41.23
	Out of nucleus chromosomes		7	3.20		-	-
	Unbalanced nuclei		62	28.31		63	55.26
	Unbalanced nuclei + cytomixis		3	1.37		-	-
	Cytomixis		11	5.02		4	3.51
MII	Normal	61	32	52.46	29	22	75.86
	Out of plate chromosomes		13	21.31		5	17.24
	Bridges		1	1.64		1	3.45
	Nucleus of restitution		15	24.59		-	-
	Cytomixis		-	-		1	3.45
TII	Normal	1137	783	68.87	166	87	52.41
	Unbalanced nuclei		24	2.11		42	28
	Multiple nuclei		-	-		3	1.81
	Micronuclei		7	0.62		-	-
	1 nucleus of restitution 2n		61	5.36		18	10.84
	Cytomixis		262	23.04		-	-
Total		2623			1407		
	% normal cells		71.26			59.77	
Sporads							
Tetrads			2202	80.22		1764	64.38
Abnormal Tetrads			314	11.44		146	5.33
Polyads			6	0.23		10	0.36
Triads			102	3.72		170	6.20
Abnormal Triads			11	0.40		9	0.32
Dyads			75	2.73		195	7.12
Abnormal Dyads			30	1.09		445	16.24
Monads			-	-		1	0.05
Abnormal Monads			5	0.18		-	-
Total			2745			2740	
	% MI		80.22			64.38	

Phases: P, prophase; M, metaphase; A, anaphase; T, telophase; I, first meiotic division; II, second meiotic division. MI, meiotic index.

and a high frequency of bivalents in intragenomic hybrids suggests strong genomic similarities between species (Singh and Moss 1984).

Multivalents have only been reported in a few *Arachis* hybrids and at low frequencies (Singh and Moss 1984, Stalker et al. 1991). Quadrivalents could occur due to the presence of reciprocal translocation heterozygotes, by which cytological differentiation and subsequent speciation develop among *Arachis* taxa (Stalker et al. 1991). According to Singh and Moss (1984), a reduction in bivalent and an increase in univalent frequency in hybrids indicate reduced homology between parental chromosomes, whereas a decrease in bivalents and the formation of multivalents indicate a repatterning of genetic material and not a reduction in homology. Therefore, both the low degree of multivalent association and the high level of bivalent association observed in this study are not conclusive to generalize chromosome structural differentiations and clearly show a high degree of homology between the B genomes of *A. ipaënsis* and *A. williamsii*.
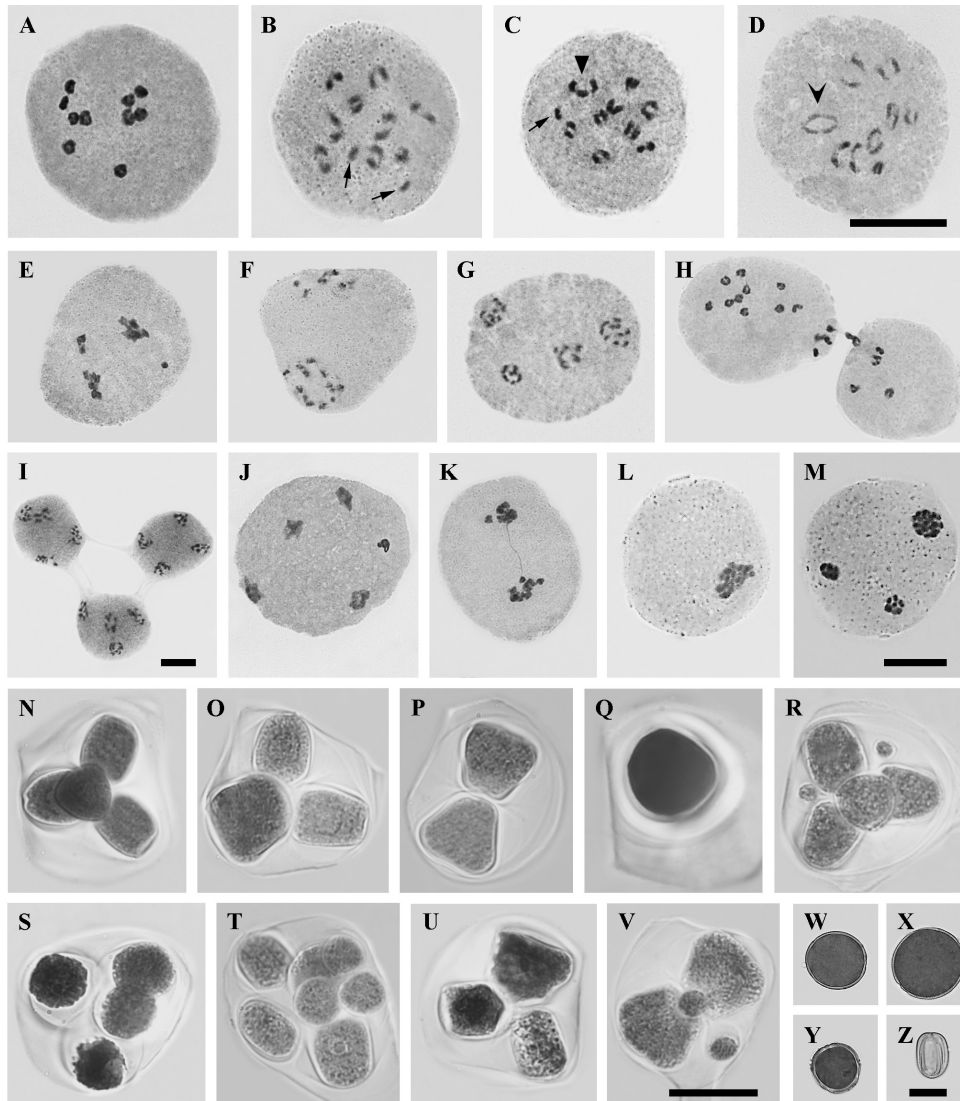
Figure 1. Meiotic analysis in hybrid individuals. Meiotic configurations: 10II (A); 9II+2I (B); 8II+1III+1I (C); 8II+1IV (D). Meiotic irregularities: MII with out-of-plate chromosomes (E); PII and TII with unbalanced nuclei (F-G); Di with cytomixis (H); TII with cytomictic channels (I); TII with multiple nuclei (J); MII with bridge (K); MII and TII with restitution nucleus (L-M). Sporads: tetrad (N); triad (O); dyad (P); monad (Q); abnormal tetrads (R-S); polyad (T); abnormal triad (U) and abnormal dyad (V). Pollen: normal (W); 2n (X); small (Y); unviable (Z). P, prophase; Di, diakinesis; M, metaphase; A, anaphase; T, telophase; I, first meiotic division; II, second meiotic division. Arrow = univalent. Triangle = trivalent. Arrowhead = tetravalent. Scale bar = 20 μm.

Chromosome behavior

The analysis of IpaWil and WilIpa hybrids revealed a rate of regularity of meiotic division in 71.26% and 59.77% of the PMCs, respectively. The irregularities detected included out-of-plate and out-of-nucleus chromosomes that were found at high frequencies in metaphases I and II, telophase I, and prophase II (Table 1, Figure 1E). These irregularities could explain the cells with micronuclei in telophase II or those with unbalanced nuclei in prophase II and telophase II (Figure 1F-G), and may also be related to the presence of cytomixis, as this phenomenon was detected in the majority of phases of meiotic division (Figure 1H-I). We observed empty cells, suggesting that they had undergone cytomixis and transferred all of the genetic material (unrecorded data). This could also explain cells with a larger number of nuclei than normally expected in telophase II (Figure 1J). Bridges were the irregularities less often found in metaphase II (Figure 1K). Finally, restitution nuclei occurred in metaphase II (Figure 1L), and cells with two haploid nuclei and a 2n restitution nucleus were found in telophase II (Figure 1M).

Meiotic irregularities have been related to the origin of aneuploid gametes (Mursalimov and Deineko 2018), which impose detrimental effects on pollen development (Shin et al. 2021). Out-of-plate and out-of-nucleus chromosomes can be caused by unequal tension from the poles (Lampson and Grishchuk 2017). Meanwhile, cytomixis is considered an additional mechanism for the origin of aneuploidy and polyploidy, since, when only a part of the genome is transferred, unbalanced and sterile gametes are formed (García et al. 2020). Similarly, unequal separation of chromosomes to the poles results in the formation of micronuclei (Zhang et al. 2018). In our analysis, micronuclei were observed at a low frequency compared to the percentage of PMCs with abnormalities; therefore, most of the irregularly segregated chromosomes were likely included in at least one of the main nuclei. Nevertheless, such inclusions do not assure the restitution of the euploid chromosome number of the main nucleus (García et al. 2020), which could explain the unbalanced nuclei observed in telophase II. The occurrence of multiple nuclei in telophase II may relate to the presence of multipolar spindles, as has been observed in other genera (Reis et al. 2016).

Restitution nuclei in PMCs in metaphase II of IpaWil hybrids could have arisen due to processes of nuclear restitution that began in the first meiotic division. During anaphase II and telophase II, these nuclei could continue the meiotic division resulting in two 2n nuclei, or undergo a second nuclear restitution, to produce 4n nuclei (not shown). In addition, processes of nuclear restitution can originate during the second meiotic division and produce a restitution nucleus and two haploid nuclei, similar to those observed in telophase II in both hybrids.

Sporad analysis

The analysis showed that IpaWil hybrids produced a high number of tetrads (Figure 1N) and consequently a high meiotic index (MI) (80.22%) with low percentages of abnormal spores, including sporads associated with the formation of unreduced gametes such as triads and dyads (Table 1). In WilIpa hybrids, the analysis revealed an intermediate MI (64.38%), likely due to a higher proportion of abnormal sporads, including triads, dyads, and monads (Table 1, Figure 1O–Q). The meiotic index (MI) allows for the assessment of the regularity of the meiotic process, since a higher MI indicates a more normal meiotic chromosome behavior (Love 1951). In our results, the MIs of hybrids were higher than the meiosis regularity values, which indicate that irregularities do not generally interfere with the normal process of pollen grain formation. In addition, the abnormalities observed in meiosis were consistent with the abnormal sporads found. For instance, the presence of irregularities in telophase II would lead to the formation of abnormal tetrads or polyads (Figure 1 R–T), and thus to the formation of abnormal gametes and the reduction of viability. The abnormalities detected in IpaWil hybrid sporads would affect not only the proportion of normal n-pollen grains, but also the production of unreduced gametes, as abnormal triads, dyads, and monads were present (Figure 1 U–V).

Regarding sporads related to the formation of unreduced gametes, the presence of cells with 2n restitution nuclei in telophase II explains the occurrence of triads. Although the meiotic mechanisms involved were not determined, the detection of dyads and monads indicated the occurrence of unreduced gametes. Hybrids between *A. duranensis* and *A. ipaënsis* have recently been shown to present tripolar spindles and cytomixis, which lead to the formation of restitution nuclei. Consequently, these failures contribute to the production of triads and dyads and ultimately 2n pollen grains (García et al. 2020).

Pollen analysis

The pollen grains of *A. ipaënsis*, *A. williamsii*, and the seven hybrids obtained were analyzed to estimate pollen viability (PV) and determine the presence of unreduced gametes based on the size and morphology of pollen grains. Both parental species had high PV (*A. ipaënsis* 98.75% and *A. williamsii,* 96.20%). Pollen viability of hybrids is a traditional tool used in the genus *Arachis* to infer genomic relationships between parental species. It is accepted that intraspecific, intragenomic, and intergenomic hybrids show pollen viability values greater than 70%, between 25% and 70%, and below 10%, respectively (Stalker et al. 1991, Krapovickas and Gregory 1994). The average PV of IpaWil was 70.19%, whereas that of WilIpa was 64.99% (Table 2). These values are in agreement with the number of normal tetrads found, as reported in hybrids of other genera (Fernandes Moreira et al. 2017). Simpson and Faries (2001) reported that hybrids from crosses between *A. williamsii* and *A. ipaënsis* had a pollen stainability of 62.9 %. These data support that *A. ipaënsis* and *A. williamsii* have similar genomes, which was confirmed by our analysis of bivalent formation. Simpson and Faries (2001) also reported that hybrids between *A. magna* (B genome) and *A. ipaënsis* had a 90.6 % PV. In contrast, hybrids between *A. valida* (B genome) and the other B genome species showed PV varying from 10.5% with *A. gregoryi* to 39.4% with *A. magna* (Wondracek-Lüdke et al. 2015). Hybrids between *A. gregoryi* and the other B genome species showed lower values, from 2.6% with *A. williamsii* to 10.45% with *A. magna* (Simpson and Faries 2001, Custodio et al. 2021). These results suggest that the B genome group has a subgroup of further related species composed of *A. ipaënsis*, *A. magna*, and *A. williamsii*, whose hybrids show pollen viability values above 62%. Thus, although B genome species form a uniform group (Bechara et al. 2010, Robledo and Seijo 2010, Moretzsohn et al. 2013), differences between their karyotypes could influence the fertility of their hybrids. The three species mentioned share the same karyotype formula, and two ribosomal loci on pair 3 are present only in *A. magna* and *A. ipaënsis*; in contrast, a distal DAPI band on pair 3 was observed in *A. gregoryi*, *A. valida*, and *A. williamsii*, which could reflect a closer relationship among these taxa (Robledo and Seijo 2010). Therefore, new crosses among all B genome species, including meiotic analysis, could better define their homology, as crossings between *A. magna* and *A. williamsii* are still lacking.

Regarding the size of viable pollen grains, a statistically significant difference was detected among all genotypes: *A. ipaënsis*, *A. williamsii*, IpaWil, and WilIpa (Kruskal-Wallis test, Dunn test, p values < 0.001). The average pollen size in *A. williamsii* was 31.48 ± 1.59 μm, varying from 26.73 to 36.13 μm, whereas that in *A. ipaënsis* was 32.19 ± 1.79 μm, varying from 27.28 to 35.80. The hybrids, particularly WilIpa, showed wider size ranges and larger pollen sizes than the parental species. The average pollen size in IpaWil was 33.02 ± 4.36 μm, varying from 20.83 to 46.11 μm, whereas that in WilIpa and 35.04 ± 6.50 μm, varying from 20.02 to 49.80 μm (Figure 2).

Pollen viability values are consistent with the high production of viable grains of different sizes, since in addition to normal pollen grains, we observed unreduced pollen grains (2n) and small size grains (Figure 1W–Y viable; Z unviable). Unreduced pollen grains were considered with a size equal to or larger than 41.27 and 43.80 μm in IpaWil and WilIpa

Table 2. Types of pollen grains found and pollen viability evaluated in hybrids

Individual	n	Viable (%)			Non-viable (%)	Viability (%)
		No	2n	Sm		
A. ipaënsis	1500	-	-	-	1.25	98.75
A. williamsii	1500	-	-	-	3.80	96.20
IpaWil						
1	1500	56.13	0.67	13.40	29.80	70.20
2	1500	56.86	0.40	11.13	31.61	68.39
6	2540	64.96	0.98	6.06	28.00	72.00
mean		59.31	0.68	10.19	29.80	70.19
WilIpa						
4	2665	57.15	1.80	5.82	35.23	64.77
5	2723	59.09	0.26	7.27	33.38	66.62
7	2847	58.90	1.23	4.18	35.69	64.31
8	2159	63.55	0.70	7.78	27.97	64.25
mean		59.67	0.99	6.26	33.08	64.99

n, number of pollen grains analyzed; No, reduced normal pollen grain; 2n, giant pollen grain; Sm, small size pollen.
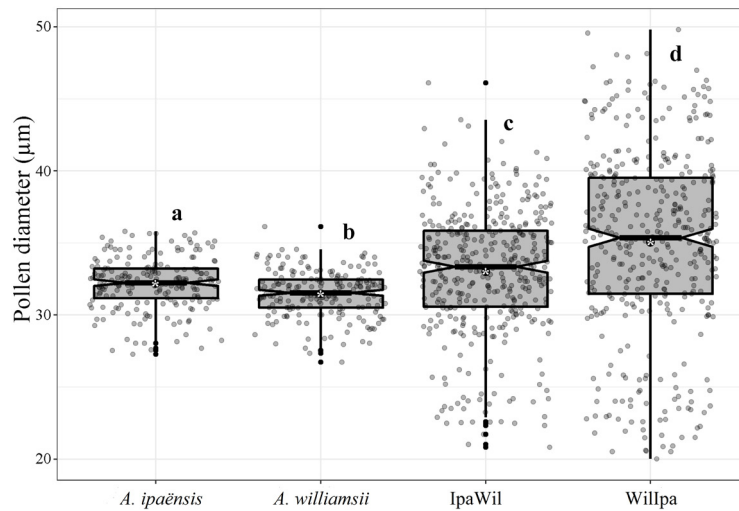
Figure 2. Comparison of pollen size data among *A. ipaënsis*, *A. williamsii*, WilIpa and IpaWil. In each boxplot, the box represents the middle 50% of scores (Q1-Q3), the horizontal line represents the median, the notches represent the 95% confidence interval for the median, the white asterisk represents the mean, and the gray dots represent the values observed. The lower whisker extends to the lowest sample item not more than 1.5xIQR (Q3 - Q1) below Q1, whereas the upper whisker extends to the largest sample item not more than 1.5xIQR above Q3. The individual black dots outside the whiskers are the outliers. Different letters indicate a statistically significant difference (Kruskal-Wallis, Dunn tests, p< 0.001). Additionally, the notches from different boxes that do not overlap show that there are significant differences between the pollen sizes compared. It is noticeable that the values of *A. ipaënsis* are higher than those of *A. williamsii*, and that the hybrids have a wider size range than parental species.

hybrids, respectively. The production of unreduced pollen grains was lower in the former but varied among individuals in both crosses (Table 2). Previous data have shown that the frequency of 2n gamete production could vary among species, cultivars, and even among flowers of an individual (Kreiner et al. 2017). Our results showed production between 0.40% and 0.98% of unreduced gametes in IpaWil, and 0.26% to 1.80% in WilIpa (Table 2). These gametes were probably the product of the restitution nuclei formed because of the non-disjunction of chromosomes in anaphase I and anaphase II. The presence of unreduced pollen grains together with dyads and triads evidenced that these hybrids can form unreduced gametes and that they could naturally generate polyploid individuals. Our data, together with a recent report by García et al. (2020), suggest that the production of 2n gametes by interspecific F$_1$ hybrids of *Arachis* is not an isolated event in this genus. The production of unreduced gametes is useful for improving crop species by enabling crosses between plants of different ploidy levels (Brownfield and Köhler 2011). Therefore, the analysis of the production and frequency of unreduced gametes in *Arachis* hybrids provides useful information that could be used for breeding programs. This is important considering that the method to detect unreduced gametes is inexpensive and rapid, and provides information on the reproductive biology of *Arachis* species hybrids. For example, the IpaWil and WilIpa hybrids studied here are sufficiently fertile to produce seeds. In addition, as they produce unreduced gametes, they would likely generate natural polyploids in subsequent generations.

In summary, we developed hybrid materials between *A. ipaënsis* and *A. williamsii*, analyzed the meiotic behavior, and confirmed the production of 2n gametes in the F$_1$ hybrid progeny. Our results on chromosome pairing and pollen viability confirm that the two species share high genomic affinity. Furthermore, the number of seeds obtained in the crosses, meiotic index, meiotic chromosome behavior, and pollen viability appear to indicate that reproductive success is higher when *A. ipaënsis* is used as the female parent. All data provided will be useful for performing evolutionary studies and for introgression programs to improve the B genome of *A. hypogaea*.

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