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Research article

Water deficit induces variation in expression of stress-responsive genes in two peanut (*Arachis hypogaea* L.) cultivars with different tolerance to drought

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Abstract

Peanut (*Arachis hypogaea* L.) is an important subsistence and cash crop in the semi-arid tropics where it often suffers from drought stress. Although its ecophysiological responses are studied, little is known about the molecular events involved in its adaptive responses to drought. The aim of this study was to investigate the involvement of membrane phospholipid and protein degrading enzymes as well as protective proteins such as ''late embryogenesis-abundant'' (LEA) protein in peanut adaptive responses to drought. Partial cDNAs encoding putative phospholipase Dα, cysteine protease, serine protease and a full-length cDNA encoding a LEA protein were cloned. Their expression in response to progressive water deficit and rehydration was compared between cultivars differing in their tolerance to drought. Differential gene expression pattern according to either water deficit intensity and cultivar's tolerance to drought were observed. A good correspondence between the molecular responses of the studied cultivars and their physiological responses previously defined in greenhouse and field experiments was found. Molecular characters, as they were detectable at an early stage, could therefore be efficiently integrated in groundnut breeding programmes for drought adaptation. Thus, the relevance of the target genes as drought tolerance indicators is discussed.

Keywords: Peanut (*Arachis hypogaea* L.); Water deficit; Reverse transcription (RT)-PCR; Drought tolerance

1. Introduction

Environmental stresses, such as drought, high salinity and extreme temperatures have adverse effects on plant productivity. Tolerant plants respond and adapt to these stresses through various morphological, physiological and molecular processes [21,16]. Most molecular processes studied in response to drought to date regard transcriptional modification of gene expression [3,30]. Gene products have been classified between two groups: those that regulate gene expression and signal transduction during stress response and those that directly protect cells against environmental stresses [30].

Phospholipases are membrane lipid hydrolases whose action generates lipid-derived second messengers such as phosphatidic acid, diacylglycerol or inositol-triphosphate. Recently, the phospholipase Dα (PLDα) isoform has been shown to be involved in the abscisic acid signal transduction pathway leading to stomatal closure during drought stress [17,29,35]. Although regulating stomatal aperture is effective to limit water loss during short drought periods, it considerably reduces the rate of photosynthetic carbon dioxide uptake which is fundamental for plant growth and development

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[7,11]. Under these nutrient-limited conditions, plant survival depends on their capacity to recycle intracellular constituents such as proteins to supply cells with amino acids necessary for the synthesis of new proteins [31,32]. In response to water deficit, increases in protease activities have been observed in several species including tropical and temperate legumes [10,15,28]. Furthermore, by comparing drought-susceptible and drought-tolerant cultivars belonging to the same species, it has been established that the latter strictly control hydrolytic processes [10,23,24,25,28], and therefore damage to cellular structures, such as membranes, are avoided. Another way of keeping the integrity of cellular constituents and membranes is through accumulation of protective molecules such as late embryogenesis-abundant (LEA) proteins. These proteins accumulate in plant tissues in response to cellular dehydration resulting from developmental events, environmental stimuli or abscisic acid treatment [5,9,16]. It is hypothesized that LEA proteins bind water in their random coil conformation and protect cellular structures from dehydration stress [16]. Thus, they are thought to be key components of dehydration tolerance acquisition.

In the semi-arid tropical regions where drought is a major constraint, peanut (*Arachis hypogaea* L.) is one of the most agriculturally valuable plants. Despite its agronomic and economic importance, very little is known about its molecular adaptive responses to drought [18,22]. In the present study, we developed an original comparative plant system comprised of two peanut cultivars with different adaptive behaviour (stress avoidance through rapid stomatal closure or stress tolerance due to high membrane stability) under both greenhouse and field conditions [8,20]. Using this system, we studied variations in gene expression corresponding to putative PLDα, cysteine protease, serine protease and LEA protein in leaf tissues of plants submitted to progressive water deficit (15 days) and rehydration. This enabled us to correlate the cultivars' molecular responses with their physiological responses previously defined under drought conditions. The variations in gene expression induced by water deficit have been discussed taking into account the tolerance level of the cultivar and the intensity of the water deficit.

2. Materials and methods

2.1. Plant material and sample treatment

Seeds from two sahelian *Arachis hypogaea* L. cultivars, one tolerant to drought (cv. Fleur 11) and the other susceptible to drought (cv. 73-30), were surface-sterilized and germinated on wet filter paper. After 48 h, seedlings with well developed roots and equivalent morphology were selected and planted individually in pots containing a mixture of fertilized peat (TKS2® instant, Floragard France) and vermiculite (1/1, v/v). Plants were grown in a greenhouse as follows: 12 h photoperiod with photon flux density ranging between 400 and 500 μmol m^{-2} s^{-1}, temperature of 25 °C and 70–80% relative humidity. They were watered three times a week with tap water. Water deficit was applied by withholding irrigation on 21-day-old plants. Leaf water potentials were measured daily using a J14 press (Campbell Scientific Inc.) [4]. When plants reached potentials of -0.50 ± 0.05 MPa (C), -1.5 ± 0.2 MPa (S_1), -2.5 ± 0.2 MPa (S_2) and -3.5 ± 0.2 MPa (S_3) corresponding respectively to control, low, moderate and severe water stress, the third fully expanded leaves from the top were harvested and used for RNA extraction. In recovery experiments, S_3 plants were rehydrated and leaves harvested when ψ_w increased back to -0.50 ± 0.05 MPa (R).

2.2. Total RNA isolation and partial cDNA sequence amplification

Total RNA from control, stressed and rehydrated leaves (≈250 mg FW) were isolated using the RNeasy Midi kit (Qiagen) after DNA digestion using the RNase free DNase kit (Qiagen), following the manufacturer's protocols. RNA concentration was determined by spectrophotometry ($\lambda = 260$ nm) and integrity confirmed by gel electrophoresis in 1.5% agarose gels (0.5× TAE).

RT-PCR reactions to amplify partial cDNA sequences were performed using the OneStep RT-PCR kit (Qiagen). Reaction mixtures contained 100 ng of total RNA from leaves of moderately stressed plants, 200 μM of each dNTP, 1 μl of enzyme mix, 5 μl of 5× buffer and 20 pmol of each primer in a final volume of 25 μl. The gradient Mastercycler (Eppendorf) cycling parameters were as follows: 50 °C 30 min; 95 °C 15 min; 35 cycles of 95 °C 45 s; Tm°C 30 s; 72 °C 30–40 s; 72 °C 10 min. Tm were determined according to primer sequences. Heterologous primers designed from alignments between cDNA sequences from dicotyledonous species were used to amplify peanut coding segments of the target genes. The amplified partial serine protease and LEA cDNA sequences were elongated by 5′ and 3′ cDNA ends extension using a 5′/3′RACE amplification kit (Roche Diagnostics), following the manufacturer's protocol.

2.3. cDNA sequence identification

RT-PCR products were cloned into pGEM-T Easy vector (Promega) according to the manufacturer's protocol and used to transform *Escherichia coli* competent cells (strain DH5α) by heat shock. Plasmid DNA of single colonies was isolated using the Wizard® Plus SV minipreps DNA Purification System kit (Promega) and restriction digests were performed to confirm proper cloning. Recombinant plasmids were sequenced on both strands, using the universal T7 and SP6 primers by ESGS (France). Searches for sequence homology and alignment of the deduced amino acid sequences with related sequences from other species were performed with the FASTA (http://www.ebi.ac.uk/fasta33/) and CLUSTALW (http://www.ebi.ac.uk/clustalw/) programs, respectively.

2.4. Analysis of gene expression

Gene expression studies were performed by RT-PCR under the conditions described above, using peanut specific primers.

Semi-quantitative RT-PCR was performed for all studied genes on total RNA isolated from control, drought-stressed and rehydrated leaves of the two cultivars. Initial estimates of the linear range for RT-PCR for each gene were determined by running reactions over a wide range of PCR cycle numbers (data not shown). Cycle numbers that consistently gave product in the linear range were used for all experiments. Final experiments were performed in duplicate independently. Amplification products were analyzed by gel electrophoresis on 1.5% agarose gels and visualized by staining with ethidium bromide. Expression levels were quantified, using the Gene Tools/Gene Snap software (Syngene).

3. Results

3.1. Isolation of peanut cDNA sequences

cDNA sequences were obtained by RT-PCR and 5′/3′ RACE-PCR amplifications on leaf total RNA extracts from peanut cv. Fleur 11 submitted to moderate stress (S_2, $\psi_w = -2.5$ MPa). Heterologous primer sequences were consistently chosen in regions specific to the corresponding protein since some of them, like phospholipase D, include several isoforms with homologous domains. They were designed as indicated in Table 1 to amplify partial ORFs sequences encoding putative phospholipase Dα (PLD), cysteine protease (CP), serine protease (SP) and LEA protein.

Protein sequences deduced from the isolated peanut cDNA fragments all contained at least one motif characterizing the protein considered (Fig. 1). AHPLD (289 aa) presented the HxKxxxxD motif characteristic of the phospholipases D catalytic site [27]. Furthermore, its sequence was highly homologous to that of phospholipase Dα (PLDα) sequences from other species (Table 2). AHCP (77 aa) corresponded to a fragment of a putative mature cysteine protease as shown by sequence comparison analysis (Table 2). It included the cysteine residue conserved in the catalytic site (Fig. 1) and the GCNGGLM amino acids conserved amongst papain-like cysteine proteases [2]. AHSP (539 aa) showed 80% identity with *A. thaliana* subtilisin-like serine protease and lower identity levels with serine proteases from other species (Table 2). Its sequence contained the conserved serine residue of the catalytic site and the protease associated domain (PA) identified in all plant subtilisins [2] (Fig. 1). AHLEA (96 aa) was identified as a putative full-length late-embryogenesis abundant (LEA) protein from group 1 as referred to identity percentages with LEA proteins from other species (Table 2). Despite its leaf origin, it included the "small hydrophilic plant seed protein" signature (Fig. 1).

Table 1
Heterologous primers derived from related sequences previously described from various species and used to amplify fragments of peanut genes coding a phospholipase Dα (PLDα), a cysteine protease (CP), a serine protease (SP) and a late embryogenesis abundant (LEA) protein

Gene	Primer sequence	Tm (°C)
PLDα	5′ CGTCCGCGAACACGAACACG 3′	58
	5′ AAGGGTGTGAGAGAGATCCG 3′	53
CP	5′ CTNCCNRAGRATTTYGATTGG 3′	54.6
	5′ CATMAAHCCDCCHTYACASCC 3′	56.6
SP	5′ CCATGGATAGCATCTGTAGCA 3′	55.9
	5′ ATCTATCAGTTCCTTTGAATTGCTGAC 3′	60.1
LEA	5′ GGCATCTAAGCAACAAAACCG 3′	57.9
	5′ TGGACTCATCAATCTCAACGC 3′	57.9

Nucleotides are indicated by IUPAC 1-letter codes (N = A + C + G + T; R = A + G; Y = C + T; S = C + G; H = A + C + T; D = A + G + T; M = A + C). GenBank accession nos. of the genes from which primers were designed for amplification of PLDα, SP and LEA cDNA fragments were ***U92656***, ***B1787695*** and ***AF479305*** respectively.

All identified sequences were deposited in the GenBank database. Accession nos. are ***DQ011882***, ***DQ011883***, ***DQ011884*** and ***DQ011885*** for putative *Arachis hypogaea* phospholipase Dα (AHPLD), *Arachis hypogaea* serine protease (AHSP), *Arachis hypogaea* cysteine protease (AHCP) and *Arachis hypogaea* LEA protein (AHLEA), respectively. *AHCP* and *AHSP* are the first cysteine protease- and serine protease-like sequences described in peanut. A LEA protein (***AF479305***; LEA1) and a PLD (***AY274834***) cDNAs have previously been isolated in peanut [13]. The deduced AHLEA (this study) differed from LEA1 by six amino acid residues over 96 (Fig. 2A). Equivalent regions of the two peanut putative PLD showed only 66% identity (Fig. 2B).

3.2. Gene expression in response to drought stress

Expression of *AHPLD*, AHCP, AHSP and *AHLEA* was studied during progressive water stress and rehydration in the leaves of drought-tolerant (Fleur 11) and drought-susceptible (73-30) peanut cultivars, using RT-PCR with primers designed from the isolated peanut cDNA sequences described above (Table 3). Results showed that the putative PLDα gene (*AHPLD*) was upregulated by water deficit in both Fleur 11 and 73-30 cultivars. However, maximum *AHPLD* transcript accumulation was observed in S_1 plants, for the susceptible cultivar 73-30 whereas in the tolerant cv. Fleur 11, transcript accumulation peaked in S_2 plants (Fig. 3). Severe drought (S_3) lowered *AHPLD* transcript accumulation compared to S_2. This was more pronounced in the susceptible cultivar 73-30. Upon rehydration, *AHPLD* transcript levels were back to control levels (tolerant cultivar) or slightly higher (susceptible cultivar) (Fig. 3).

In both cultivars, low water deficit (S1) induced a decrease in *AHCP* transcript accumulation. In the tolerant cultivar Fleur 11, cysteine protease-like transcripts accumulated above control levels under S_2 and S_3 water stress conditions, whereas this occurred only in S_2 leaves in the sensitive cultivar 73-30 (Fig. 3; Table 4). In both cultivars, rehydration restored steady-state *AHCP* transcript accumulation approximately to S_1 levels rather than to control levels.

In response to water deficit, expression of the putative serine protease gene (*AHSP*) was downregulated in both cultivars. However, the decrease in expression was more pronounced in the tolerant cv. Fleur 11 than in the susceptible cv. 73-30. In both cultivars, *AHSP* expression was restored after rehydration. In rehydrated cv. Fleur 11 plants, *AHSP* expression

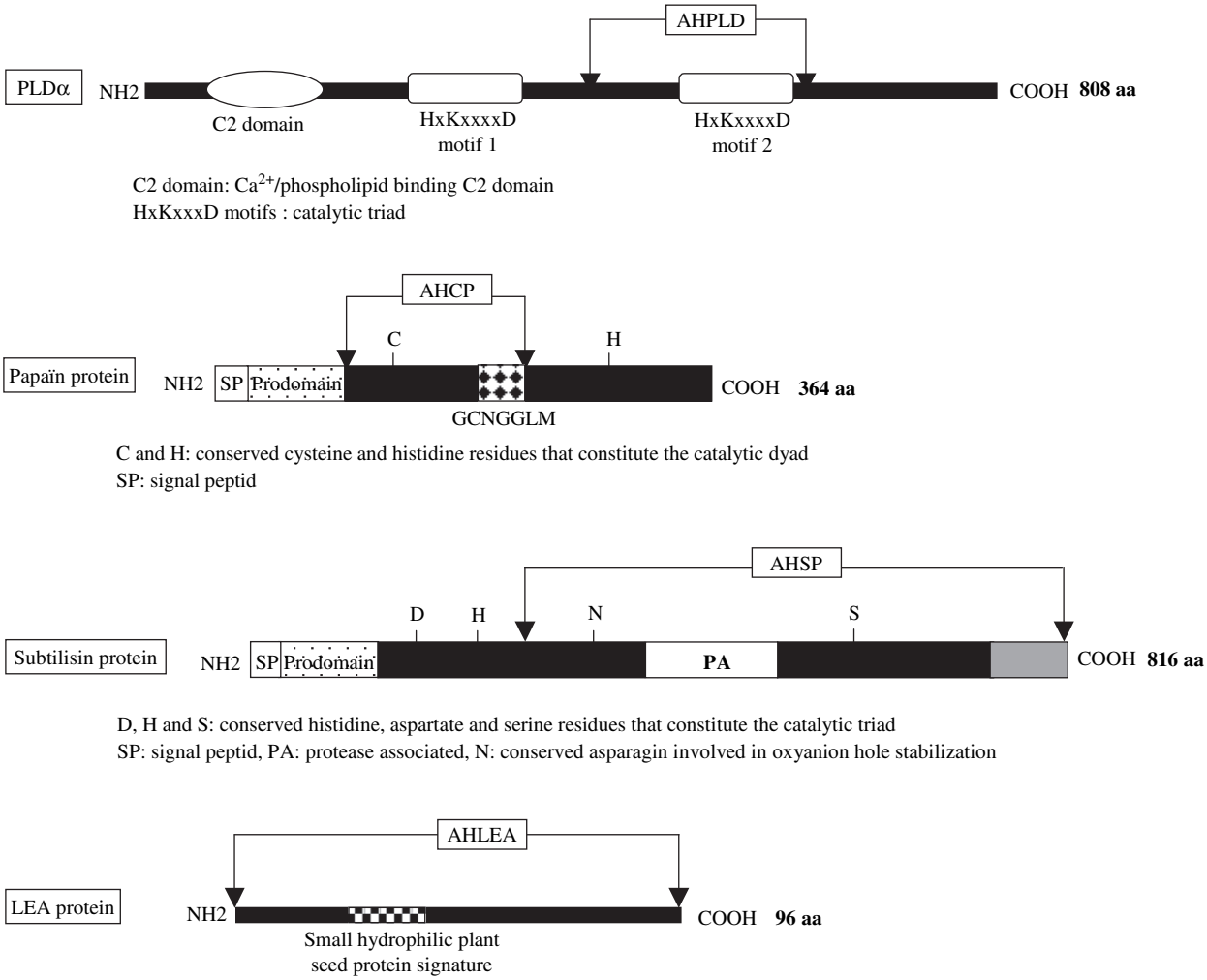

Fig. 1. Schematic representation of target gene products. Regions spanning between the vertical arrows correspond to the deduced proteins from peanut sequences isolated here. AHPLD, *Arachis hypogaea* phospholipase Dα (289 aa); AHCP, *Arachis hypogaea* cysteine protease (77 aa); AHSP, *Arachis hypogaea* serine protease (539 aa); and AHLEA, *Arachis hypogaea* late embryogenesis abundant protein (96 aa). Entire proteins correspond to PLDα, papain-like cysteine protease, subtilisin-like serine protease and LEA protein isolated from *Ricinus communis* (U72693, 808 aa), *Vigna mungo* (AB038598, 364 aa), *Arabidopsis thaliana* (AY139780, 816 aa) and *Arachis hypogaea* (AF479305, 95 aa) respectively.

exceeded that of control plants, whereas in rehydrated cv. 73-30 plants, expression was equivalent to that of S_1 plants (Fig. 3).

In both cultivars, putative LEA protein gene (*AHLEA*) expression was induced by water deficit. In cv. Fleur 11, transcripts accumulated mostly at S_2 whereas in cv. 73-30, their accumulation remained mostly unchanged regardless of stress intensity. After rehydration, only the susceptible cultivar showed *AHLEA* transcript accumulation (Fig. 3).

4. Discussion

In order to obtain useful information regarding the molecular responses of peanut growing under drought conditions, well characterized plant material with distinct tolerance levels to drought was chosen based on previous agronomical and physiological studies carried out under both field and greenhouse conditions [8,20]. Under our experimental conditions, plant leaves reached a water potential of approximately −3.5 MPa without showing any drought-induced senescence symptoms (yellowing, abscission), after 13−15 days without irrigation. When re-watered at this stage, flaccid tissues of cultivar Fleur 11 recovered full turgescence more readily than tissues from cultivar 73-30, suggesting the existence of more efficient protection and repair mechanisms in the former and confirming its better tolerance to drought-stress [8,20].

In response to progressive dehydration imposed on the plants, drought-responsive cDNAs encoding a putative phospholipase Dα (PLDα), papain-like cysteine protease (CP), subtilisin-like serine protease (SP) and LEA protein were identified in the leaves of both cultivars. Furthermore, variations in the accumulation levels of the corresponding transcripts were observed that were cultivar-specific and appeared to correlate with their previously established drought-tolerance abilities. In the susceptible cultivar (73-30), an increase in PLDα expression was observed in S_1 leaves whereas in the drought-tolerant cultivar (Fleur 11) it occurred in S_2 leaves. Equivalent differences between drought-sensitive

Table 2
Percent identity of deduced peanut partial *AHPLD*, *AHCP*, *AHSP* and full-length *AHLEA* amino acid sequences with the corresponding regions of related sequences from other species

Putative proteins	Species	GenBank accession no.	Amino acid (% identity)
AHPLD (289 aa)	*Ricinus communis*	***U72693***	90
	Vigna unguiculata	***U92656***	87
	Lycopersicon esculentum	***AY013252***	86
	Craterostigma plantagineum	***AJ133000***	86
	Nicotiana tabacum	***Z84822***	86
AHCP (77 aa)	*Vigna mungo*	***AB038598***	90
	Phaseolus vulgaris	***Z99953***	90
	Glycine max	***U71379***	90
	Vicia sativa	***Z30338***	88
	Vicia faba	***U59465***	87
AHSP (539 aa)	*Arabidopsis thaliana*	***AY139780***	80
	Oriza sativa	***AP004278***	69
	Lycopersicon esculentum	***U80583***	46
	Lilium longiflorum	***D21815***	44
	Glycine max	***AY277949***	35
AHLEA (96 aa)	*Arachis hypogaea*	***AF479305***	94
	Phaseolus aureus	***U31211***	83
	Robinia pseudoacacia	***U40821***	83
	Glycine max	***U66317***	83
	Arabidopsis thaliana	***AF360157***	80

Five of the best identity matches are presented.

and resistant peanut lines were recently observed in a greenhouse and field study [13]. Furthermore, these results are concurrent with those obtained on cowpea plants where the drought sensitive cultivar accumulated PLDα transcripts at the onset of the drought treatment (S_1) and not the tolerant one [23]. Due to efficient stomatal regulation occurring at light stress levels, it has previously been observed that 73-30 maintained higher relative water contents (RWC) than Fleur 11 in its foliar tissues [8]. Since PLDα isoforms have been shown to be implicated in stomatal closure via ABA signalling [17,29,35], the increase in PLDα transcripts under S1 conditions could be part of the regulating events leading to maintenance of high water status in the sensitive cv. 73-30.

Other PLD isoforms are involved in bulk hydrolysis of membrane lipids in response to water deficit. Such degradative phenomena have been shown to be very important in drought-susceptible cultivars of other tropical legume species [23,25]. It is not excluded that the putative peanut PLDα studied here participates in this process since massive breakdown of membrane lipids has been observed in the leaves of drought-stressed 73-30 plants [8,20]. Upon rehydration, peanut PLDα could also be involved in membrane repair and rearrangement as was shown for a dehydration-inducible *Craterostigma plantagineum* PLDα [12] since PLDα transcript levels were higher than in control plants for both cultivars.

Protein breakdown and recycling are essential processes of plant responses undergoing reduced photosynthetic activities as induced by drought [31,32]. In this study, a partial peanut cysteine protease cDNA was isolated and its sequence analysis showed that it is closely related to the *Vigna mungo* membrane-associated cysteine protease (MCP). The MCP is involved in both the degradation of storage proteins and the processing of a protease responsible for the degradation of storage proteins from cotyledon cells [26]. Characterization of the partial putative *CP* peanut cDNA in response to water deficit showed that transcript accumulation was enhanced under moderate stress conditions (S_2), in the sensitive cultivar 73-30, and under both moderate and severe stress conditions (S_2 and S_3) in Fleur 11. Contrary to peanut, tomato and *Arabidopsis thaliana* lines showed induction of cysteine protease gene expression in response to low water stress [14,19], suggesting a better tolerance to light drought conditions for the

A

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AHLEA     MASKQQNRQELDERAKQ GETVVPGGT GGKSLEAQEHLAEGRSKGGQTRREQLGTEGYQEMGRKG 64
LEA1      MASKQQNRQELDERAKQ GETVVPGGT GGKSLEAQEHLAEGRSKGGQTRREQLGTEGSKRC-RKG 63

AHLEA     GFSTMEKSGEERAEEEGVEIDESKFATKNQNK 96
LEA1      GFSTMEKSGGERAEEEGVEIDESKFATKNQNK 95
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B

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AHPLD     HHDDFHQPNFAGASIMKGGPREPWHDIHSRLEGPIAWDVLFNFEQRWRKQGGKD-LLIPLREL  62
AY274834  HHDDFYQPSISGSAIEKGGPREPWHDIHCKLEGPIAWDVYSTFVQRFRKQGTDQGMLLSEEKL 416

AHPLD     EDVIIPPSPVTFAEDQE-TWNVQLFRSIDGGAAFGFPETPEDAARAGLVSGKDNIIDRSIQDA 124
AY274834  KDFIVAPSQVTNPDDDDDTWNVQLFRSIDDTATLGFPETAKEALNTGLVQWENKMIDRSIQDA 479

AHPLD     YIHAIRRAKNFIYIENQYFLGSCFGWSPDDIKPEDIGALHLIPKELSLKIVSKIEAGERFTVY 187
AY274834  YINAIRRAKNFIYIENQYFIGSAFGWS---VDSTEFDAVHLIPKELSLKIVSKIKAKEKFMVY 539
                      box B

AHPLD     IVVPMWPEGFPE---SGSVQAILDWQRRTMEMMYKDIVEALNAKGIVE-DPRNYLTFFCLGNR 246
AY274834  VVIPMWPEGVPINKTTGTVQKILYLQRRTIEMMYKDIVEALKEEKI-EQDPRKYLSFFCLGNR 601

AHPLD     EVKKPGEYEPSERPDPDSDYIKAQENRRFMIYVHAKMMIVDDE 289
AY274834  EAKKYGEYVPPQRPKQGSDYQKAQEARRFMIYVHSKMMIVDDE 644
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Fig. 2. Comparison of deduced amino acid sequences of cloned peanut *AHPLD* and *AHLEA* gene fragments with other related peanut sequences. (A) Alignment of AHLEA with previously isolated LEA1 (***AF479305***). (B) Alignment of AHPLD fragment with equivalent portion of previously isolated PLD (***AY274834***). Bold residues represent the pfam "small hydrophilic plant seed protein" signature (GETVVPGGT) in LEA protein. Conserved PLDα motifs are underlined. Residues differing between peanut sequences are in grey.

Table 3
Homologous primers designed from each peanut partial cDNA sequence and used to study *AHPLD*, *AHCP*, *AHSP* and *AHLEA* gene expression

Gene	Primer sequence	Tm (°C)	Number of cycles	Product size (bp)
AHPLD	5′ TTGCCGGTGCTTCCATCATGA 3′	59.8	25	524
	5′ AACCTCTCCCCGGCTTCGATT 3′	61.8		
AHCP	5′ ATTGGCGTGACAAGGGAGCTG 3′	61.8	30	203
	5′ TTCACAGCCTGCGTCACATGC 3′	61.8		
AHSP	5′ GCTGCAATTGATGATCGTAG 3′	55.3	30	389
	5′ CTACGATAACAATCTCGAGA 3′	53.2		
AHLEA	5′ GGCATCTAAGCAACAAAACCG 3′	57.9	25	261
	5′ TGGACTCATCAATCTCAACGC 3′	57.9		

tropical legume. Peanut putative *CP* expression profiles suggested that proteolytic episodes lasted longer into the drought treatment for the tolerant cultivar Fleur 11 than they did in the susceptible one. This feature could allow for more efficient mobilization of protein precursors to overcome drought-induced nutrient starvation.

In contrast with *AHCP*, expression of *AHSP* (encoding a serine protease) decreased with the intensity of water deficit. Therefore, the corresponding enzyme is not likely implicated in adaptive protein breakdown in response to drought-stress conditions. The metabolic functions of plant serine proteases are diverse and include differentiation, senescence and protein processing [1,2]. Characterization of senescence-associated proteases in broccoli florets has shown the contribution of serine proteases to early and late stages of senescence [33]. In drought-tolerant *Phaseolus vulgaris* cultivars, abundance of a 65 kDa leaf protein with serine protease activity decreased during drought treatment. This decrease was thought to be associated with a protection mechanism against premature drought-induced senescence [15]. Similarly, the down-regulation of *AHSP* expression could be a drought tolerance mechanism to reduce serine protease activity and delay drought-induced senescence. Under this assumption, tolerant cultivar Fleur 11 presents better control over leaf cellular proteolysis than sensitive cultivar 73-30.

Tolerance to dehydration involves accumulation of LEA proteins that protect cells from damage [5,9,16]. In peanut leaves, *AHLEA* was fully induced by water deficit, no visible transcript accumulation was observed in well watered plants. Since expression of a barley LEA protein (HVA1) conferred increased tolerance to water deficit and salt-stress to transgenic rice plants [6,34], it can be suggested that in the leaves of drought-stressed peanut plants, LEA transcript accumulation is associated with tolerance acquisition mechanisms. Observation of higher *AHLEA* transcript levels in the drought-tolerant cultivar compared to the susceptible one is concurrent with this hypothesis.

Present data accumulation on some of the molecular changes occurring in the leaves of peanut plants submitted to progressive drought stress has shown a good correlation with the agronomical and physiological responses previously described. Even though transcriptional variations remain to be confirmed at protein level, this study contributes to a better understanding of how peanut plants cope with water deficit.

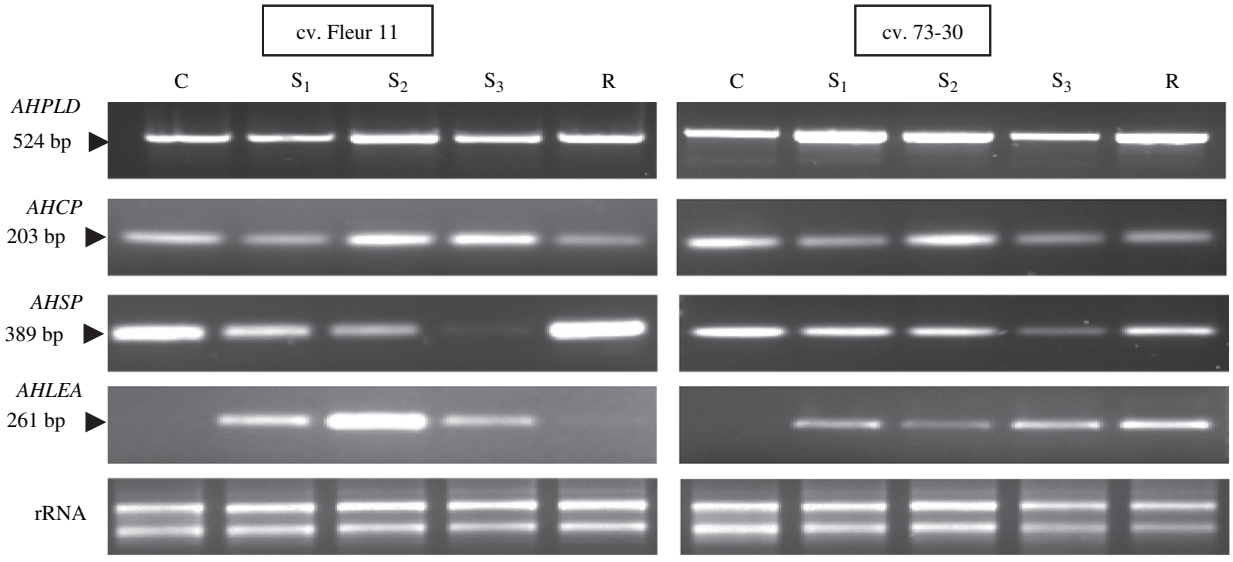

Fig. 3. RT-PCR analysis from a representative experiment showing expression of *AHPLD*, *AHCP*, *AHSP* and *AHLEA* genes in the leaves of two peanut cultivars (Fleur 11, drought-tolerant and 73-30, drought-susceptible) submitted to various levels of water deficit. C (control): $\psi_w = -0.25$ MPa; S_1 (low stress): $\psi_w = -1.5$ MPa; S_2 (moderate stress): $\psi_w = -2.5$ MPa; S_3 (severe stress): $\psi_w = -3.5$ MPa; R (rehydration): $\psi_w = -0.5$ MPa. Uniformity in template RNA amounts was checked with ribosomal RNA (rRNA).

Table 4
Relative percent changes in gene expression between control and drought stressed plants

		Percent difference between control and drought stressed plants			
		AHPLD	*AHCP*	*AHSP*	*AHLEA*[a]
cv. Fleur 11	S_1	8 ± 3	-37 ± 3	-32 ± 1	NA
	S_2	40 ± 4	17 ± 1	-51 ± 2	102 ± 2
	S_3	5 ± 1	22 ± 1	-93 ± 2	-10 ± 1
	R	10 ± 4	-44 ± 8	26 ± 1	ND
cv. 73-30	S_1	51 ± 2	-40 ± 1	-9 ± 1	NA
	S_2	17 ± 5	11 ± 2	-28 ± 5	-26 ± 5
	S_3	-12 ± 1	-50 ± 2	-75 ± 3	14 ± 2
	R	31 ± 3	-38 ± 2	-26 ± 1	29 ± 2

Results were obtained by quantification of RT-PCR results shown in Fig. 3. Data are means of percent variations according to control plants.
[a] Variations were determined according to S1 plants; NA, not applicable; ND, not detected.

Increases in PLDα and LEA transcripts accumulation could contribute to reduced water loss and protection of cellular components. Prolonged expression of AHCP during drought stress could allow mobilization of precursors for the synthesis of new proteins. Decrease in AHSP expression could result in delaying drought-induced senescence. Since, target gene expression is differentially regulated according to the drought tolerance level of the cultivars, their use as screening genes for drought tolerance in peanut is possible, particularly those encoding the LEA protein and PLDα, as has previously been suggested [13].

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