

Gene expression profile, physiological and morphological characterization of wild *Arachis spp.* under drought stress

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INTRODUCTION

Wild *Arachis* species are a rich source of new alleles for peanut improvement, and have the polymorphism necessary for the genetic characterization of these alleles. An integrated approach associating leaf morphology, plant physiological behavior and transcriptome characterization of wild species of *Arachis* is underway in order to identify species harboring drought tolerance. *A. magna*, a wild type BB diploid species, showed higher tolerance under low water conditions. In this study, the transcriptome of *A. magna* accession KG30097, was analyzed under hydric stress aiming to identify genes related to drought response. In addition, the responses of leaf gas exchange under progressive soil drying in wild, cultivated and synthetic amphidiploids of *Arachis* were evaluated alongside the effects of polyploidization on epidermis organization.

MATERIAL AND METHODS

Dry-down experiments

Physiological traits such as transpiration behavior, TE (transpiration efficiency or soil water extraction capability or improvement in water use efficiency (WUE)), SLA (specific leaf area) and SCMR (SPAD chlorophyll meter reading) were determined under a gradual water stress in wild *Arachis* species - *A. ipaënsis* (K30076) and *A. duranensis* (V14167), cultivated *Arachis* - peanuts, and in a synthetic amphidiploid ((V14167 x K30076) x *A. hypogaea*).

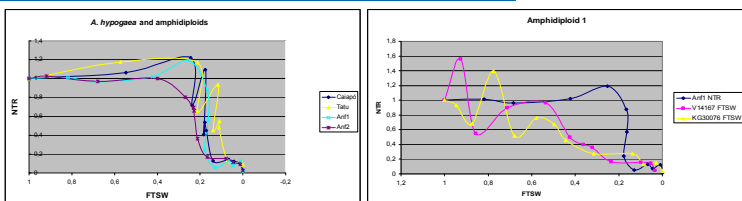
Leaf morphology

Morphological features such as stomata type, length and type, stomata index (SI = [# of epidermal cells / ([# of epidermal cells + [# of stomata]) x 100] and epidermis thickness and composition was determined on the plants above regularly irrigated.

SSH cDNA libraries

A. magna plants were submitted to a gradual dry down assay where the Normalized Transpiration Ratio (NTR) was controlled daily. Two subtractive libraries were constructed using cDNA from leaf tissues of stressed and well-watered control plants according to supplier's instructions (PCR-Select cDNA Subtraction Kit - Clontech).

RESULTS AND DISCUSSION



Figures 1, 2. NTR x FTSW of 1) *A. hypogaea* cv IAC-Catalpó, *A. hypogaea* cv Tauá, and the two amphidiploids, Anf1 (V14167 x KG30076); Anf 2 (V6389 x V9401) and 2) of two wild accessions (V14167 and K.30076) and the amphidiploid (V14167 x KG30076).

The responses to progressive water deficit in wild, synthetic and cultivated peanut were investigated. Although the transpiration behavior of synthetics was observed as being distinct from their wild parents, transpiration efficiency was similar, showing that direct screening of wild species for desirable drought responses needs to be interpreted with caution. Large variations of transpiration response were found between different wild species, and a surprisingly high variation in cultivated peanut was observed. In general, wild accessions had a "conservative" behavior: transpiration decreasing dramatically when the fraction of transpirable soil water was high (0.8 – 0.6). On the other hand, the transpiration of cultivated peanut varieties declined at lower soil water content (FTSW c.0.2), showing a more "opportunistic" behavior regarding water use (Fig.1). The transpiration response and the epidermis structure of the amphidiploid showed to be more similar to the tetraploid cultivated *Arachis* than to the diploid parents (Fig. 2). This suggests that tetraploidization could be affecting more these traits than the parental heritage (Fig.3 and 4).

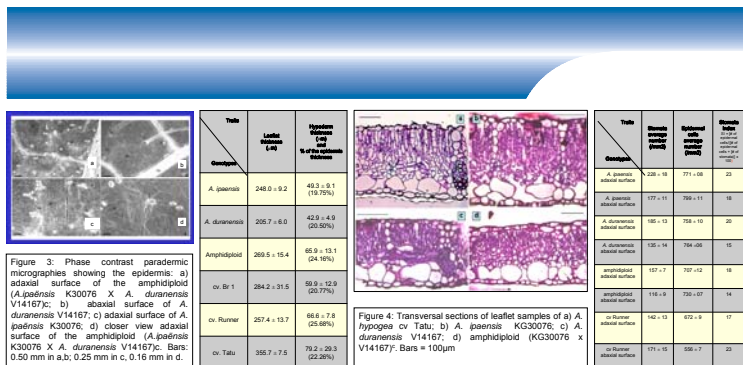


Figure 3: Phase contrast micrographs showing the epidermis: a) adaxial surface of the amphidiploid (*A. ipaënsis* K30076 X *A. duranensis* V14167); b) abaxial surface of *A. duranensis* V14167; c) adaxial surface of *A. ipaënsis* K30076; d) closer view adaxial surface of the amphidiploid (*A. ipaënsis* K30076 X *A. duranensis* V14167). Bars: 0.50 mm in a,b; 0.25 mm in c, 0.16 mm in d.

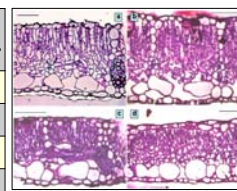


Figure 4: Transversal sections of leaflet samples of a) *A. hypogaea* cv Tauá; b) *A. ipaënsis* KG30076; c) *A. duranensis* V14167; d) amphidiploid (KG30076 x V14167). Bars = 100µm



Figure 5 - *A. magna* plants under drought stress (left) and watered control (right).

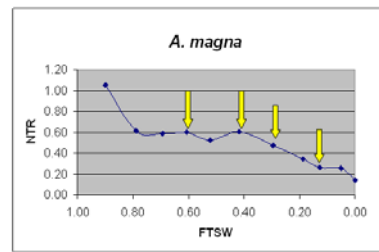


Figure 6 - NTR x FTSW of *A. magna* KG30097

During dry-down experiments, parameters as FTSW (fraction of transpirable soil water) and transpiration efficiency (TE) were calculated. A curve indicating the transpiration profile of a wild type BB diploid species, *A. magna* accession KG30097, showed high adaptability to water stress conditions (Fig. 6). This accession was chosen for transcriptome analysis and the distribution of the sequences of the SSH libraries are shown in Fig. 7.

Table 1: In silico analysis of SSH cDNA libraries

Blast hit	Reads/Contig
Drought inducible protein_S. officinarum	09
Carbonic anhydrase	07
Disease resistance protein (<i>A. hypogaea</i>)	37
EST Populus-drought stressed leaves	06
No hit	13
Glycolate oxidase	05
No hit	04
Glycine decarboxylase complex	04
No hit	04
All induced	04
Metallothionein (MT1)	04
Drought induced thioisulfide exchange intermediate	04
Populus EST - Drought induced	07
Drought induced thioisulfide exchange intermediate <i>A. thaliana</i>	04

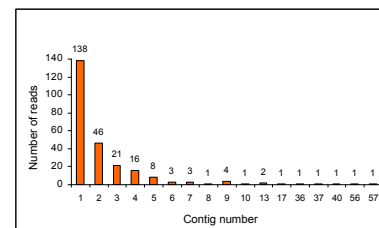


Figure 7 - Sequence distribution in contigs.

Subtractive hybridization was performed in both directions: i.e., using cDNA from stressed plants first as driver and then as tester against the cDNA from control plants. This allowed the enrichment of the genes either induced or inhibited during osmotic stress. *In silico* analysis revealed 759 reads, which were grouped into 249 clusters (138 singlets and 111 contigs), with a novelty index of 32.8%. Several genes that were up or down-regulated exclusively in the stressed or control conditions were identified. Numerous sequences related to biotic and abiotic stress were revealed, such as drought induced proteins, disease resistance proteins and catalases (Table 1). This is to date, the first report on the analysis of transcriptome of a wild relative of peanut under hydric stress. The ESTs produced in this study are a valuable resource for gene discovery, the characterization of new wild alleles, and for marker development.