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The effect of tetraploidization of wild *Arachis* on leaf morphology and other drought-related traits

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ABSTRACT

Cultivated peanut is an allotetraploid (genome type AABB) with a very narrow genetic base, therefore wild species are an attractive source of new variability and traits. Because most wild species are diploid, the first step of introgression usually involves hybridization of wild species and polyploidization to produce a synthetic allotetraploid (AABB) that is sexually compatible with peanut. This study investigates drought-related traits such as leaf morphology, transpiration profile, chlorophyll meter readings (SCMR), specific leaf area (SLA) and transpiration rate per leaf area for two wild diploids (*Arachis duranensis* and *Arachis ipaënsis*) that could be of interest for improvement of the peanut crop. Furthermore, the inheritance of the traits from the diploid to the tetraploid state was investigated. Results showed that whilst some diploid traits such as SCMR, are maintained through hybridization and polyploidization, most characters, such as the leaf area, stomata size, trichome density and transpiration profile, are substantially modified. The study concludes that direct evaluations of drought-related traits in wild diploids may be useful for evaluation of wild species to be used in introgression. However, evaluations on wild-derived synthetic tetraploids are likely to be more informative.

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1. Introduction

Cultivated peanut (*Arachis hypogaea*) is an allotetraploid (AABB) which most probably originated via hybridization of two diploid wild species followed by a spontaneous duplication of chromosomes or fusion of unreduced gametes (Halward et al., 1991). Due to difference in ploidy from the diploid wild species, peanut became reproductively isolated from its wild relatives. This led to lack of diversity for some traits of agricultural interest and very low DNA polymorphism. Consequently the wild diploid species of *Arachis* harbor many "lost alleles" and have potential for broadening the genetic base of the peanut crop.

Historically, for peanut, the transfer of genes from wild species by sexual crossing was hindered by the ploidy differences. This can be overcome by artificial hybridizing A and B genome wilds followed by induced chromosome duplication to restore fertility through the tetraploid or hexaploid route (Stalker and Wynne, 1979; Simpson et al., 1993). Furthermore, improved knowledge of *Arachis* species relationships has been gained in recent years by more detailed cytogenetic and molecular phylogenetic studies (Kochert et al., 1996; Burow et al., 2001; Robledo and Seijo, 2010). These have provided a much better understanding of the relationships of the wild and cultivated species. Linkage drag and difficulties in confirming hybrid identities and tracking introgressed segments have also hindered progress. However, over the last few years very significant advances have been made in the development of the genetic tools needed to overcome these problems.

Many wild *Arachis* species have been found to be resistant to biotic stresses (Leal-Bertioli et al., 2010; Nelson et al., 1989). At least in principle, the introgression of disease resistances from wild relatives into crop plants is relatively simple, because generally, one or a few genes confer disease resistance (Hajjar and Hodgkin, 2007).

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So far, genetically characterized wild chromosome segments that confer improved nematode, leaf-spot and rust resistance have been introgressed into peanut (Simpson and Starr, 2001). With active research in this area we can expect more successes in the coming years (Khedikar et al., 2010).

Peanut is widely cultivated in the tropics, where drought is one of the most limiting factors for production. This together with concerns about climate change and yield stability has led to increased interest in improved drought tolerance. The wild species of peanut are found in extremely diverse environments, ranging from swamps to grasslands, to rocky ground in semi-arid conditions (Krapovickas and Gregory, 2007; Bertioli et al., 2011). Therefore, it is possible that wild species do harbor genes that could confer improved performance under certain water limited conditions. However, the inheritance of characters associated with drought adaptation is likely to be genetically complex, and therefore unlikely to be tractable with alternating cycles of backcrossing and phenotyping/genotyping. Perhaps because of this, so far, little effort has been made towards the use of wild species to improve this aspect of the peanut crop.

One promising route for the introgression of improved drought-related traits using synthetic allotetraploids could be the construction of chromosome substitution lines (Foncéka et al., 2009). Such lines are powerful tools for revealing cryptic beneficial alleles from the wild species but are very labor intensive to construct, and only possible to make with a limited number of wild accessions/species. Considering the resources needed to obtain new synthetics and backcrossed lines, a pertinent question is what wild diploid parentals to choose and whether their direct evaluations can be used to identify desirable drought adaptation traits that have potential for improving cultivated peanut. Potentially, wild diploid species identified by such evaluations (Nautiyal et al., 2008; Upadhyaya et al., 2011) could be used for the production of synthetic allotetraploids and introgression.

In this study, we initiated an investigation on drought-related characters in wild *Arachis* accessions. Focus was placed on two accessions of *Arachis ipaënsis* and *Arachis duranensis*, originated from regions with relatively low rainfall and their derived synthetic, using cultivated peanut as a parameter for comparison. The overall aim of the study was to investigate some drought-related characters and how far direct evaluations of wild diploid species may be useful to identify desirable traits that could be introgressed into cultivated peanut to improve its drought tolerance.

2. Materials and methods

2.1. Plant material

Arachis seeds were obtained from the Active Germplasm Bank of Embrapa Genetic Resources and Biotechnology (Cenargen, Brasília, Brazil). Seeds were bulked up in greenhouse conditions. An initial experiment of progressive water deficit was carried out with 10 wild diploid accessions, two cultivated tetraploid genotypes and a synthetic allotetraploid (*A. duranensis* V14167 × *A. ipaënsis* KG 30076)$^{4\times}$ (Fávero et al., 2006) referred to here as synthetic, all annual genotypes (Supplementary File 1). Subsequently, the following genotypes were chosen for more detailed study: *A. duranensis* V14167; *A. ipaënsis* KG30076, the synthetic and *A. hypogaea* subsp. *hypogaea* var. *hypogaea* 'IAC-Runner' (referred here as 'Runner'). Seeds were germinated in germitex paper, with 2% Ethrel (2-chloroethylphosphonic acid) to break dormancy and 0.05% Thiram® to prevent fungal contamination. Plantlets were transferred to pots of 15 cm diameter and 1200 g of dry soil capacity. Wild accessions show greater seed dormancy and initiate growth at a lower rate; therefore they were planted two weeks before the cultivated genotype and one week before the synthetic. All plants were kept in greenhouse conditions. Plants were periodically treated for mites and fungal diseases prior to water stress imposition. Temperature and relative humidity were recorded hourly using Electronic Datalogger Sato SK-L200TH II (Sato, Japan).

2.2. Leaf morphology

Features observed on the abaxial and adaxial surfaces included stomata type according to Metcalfe and Chalk (1950), stomata length and width, stomata and epidermal cells density (number of stomata or epidermal cells mm^{-2}), number of trichomes, leaflet and thickness of water storage cells layer. Leaves from ninety-day old plants were used. Portions of two leaflets of first expanded leaf from five plants of each genotype were dissociated (Berlyn and Miksche, 1976). Six epidermal dissociations (three for adaxial and three for abaxial surface observations) were mounted for each plant with glycerinated gelatin and observed in Zeiss Axiophot phase contrast microscope (Carl Zeiss, Germany). Images were recorded in AxioCam Zeiss system (Carl Zeiss, Germany).

Features observed on the abaxial and adaxial surfaces included stomata length and width, stomata and epidermal cells density (number of stomata or epidermal cells mm^{-2}), number of trichomes, leaflet and spongy parenchyma thickness. To determine stomata density, the numbers of epidermal cells and stomata were determined in three observations of each surface, of each of the leaflets of five plants in an area of 0.33 mm^2. Stomatic index was calculated as: $IE = [NE/(CE + NE)] \times 100$, where NE corresponds to the number of estomata and CE to the number of epidermal cells (Cutter, 1986). Other samples were collected and processed for JB4® resin embedding. Transversal astra blue and safranin stained semithin sections (2–4 μm thick) were analyzed to determine leaf cell types and estimate leaflet and hypodermis thickness. Leaf length and width were determined using a digital caliper and leaf area was estimated considering that leaflets are ellipse-like. Statistical analyses for genotype comparisons were performed using multiple observations with the non-parametric test of Tukey with significance levels of 5%. For biplot of multivariate data based on Principal Components Analysis (PCA), the method of Principal Components with GH factoring column metric preserving was used (Gabriel, 1971). Analyses were performed using the statistical software *R* (R Development Core Team, 2010).

2.3. Drought-related traits

2.3.1. Transpiration profile (dry-down)

Evaluations of transpiration profile in the initial experiment of progressive water deficit experiment—dry down (Sinclair and Ludlow, 1986) in *Arachis* genotypes (Supplementary File 1) were conducted in June 2007 in temperature-controlled greenhouse. Further evaluations of four selected genotypes were conducted in February/March 2010. Dry-down experiments were initiated at 8, 9, and 10 weeks after sowing the cultivated, synthetic, and wild diploid genotypes, respectively, after plants had initiated reproductive stage. At field capacity (FC), pots contained approximately 1200 g of dried soil and 350 g of water. Until water stress imposition plants were grown under well-watered conditions. Plants were divided into different sets of five repetitions of each genotype: one set was harvested to assess biomass at the time of stress imposition, one set was used as control with well-watered treatment (WW), and the last set was used for dry-down (DS).

During the experiment, all plant pots were weighed every day at around 9 AM. WW plants were kept at approximately 70% FC by compensating water losses due to transpiration. For DS plants, a loss of no more than 10 g of water per day was allowed, so that stress imposition was gradual. Daily transpiration rate (TR) was