FEBS Letters 491 (2001) 188–192 FEBS 24637

A novel patatin-like gene stimulated by drought stress encodes a galactolipid acyl hydrolase

Ana Rita Matos[a,*], Agnès d'Arcy-Lameta[a], Marcel França[a], Stéphane Pêtres[d], Léna Edelman[d], Jean-Claude Kader[b,c], Yasmine Zuily-Fodil[a], Anh Thu Pham-Thi[a,c]

[a] *Laboratoire de Biochimie et Physiologie de l'Adaptation Végétale, Université Denis Diderot, case 7019, 2 Place Jussieu, 75251 Paris Cedex 05, France*
[b] *Laboratoire de Physiologie Cellulaire et Moléculaire des Plantes, Université Pierre et Marie Curie, 4 Place Jussieu, 75252 Paris, France*
[c] *UMR 7632 CNRS, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France*
[d] *Laboratoire de Technologie Cellulaire, Département des Biotechnologies, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France*

Received 28 November 2000; revised 29 January 2001; accepted 29 January 2001

First published online 9 February 2001

Edited by Ulf-Ingo Flügge

Abstract A cDNA (*Vupat1*) encoding a predicted 43 kDa protein was isolated from drought-stressed cowpea (*Vigna unguiculata*) leaves. It has homology with patatin, a potato tuber storage protein with lipolytic acyl hydrolase activity. The recombinant protein VUPAT1 expressed in the baculovirus system displays preferentially galactolipid acyl hydrolase activity. Phospholipids are very slowly hydrolyzed and apparently triacylglycerols are not deacylated. *Vupat1* promoter contains putative drought-inducible sequences. Northern blots showed that gene expression is stimulated by drought stress and is more pronounced in a drought-sensitive cultivar than in a drought-tolerant one. An involvement in drought-induced galactolipid degradation is proposed for VUPAT1.

Key words: Drought stress; Lipolytic acyl hydrolase; Galactolipase; Patatin; Cowpea (*Vigna unguiculata* L. Walp)

1. Introduction

Galactolipids are main components of chloroplast membranes accounting for more than 60% of total polar lipids in photosynthetic tissues. Their hydrolysis is stimulated by drought stress [1,2], chilling [3] and senescence [4]. In 1964 Sastry and Kates [5] reported for the first time the existence in the leaves of *Phaseolus multiflorus* of an enzyme able to catalyze the hydrolysis of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). This enzyme capable of removing fatty acids (FA) from both *sn* positions was designated galactolipase (EC 3.1.1.26). The majority of the galactolipid hydrolyzing enzymes purified from plant leaves [6–12] were also active towards phospholipids but not triacylglycerols so the term lipolytic acyl hydrolase (LAH) is usually applied to those enzymes only described in the plant kingdom. Despite the research done on leaf LAHs the corresponding genes remain unknown.

*Corresponding author. Fax: (33)-142276068.
E-mail: matos@ccr.jussieu.fr

Abbreviations: LAH, lipolytic acyl hydrolase; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol; PC, phosphatidylcholine; PLA_2, phospholipase A_2; FA, fatty acids

Galliard [13] first demonstrated the LAH activity of patatin, the major reserve glycoprotein from potato tubers. Recombinant patatin is preferentially active towards phospholipids and moderately active towards galactolipids [14]. Patatin-like cDNAs were cloned from cucumber seedlings [15], *Hevea brasiliensis* latex [16] and tobacco leaves [17]. Cucumber and tobacco proteins were shown to display phospholipase A_2 (PLA_2) activity and the latex allergen was able to hydrolyze *p*-nitrophenyl palmitate. Several other patatin-like genes are published in sequence databases but remain putative.

In cowpea (*Vigna unguiculata*) leaf extracts, LAH activity increases with water deficit and is more pronounced in drought-sensitive cultivars (cvs) [1]. From the same plant a soluble LAH hydrolyzing both galacto and phospholipids was purified [2]. In this work a patatin-like cDNA designated *Vupat1* was isolated from drought-stressed cowpea leaves. A genomic clone containing the *Vupat1* promoter and coding regions was also isolated. The recombinant protein was obtained by expression in the baculovirus system and its ability to hydrolyze several lipid substrates, extracted from cowpea leaves, was investigated. The expression of *Vupat1* in cowpea leaves, in response to progressive drought stress, re-hydration, rapid desiccation and abscisic acid (ABA), was analyzed in drought-sensitive and drought-tolerant cvs.

2. Materials and methods

2.1. Plant material and stress treatments

Two *V. unguiculata* L. Walp cvs differing in drought stress tolerance, cv EPACE-1 (drought-tolerant) and 1183 (drought-sensitive), were grown in greenhouse as previously described [18]. Drought stress was induced by withholding watering for 7–10 days in 21 days old plants. Stress levels are based on leaf water potentials measured in a pressure chamber [19] (PMS, ECS Instruments). ABA treatments were performed on detached leaves incubated in a 0.1 mM ABA solution (ABA^+) or water (ABA^-) for 24 h. Desiccation was also assayed on detached leaves left to dry at room temperature and daylight for 5 h.

2.2. cDNA cloning and sequence analysis

Primers corresponding to patatin consensus regions were used in PCR amplifications, the sequences were GAYTAYTTYGAYRTNATVG for sense oligonucleotide and GGRKSNCCNAARATHTTYCC (H = A/T/C, K = G/T, N = A/C/G/T, R = A/G, S = G/C, V = G/A/C, Y = C/T) for the antisense one. Template cDNAs were previously prepared by [18] from leaves of *V. unguiculata* cv EPACE-1 plants submitted to mild drought stress (S2). The fragment obtained was used to screen a λ Ziplox cDNA library constructed from the same cDNAs [18]. A positive clone (*Vupat1*) was isolated, plasmid DNA was extracted with a Maxi-prep kit (Qiagen) and sequenced

PII: S0014-5793(01)02194-9

on both strands (ESGS, France). Sequence analysis was performed using PCgene software (Intelligenetics) and programs available at internet sites detailed in Section 3.

2.3. Genomic library construction and screening

The extraction of genomic DNA from *V. unguiculata* cv EPACE-1 leaves was performed as described by [20]. DNA was purified in a CsCl gradient according to [21] and partially digested by *Sau*3AI (Appligene). Suitable size fragments were separated in a sucrose gradient as detailed in [22]. The fragments were ligated to the vector lambda DASH/*Bam*HI arms (Stratagene) with T4 DNA ligase (Appligene) and packaged with Gigapack III Gold Packaging extract (Stratagene) according to the manufacturer's instructions. Recombinant phages were propagated on *Escherichia coli* XL1-Blue MRA (P_2) (Stratagene). Nylon filters (Hybond-N, Amersham) were prepared and hybridized in high stringency conditions as described by [22] with a full-length *Vupat1* cDNA radiolabeled with the kit Prime-a-gene (Promega). Positive clones were isolated, the DNA was purified with a lambda Midi kit (Qiagen) and sequenced on both strands (ESGS, France). Searching for putative *cis*-acting sequences was performed using PLACE database [23].

2.4. Expression in the baculovirus system

The coding region of *Vupat1* cDNA was cloned in the vector pAcGP67 (Pharmingen). Recombinant baculoviruses were generated by co-transfecting the recombinant plasmid and linearized baculovirus DNA (Baculogold, Pharmingen) into *Spodoptera frugiperda* (Sf-9) cells, for details see [24]. The recombinant protein was produced by BTI-TN-5B1-4 cells derived from *Trichoplusia ni* egg (Invitrogen) infected with recombinant baculovirus at a multiplicity of infection of 10 and cultured in TC100 medium (Life Technologies) in 150 cm^2 culture flasks (Corning). Culture medium was harvested 3 days after infection, centrifuged at $2000\times g$ for 5 min and the supernatant containing the recombinant protein was kept at −20°C until needed. Protein concentration was measured according to Bradford's method [25] with the protein assay kit (Bio-Rad), using bovine serum albumin as a standard. The presence of the recombinant protein in the culture medium was assessed by immunoblots with anti-VUPAT1 polyclonal antibodies produced against recombinant VUPAT1 expressed in *E. coli* (Matos et al., unpublished) performed according to [26] and enzymatic assays. Negative controls corresponding to culture medium from cells infected with non-recombinant baculovirus were also tested.

2.5. Lipolytic activity assays

Lipid substrates were prepared from *V. unguiculata* leaves fed for 24 h with [^{14}C]sodium acetate as described in [27]. Reaction mixtures contained a volume of substrate corresponding to 0.3 μmol hydrolyzable bonds, dried under a nitrogen stream and suspended in 10 μl methanol, 10 μg total protein (50 μl culture medium) in a final volume of 480 μl Tris–HCl pH 7 containing 0.3% Triton X-100. Reactions were carried at 30°C for 90 min. Lipids were extracted and separated by thin layer chromatography on silica-gel plates using Mangold solvent system [28], bands corresponding to free FA were scraped off and counted in a liquid scintillation spectrometer (1600 CA, Packard). Leaf proteins extracted as in [2] were used as a positive control.

2.6. Northern blot analysis

Extraction of total RNA was performed according to [29]. Poly-$(A)^+$ mRNA was isolated using Oligotex columns (Qiagen) following the manufacturer's instructions. 4 μg mRNA of each treatment was separated on a 1% agarose–formaldehyde gel and transferred to nylon membranes (Hybond-N, Amersham) as described by [22]. Hybridizations were carried out as described for the library screening except that the probe used was a 186 bp fragment from the *Vupat1* 3′ untranslated region.

3. Results

3.1. cDNA cloning and sequence analysis

Degenerated primers corresponding to patatin consensus sequences (DYFDV/IA/G and H/NC/A/GPKIFP) were used in PCR amplifications. The 143 bp fragment obtained was used as a probe to screen a cDNA library constructed from cowpea leaves submitted to mild drought stress (S2). A full-length clone of 1570 bp was isolated, sequenced and designated *Vupat1* (GenBank accession AF193067). It encodes a predicted polypeptide of 400 amino acid residues with calculated p*I* of 8.55 and deduced molecular mass of 43 481 Da. The 5′ untranslated region contains an in-frame stop codon 93 bp before the first methionine and the 3′ untranslated region is 261 bp long. VUPAT1 shares 48% amino acid identity with potato patatin [14], 53% with Hev b 7 latex allergen

```
                        P                                                    ++++++++++++
VUPAT1  ----------MAATQTPSKVD---DGALITVLSIDGGGIRGIIPGILLAFLESELQKLDGA-DARLADYFDVIAGTSTGGLVTAMLTAPNENNRPLYAAK  86
HEVb7   ------------MATGSTTLT---QGKKITVLSIDGGGIRGIIPGIILASLESKLQDLDGP-DARIADYFDIIAGTSTGGLITTMLTAPNEDKKPIYQAK  84
CUCUMIS -PRVRFLSLFVLLTMVADFA----KGKMITILSIDGGGIRGIIPSIILAFLESKLQELDGP-DVRIADYFDVIAGTSTGGLVTSMLTAPDKNNRPLYSAS  94
PATATIN MATTKSFLILFFMILATTSSTCATLGEMVTVLSIDGGGIKGIIPAIILEFLEGQLQEVDNNKDARLADYFDVIGGTSTGGLLTAMITTPNENNRPFAAAK 100
                        .. .    *  .*.********.****.*.*. **..**..*.  *.*.*****.*.*******.*.*.*.*.....*.  *.

                  P                                       P
VUPAT1  DIKDFYLEHTPKIFPQSSSWNLIATAMKKGRSLMGPQYDGKYLHKLVREKLGNTKLEHTLTNVVIPAFDIKNLQPAIFSSFQVKKRPYLNAALSDICIST 186
HEVb7   DIKDFYLENCPKIFPKES------RDTYDPIHSIGPIYDGEYLRELCNNLLKDLTVKDTLTDVIIPAFDIKLLLPVIFSSDDAKCNALKNARLADVCIST 178
CUCUMIS DLALFYIEHAPKIFPQRNYFLCSLVNFFGKV--MGPKYNGLYLRSLIKGLLGDITLKQTLSQVVIPAFDIKLLQPVIFTTIEAKCSELKNPKLADVCIST  92
PATATIN DIVPFYFEHGPHIFNYRG-------------SIFGPRYDGKYLLQVLQEKLGETRVHQALTEVAISSFDIKTNKPVIFTKSNLAESPQLDAKMYDICYST 187
        *.  **.*. *.**   .              .** *.* **  . .. * .  ....*..* *..****   *.**.. .   .  .     *.* **

                        P                                       P
VUPAT1  SAAPTYLPAHCFETKTSTASFK-FDLVDGGVAA-NNPALVAMAEVSNEIRN-EGSCASLKVKPLQYKKFLVISLGTGSQ-QHEMRYSADKASTWGLVGWL 282
HEVb7   SAAPVLLPAHSFTTEDDK-NIHTFELIDGGVAA-ANPTLLALTHIRNE--IIRQNPRFIGANLTESKSRLVLSLGTGKS-EYKEKYNADMTSKWRLYNWA 273
CUCUMIS SAAPTFLPGYEFQTKDSKGNIRNYEMVDGGVAA-NNPTLAAMTHVTKEMSILRHRSELLKIKPMETERMLVLSLGTGTP-KNDEKYSAAKASKWGMLDWV 290
PATATIN AAAPIYFPPHHFVTHTSNGATYEFNLVDGAVATVGDPALLSLSVAT---RLAQEDPAFSSIKSLDYKQMLLLSLGTGTNSEFDKTYTAEEAAKWGPLRWM 284
        .***. .* . * *.... .   ....**.**. ..*.* ... .  . .                  *..*****.  . .  *.*. ...*    *

                                    P                       P                                   G P
VUPAT1  SSSGGTPLIDVFSHASSDMVDFHISSVFQARHAEQNYLRIQDDTLTGDLGSVDVATEKNLNGLVQVAEALLKKPVSKINLRTGIHEPVESNETNAEALKR 382
HEVb7   LYNGNSPAVDIFSNASSDMVDFHLSALFKSLDCEDYYLRIQDDTLTGEESSGHIATEENLQRLVEIGTELLEKQESRINLDTGRLESIPGAPTNEAAIAK 373
CUCUMIS YHGGGTPIVDIFSDASADMVDYHISSIFQSDHCHKNYLRIQDDTLSGEVSSVDIATEENLLNLIYVGENLLKKPLSRVNLESGKFEPLDAKGTNEQALAE 390
PATATIN L-----AIQQMTNAASSYMTDYYISTVFQARHSQNNYLRVQENALTGTTTEMDDASEANMELLVQVGETLLKKPVSK----------DSPETYEEALKR 368
              .  .. . **. *.*...*..*.. . .. ***.*...*.*. . .  *.* *.  *. ....**.*. *.           .  * ..*.


VUPAT1  FAARLSNQRRFRKSQTFA 400
HEVb7   FAKLLSEERKLRQLK--- 388
CUCUMIS FAKMLSNERKLRLSP--- 405
PATATIN --KLLSDRKKLRANKASH 384
            **. ...*
```

Fig. 1. Amino acid sequence comparison of VUPAT1, potato patatin (GenBank accession no. M21879), cucumber patatin-like protein (Y12793) and Hev b 7 latex allergen (AJ223039). Similar amino acids (*), well conserved amino acids (.). Putative sites of phosphorylation (P), glycosylation (G) and α/β fold hydrolase motif (+) in VUPAT1 sequence.

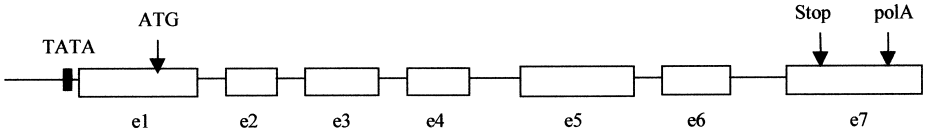

Fig. 2. Schematic representation of *Vupat1* gene. Putative transcription start site and TATA box are located at positions −172 bp and −195 bp, respectively. The seven exons are represented by boxes and are 300, 191, 193, 159, 306, 176 and 305 bp long; the six introns are represented by lines and are 74, 99, 108, 138, 93 and 226 bp long.

[16] and 56% with cucumber patatin-like protein [15] (Fig. 1). According to the hydropathy profile determined by PRED-TMR2 method (http://o2.biol.uoa.gr) no transmembrane segment was determined for VUPAT1. Sequence analysis by the program pSORT (http://psort.nibb.ac.jp) reveals that VUPAT1 seems to have an uncleavable N-terminal signal sequence, however no chloroplast targeting peptide is predicted by this program. Potential protein kinase C phosphorylation sites are found at positions 96, 206, 241 and 338; casein kinase II phosphorylation sites at positions 8, 140 and 375; one tyrosine kinase phosphorylation site at position 313 and one *N*-glycosylation site at position 373 (PROSITE, PCgene) [30]. An α/β fold hydrolase motif [31] with the characteristic signature (GXSXG) is present in positions 61–72 (eMotif, http://dna.stanford.edu) [32] (Fig. 1).

3.2. Gene cloning and analysis

The screening of a cowpea cv EPACE-1 genomic library allowed the isolation of a genomic clone (GenBank accession AF318315) containing the promoter and the coding regions of *Vupat1*. The coding region extends through 2368 bp and is composed of seven exons and six introns bordered by canonical sequences (GT/AG) (Fig. 2). The 3105 bp sequenced upstream the start ATG of the gene were searched for the binding sites of transcription factors and transcriptional regulatory elements. A putative TATA box (TATATAA) is located at −195 bp from the first Met and the plant consensus transcription starting sequence (CTCATCA) is found at position −172 bp. Among the putative *cis* sequences identified in *Vupat1* promoter are the binding sites for MYB (TAACTG at position −456) and MYC (CACATG at positions −1675, −2251, −2289 and −2950), proteins that are known to be induced by drought and ABA and bind to promoters of dehydration responsive genes in *Arabidopsis thaliana* [33]. The LTRE core (CCGAC at position −1088), besides the low temperature responsiveness observed in *Brassica napus* [34] genes is also induced by drought and ABA in *A. thaliana* genes [35]. Other transcription factors binding sites involved in phythormone and light responses are also present in *Vupat1* promoter.

3.3. Expression in the baculovirus system and lipolytic activity assays

Recombinant VUPAT1 was produced in insect cells with an N-terminal peptide that mediates the forced secretion of the protein and is cleaved during transport across cell membrane. Culture medium from cells infected with recombinant baculovirus was tested for the presence of recombinant protein. VUPAT1 could not be detected in polyacrylamide gels after Coomassie staining, but through immunodetection with a specific polyclonal antibody (Matos et al., unpublished) a single band was revealed (Fig. 3). The band presents the expected M_r (approximately 43 kDa) and is absent in negative control.

The culture medium was assayed for lipolytic activity towards several lipid substrates radiolabeled and extracted from cowpea leaves. In the conditions described in Section 2 the enzyme degrades preferentially the galactolipids MGDG and DGDG and the sulpholipid sulphoquinovosyldiacylglycerol (SQDG) (Fig. 4). Phospholipids are very slowly hydrolyzed and apparently triacylglycerols are not deacylated. The optimum pH for hydrolysis of MGDG is around 7 (data not shown).

3.4. Northern blot analyses

Fig. 5 shows *Vupat1* expression pattern in response to drought stress, desiccation and ABA in two cowpea cvs.

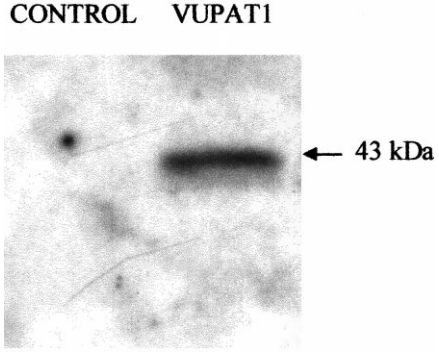

Fig. 3. Immunochemical detection of the recombinant VUPAT1. Insect cell culture medium corresponding to 0.12 mg total protein was separated in each lane by SDS–PAGE and transferred to a Hybond-ECL membrane (Amersham). The protein was detected with an anti-VUPAT1 antibody followed by a rabbit anti-rat HPR-conjugated antibody revealed with ECL reagents (Amersham).

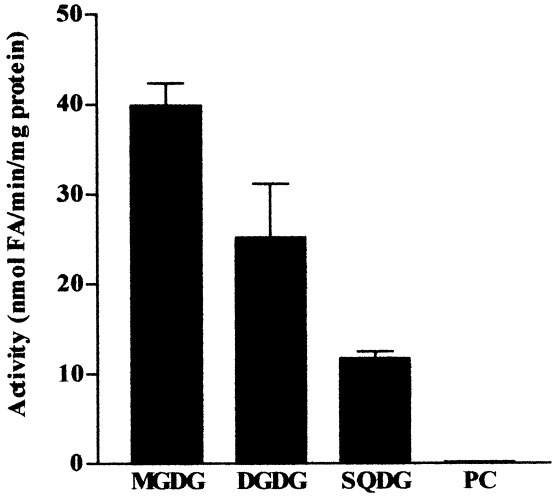

Fig. 4. Hydrolysis of DGDG, MGDG, SQDG an PC by the recombinant protein VUPAT1. The activity is expressed in nmol FA released per mg total protein per minute.

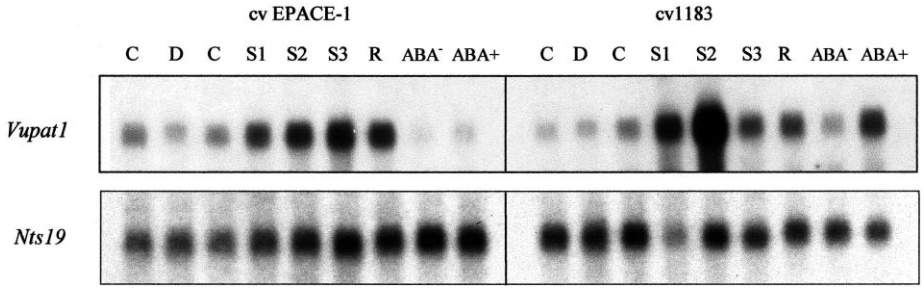

Fig. 5. Northern blot analyses of *Vupat1* mRNA in *V. unguiculata* leaves cvs EPACE-1 (drought-tolerant) and 1183 (drought-sensitive). Control (C), water potentials of −1.0 MPa (S1), −1.5 MPa (S2), −2.0 MPa (S3), 24 h S2 re-hydrated plants (R), 5 h desiccated leaves (D) and leaves incubated in 0.1 mM ABA (ABA^+) or in water (ABA^-) for 24 h. Equal amounts of mRNA in each lane were checked with a *Nicotiana tabacum* ribosomal protein *Nts19*.

Vupat1 is expressed in well watered plants (control) and is stimulated by drought in both cvs. In the drought-sensitive cv 1183, the transcripts show maximal accumulation at mild drought stress (S2, −1.5 MPa) and decrease at severe water deficit (S3, −2.0 MPa) probably due to a generalized degradation of the cellular components. The tolerant cv EPACE-1 shows a less pronounced and slower response, with maximal accumulation at severe water deficits (S3, −2.0 MPa). For both plants a period of 24 h re-hydration is not enough to reduce transcription to control levels. Rapid desiccation of detached leaves does not induce an increase in *Vupat1* expression. A 24 h incubation in ABA seems to slightly induce gene expression in the case of cv 1183.

4. Discussion

Little is known about galactolipid hydrolyzing enzymes; after the first report by Sastry and Kates [5], other authors tried to purify the enzyme from leaves, however no corresponding gene has been isolated. The majority of the purified enzymes including the one from cowpea were able to hydrolyze both galactolipids and phospholipids [8–12]. The only authors who clearly demonstrated the existence of an enzyme specific to glycolipids were Burns and his co-workers [6,7]. They partially purified two LAHs, one specific to MGDG, DGDG, SQDG and monolein and another one that acted on phosphatidylcholine (PC) and monolein. The molecular weights were estimated to be in the range of 70–90 kDa and the authors pointed out the difficulties to purify further those proteins. The work of Matsuda et al. [11] in potato leaves also highlighted the presence of several LAH isoforms sharing biochemical properties that render difficult their purification to homogeneity.

In contrast to leaf LAH, potato tuber patatins are well studied. They are a group of 40 kDa vacuolar glycoproteins with LAH activity that account for 40% of soluble protein in tubers [36,37]. They are encoded by a family of genes which are present at 10–18 copies per haploid genome [38]. The expression of one of those genes in the baculovirus system [14] showed that it encoded an enzyme active with phospholipids, monoacylglycerols and moderately active with galactolipids. The correspondence between patatin-like genes and the leaf LAH has never been established.

In this work a patatin-like gene expressed in cowpea leaves was cloned and through the use of substrates extracted from the same biological source as the cDNA it was clearly shown that it encodes a protein displaying high activity towards MGDG, DGDG and SQDG and very little activity towards PC. The reaction products are free FA, therefore the designation galactolipid acyl hydrolase is proposed. The results obtained for the recombinant VUPAT1 indicate that in vivo the native protein may be involved in galactolipid degradation. Nevertheless it is important to underline that besides VUPAT1 other enzymes might contribute to galactolipid hydrolysis observed in leaf extracts [2]. The question whether they are encoded or not by patatin-like genes remains to be investigated.

Since galactolipids are restricted to plastids and are major lipids in the thylakoids and in the inner envelope [39], it could be expected that this protein had a consensus chloroplast targeting sequence. The absence of such a peptide does not exclude the hypothesis of a chloroplastic localization, that should be further investigated, but the cytoplasmic localization could explain why galactolipids are not rapidly hydrolyzed in vivo despite the considerable galactolipase activity in leaf homogenates. Those proteins probably reach their substrates only after a loss of compartmentation carried out by other enzymes. Additionally modulation of the activity by kinase phosphorylation should also be considered by analogy to animal PLA_2. Those enzymes are known to be regulated by phosphorylation and stimulated by calcium which triggers their translocation to membranes [40]. There is no direct evidence for a same mode of action for LAH but a stimulation by calcium [12] and calmodulin [41] was reported and VUPAT1 has several putative phosphorylation sites.

The physiological role of patatin-like proteins is still unclear. The existence of such a group of proteins sharing amino acid identities but with different substrate specificity and present in different organs and at different stages of development indicates that probably they are important in many aspects of plant physiology. In this work it is shown that a progressive drought stress stimulates the expression of *Vupat1*. The sensitive cv shows a rapid increase in *Vupat1* expression at mild water stress while the tolerant one is able to maintain lower levels of transcripts. *Vupat1* expression pattern correlates with galactolipid content and degradation previously reported [1,2] since the drought-sensitive cv shows a strong reduction of galactolipid content and high galactolipase activity at mild water deficit while the drought-tolerant is only significantly affected by severe water deficit. Furthermore the analysis of *Vupat1* promoter region indicates the presence of several putative *cis*-acting elements associated with water deficit response corroborating the results obtained with Northern blots. Taken together, all those results allow to

suggest that VUPAT1 might be involved in membrane degradation induced by water stress.

Several other putative regulatory sequences including phythormone response were detected in *Vupat1* promoter, which suggests that this protein is involved in a wide variety of cellular and developmental responses.

An involvement in the oxylipin pathway should also be considered. The release of linolenic acid from membranes is a key step in the formation of signalling molecules like jasmonic acid that induce transcriptional activation of water deficit [42], wound and pathogen responsive genes [43]. Patatin-like proteins with PLA_2 activity were proposed to be involved in such transduction pathways [17,44,45] and recently it was suggested that a galactolipid 'lipase' must provide the polyunsaturated FA that undergo peroxidation by enzymes located on the chloroplast envelope [46,47].

Despite the growing interest in the fields of stress and senescence only a few reports exist on lipid deacylating enzymes in such physiological conditions. In this work are presented the cloning and expression of a gene encoding a galactolipid acyl hydrolase that is stimulated by drought stress. Since VUPAT1 exhibits different properties from those of the patatin-like proteins analyzed so far it would be of great interest to characterize in detail the biochemical and molecular properties of this family.

Acknowledgements: This work was supported by the program PRAXIS XXI (FCT, Portugal).

References

[1] Monteiro de Paula, F., Pham Thi, A.T., Zuily-Fodil, Y., Ferrari-Iliou, R., Vieira da Silva, J. and Mazliak, P. (1993) Plant Physiol. Biochem. 31, 707–715.
[2] Sahsah, Y., Campos, P., Gareil, M., Zuily-Fodil, Y. and Pham-Thi, A.T. (1998) Physiol. Plant. 104, 577–586.
[3] Kaniuga, Z. and Gemel, J. (1984) FEBS Lett. 171, 55–58.
[4] Engelman-Silvestre, I., Bureau, J., Trémolières, A. and Paulin, A. (1989) Plant Physiol. Biochem. 27, 931–937.
[5] Sastry, P.S. and Kates, M. (1964) Biochemistry 3, 1280–1287.
[6] Burns, D.D., Galliard, T. and Harwood, J.L. (1979) Phytochemistry 18, 1793–1797.
[7] Burns, D.D., Galliard, T. and Harwood, J.L. (1980) Phytochemistry 16, 651–654.
[8] Helmsing, P.J. (1969) Biochim. Biophys. Acta 178, 519–533.
[9] Anderson, M.M., Mc Carty, R.E. and Zimmer, E.A. (1974) Plant Physiol. 53, 699–704.
[10] Matsuda, H. and Hirayama, O. (1979) Biochim. Biophys. Acta 573, 155–165.
[11] Matsuda, H., Tanaka, G., Morita, K. and Hirayama, O. (1979) Agric. Biol. Chem. 43, 563–570.
[12] Sahsah, Y., Pham-Thi, A.T., Roy-Macauley, H., d'Arcy-Lameta, A., Repellin, A. and Zuily-Fodil, Y. (1994) Biochim. Biophys. Acta 1215, 66–73.
[13] Galliard, T. (1971) Biochem. J. 121, 379–390.
[14] Andrews, D.L., Beames, B., Summers, M.D. and Park, D. (1988) Biochem. J. 252, 199–206.
[15] May, S., Preisig-Müller, R., Höne, M., Grau, P. and Kindl, H. (1998) Biochim. Biophys. Acta 93, 267–276.
[16] Sowka, S., Wagnar, S., Krebitz, M., Arija-Mad-Arif, S., Yusof, F., Kinaciyan, T., Brehler, R., Scheiner, O. and Breiteneder, H. (1998) Eur. J. Biochem. 225, 213–219.
[17] Dhondt, S., Geoffroy, P., Stelmach, B.A., Legrand, M. and Heitz, T. (2000) Plant J. 23, 431–440.
[18] El Maarouf, H., Zuily-Fodil, Y., Gareil, M., d'Arcy-Lameta, A. and Pham-Thi, A.T. (1999) Plant. Mol. Biol. 39, 1257–1265.
[19] Scholander, F., Hammel, H.T., Hemmingsen, E.A. and Bradstreet, E.D. (1964) Proc. Natl. Acad. Sci. USA 52, 119–125.
[20] Rogers S.O. and Bendich, A.J. (1991) Extraction of DNA from plant tissues, in: Plant Molecular Biology Manual (Gelvin, S.A. and Schilperoort, R.A., Eds.), pp. A6/1–A6/10, Kluwer Academic Publishers, Dordrecht.
[21] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1994) Current Protocols in Molecular Biology, Vol. 1, unit 2.3, Preparation and Analysis of DNA, John Wiley and Sons.
[22] Sambrook, J., Fritsch, E.F. and Maniatis, T.(1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[23] Higo, K., Ugawa, Y., Iwamoto, M. and Korenaga, T. (1999) Nucleic Acids Res. 27, 297–300.
[24] Edelman, L., Margaritte, C., Chaabihi, H., Monchatre, E., Blanchard, D., Cardona, A., Morin, F., Dumas, G., Petres, S. and Kaczorek, M. (1997) Immunology 91, 13–19.
[25] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[26] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[27] El-Hafid, L., Pham-Thi, A.T., Zuily-Fodil, Y.A. and Vieira da Silva, J. (1989) Plant Physiol. Biochem. 27, 495–502.
[28] Mangold, H.K. (1961) J. Am. Chem. Soc. 47, 762–773.
[29] Hall, T.C., Ma, Y., Buchbinder, B.U., Pyne, J.W., Sun, S.M. and Bliss, F.A. (1978) Proc. Natl. Acad. Sci. USA 75, 3196–3200.
[30] Hofmann, K., Bucher, P., Falquet, L. and Bairoch, A. (1999) Nucleic Acids Res. 27, 215–219.
[31] Ollis, D.L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franken, S.M., Harel, M., Remington, S.J., Silman, I., Schrag, J., Sussman, J.L., Verschueren, K.H.G. and Goldman, A. (1992) Protein Eng. 5, 197–211.
[32] Attwood, T.K., Avison, H., Beck, M.E., Bewley, M., Bleasby, A.J., Brewster, F., Cooper, P., Degtyarenko, K., Geddes, A.J., Flower, D.R., Kelly, M.P., Lott, S., Measures, K.M., Parry-Smith, D.J., Perkins, D.N., Scordis, P., Scott, D. and Worledge, C. (1997) J. Chem. Inf. Comput. Sci. 37, 417–424.
[33] Abe, H., Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Husowaka, D. and Shinozaki, K. (1997) Plant Cell 9, 1859–1868.
[34] Jiang, C., Iu, B. and Singh, J. (1996) Plant Mol. Biol. 30, 679–684.
[35] Baker, S.S., Wilhem, K.S. and Thomashow, M.F. (1994) Plant Mol. Biol. 24, 701.
[36] Paiva, E., Lister, R.M. and Park, W.D. (1983) Plant Physiol. 71, 161–168.
[37] Tweel, D. and Ooms, G. (1988) Mol. Gen. Genet. 212, 325–336.
[38] Sonnewald, U., Studer, D., Rocha-Sosa, M. and Willmitzer, L. (1989) Planta 178, 176–183.
[39] Joyard, J., Marechal, E., Block, M.A. and Douce, R. (1996) Plant galactolipids and sulfolipid: structure, distribution and biosynthesis in membranes, in: Specialized Functions in Plants (Smallwood, M., Knox, J.P. and Bowles, D.J., Eds.), BIOS Scientific Publishers Limited.
[40] Munnik, T., Irvine, R.F. and Musgrave, A. (1998) Biochim. Biophys. Acta 1389, 222–272.
[41] Moreau, R.A. and Isett, T.F. (1985) Plant Sci. 40, 95–98.
[43] Creelman, R., Tierney, M.L. and Mullet, J.E. (1992) Proc. Natl. Acad. Sci. USA 89, 4938–4941.
[42] Creelman, R. and Muller, M.J. (1992) Proc. Natl. Acad. Sci. USA 92, 4114–4119.
[44] Senda, K., Yoshioka, H., Doke, N. and Kawakita, K. (1996) Plant Cell Physiol. 37, 347–353.
[45] Jung, K.M. and Kim, D.K. (2000) Plant Physiol. 123, 1057–1067.
[46] Rustérucci, C., Montillet, J., Agnel, J., Battesti, C., Alonso, B., Knoll, A., Bessoule, J., Etienne, P., Suty, L., Blein, J. and Triantaphylidès, C. (2000) J. Biol. Chem. 274, 36446–36455.
[47] Blée, E. and Joyard, J. (1996) Plant Physiol. 110, 445–454.