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Karyotypic studies in wild species of *Arachis* (Leguminosae) belonging to sections Erectoides, Procumbentes and Rhizomatosae

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Summary: Karyotypes of three diploid species belonging to sections Erectoides (*Arachis hermannii*), Procumbentes (*A. rigonii*) and Rhizomatosae (*A. burkartii*) were analyzed by Feulgen's technique. The karyotype formula was different in each of the taxa analyzed: 2n=2x=16m+4sm in *A. hermannii*, 2n=2x=18m+2sm in *A. rigonii*, and 2n=2x=20m in *A. burkartii*. All species had a pair of satellited chromosomes, which corresponded to type 2 in *A. hermannii*, type 9 in *A. rigonii*, and type 8 in *A. burkartii*. *Arachis hermannii* and *A. rigonii* presented chromosomal features similar to those of the other species included in their respective sections. However, *A. burkartii* showed chromosome characteristics different from those found in the rest of the species of section Rhizomatosae.

Key words: *Arachis hermannii*, *A. rigonii*, *A. burkartii*, chromosomes, phylogenetic relationships.

Resumen: Estudios cariotípicos en especies silvestres de *Arachis* (Leguminosae) pertenecientes a las secciones Erectoides, Procumbentes y Rhizomatosae. Los cariotipos de tres especies diploides pertenecientes a las secciones Erectoides (*A. hermannii*), Procumbentes (*A. rigonii*) y Rhizomatosae (*A. burkartii*) fueron analizados mediante la técnica de Feulgen. Las fórmulas cariotípicas obtenidas son diferentes en los taxones analizados, 2n=2x=16m+4sm en *A. hermannii*, 2n=2x=18m+2sm en *A. rigonii*, y 2n=2x=20m en *A. burkartii*. Las tres especies presentaron un par de cromosomas con satélite, en *A. hermannii* tipo 2, en *A. rigonii* tipo 9 y en *A. burkartii* tipo 8. *Arachis hermannii* y *A. rigonii* presentaron características cromosómicas similares a las especies incluidas en sus respectivas secciones. Sin embargo, *A. burkartii* no comparte características cromosómicas con el resto de las especies de la sección Rhizomatosae.

Palabras clave: *Arachis hermannii*, *A. rigonii*, *A. burkartii*, cromosomas, relaciones filogenéticas.

Introduction

Arachis is an exclusively South American genus presenting around eighty annual or perennial species (Krapovickas & Gregory, 1994; Valls & Simpson, 2005), distributed east of the Andes, from southern Amazon River to the north of the Plata River. They are organized in nine sections (*Trierectoides*, *Erectoides*, *Extranervosae*, *Triseminatae*, *Heteranthae*, *Caulorrhizae*, *Procumbentes*, *Rhizomatosae* and *Arachis*), according to their morphology, chromosome characteristics, and cross-compatibility relations (Krapovickas & Gregory, 1994).

Peanut (*A. hypogaea* L.) is one of the most important sources of dietary protein in the world. However, considering its productivity, this crop remains underexploited because of its susceptibility to pests and diseases. The main constraint to the genetic improvement of peanut is the narrow genetic base of the extant cultigen. Wild *Arachis* species, by contrast, are diverse and have the genetic variability and agronomically useful characters needed to improve the cultigen (Holbrook & Stalker, 2003) and constitute valuable resources for the genetic upgrading of peanut. In this sense, information on the cytogenetics and phylogenetic relationships among wild species and between these species and the cultigen is critical to the rational development of

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breeding programs and complete utilization of the wild materials.

Chromosomal data have played a pivotal role in accelerating crop improvement (Jauhar, 2006) and our understanding of the phylogenetic relationships between wild and cultivated species (Cao, 2003). On the other hand, cytological data are of great importance in the study of plant evolution and diversification (Stebbins, 1950; 1971; Hong, 1990; Stace, 2000). Moreover, the analysis of karyotype characteristics has contributed valuable data for inferring evolutionary trends within particular plant groups and analyzing traits such as changes in chromosome numbers (Mercado-Ruaro & Delgado-Salinas, 1998), karyotype symmetry, and chromosome length (Poggio *et al*., 2007).

The most comprehensive work on cytogenetics in *Arachis* was carried out by Fernández & Krapovickas (1994), who analyzed species belonging to different sections. Chromosome numbers are currently known for 95% species of *Arachis*. Most of them are diploids based on x=10 (67), few (4) are diploids based on x=9, and some (5) are tetraploids with x=10 as base number (Peñaloza *et al*., 1996; Lavia, 1998; 2000; Peñaloza & Valls, 2005). All diploids with x=18 belong to section Arachis (*A. decora*, *A. palustris* and *A. praecox*), except *A. porphyrocalyx,* which is included in section Erectoides.

Phylogenetic relationships among them have been investigated by molecular analysis, crossability experiments and cytogenetic studies (Lavia *et al*., 2008 and references therein; Lavia *et al.,* 2009). However, the chromosome characteristics of each *Arachis* species are not yet described. Therefore, to increase the cytogenetic knowledge of this genus, this work presents the karyotype characteristics of the three diploid species belonging to the sections Erectoides (*A. hermannii* Krapov. & W.C. Gregory), Procumbentes (*A. rigonii* Krapov. & W.C. Gregory) and Rhizomatosae (*A. burkartii* Handro).

Materials and Methods

The material studied is presented in Table 1. Voucher specimens were deposited in the herbaria of the Instituto de Botánica del Nordeste (CTES), Corrientes, Argentina, and of the Centro Nacional do Recursos Genéticos e Biotenología (CEN), Brasilia, Brazil.

The material used for this study was provided by the Instituto de Botánica del Nordeste, Corrientes, Argentina. Mitotic preparations were obtained from root tips. After a pretreatment of 3 h in 0.002 M 8-hydroxyquinoline solution at room temperature, the material was fixed in ethanol:acetic acid (3:1), stained following Feulgen`s technique, and then squashed in a drop of 2% acetic orcein.

At least three plants per species and five metaphase plates per individual were used for chromosome measurements using the free version of the MicroMeasure 3.3 program (http://www.colostate.edu/Depts/Biology/MicroMeasure/).

For the numerical characterization of the karyotypes, the following parameters were calculated: total chromosome length (**TCL**), mean length of the chromosomes (**ML**), mean centromeric index (**CI**), intrachromosomal asymmetry index ($\mathbf{A_1}$) and interchromosomal asymmetry index ($\mathbf{A_2}$) (Romero Zarco, 1986). SAT chromosomes were classified according to Fernández & Krapovickas (1994) and Lavia (2000).

The nomenclature followed for karyotype description was proposed by Levan *et al*. (1964).

Table 1. Studied material.

Species	Collector and locality
Sect. Erectoides Krapov. & W.C. Gregory	
A. hermannii Krapov. & W.C. Gregory	VRGeSv 7560. Brasil, Mato Grosso do Sul, Coxim.
Sect. Procumbentes Krapov. & W.C. Gregory	
A. rigonii Krapov. & W.C. Gregory	GKP 10034. Bolivia, Santa Cruz, Santa Cruz de las Sierras.
Sect. Rhizomatosae	
A. burkartii Handro	SeSo 2865. Argentina, Corrientes, Dpto. Concepción.

Colectors: G=W.C. Gregory, Ge=M.A.N. Gerin, K=A. Krapovickas, P=J.R. Pietrarelli, R=V.R. Rao, Se=J.G. Seijo, So=V. Solís Neffa, Sv=G.P. Silva, V= J.F.M. Valls.

Chromosome morphology was determined using the centromeric index (short arm x 100/total length). Accordingly, chromosomes were classified as metacentric (m) = 50-37.5 and submetacentric (sm) = 37.5-25. Mean karyotype values for each species were represented as haploid complements in the idiograms. Chromosomes were ordered primarily by morphology and then by decreasing size.

A cluster analysis of the karyotype data was carried out to examine karyotype similarity among species and sections. A data matrix of 16 operational taxonomic units (OTUs) x 2 variables (the mean chromosome length and type of SAT chromosome, Fig. 3B) was constructed using data obtained in this paper and those obtained by Fernández & Krapovickas (1994) and Lavia (2001). Clustering was performed using the unweighted pair-group method (UPGMA) with the software InfoStat (2008). The cophenetic correlation was 0.93 indicating a good fit between the cophenetic value matrix and the mean taxonomic distance matrix.

Results and Discussion

Mitotic chromosomes and idiograms are shown in Figs. 1 and 2. Karyotype formula and the parameters analyzed for the species are summarized in Table 2. Firstly, we describe general chromosome characteristics and secondly we discuss each particular species.

The karyotypes of *A. hermannii* and *A. burkartii* are reported for the first time here, while the karyotype of *A. rigonii* was presented previously in Cai *et al*. (1987). As a whole, the karyotypes were symmetrical, and most chromosomes were metacentric. However, the karyotype formula was different in the three entities: 2n=20=16m+4sm in *A. hermannii* (Figs. 1A and 2), 2n=20=18m+2sm in *A. rigonii* (Figs. 1B and 2) and 2n=20=20m in *A. burkartii* (Figs. 1C and 2). Previously, Cai *et al*. (1987) found 12m+6sm+2st in *A. rigonii*. However, the same accession was used in both studies since this species is known only from the type locality, Santa Cruz de la Sierra in Bolivia. Therefore, the higher number of submetacentric chromosomes and even the presence of subtelocentric chromosomes found by Cai *et al*. (1987) could be due to the different conditions of the root pretreatment. In this study, roots were pretreated for 3 h at 25ºC, whereas in other work, roots were pretreated for 4-6 h at 0-4ºC. These different pretreatments may be responsible for differential chromosome condensation resulting in different chromosome morphology.

All the chromosomes analyzed belong to the small category according to Lima de Faria (1980), because the average of chromosome length varied from 1.56 to 2.94 µm. Among these species, *A. hermannii* presented the longest chromosome length (51.77 µm), while *A. rigonii* (46.82 µm) and *A. burkartii* (45.56 µm) presented intermediate chromosome lengths.

Arachis hermannii presented a mean chromosome

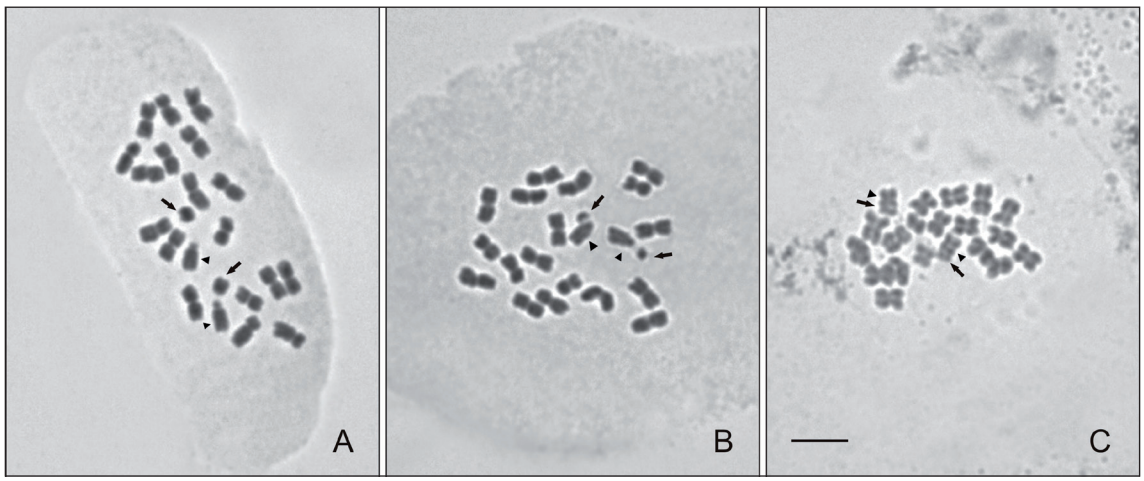


Fig. 1. Mitotic chromosomes of *Arachis* species. **A**: *A. hermannii*. **B**: *A. rigonii*. **C**: *A. burkartii*. SAT chromosomes: ▶ arm 1 + proximal segment, ➞ satellite. Bar = 5 µm.

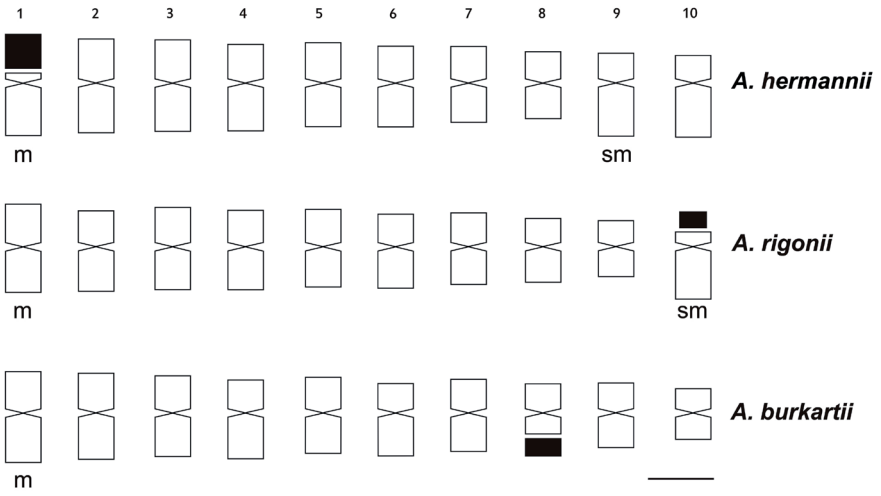


Fig. 2. Idiograms of *Arachis* species. **A**: *A. hermannii* (Section Erectoides). **B**: *A. rigonii* (Section Procumbentes). **C**: *A. burkartii* (Section Rhizomatosae). ■ Satellite. Bar = 2 µm.

length of 2.59 µm, and a pair of SAT chromosomes type 2 (Figs. 1A and 2). This type of SAT chromosomes has also been found in other species of section Erectoides and suggested to be one of the most primitive of the genus (Fernández & Krapovickas, 1994; Lavia, 2001). Inside section Erectoides it has been observed that the species could be included in two groups with different morphological (Fernández & Krapovickas, 1994) and cytogenetic (Lavia, 2001) characteristics: one with shorter chromosomes (between 2.11 and 2.34 µm) and a pair of SAT chromosomes type 3 or 4, and the other with larger chromosomes (between 2.80 and 2.96 µm) and SAT chromosomes type 2 (Fig. 3A). Therefore, *A. hermannii* should be included in the second group (Fig. 3A). However, since only 28.57 % of species of section Erectoides have been karyotypically analyzed, it would be necessary to study a higher number of species of the section to clarify the intrasectional relationships.

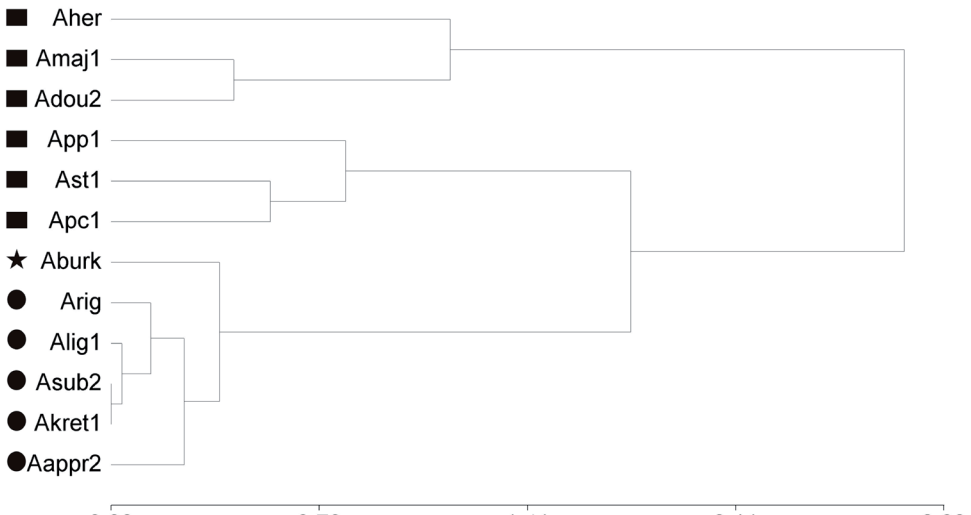
Arachis rigonii presented a mean chromosome length of 2.34 µm and a pair of SAT chromosomes type 9 (Figs. 1B and 2). This type of satellite and mean chromosome lengths between 2.30 and 2.38 µm have been proposed as differential chromosomal characteristics of species of section Procumbentes (Fernández & Krapovickas, 1994; Lavia, 2001); therefore, *A. rigonii* shares the characteristics of this section (Fig. 3). On the other hand, *A. rigonii* can produce hybrids with other species of section Procumbentes (Krapovickas & Gregory, 1994) and presents low values of asymmetry indices (A1=0.12 and A2=0.13). These two features suggest that this species could be considered as one of the most primitive of the section Procumbentes.

Arachis burkartii presented a pair of SAT chromosomes type 8 (Figs. 1C and 2). However, this type of SAT is not found in the rest of the species of section Rhizomatosae, which are tetraploids

Table 2. Karyotype parameters for *Arachis* species. Karyotype, SAT chromosome type (SAT), total chromosome length (TCL), mean chromosome length (ML), size range, centromeric index (CI), intrachromosomal asymmetry index (A_1), interchromosomal asymmetry index (A_2). TCL, ML and size range in µm.

Species	Karyotype formula	SAT	TCL	ML	Size range	CI	A1	A2
A. hermannii	16m+4sm	2	51.77	2.59	2.94-2.06	44.37	0.16	0.10
A. rigonii	18m+2sm	9	46.82	2.34	2.71-1.72	45.71	0.12	0.13
A. burkartii	20m	8	45.56	2.28	2.79-1.56	45.15	0.17	0.15

A)



B)

	Aburk	Arig	Aher	Akret1	Alig1	Aappr2	Asub2	Amaj1	Amaj1	Amaj1	Adou2	App1	App1	Apc1	Ast1	Ast1
SAT	8	9	2	9	9	9	9	2	2	2	2	4	4	3	4	4
ML	2.28	2.34	2.59	2.30	2.31	2.38	2.30	2.76	3.09	2.69	2.96	2.34	2.24	2.02	2.11	2.17

Fig. 3. A) Phenogram of unwighted-pair group method (UPGMA) based on the mean chromosome length (ML) and type of SAT chromosome for eleven *Arachis* diploid species belonging to sections Erectoides (▬), Procumbentes (●) and Rhizomatosae (★). **B**) Values of the mean chromosome length (ML) in µm and type of SAT chromosome of these species. Abbreviations: Aher = *A. hermannii*, Amaj = *A. major,* Adou = *A. douradiana*, App = *A. paraguariensis* subsp. *paraguariensis*, Ast = *A. stenophylla*, Apc = *A. paraguariensis* subsp. *capibariensis*, Abur = *A. burkartii*, Arig = *A. rigonii*, Alig = *A. lignosa*, Asub = *A. subcoriacea*, Akret = *A. kretschmeri*, Aappr = *A. appressipila*. **1** Fernández & Krapovickas (1994), **2** Lavia (2001).

and have SAT chromosomes type 3 (Peñaloza & Valls, 2005; Ortiz, 2012). Previous studies have found genetic differentiation between species with different ploidy level of section Rhizomatosae, since *A. burkartii* (2x) clusters in a group different from that of 4x species (Bechara *et al*., 2010; Wang *et al*., 2011). Therefore, it would be necessary to carry out more cytogenetic studies to bring light over the phylogenetic relationships between the species of this section.

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