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RESEARCH

Identification of Smut Resistance in Wild *Arachis* Species and Its Introgression into Peanut Elite Lines

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ABSTRACT

Peanut smut caused by *Thecaphora frezii* Carranza & Lindquist has been an issue for farmers and the peanut industry (*Arachis hypogaea* L.) in Argentina since the mid-1990s. This disease causes pod malformation due to hypertrophy of seed tissues; in addition, colonized cells filled with teliospores give seeds a smutted mass appearance. Incidence may reach up to 52% in commercial plots, with up to 35% yield losses. Cultural management strategies and chemical treatment have not been effective; therefore, growing resistant varieties is likely to be the most effective control method for this disease. This study is aimed to identify sources of resistance in wild *Arachis* and to develop pre-breeding materials for transferring the trait to cultivated peanut. After 3 yr of field trials using a randomized complete block design, the seven accessions of wild species assayed were resistant to smut. An amphidiploid [*A. correntina* (Burkart) Krapov. & W.C. Greg. × *A. cardenasii* Krapov. & W.C. Greg.] × *A. batizocoi* Krapov. & W.C. Greg.)$^{4\times}$ was obtained and subsequently crossed with and experimental line of *A. hypogaea* for the development of a recombinant inbred line (RIL) population (89 lines). The RIL population showed a high phenotypic variability for resistance to peanut smut. The amphidiploid and 22 RILs were highly resistant, illustrating the effective transmission of resistance to peanut smut from the wild diploids into *A. hypogaea*. The development of RILs with resistance derived from wild species is a significant step towards the development of new peanut cultivars with different sources of resistance to peanut smut.

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Abbreviations: DI, disease index; RIL, recombinant inbred line; SSR, simple sequence repeat; UPGMA, unweighted pair group method with arithmetic mean.

Peanut (*Arachis hypogaea* L.) is an important oilseed and cash crop worldwide, and one of the primary sources of vegetable oil and protein in developing countries. Argentina is one of the largest peanut exporters in the world, with approximately 1 million tons being traded annually (FAOSTAT, 2016). Cultivated peanut was subjected to intensive selection, resulting in favorable changes in yield, biochemical composition, and other agronomic traits (Holbrook and Stalker, 2003; Anderson et al., 2006; Mallikarjuna

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and Varshney, 2014). However, many of the commercial cultivars share common ancestors, which generate a narrow genetic basis for the development of new varieties (Isleib and Gorbet, 2001; Ren et al., 2014).

Peanut smut has become the main production concern for the industry in Argentina because of its high impact on quality and yield. Peanut smut is a soil-borne disease caused by the fungus (*Thecaphora frezii* Carranza & Lindquist); it was first identified in wild peanut samples from Aquidauana, Matto Grosso do Sul, Brazil, in 1962 (Carranza and Lindquist, 1962). Later, infected fruits were observed in accessions of *A. kempff-mercadoi* Krapov., W.C. Greg. & C.E. Simpson from Santa Cruz de la Sierra, Bolivia (Soave et al., 2014). Infection occurs at pegging; when peanut pegs penetrate the soil, their exudates promote spore germination, triggering local infections (Marraro Acuña et al., 2013). The pathogen causes pod malformation due to hypertrophy of seed tissues. In addition, colonized cells are filled with teliospores in the reproductive stage of the fungus, giving seeds a smutted mass appearance (March and Marinelli, 2005; Astiz Gassó and Marinelli, 2013; Marraro Acuña et al., 2013).

During the 1995 harvest season, seeds with smut were detected in the central-northern crop area of Córdoba, Argentina (Marinelli et al., 1995); this was the first report of the disease in commercial plots. Since then, the disease has spread throughout the whole peanut growing area in Argentina covering >2000 km^2. Smut disease incidence increases with increasing soil inoculum (Rago et al., 2017). Recent surveys recorded incidence values of up to 52% in commercial plots, with records of 35% yield losses (Cazzola et al., 2012; Paredes et al., 2016).

All commercial cultivars planted in the peanut cultivation area of Argentina are susceptible to peanut smut. Different alternatives for disease control with fungicides, rotations, and other strategies have been tested, but they were not sufficiently effective to control the disease and they increased production costs significantly (Rago et al., 2017). Therefore, growing resistant varieties is likely to be the most effective control method for this disease. However, genotypes resistant to peanut smut and the nature of the trait inheritance still need to be identified.

The limited genetic variability found within cultivated peanut has been attributed to genetic bottleneck deriving from a single domestication event (Halward et al., 1991, 1992; Grabiele et al., 2012) that occurred ~10,000 yr ago (Bertioli et al., 2016). By contrast, wild *Arachis* species present high interspecific genetic variability and are important sources of resistance to many pests and diseases (Stalker, 2017). Moreover, some of those resistances have been successfully transferred to commercial genotypes (Stalker and Moss, 1987; Pasupuleti et al., 2013; Stalker, 2017). Crosses with wild species allowed the selection of cultivated materials resistant to early leaf spot (caused by *Cercospora arachidicola* S. Hori), late leaf spot [caused by *Cercosporidium personatum* (Berk. and Curt.) Deighton] (Moss et al., 1981; Wynne and Halward, 1989; Stalker and Wynne, 1979), Sclerotinia blight [caused by *Sclerotinia minor* Jagger and *S. sclerotiorum* (Lib.) de Bary] (Isleib et al., 2006; Tallury et al., 2014), Cylindrocladium black root [caused by *Cylindrocladium crotalariae* (C.A. Loos) D.K. Bell & Sobers] (Tallury et al., 2014), nematodes (Simpson and Starr, 2001; Simpson et al., 2003), and several insect pests (Stalker and Lynch, 2002; Stalker et al., 2002; Michelotto et al., 2017). Therefore, the use of wild *Arachis* as a source of resistance to peanut smut is a promising option.

The main difficulty in using wild materials in peanut breeding is that most of the species of section *Arachis* are diploid ($2n = 2x = 20$, $2n = 2x = 18$) and bear A, B, D, F, G, or K genomes, whereas peanut is allotetraploid ($2n = 2x = 40$), with an AABB genome formula. Therefore, the development of synthetic amphidiploids chromosomally compatible with *A. hypogaea* is necessary for the successful transfer of agronomical traits from wild species to cultivated peanut (Simpson, 1991; Simpson and Starr, 2001; Fávero et al., 2006).

The objective of this paper was to identify phenotypic resistance to peanut smut in wild *Arachis* species, to generate a compatible amphidiploid complex, and to transfer the trait to an experimental elite line of *A. hypogaea* by developing a recombinant inbred line (RIL).

MATERIALS AND METHODS

Screening of Wild *Arachis* Species

Seven wild *Arachis* species were screened for peanut smut, and the commercial variety 'Tegua' of *A. hypogaea* was used as susceptible control (Table 1). Field trials were conducted sowing each accession in two plots of 5 × 1 m. Trials were placed in the nursery of Criadero El Carmen in General Cabrera, Córdoba, Argentina (32°49′46″ S, 63°52′12″ W), during three crop seasons (2003–2005). The area has a historical record of peanut smut, with an average of 1.5 ×10^3 teliospores g^{-1} of soil. Pods and seeds were assessed for peanut smut, using three samples of 100 pods per plot in each season during the assay. Pods were manually opened, and the smutted seeds were counted.

Interspecific Hybridization

In February 2004, three *A. cardenasii* Krapov. & W.C. Greg. individuals were used as female parents and one *A. correntina* (Burkart) Krapov. & W.C. Greg. individual was used as the male donor in a crossing program. A total of 21 flowers were cross-pollinated; four hybrid seeds were obtained, which were harvested in April 2004. Two of the four F_1 hybrid seeds were germinated in November 2005, and 28 flowers were cross-pollinated in the hybridizations with *A. batizocoi* Krapov. & W.C. Greg. in February 2006. All crosses were performed as described by Simpson (1991). Hybrid plants were initially identified by their aggregated pollen masses and absence of peg production (Gregory and Gregory, 1979; Krapovickas and Gregory, 1994; Leal-Bertioli et al., 2015) and further

Table 1. Species, accessions, ploidy level, genome, and life cycle of the materials used in the smut assay during the crop season of 2003 to 2005.

Species	Accession	Ploidy (*x*)	Genome†	Life cycle‡
A. batizocoi Krapov. & W.C. Greg.	K 9484	2	K	A
A. cardenasii Krapov. & W.C. Greg.	KSSc 36015	2	A	P
A. correntina (Burkart) Krapov. & W.C. Greg.	K 11905	2	A	P
A. duranensis Krapov. & W.C. Greg.	K 8010	2	A	A
A. hypogaea	var. Tegua	4	AB	A
A. ipaënsis Krapov. & W.C. Greg.	GKPSBSc 30076	2	B	A
A. magna Krapov., W.C. Greg. & C. E. Simpson	KGSSc 30097	2	A	A
A. monticola Krapov. & Rigoni	S.J. 99004	4	AB	A
A. villosa Benth.	Seijo & Solís Neffa 2869	2	A	P

† Genome assignation after Robledo et al. (2009) and Robledo and Seijo (2010).

‡ A, annual; P, perennial.

confirmed by simple sequence repeat (SSR) markers. The four sterile F_1 diploid hybrids obtained were treated with colchicine, following Torres et al. (2012), to induce chromosome doubling. Only one colchicine-induced tetraploid was obtained and incorporated in the Criadero El Carmen germplasm collection with the accession number JS1806; this material will be hereafter referred to as the amphidiploid.

Development of an RIL Population

Recombinant inbred lines were derived from 33 crosses between the artificial amphidiploid (male) and a susceptible high-oleic *A. hypogaea* experimental elite line JS17304-7-B (female), hereafter referred to as the cultivated parent. Hybridizations were performed in February 2010. The F_6 to F_8 89-RIL population was developed using the single seed descent method from all the F_2 seeds.

Smut Resistance Assays

To evaluate smut resistance, RILs were annually planted following a randomized complete block design with three replications per line in the Criadero El Carmen nursery (General Cabrera, Córdoba) from 2015 to 2017. Each plot was represented by each genotype assessed. The experimental unit (plot) was composed of 25 plants per RIL sown in 2.5-m rows. The F_6:F_8 RIL generation was evaluated in field trials for 3 yr (2012–2015). Field inoculum of *T. frezii* was increased with a water suspension of 1×10^4 teliospores mL^{-1}, sprayed with a manual sprayer over the plots until an average of 1.2×10^4 teliospores g^{-1} of soil was reached in the first-year trial. The inoculum was increased to 1.6×10^4 teliospores g^{-1} of soil in the second and third years of the assay with successive applications of teliospores. Inoculum density in the experimental plots was 3.5 (average for the 3 yr of assay) times higher than the highest value recorded in the cultivated area (Rago et al., 2017). The amphidiploid and the cultivated parent used to develop the RIL population were included in the assay as controls.

Phenotypic observations of the RIL population and parental genotypes were recorded in the three growing seasons. The damage caused by smut was recorded in a sample of 100 pods per plot. Pods were manually opened and the number of smutted seeds was determined, as well as the level of smutted mass per seed. Accordingly, the disease severity was evaluated as the level of infection using a scale of 0 to 4 (Astiz Gassó et al., 2008), where 0 = healthy pods, 1 = normal pod with incipient affection in a single seed, 2 = deformed or normal pod with one completely smutted seed, 3 = deformed pod with one completely smutted seed and the other with an incipient affection, and 4 = deformed pod with the two seeds completely smutted (Fig. 1). Severity was used to calculate a disease index (DI) for each RIL using the following equation:

$$\mathrm{DI} = \frac{1x_1 + 2x_2 + 3x_3 + 4x_2}{\text{total pods} \times 4}$$

where x_n is the number of pods with n severity grade. Affected pods were recorded as the pods with any level of smut symptoms. Incidence was calculated as the ratio of affected pods to the total number of pods in the sample:

$$\text{incidence} = \frac{\text{affected pods}}{\text{total pods}}$$

The DI and incidence were analyzed using a general linear model on the scale suggested by the Box–Cox transformation to a normal distribution of error terms (square root). The general linear model included genotype, year, and genotype- × year as fixed effects. Genotype means were compared ($\alpha = 0.05$) using the Scott and Knott procedure (Scott and Knott, 1974). Data were statistically analyzed using the InfoStat software (Di Rienzo et al., 2017). Additionally, variance component analysis was done by fitting a linear mixed model from the glmm package (Knudson and Geyer, 2018) in R. Results were used to calculate broad-sense heritability using the following equation:

$$H^2 = \frac{\sigma^2_g}{\left(\sigma^2_g + \sigma^2_{g\times y}/m + \sigma^2_e/rm\right)}$$

where σ^2_g, $\sigma^2_{g\times y}/m$, and σ^2_e/rm stand for the genetic, genotype × year interaction, and residual variance components, respectively, m is the number of years, and r is the number of replications.

SSR Markers

A first screening using 373 SSR markers was performed including *A. cardenasii* (KSSc 36015), *A. correntina* (K 11905), *A. batizocoi* (K 9484), and the progenitors of the RIL population: *A. hypogaea* (JS17304-7-B) and the amphidiploid. The set of 373 molecular markers was composed of 288 newly developed SSRs (Arias et al., 2018), 12 insertion-deletion (InDel)

Fig. 1. Peanut smut severity scale: 0 = healthy pods; 1 = normal pod with incipient affection in a single seed; 2 = deformed or normal pod with one seed completely smutted; 3 = deformed pod with one completely smutted seed and the other with incipient affection; and 4 = deformed pod with the two seeds completely smutted.

markers, and 73 SSRs reported in the literature (Moretzsohn et al., 2005; Proite et al., 2007; Leal-Bertioli et al., 2009). Total genomic DNA was extracted from young leaves of all the genotypes using the DNeasy PowerPlant Pro Kit (Qiagen) and CTAB method (Doyle and Dickson, 1987). Forward primers were 5′ tailed with the sequence 5′-CAGTTTTCCCAGTCACGAC-3′ (Waldbieser et al., 2003) and reverse primers were tailed at the 5′-end with the sequence 5′-GTTT-3′ to promote nontemplate adenylation (Brownstein et al., 1996). Primer 5′-CAGTTTTCCCAGTCACGAC-3′ labelled with 6-carboxy-X-rhodamine (ROX) (IDT-Technologies) was used to amplify 10 ng DNA using Titanium Taq DNA Polymerase (Clontech) as previously reported (Arias et al., 2018). Fluorescently labelled polymerase chain reaction (PCR) fragments were analyzed by capillary electrophoresis on an ABI 3730XL DNA analyzer (Applied Biosystems), and data were processed using Gene Mapper software 4.0 (Applied Biosystems, 2005).

Scoring and Analysis

The scoring of the amplified sequences was recorded as base pair allele size and transformed into allelic frequency and binary data for use in the following analysis. The markers were kept for the analysis when missing values were <5%. Novel alleles found in the amphidiploid were kept as informative and considered as newly arisen by genetic instability caused by the hybridization and chromosome doubling (Song et al., 1993; Liu et al., 1998; Zhang et al., 2005). To confirm amphidiploid hybridization, the amplified species-specific fragments and those shared between the amphidiploid and wild relatives were recorded. The contribution of alleles from the wild species to the amphidiploid genome was calculated as the percentage of specific alleles amplified on each wild species and common alleles between two wild species that were found and scored in the amphidiploid. Genetic diversity, heterozygosis, polymorphic information content, and the number of alleles per locus were calculated.

Euclidean distances between the amphidiploid and the wild species were calculated using a dataset of 235 amplified sequences. Cluster analysis was done using the unweighted pair group method with arithmetic mean (UPGMA) for wild species and the amphidiploid. The molecular dataset was also analyzed by multidimensional scaling and analysis of molecular variance (Excoffier et al., 1992).

RESULTS

Phenotypic Evaluation and RIL Development

The analysis of seven wild *Arachis* accessions cultivated in the nursery field with 1.5×10^3 teliospores g^{-1} of soil did not show any symptoms of smut infection in any of the 3 yr of observation. Therefore, all the wild diploid species of the A (*A. cardenasii*, *A. correntina*, and *A. villosa* Benth.), B (*A. magna* Krapov., W.C. Greg. & C. E. Simpson and *A. ipaënsis* Krapov. & W.C. Greg.), and K genomes (*A. batizocoi*) and the wild tetraploid AABB genome (*A. monticola* Krapov. & Rigoni) were considered resistant to peanut smut. The resistance in wild species was observed irrespective of the life cycle and ploidy level (Table 1). The incidence for the cultivated control was 0.38.

The interspecific crossing of three of the wild species produced a sterile diploid hybrid (AK), whose chromosome number was successfully doubled by colchicine. The obtained amphidiploid [(*A. cardenasii* × *A. correntina*) × *A. batizocoi*]$^{4\times}$ partially recovered the fertility and was resistant to peanut smut, as its parental species. The 33 cross-pollinations performed between the amphidiploid and the peanut experimental line JS173047-B produced five F_1 hybrid seeds that were planted and pods were collected separately. Each of the five plants produced 93, 115, 123, 62, and 60 seeds, totaling 453 seeds. These seeds were bulked and sown individually to generate the F_3 generation.

The development of the RIL population by single-seed descent was started from the F_2. Plants were harvested and the progeny were individually grown to produce the F_6:F_8 RIL population. During this process, many lines produced nonviable seeds or did not produce seeds at all. The recorded sterility caused the loss of almost 80% of the lines, and therefore the F_6 population was at last composed of 89 fertile RILs.

The phenotypic characterization of peanut smut disease in the F_6:F_8 RIL population with a high inoculum density ($1.2–1.6 \times 10^4$ teliospores g^{-1} of soil) showed a mean incidence of 8% (with a range from 0 to 50% over the three study years) and a mean DI of 0.05 (with a range from 0 to 0.4) (Fig. 2). Statistically significant interannual differences ($P = 0.0001$) were recorded for DI (averages of 0.03, 0.05, and 0.07 for 2015, 2016, and 2017, respectively) and incidence of smut disease (averages of 0.05, 0.08, and 0.10 for 2015, 2016, and 2017, respectively). The genetic variance was higher than the variance of genotype × year interaction (78 [$P > 0.0001$] vs. 12% [$P = 0.0038$] for DI, and 59 [$P > 0.0001$] vs. 12% [$P = 0.0017$] for incidence]. Broad-sense heritability was high ($H_2 = 0.94$ for DI and $H_2 = 0.96$ for incidence). Therefore, despite the differences of DI and incidence among years, the ranks of the lines regarding resistance to peanut smut remained the same during the study years. For the susceptible parent, the interanual average incidence of peanut smut was 56% and the DI was 0.44, whereas in the amphidiploid, incidence and DI were 0%. A wide range of plant response to the disease was observed in the F_6:F_8 RIL population. The genotypic means of incidence were highly correlated with the means of DI ($r = 0.99$, $p < 0.0001$). The pairwise

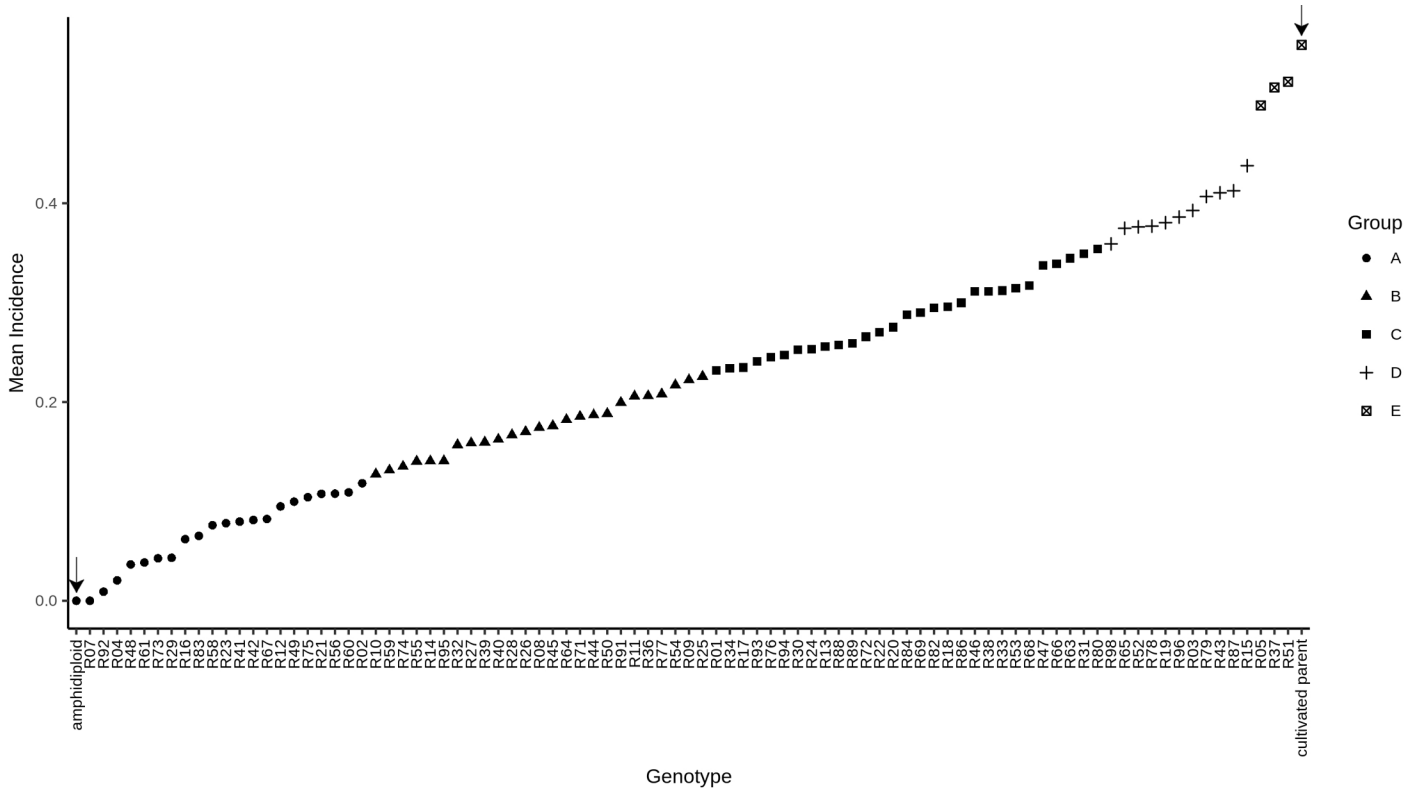

Fig. 2. Clusters of recombinant inbred lines (RILs) according to the mean incidence per genotype obtained by Scott and Knott analysis. Clusters are defined as Groups A, B, C, D, and F. Arrows indicate the amphidiploid (AM) and the cultivated parent (CP).

mean comparison by Scott and Knott test partitioned the assayed RILs into five groups (Fig. 2). Twenty-one lines had an incidence record <0.12 and did not show significant differences from the amphidiploid (resistant control line). The average incidence of these lines was 0.06 and ranged between 0 and 0.12. Among them, three lines (04, 07, and 92) were highly resistant with an average incidence value <0.02. On the other hand, three lines presented an incidence score >0.5 and did not show significant differences from the susceptible parental line JS173047-B.

Molecular Characterization of the Amphidiploid

The screening of the two RIL parents and the amphidiploid progenitors using 373 markers showed good amplification and polymorphism. A few primers failed to amplify in all the samples, showed multiple stutters, or had very low fluorescence in one or more samples and were discarded. A set of 235 out of 373 markers presented specific alleles for each wild accession assessed, and most of them (70%) were shared between the amphidiploid and its wild progenitors, detailed in Supplemental Table S1. Eight markers amplified 24 alleles in the amphidiploid that could not be attributed to any of the wild progenitors. Species-specific alleles of the three diploid parental species were detected in the amphidiploid genome (Table 2), and the percentage of allele contribution to the genomic constitution of the amphidiploid is shown in Fig. 3. A total of 566 alleles amplified selectively on the K genome (*A. batizocoi*), and 1072 alleles amplified selectively on the A genome (*A. cardenasii* and *A. correntina*). The set of 235 markers showed a good transferability between the wild species tested in this work.

The UPGMA analysis of the amphidiploid and the wild species was consistent with the alleles amplified in each wild species and showed that *A. batizocoi* (K genome) remained outside the cluster that included all the A genome species and the amphidiploid. Within the latter, the amphidiploid was more closely related to *A. cardenasii* than to *A. correntina*.

DISCUSSION

Peanut smut has been described as a disease causing important peanut production losses, with the amount of inoculum and yield losses being highly correlated (Cazzola et al., 2012). Genetic improvement by transfer of resistance from alien materials appears as the most promising strategy for a long-term sustainable control of this disease (Singh et al., 1991; Rago et al., 2017). For this purpose, the identification of diverse sources of resistance to the disease and the development of materials compatible with cultivated peanut are fundamental steps in any resistance breeding program. In this study, we report the identification of peanut smut resistance in wild species of *Arachis* and its stable introgression into advanced tetraploid experimental breeding lines.

Table 2. Species-specific alleles of the three diploid parental species that were detected in the amphidiploid genome.

Species	Markers†
A. correntina	NPRL-Ah1TC6E01, NPRL-AHBGSI1002B03, NPRL-cont00058a, NPRL-cont00095a, NPRL-cont00151a, NPRL-cont00176b, NPRL-cont00250a, NPRL-cont00710a, NPRL-cont00834a, NPRL-cont00981b, NPRL-cont01078a, NPRL-cont01356a, NPRL-cont01409a, NPRL-cont01663a, NPRL-cont02426a, NPRL-Indel-003, NPRL-RN2C06
A. batizocoi	NPRL-ABCLW, NPRL-Ah-229, NPRL-cont01065a, NPRL-cont02904a, NPRL-gi-832, NPRL-RN2F12
A. cardenasii	NPRL-Ah1TC11A02, NPRL-Ah1TC9F04, NPRL-cont00125a, NPRL-cont00318a, NPRL-cont00523a, NPRL-cont00629b, NPRL-cont00644a, NPRL-cont00843a, NPRL-cont01310a, NPRL-cont01611a, NPRL-cont01924a, NPRL-cont02125a, NPRL-gi-30419385, NPRL-gi-560, NPRL-Indel-020, NPRL-RM15C11

† NPRL, National Peanut Research Laboratory.

Identification of Sources of Smut Resistance and Development of the Amphidiploid

All the accessions of wild *Arachis* species were highly resistant to peanut smut, as demonstrated in the field assays. This result confirmed the importance of wild species as sources of genes for developing improved cultivars with pest and disease resistance (Sharma et al., 2013; Oddino et al., 2017; Stalker, 2017). Moreover, the fact that smut resistance was identified in diploid species with different genomes and life cycles provide the opportunity for pyramidizing resistance genes in the A and B genomes of the cultivated peanut. The resistance found in *A. monticola* is also very significant, since it is cross compatible with *A. hypogaea*. The finding of many sources of resistance in wild species is relevant for peanut breeding, since there is an emerging concern about the probably genetic variability present in *T. frezii* (Rago et al., 2017), a fact that would reduce the durability of resistance from a single source.

The complex hybrid developed from three wild species in this work partially recovered the fertility after chromosome doubling, as previously reported for other *Arachis* complex hybrids through hybridization of wild species bearing different genomes (Simpson, 1991; Fávero et al., 2015). The five F_1 seeds obtained here by the crossing of this AAKK amphidiploid with the cultivated peanut supports the homologous pairing suggested by Leal-Bertioli et al. (2015) (i.e., crossing the A genome chromosomes of the cultivated parent with those of the same genome in the amphidiploid, and those of the B genome of the cultivated parent with those of the K genome of the amphidiploid). This phenomenon is significant for peanut breeding, since it reinforces the potential sources (K genome) for providing new gene sources to be introgressed into the peanut genome, as reported previously (Simpson et al., 1993; Burow et al., 2001; Simpson and Starr, 2001; Fávero et al., 2015).

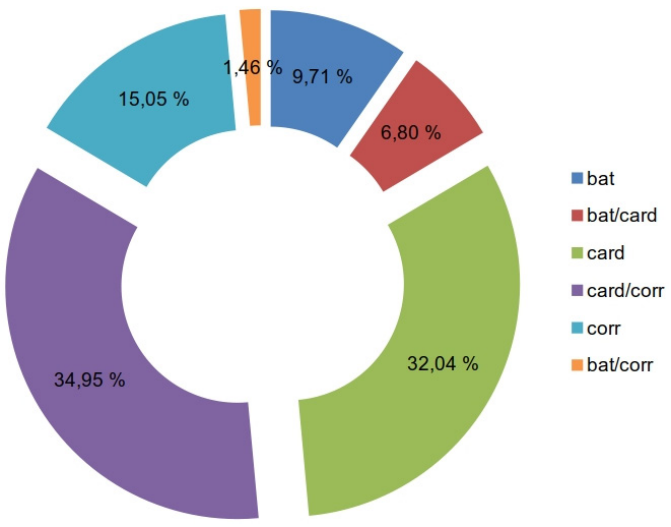

Fig. 3. Ring plot showing the percentage of allele contribution of the wild species to the amphidiploid assessed using 235 molecular markers. The combinations bat/card (7%), card/corr (35%), and bat/corr (1%) show the contribution of alleles common to each pair of wild species. Abbreviations: bat, *A. batizocoi*; card, *A. cardenasii*; corr, *A. correntina.*

The drastic reduction in the number of inbreeding lines from F_2 to F_5, due to the production of few or nonviable seeds, suggests that recombination in each generation produced gametes with unbalanced chromosomes or genes that compromised the survival of the following generation. This phenomenon recalls the classical genetic dysgenesis phenomenon that occurs as a postzygotic barrier in many plant groups (Grant, 1981) and was noted early in interspecific hybrids of *Arachis* by Simpson (1991). However, the production of abundant seeds during several generations in the remaining 89 lines suggests that they have more stable genome combinations of alleles and constitute valuable materials for disease evaluation and introgression of new traits into peanut cultivars.

Detection of Stable Resistance to Peanut Smut in the RIL Population

The phenotypic characterization evidenced a wide range of disease resistance within the RIL population. Considering the high inoculum load used in the experimental field ($1.2–1.6 \times 10^4$ teliospores g^{-1} of soil), records of stable incidence values close to 0% (from 0 to 0.07%) in nine RILs are very important for the future development of commercial varieties with resistance to peanut smut. Moreover, the fact that 85% of the RILs presented lower incidence values than those reported by Capello and Dignani (2014) in commercial plots (with soil inoculum almost three times lower than that used in the present work) demonstrated that resistance found in the wild species was transferred to a sizeable proportion of the RILs, although with different degrees. More importantly, the transferred resistance was stable at extremely high inoculum density throughout the three study years. The high values of broad-sense heritability of

DI and incidence are consistent with the high correlation between RILs' ranks regarding the high resistance stability. The results suggest that the characteristic is governed by few genes with additive effects.

Molecular Characterization

The smaller genetic distance of the amphidiploid with the A genome species than with *A. batizocoi* evidenced that, despite the balanced number of alleles amplified on the wild species, the allele contribution of the A genome species was higher than that of the K genome. Differential contribution of alleles has been explained by several mechanisms such as uneven meiotic segregation, meiotic drive, genome instability, and gene conversion (Buckler et al., 1999; Schommer et al., 2003; Wijnker et al., 2013; Lindholm et al., 2016); however, further studies are needed to understand the mechanism involved. Despite the differences in the number of alleles amplified for each genome, the overall analysis of the markers demonstrated that the three parental species contributed to the amphidiploid genome. The detection of novel alleles in the amphidiploids developed here is not unusual in *Arachis* complex hybrids. The percentage of novel alleles (4.15%) detected here was almost equal to the percentage (5%) detected in a BC_1 derived from a cross between *A. hypogaea* and the complex hybrid TxAG-6 (Burow et al., 2001). Different mechanisms related to the genomic restructuring that is triggered by allopolyploidization have been cited to explain the origin of the new alleles in amphidiploids (Song et al., 1995; Liu et al., 1998; Zhang et al., 2005). However, additional research is needed in *Arachis* complex polyploids to better define the mechanism of genomic restructuring.

CONCLUSION

We identified resistance to peanut smut in several wild *Arachis* species. The amphidiploid obtained by crossing three of these wild *Arachis* species and some of the RILs developed from the crossing of *A. hypogaea* with the amphidiploid behaved similarly to the wild species. Thus, an effective transfer of resistance from the wild diploid to materials completely compatible with peanut was achieved. The development of these new breeding materials is a very important step in the process aiming to obtain peanut commercial cultivars resistant to peanut smut caused by *T. frezii*.

Supplemental Material

A supplemental table is available online that shows the unique alleles for the amphidiploid and *Arachis* wild species and the number of those alleles shared between wild relatives and the amphidiploid.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Supplemental Table S1. Unique alleles for the amphidiploid and *Arachis* wild species and number of those alleles shared between wild relatives and the amphidiploid

	A. batizocoi	***A. cardenasii***	***A. correntina***	**Amphidiploid**
Total of unique alleles	121	58	61	24
Alleles unique to wild spp. and present in the amphidiploid	20	66	31	