A multicystatin is induced by drought-stress in cowpea (*Vigna unguiculata* (L.) Walp.) leaves ☆

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Received 15 June 2004; revised 12 July 2004; accepted 5 October 2004

Available online 20 October 2004

Edited by Valdimir Skulachev

Abstract Cystatins are protein inhibitors of cystein proteinases belonging to the papain family. In cowpea, cystatin-like polypeptides and a cDNA have been identified from seeds and metabolic functions have been attributed to them. This paper describes *VuC1*, a new cystatin cDNA isolated from cowpea leaves (*Vigna unguiculata* (L.) Walp.). Sequence analysis revealed a multicystatin structure with two cystatin-like domains. The recombinant VUC1 protein (rVUC1) was expressed in an heterologous expression system and purified to apparent homogeneity. It appeared to be an efficient inhibitor of papain activity on a chromogenic substrate. Polyclonal antibodies against rVUC1 were obtained. Involvement of the *VuC1* cDNA in the cellular response to various abiotic stresses (progressive drought-stress, dessication and application of exogenous abscissic acid) was studied, using Northern blot and Western blot analysis, in the leaf tissues of cowpea plants corresponding to two cultivars with different capacity to tolerate drought-stress. Surprisingly, these abiotic stresses induced accumulation of two *VuC1*-like messages both translated into VUC1-like polypeptides. Difference in the transcript accumulation patterns was observed between the two cultivars and related to their respective tolerance level. Presence of multiple cystatin-like polypeptides and their possible involvement in the control of leaf protein degradation by cysteine proteinases is discussed.

Keywords: Abiotic stress; Cowpea (*Vigna unguiculata* (L.) Walp.); Cystatin; Cysteine proteinase; Drought tolerance

1. Introduction

Cystatins are proteinaceous reversible inhibitors of cysteine proteinases, such as papain and cathepsin H. Originally identified from mammalian systems, they have recently been characterized in plants [1,2]. Like all members of the cystatin super-family, phytocystatins contain the three conserved regions interacting with cysteine proteinase molecules: G at the N-terminus, QxVxG and W at the C-terminus [3]. Moreover, phytocystatins differ from non-plant cystatins due to the presence of a plant-specific sequence, or PSS, [LVI]-[AGT]-[RKE]-[FY]-[AS]-[VI]-x-[EDQV]-[HYFQ]-N located in a region corresponding to a predictable amino-terminal α-helix [4].

Involvement of phytocystatins in plant defensive strategies against various pests and predators, such as coleopteran insects [5–7] and fungi [8,7], is widely accepted. The ability of phytocystatins to inhibit exogenous cysteine proteinases has been used to produce phytocystatin overexpressing transgenic plants with enhanced resistance to insects [9,10], nematodes [11,12] and potyviruses [13]. In addition to protective functions, some cystatins may also have metabolic roles, such as the regulation of cysteine proteinase activities during the mobilization of storage proteins, in seeds particularly [14,15]. More recently, it has appeared that cystatins could act as modulators in programmed cell death [16]. Also, participation of cystatins in plant responses to abiotic stresses has been questioned. Cystatin mRNA accumulate in the vegetative tissues of barley plants submitted to anaerobiosis, darkness and cold-shock [17]. In addition to cold, saline- and heat-shocks induced cystatin message accumulation in the leaves and roots of chestnut plantlets [18]. In rice seedlings, exposure to the gaseous air pollutant SO_2 led to changes in phytocystatin-like proteins [19].

The present study reports the isolation and characterization of a cDNA coding for a putative cystatin in cowpea (*Vigna unguiculata* (L.) Walp.) leaves. Analysis of the deduced amino acid sequence indicated that the predicted polypeptide was a multicystatin, the first one to be reported from leaves. In response to progressive drought, desiccation and application of exogenous ABA, variations in the expression patterns of two cystatin messages and proteins have been observed. Using a unique plant system including two cowpea cultivars differing in their capacity to tolerate drought, the role of cystatin in

☆ *VuC1* GenBank Accession No.: AF278573.

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Abbreviations: ABA, abscissic acid; DTT, dithiothreitol; Ni–NTA, nickel–nitrilotriacetic acid; ψ_w, leaf water potential; PCR, polymerase chain reaction; PMSF, phenylmethane sulfonyl fluoride; PVP, polyvinylpyrrolidone; TCA, trichloroacetic acid; *Vu*, *Vigna unguiculata*

0014-5793/$22.00
doi:10.1016/j.febslet.2004.10.014

drought-stress tolerance at the cellular level has been addressed.

2. Materials and methods

2.1. Plant culture and treatments

Cowpea (*V. unguiculata* (L.) Walp.) plants (cultivars EPACE-1, drought-tolerant and IT83, drought-sensitive) were grown in a greenhouse, as previously reported [20,21]. Water status was measured using a pressure-chamber (PMS, ECS Instruments) [22]. When plants were 21-day-old, controlled water deficit was induced, by withholding watering. Fresh leaf tissue (approximately 6 g) was sampled from control plants (C; $\psi_w = -0.3$ MPa), mildly stressed plants (S1; $\psi_w = -1.0$ MPa), moderately stressed plants (S2; $\psi_w = -1.5$ MPa), severely stressed plants (S3; $\psi_w = -2.0$ MPa) and plants rehydrated for 24 h (R; $\psi_w = 0.3$ MPa). Sampled tissues were frozen in liquid nitrogen and stored at −80 °C. Abscissic acid (ABA) treatment was performed on detached leaves, by soaking petioles for 24 h in a buffer (10 mM Tris–Cl, pH 7.0) containing 0.1 mM ABA (or no ABA for the controls). Dessication was obtained by air-drying excised leaves on a laboratory bench. All experiments were carried out on the second fully expended leaves.

2.2. cDNA cloning and sequence analysis

Degenerate primers (Fig. 1) 5′-CGYTTYGCYGTBGARGA-3′ (sense) and 5′-CCCAVACYTTDGCYTCRTA-3′ (antisense) (B = C/G/T, D = A/G/T, R = A/G, V = G/A/C, Y = C/T), designed from consensus regions identified in cystatin sequences from rice (GenBank Accession No. S49967), soybean (GenBank Accession No. D31700) and cowpea seeds (GenBank Accession No. Z21954), were used in PCRs using EPACE-1 leaf cDNA, as a template (cDNA Synthesis Kit, Amersham). The 160-bp amplified cDNA fragment (*Vup1*) was purified (Wizard PCR Prep, Promega), cloned in the pCR II plasmid (T/A cloning kit, Invitrogen) and used to screen a cDNA library prepared from EPACE-1 leaves [23]. A positive clone (*VuC1*) was isolated and sequenced on both strands (ESGS, France). Sequence analysis was performed using Internet site programs detailed in Section 3.

2.3. Purification of a recombinant cowpea cystatin

The *VuC1* leaf cDNA was cloned in a bacterial expression vector (QE-30; Qia-Expressionist, Qiagen) and used to transform *Escherichia coli* cells (strain M15; Qiagen). Transformed cells were cultivated in LB medium at 37 °C and pelleted at $4000 \times g$ for 20 min. The recombinant protein in the supernatant was purified by chromatography on Ni–NTA affinity columns (Qiagen) and denoted ''cystavin''.

2.4. Extraction and determination of V. unguiculata leaf soluble protein

V. unguiculata leaf soluble proteins were extracted from frozen material in extraction buffer (60 mM Tris–HCl, pH 6.8; 0.5 mM DTT; 0.5 mM PMSF; insoluble PVP 120 mg g^{-1} leaf fresh weight). Homogenates were centrifuged at $1100 \times g$ for 20 min. Primary supernatants were further centrifuged at $48\,400 \times g$ for 40 min. Secondary supernatants represented the soluble extracts. Protein concentration was measured according to Bradford [24] using a protein assay kit (BioRad, Richmond, USA) and bovine serum albumin as a standard.

2.5. Protein separation

SDS–PAGE separation of leaf soluble proteins was carried out using a Phast-System apparatus (Amersham Pharmacia Biotech, Sweden) and 4–15% polyacrylamide gradient gels, according to [25].

2.6. Production of polyclonal antibodies against a recombinant cowpea cystatin and Western blot analysis

150 μg of the purified recombinant ''cystavin'' was injected into rats to raise anti-cystatin polyclonal antibodies (Technofarm; France). Their immuno specificity was tested in dot-blot experiments.

For Western blot analysis, SDS–PAGE separated leaf soluble proteins (1 μg) were transferred onto nitrocellulose membranes (ECL, Amersham Pharmacia Biotech) in transfer buffer (25 mM Tris–HCl, pH 8.4; 192 mM glycine and 20% ethanol v/v). Cystatin-like polypeptides were detected using the ECL immunodectection kit (Amersham Pharmacia Biotech) with the primary anti-cystatin serum (1:400) in Tween-20 Tris-buffer saline.

2.7. Recombinant cystatin blocking activity assay

Enzymatic activity of recombinant *V. unguiculata* cystavin was measured as its inhibitory capacity on papain (EC 3.4.22.2) activity, with azocasein as a substrate. Azocasein is a combination of casein with an azo-residue through a peptidic bond. It can be broken down by cysteine proteinases, such as papain. The reaction leads to the removal of the azo-residue in the solution and to an increase of the absorbance read at 340 nm ($A_{(340\,nm)}$).

Cystavin blocking assays (500 μL) included 0.25 μg μL^{-1} papain (Sigma), 1.2 μg μL^{-1} purified recombinant cystavin (absent in control assays) and 1% azocazein (Sigma) w/v in buffer (0.5 M Tris–HCl, pH 6.0 and 25 mM β-mercaptoethanol). The mixtures were incubated at 37 °C for 15 min and the reactions were stopped by addition of TCA (10%; w/v) at 5 °C for 30 min. After centrifugation at $2000 \times g$ for 5 min, supernatant absorbance was read at 340 nm. Variations in $A_{(340\,nm)}$ between the control and non-control assay mixtures were considered as evidence for the inhibitory capacity of the recombinant cystatin on the cysteine proteinase activity of papain.

2.8. mRNA extraction and Northern blot analysis

Poly(A)$^+$ RNA were prepared from the leaves of EPACE-1 and IT83, as previously described [26]. For each plant treatment, 3 μg of poly(A)$^+$ RNA were separated on 1% agarose formaldehyde gels, transferred onto nylon membranes (Hybond-N, Amersham Pharmacia Biotech) and hybridized with the ^{32}P-labeled *Vup1* cDNA probe (Prime-a-gene® Labeling System; Promega), as previously described [23]. RNA loading was checked using a cDNA fragment (*nt*S19) coding for the S19 ribosomal protein from *Nicotiana tabacum* [26,27].

3. Results

3.1. Isolation and sequence analysis of a cDNA encoding a putative multicystatin from cowpea (Vigna unguiculata (L.) Walp.) leaves

A 160 bp-long cystatin-specific cDNA fragment (*Vup1*) was PCR-amplified (Fig. 1) and used as a probe to screen a cDNA library constructed from EPACE-1 leaves [23]. An 857 bp-long cDNA clone was isolated, sequenced and referenced as *VuC1*.

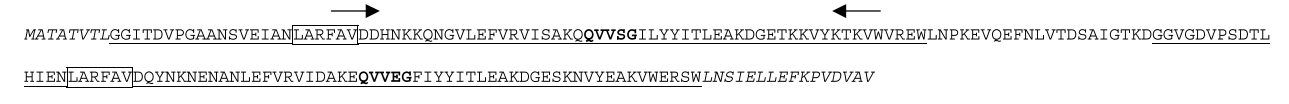

Fig. 1. Amino-acid sequence deduced from the *VuC1* cDNA isolated from a cowpea leaf cDNA library. The two cystatin-like domains (D1 and D2) in VUC1 are underlined. The amino acid residues essential to the activity of cystatins are in bold. The plant specific sequences are boxed. The N-terminal extension with the putative translation initiation codon and the C-terminal extension are in italics. The interdomain connection region is in black. The two arrows indicate the locations of the degenerate primers used to amplify the *Vup1* cDNA fragment used as a probe to screen the library.

In *VuC1*, the longest open reading frame was 588 bp-long, spanning from a putative ATG start-point at position 76 to a TGA stop codon at position 661. The ORF was flanked by a 75 nucleotide-long 5′-untranslated region (UTR) and by a 194 nucleotide-long 3′-UTR. The latter included the highly conserved AAUAAA polyadenylation signal at position 804, as well as the poly(A) tail.

The ORF encoded a predicted polypeptide with 195 amino acid residues and calculated M_r and p*I* of 21 783 and 5.1, respectively. Analysis of the deduced *VuC1* amino acid sequence (http://hits.isb-sib.ch/cgi-bin/PFSCAN_parser) showed the presence of two cystatin-like domains, denoted domains 1 and 2 (Fig. 1). Each included cystatin-specific motifs: G-1, Q-V-V-S/E-G (residues 45–49) and W-76 in VUC1 domain 1 numbering. In addition, each domain included the plant cystatin-specific sequence (PSS), L-A-R-F-A-V-D-[D/Q]-[H/Y]-N, located in the N-terminal region. Another motif identified (http://www.infobiogen.fr/services/analyseq/cgi-bin/signpt) was a putative myristoylation site G-T-K-D-G-G (residues −4 to +2 in VUC1 domain 1 numbering). There was no evidence for the presence of a secretory pre-peptide at the N-terminus of the deduced sequence. At the protein level, the two cystatin-like domains shared 66% identity, over the 76 and 74 residues within them. When compared with other cystatin domains, VUC1 domains 1 and 2 showed 50–68% and 43–58% identity, respectively, with sequences from other plant species. However, identity between VUC1 domains 1 and 2 and the equivalent region in a human stefin-like protein [28] was 18% and 19%, respectively.

Additional sequences to the cystatin-like domains included: (1) an eight-amino acid residue extension at the N-terminus with the putative translation initiation codon; (2) a 16-amino acid residue extension at the C-terminus and (3) a 21-amino acid residue interdomain connection. The latter shared 25–42% identity with the seven and the two interdomain connection regions found in the potato [5] and sunflower multicystatins (GenPept Accession no. BAA95416), respectively.

3.2. Accumulation of cystatin-like transcripts in response to drought-stress, dessication and ABA treatment, in cowpea leaves

Using Northern techniques and cDNA fragment *Vup1* as a probe, *VuC1*-like transcript accumulation was studied in the leaves of EPACE-1 and IT83 plants submitted to drought-stress and rehydration, ABA treatment and dessication. In all the controls, no cystatin-like signal was detected (Fig. 2). In response to the various stress treatments, two signals were observed, at approximately 0.9- and 1.3-kb (Fig. 2). Accumulation of the large transcript always was stronger than that of the short one. In response to drought-stress, cystatin-like transcripts accumulated differently in EPACE-1 and IT83 plants. In EPACE-1, expression levels of the large transcript remained unchanged in S1–S3 plants, whereas the short transcript gradually accumulated between S1 and S3. In IT83 plants, signals for both transcripts were weak at S1, maximum at S2 and reduced to S1 levels in S3 plants. For both cultivars, re-watering induced a drastic decrease in cystatin-like transcript accumulation (Fig. 2).

In response to the other two stress treatments (dessication and application of exogenous ABA), cystatin-like transcript accumulation was less than in response to drought-stress. Both transcripts were present, mainly in dessicated IT83 plants (Fig. 2).

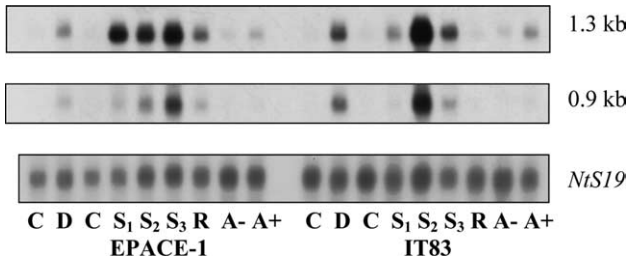

Fig. 2. Expression analysis of *VuC1*-like transcripts in the leaves of *Vigna unguiculata* (L.) Walp., cultivar EPACE-1 (drought-tolerant) and IT83 (drought-sensitive). Top and middle, Northern blot using the *Vup1* PCR product as a probe. C (control plants; $\psi_w = -0.3$ MPa); S1 (mildly stressed plants; $\psi_w = -1.0$ MPa); S2 (moderately stressed plants; $\psi_w = -1.5$ MPa); S3 (severely stressed plants; $\psi_w = -2.0$ MPa); R (plants rehydrated for 24 h; $\psi_w = 0.3$ MPa); D (excised leaves air-dessicated for 5 h); A (detached leaves in Tris buffer for 24 h with 0.1 mM ABA (A+) or Tris buffer (A−)). Bottom, Total RNA hybridization with *nt* S19, a cDNA fragment coding for *Nicotiana tabacum* S19 ribosomal protein.

3.3. Purification and in vitro activity of a recombinant cowpea cystatin

A recombinant protein, corresponding to *VuC1* cDNA, was overexpressed in *Escherichia coli* cells and was denoted "cystavin". It was purified to apparent homogeneity by affinity chromatography, as shown by the single band detected in Coomassie Blue stained polyacrylamide gels (Fig. 3A). The apparent M_r of the corresponding polypeptide was approximately 26 kDa. Activity of the recombinant cystavin was assayed in vitro by testing its blocking capacity on the proteolytic activity of papain, a cysteine proteinase. Hydrolysis of azocasein, a chromogenic substrate, by papain led to an increase in $A_{(340\,nm)}$ (Table 1). In the presence of recombinant cystavin, $A_{(340\,nm)}$ values were equivalent to those corresponding to intact azocasein, suggesting a total inhibition of papain activity (Table 1).

3.4. Immunodetection of cystatin-like polypeptides in the leaves of drought-stressed cowpea plants

The immuno-specificity of rat polyclonal antibodies raised against recombinant cystavin was tested on purified cystavin, as well as on control and S3 leaf protein extracts. Signal strength confirmed the anti-cystavin specificity of the rat antibodies (Fig. 3B). They were used to study cystatin accumulation in the leaves of control, S_1, S_2 and S_3 plants from cultivars EPACE-1 and IT83 (Fig. 3C). No cystatin was detected in control plants. Conversely, two cystatin-like signals were detected at approximately 25 kDa and 39 kDa, in response to drought-stress. Accumulation of the largest polypeptide always was more intense than that of the shortest. For both polypeptides, accumulation increased with the intensity of drought-stress. Compared to IT83, EPACE-1 accumulated lesser amounts of both polypeptides, at S1 and S2. However, cystatin contents were equivalent in both cultivars, at S3 (Fig. 3C).

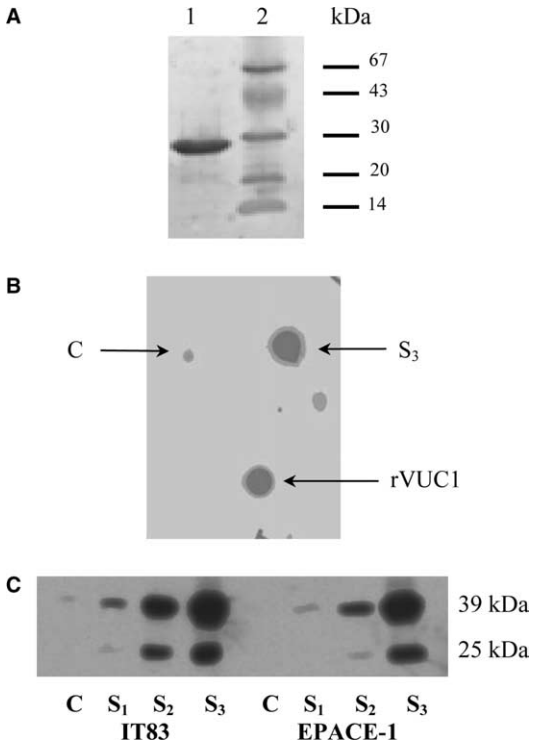

Fig. 3. Protein analysis. (A) SDS–PAGE on the eluate from a Ni–NTA affinity chromatography column. The column was loaded with total *Escherichia coli* cell extracts obtained from bacterial cells over-expressing the *VuC1* cDNA, in an inducible system. Proteins were visualized with Coomassie blue. Lane 1, column eluate; lane 2, standards. Molecular mass (kDa) is indicated on the right; (B) Analysis of the immunospecificity of rat polyclonal antibodies raised against recombinant *V. unguiculata* cystatin (rVUC1). Dot blot experiments were performed on the recombinant cystatin (16 ng) and on cowpea leaf extracts (2 μg) obtained from control (C) and S3 plants (S3) of *V.u.* cultivar IT83. The immunoblot was developed with antiserum raised against the recombinant cystatin (dilution 1:400); (C) Effects of controlled drought-stress on the expression of cystatin-like polypeptides in the leaves of *V.u.* cvs EPACE-1 (drought-tolerant) and IT83 (drought-sensitive) plants. C, S1, S2 and S3 as for Fig. 2. The amount of total leaf soluble proteins loaded in each well was 1.4 μg. The immunoblot was developed with antiserum raised against the recombinant cystatin (dilution 1:400).

Table 1
Effects of papain and recombinant VUC1 cystatin (rVUC1) on the hydrolysis of azocasein, as measured by the variation of absorbance read at 340 nm ($A_{(340\ nm)}$)

Papain	rVUC1	Azocasein	$A_{340\ nm}$
−	−	+	0.338 ± 0.027
−	+	+	0.336 ± 0.019
+	−	+	0.554 ± 0.038
+	+	+	0.343 ± 0.012

Experimental conditions were as described in Section 2.

4. Discussion

An 857-bp cDNA encoding a putative cystatin was isolated from a previously constructed cowpea leaf cDNA library [23] and referenced as *VuC1*. The nucleotide sequence of the isolated cDNA included an 588-bp ORF with an in-frame ATG codon at position 76. The context sequences surrounding this putative translation start perfectly matched the consensus sequence identified in dicotyledonous plants, i.e., A at positions −2 and −3, G and C at positions +4 and +5, respectively [29]. The ORF terminated with a TGA stop codon. Down-stream from it, a polyadenylation signal and a poly(A) tail were also identified. Together, these elements suggested that the *VuC1* cDNA was full-length. The ORF of *VuC1* encoded a deduced protein of 195 amino acid residues with a calculated molecular mass of approximately 22 kDa. Analysis of VUC1 amino acid sequence revealed the presence two cystatin-like domains (referenced as domains 1 and 2), whereas plant cystatins typically are smaller proteins (12–16 kDa) with single cystatin-like domains [4]. In plants, protein or nucleotide sequences corresponding to single-domain cystatins have previously been identified from leaves [30,18], flowers [31] and seeds [32–35]. In cowpea, a single-domain cystatin has been identified and characterized in the seeds [36]. However, multicystatins also have been characterized: from potato tuber [37,5] including eight cystatin-like domains, from tomato [38] and sunflower seeds [39], including eight and three cystatin-like domains, respectively.

In VUC1, recognition of domains 1 and 2 as cystatin-like domains was based on the identification of the cystatin signature: QxVxG [40]. In addition, all the amino acid residues involved in the formation of the tripartite wedge-shaped structure responsible for the inhibition of cysteine proteinase activity were present in each domain [41]. Moreover, both domains also included the PSS sequence, located in the N-terminal region, as typically found in phytocystatins [4]. Overall, domains 1 and 2 shared high homology with each other (66% identity) and with other plant mono-cystatin domains (43–58% identity when compared with ten sequences). The length and the sequence of the interdomain connection region also appeared to be well preserved between VUC1 and the three multicystatins sequenced to date. Within these regions, one conserved lysine residue could account for a susceptibility to cleavage by trypsin, as was established for the potato multi-cystatin [5]. In cowpea leaves, Western blot analysis using antibodies raised against recombinant VUC1 (cystavin) led to the detection of a polypeptide with a molecular mass corresponding to that of the deduced VUC1 protein. This indicated that the *VuC1* gene was expressed and that cowpea leaf double-cystatin was not subjected to post-translational cleavage. All three multicystatins identified to date have been shown to accumulate as multimers [5,38,39]. In VUC1, the presence of all the amino acid residues susceptible to interact directly to the active site clefts of the cysteine proteases of the papain family [40] suggested that it was active. This was confirmed by activity tests showing that recombinant VUC1 (cystavin) efficiently inhibited papain activity in vitro, on azocasein as a substrate. Together, these results suggested that the double VUC1 cystatin could be active in the leaf tissues of cowpea plants, as was the case for the tomato multicystatin characterized as a potent inhibitor of papain in the leaf tissues [39].

To elucidate the role of cowpea leaf cystatin in response to abiotic stresses, *V. unguiculata* plants of two different origins (EPACE-1, drought-tolerant and IT83, drought-sensitive) were submitted to controlled drought-stress, dessication and exogenous ABA. Expression of *VuC1*-like genes was studied at the transcriptional and transductional levels, using Northern blot and Western blot analysis. Results revealed the presence of two transcripts translated into two polypeptides, in the leaves of stressed plants. The shortest signals, also the weakest,

corresponded in size to the *VuC1* cDNA and its deduced polypeptide (the two-domain cystatin VUC1). Identity of the longest signals was not determined in this study. However, one hypothesis was that they corresponded to a three-domain cystatin, since the apparent molecular mass of the largest VUC1-like protein matched that of the sunflower seed multicystatin including three domains [39]. In cowpea seeds, a multiplicity of minor cystatin-like polypeptides with M_r ranging between 13 600 and 39 000 has been identified in addition to the major cystatin-like polypeptides at 25 kDa [15]. The authors concluded that this multiplicity of forms was related to a multiplicity of biological roles [15], as was also the case in rice [32]. Consistent with this, our results allowed us to hypothesize that the two polypeptides identified in the cowpea leaf tissues were the cystatin isoforms specifically involved in the responses to drought-stress.

Accumulation of both VuC1-like transcripts occurred mostly in response to controlled drought and dessication. Application of exogenous ABA did not induce high levels of VuC1-like gene expression. In severely drought-stressed plants, a clear difference in the accumulation patterns of the two *VuC1*-like messages was observed between the two cultivars. The abrupt decrease in *VuC1*-like transcripts in severely dehydrated leaves of the sensitive cultivar probably resulted from a complete loss in cell homeostasis. At the same dehydration level, leaf cells in the tolerant cultivar were able to express *VuC1* genes, suggesting that they still were physiologically functional. Similar differences between these two cultivars were observed with regards to a patatin-like gene encoding a galactolipid hydrolase [27]. The differences between the cultivars observed at the genetic level were not consistent at the protein level, since both polypeptides accumulated progressively with increasing stress intensity. This phenomenon could be explained by transcriptional and/or post-transcriptional regulation mechanisms.

Previous studies on cowpea leaf responses to drought-stress have shown that the protein content decreased in these tissues and particularly in the chloroplastic compartment, as a result of both a reduction in protein synthesis and a stimulation of several endoproteasic activities [42,21]. Amongst these, cysteine proteases may be involved, since accumulation of cysteine proteinase messages has been observed in the leaves of tomato plants submitted to drought-stress [43] and in *Arabidopsis* submitted to dehydration and wilting [44,45]. Furthermore, in tomato, the corresponding protein was found mainly in the nuclei and the chloroplasts of the stressed leaf cells [43]. To control protein degradation susceptible to result from such a response, involvement of cysteine proteinase-specific inhibitors, i.e., cystatins, is a possibility. In agreement with this hypothesis, our results indicated for the first time that two cowpea cultivars (a tolerant and a sensitive one) developed an inhibition-based blocking (or control) strategy towards cystein proteinase activities, in response to drought-stress. As compared with the sensitive cultivar, the tolerant one managed to carry out this strategy further, in terms of water deficit intensity. Together with previous results obtained from cystatin expression studies during germination of cowpea seeds, it appears that control of protein degradation through the use of protease-specific inhibitors is common to metabolic processes and to adaptive ones, including adaptation to drought-stress in this crop. To better elucidate the role of cowpea leaf cystatins in vivo, studies are now in progress to generate transgenic plants over- and under-expressing these proteins.

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