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An elicitor- and pathogen-induced cDNA from potato encodes a stress-responsive cyclophilin

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Abstract

Differential mRNA display was used to identify pathogen-responsive, stress-related genes in potato cell suspensions treated with salicylic acid and a cell wall-derived elicitor from *Phytophthora infestans*. Among the positive clones identified, one was found to be expressed at a significantly higher level in elicited cells than in control cells. DNA sequencing of this amplicon revealed high homology and identified it as a potato cyclophilin cDNA. The maximum amount of the cyclophilin mRNA was found 9 to 12 h after elicitation. Cyclophilin (CyP) mRNA synthesis was also up-regulated from 12 to 24 h in potato leaves locally infected with zoospores from *Phytophthora infestans*. However, untreated leaves responding systemically to the pathogen showed only a weak, delayed response at 24 h post infection. The observed accumulation of potato CyP mRNA in response to salicylic acid, *P. infestans* elicitor and *P. infestans* infection, suggest that CyPs play an important role in plant stress responses.

Additional key words: chaperone, *Phytophthora infestans*, *Solanum tuberosum*, stress response.

Introduction

Plant cyclophilins (CyPs), part of the immunophilin family, are 18 kDa proteins with peptidyl-prolyl cis-trans isomerase (PPI) activity that catalyzes rotation of X-Pro peptide bonds. These enzymes (E.C. 5.2.1.8) are also known as rotamases and facilitate the *in vivo* and *in vitro* folding of proteins, normally a rate-limiting step in biosynthesis (Marivet *et al.* 1994).

A high degree of conservation is found in CyP primary sequences of distantly related organisms that is indicative of strong selective pressure for maintenance of the structure of these proteins during evolution and suggests an important cellular function for cyclophilins. In addition to their chaperone role in facilitating *de novo* folding of target proteins, cyclophilins have also been postulated to act as cellular receptors, play in role in protein transport across membranes and intracellular trafficking, triggering of membrane channels, the formation of multisubunit protein complexes (Küllertz *et al.* 1999), mRNA processing, protein degradation and signal transduction (Romano *et al.* 2004).

cDNAs for cytosolic CyPs have been isolated from various higher plants, *e.g.* maize, tomato, *Brassica napus* (Gasser *et al.* 1990), *Arabidopsis thaliana* (Chou and Gasser 1997, He *et al.* 2004, Romano *et al.* 2004) and *Phaseolus vulgaris* (Marivet *et al.* 1992). Cytosolic CyPs have been found in all organs investigated as well as organelle-specific CyPs in mitochondria and chloroplasts (Breiman *et al.* 1992, Luan *et al.* 1994, Schubert *et al.* 2002). CyPs are encoded by a small gene family in rice (Buchholz *et al.* 1994); in contrast the *Arabidopsis* CyP protein family comprises 29 members (Chou and Gasser 1997, He *et al.* 2004, Romano *et al.* 2004).

CyPs are ubiquitous and constitutively expressed. However, they are also stress-responsive proteins, and up-regulated gene expression have been reported in response to abiotic stresses such as heat, cold, salt, wounding; as well as virus infection and during chemically induced defense (Marivet *et al.* 1992, 1994,

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Abbreviations: CyP - cyclophilin; DDRT-PCR - differential display reverse transcription polymerase chain reaction; PPI - peptidyl-prolyl cis-trans isomerase.

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Luan *et al.* 1994, Droual *et al.* 1997). The identification of cyclophilin genes, the determination of their expression through transcriptomic analysis and investigations into their biochemical functions are now at the center of plant cyclophilin research.

In order to identify genes involved in the response of potato to late blight disease, a differential display analysis of induced gene expression in response to a pathogen-derived elicitor was conducted. In this communication we report on the up-regulation of CyP gene expression in potato cell suspensions and intact potato leaves that is responsive to treatment with salicylic acid, cell wall-derived elicitors and the pathogen, *Phytophthora infestans.*

Materials and methods

Plants, callus and cell suspensions: *Solanum tuberosum* L. cv. BP1, susceptible to late blight disease, was obtained from the ARC-VOPI, South Africa, and grown under a 16-h photoperiod. Callus was initiated from sprouts on agar medium containing Murashige and Skoog (MS) salts and vitamins plus 2 mg cm^{-3} 2,4-dichlorophenoxy acetic acid (2,4-D) and 0.5 mg cm^{-3} kinetin, pH 5.8 (Murashige and Skoog 1962). Friable calli was placed in liquid MS medium containing the same composition plus 2 g dm^{-3} casein hydrolysate and subcultured every 7 d.

Pathogen, zoospores and elicitor: Cultures of *Phytophthora infestans*, A1 mating type (McLeod *et al.* 2001), was obtained from ARC-VOPI, South Africa, and cultivated in liquid malt extract medium. The *P. infestans* elicitor was prepared from the heat-released fraction of the mycelial cell walls (Ayers *et al.* 1976). Zoospores were harvested from mycelial cultures grown on oatmeal agar at 4 °C with sterile distilled water, vortexed and counted in a counting chamber. The spores were collected by centrifugation at 1500 *g* and suspended in sterile distilled water to a concentration of 1×10^6 spores cm^{-3}.

Induction of defense response: Cell suspensions were treated with 300 μM salicylic acid or *P. infestans* elicitor to a final concentration of 50 μg cm^{-3} three days after subculture. Cells were harvested by centrifugation and filtration at 0, 6, 9, 12 and 24 h following elicitation and used for preparation of RNA. Potato plants (± 20 cm height) were spray inoculated on two lower leaves with the *P. infestans* zoospore suspensions. Control plants were treated with distilled water. Following infection, the plants were placed in plastic chambers to ensure high humidity. Leaf tissue from the treated leaves and upper non-treated leaves were harvested at various time intervals and used for RNA isolation.

RNA extraction and analysis: RNA from treated and control cell suspension was isolated with the *FastPrep*™ *PlantGreen* system (*Q-Biogene*, Cambridge, UK). RNA was quantified spectrophotometrically (*Shimadzu UV-160A*, Tokyo, Japan) at A_{260} and A_{280} as well as fluorometrically (*Fluoroskan Ascent*, *Labsystems*, Helsinki, Finland) using *RiboGreen*™ (*Molecular Probes*, Eugene, OR, USA). RNA quality was verified in a 1.2 % denaturing agarose gel (m/v) in 1× MOPS/EDTA buffer (0.02 M 3-[N-morpholino] propanesulphonic acid, 5 mM EDTA, pH 7.0).

Differential display analysis: Synthesis of cDNA was performed with the *RNAimage*® kit (*GenHunter Corporation*, Nashville, TN, USA) on Dnase I treated total RNA. A one-base anchor oligo-dT primer (5'-$AAGCT_{11}$M-3', where M = A/G/C) was used in combination with the arbitrary primer 5'-$AAGCCT_3GCAC$-3'. The cycling conditions consisted of pre-denaturing at 94 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, 40 °C for 90 s and 72 °C for 60 s (increasing in increments of 20 s per cycle to 540 s). The ^{35}S-labelled PCR amplicons were visualised with 6 % denaturing polyacrylamide electrophoresis for 1.5 h at 45 W at 52 °C. The gel was fixed, washed and exposed to a *BioMAX MR* film (*Kodak,* New York, USA). Bands on autoradiographs were visually analysed and compared between control, salicylic acid- and elicitor-treated cell suspension cultures. Bands were cut from the dried gel, eluted in 0.1 cm^{-3} dH_2O, and used as templates for PCR reamplification. Cycling conditions used were the same as for differential display PCR. The successful reamplification of the excised cDNA was verified in a 1.5 % (m/v) agarose gel in 1× TBE (0.09 M Tris base, 0.09 M boric acid, 2 mM EDTA, pH 8) for 1 h at 10 V cm^{-1} and the size of the cDNA band determined by comparison to a 100 bp ladder (*Promega*, Madison, USA). Ethidium bromide-free cDNA was recovered from stained agarose gels with *GenElute*™ *Minus EtBr* spin columns (*Sigma*, St. Louis, MO, USA) and the reamplified DNA assessed for quality on agarose gels. cDNA was quantified using fluorometry (bizbenzimide H33258 fluorochrome), according to the manufacturer's instructions (*Polysciences Inc.*, Warrington, PA, USA).

Cloning and sequencing of differentially displayed PCR amplicons: The PCR products were ligated to the T-tailed *pGem-Easy* vector and used to transform JM 109 competent cells (*Promega*). For verification of the presence of inserts, colony PCR was performed with T7 and SP6 primers and the products analysed on a 1.5 % (m/v) agarose gel. Positive clones were grown overnight

at 37 °C in Luria Bertani (LB)-broth (Ausubel *et al.* 1995) with 100 μg cm^{-3} ampicillin. Sequencing was performed according to *DYEnamic ET* dye terminator cycle sequencing kit for *MegaBACE* in a *GeneAmp PCR System 9 700* (*Perkin Elmer*, *Applied Biosystems*, Foster City, CA, USA).

Database searches were carried out using *Blastn* and *Blastx* search algorithms provided by the National Centre of Biotechnology Information (NCBI) at National Institutes of Health (NIH) using basic local alignment search tool (*BLAST*) (http://www.ncbi.nlm.nih.gov/BLAST/).

RNA blots: PCR-amplified cDNA inserts (500 ng) were denatured and blotted onto *MagnaCharge* nylon membranes (*Osmonics*, Westborough, MA, USA), washed and immobilized by baking at 80 °C for 1 h under vacuum. The cDNA probes were synthesised from 10 μg of each of the total RNA samples from untreated cell suspension cultures (control RNA) and elicitor treated cell suspension cultures (9 h) by reverse transcription and labelling in a reaction containing 1.8 MBq [α-^{32}P] dCTP and dATP (111 TBq $mmol^{-1}$, *Amersham BioSciences*, Chalfont, UK). Unincorporated dNTPs were removed with the QIAquick PCR purification kit (*Qiagen*, Hilden, Germany). Probes were denatured at 95 °C for 5 - 10 min and placed on ice, until added. Membranes were hybridised in Rapid-hyb (*Amersham BioSciences*) buffer. Membranes were autoradiographed at -80 °C with *BioMAX MS* film (*Kodak*) for 2 d. Northern analysis was performed with the *North2South® Direct HRP* labelling and detection kit (Pierce, Rockford, IL, USA). The CyP amplicon obtained from the RT-PCR reactions was used as a probe. Equal amounts of total RNA (5 μg), isolated from local and systemic leaves were separated on a formaldehyde RNA gels and blotted to a charged nylon membrane using capillary transfer with 20× SSC (3 M NaCl, 0.3 M trisodium citrate, pH 7.0). Hybridisation with 10 ng cm^{-3} of labelled probe at 55 °C for 4 h, stringency washes and chemiluminescence detection was according to the prescribed protocol. The membrane was exposed to X-ray film (*AGFA CP-BU*).

Results and discussion

The interaction between potato and *Phytophthora infestans*, is a well established model of plant microbe interactions (Kombrink *et al.* 1996, Birch *et al.* 1999). A differentially expressed cDNA of 525 bp was initially identified in a differential display analysis of potato cell suspensions treated independently with 50 μg cm^{-3} elicitor and 300 μM salicylic acid (Fig. 1). Up-regulation in response to a pathogen-derived elicitor and salicylic acid, an endogenous transducer/activator of defence responses, was indicative of a possible role in plant defence. In order to verify that the cDNA amplicon truly represents a differentially expressed mRNA, a reverse Northern dot blot screening was performed (results not shown).

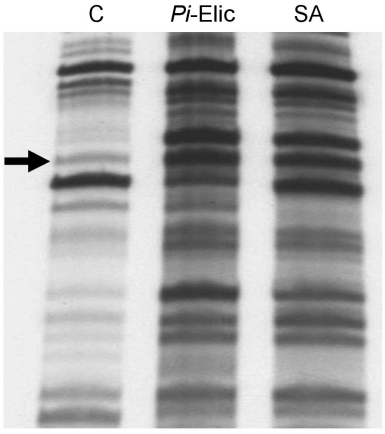

Fig. 1. DDRT-PCR analysis of differentially expressed mRNAs in control and elicitor treated *S. tuberosum* cell suspensions. C - control cells, *Pi*-Elic - cells treated with 50 μg cm^{-3} *P. infestans* elicitor for 9 h, SA - cells treated with 300 μM salicylic acid for 9 h. RT-PCR was performed with isolated total RNA using the 5'-AAGCCT$_3$GCAC-3' arbitrary primer in combination with the anchor primer H-T$_{11}$A/G/C.

Sequence analysis of the positive, differentially expressed cDNA clone and subsequent *in silico* homology searches identified the gene as a *Solanum tuberosum* cyclophilin. Several cyclophilin sequences from *S. tuberosum* are deposited in Genbank (BI 176350, AJ235744, AF126551, BF153634, but only the full coding sequence of the cv. Spunta (AF126551, Godoy *et al.* 2000) and *S. commersonii* (U92087, Meza-Zepeda *et al.* 1998) is known. The EST sequence from this study (GenBank: AY972080; cv. BP1), starting 150 bp down-stream from the ATG translational start site, was near identical to that reported for the cv. Spunta over the coding sequence but nucleotide substitutions and deletions were found in the 3'non-coding sequence (Fig. 2). Following identification of the induced transcript, differential display was repeated on RNA prepared from cell suspensions at different time intervals (0, 6, 9, 12 and 24 h post elicitation) in order to evaluate the induction kinetics of the gene (Fig. 3). The results indicate that the transcripts are maximally induced at 9 h after exposure to the elicitor and that the levels stay high until 24 h, the last time point investigated.

In order to extrapolate the results obtained with the elicitor and cell suspension interaction, leaves from intact potato plants were spray inoculated with zoospores from *P. infestans*. Total RNA was isolated from local treated

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AY972080  1    *                                                                    0
AF126551  1   ATGGCAAATCCAAGGGTTTTCTTTGACCTTACCATCGGCGGTGCACCAGCTGGCCGCGTGGTGATGGAGCTCTTCGCC 78
BF153634  1                                                ACCAGCCGGCCGTGTGGTTATGGAGCTCTTCGCC 34
BI176350  1                                      GGCGGTGCACCAGCTGGCCGCGTGGTGATGGAGCTCTCCGCC 42

AY972080  1                                                                    AAGCCT 6
AF126551  79  GATACCACTCCCAAAACCGCTGAGAACTTCCGAGCTCTCTGTACCGGTGAAAAAGGTGTTGGAAAGATGGGTAAGCCT 156
BF153634  35  GATACCACTCCCAAGACCGCTGAGAACTTCCGAGCTCTCTGTACCGGTGAAAAAGGTATTGGAAAGATGGGTAAGCCT 112
BI176350  43  GATACCACTCCCAAAACCGCTGAGAACTTCCGAGCTCTCTGTACCGGTGAAAAAGGTGTTGGAAAGATGGGTAAGCCT 120

AY972080  7   TTGCACTACAAGGGCTCAACCTTCCACCGTGTGATCCCAGGGTTCATGTGTCAAGGAGGTGATTTCACCGCCGGAAAC 84
AF126551  157 TTGCACTACAAGGGCTCAACCTTCCACCGTGTGATCCCAGGGTTCATGTGTCAAGGAGGTGATTTCACCGCCGGAAAC 234
BF153634  113 TTGCACTACAAGGGCTCAACCTTCCACCGTGTGATTCCAGGGTTCATGTGTCAAGGAGGTGATTTCACCGCCGGAAAC 190
BI176350  121 TTGCACTACAAGGGCTCAACCTTCCACCGTGTGATCCCAGGGTTCATGTGTCAAGGAGGTGATTTCACCGCCGGAAAC 198

AY972080  85  GGTACCGGAGGAGAGTCGATCTACGGCGCCAAATTCAAGGACGAGAACTTCGTGAAGAAGCACACCGGCACTGGAATT 162
AF126551  235 GGTACCGGAGGAGAGTCGATCTACGGCGCCAAATTCAAGGACGAGAACTTCGTGAAGAAGCACACCGGCACTGGAATT 312
BF153634  191 GGTACCGGAGGGGAGTTGATCTACGGAGCCAAATTCAAGGACGAGAACTTCGTCAAGAAGCACACCGGCGCTGGAATT 268
BI176350  199 GGTACCGGAGGAGAGTCGATCTACGGTGCCAAATTCAAGGATGAGAACTTCGTCAAGAAGCACACCGGCGCTGGAATT 276

AY972080  163 CTCTCCATGGCTAATGCTGGACCTGGAACCAACGGTTCTCAGTTCTTAAGCTGTACCGCTAAGACTGAATGGCTCGAT 240
AF126551  313 CTCTCCATGGCTAATGCTGGACCTGGAACCAACGGTTCTCAGTTCTTCATCTGTACCGCTAAGACTGAGTGGCTCGAT 390
BF153634  269 CTTTCCATGGCTAATGCTGGACCTGGAACCAACGGTTCTCAGTTCTTCATCTGTACCGCTAAGACTGAGTGGCTCGAT 346
BI176350  277 CTCTCCATGGCTAATGCTGGACCTGGAACCAACGGTTCTCAGTTCTTCATCTGTACCGCTAAGACTGAGTGGCTCGAT 354

AY972080  241 GGAAAGCACGTCGTGTTTGGACAAGTTGTTGAAGGCTTGGATGTGATTAAGAAGGCCGAGGCTGTTGGATCTAGTTCT 318
AF126551  391 GGAAAGCACGTCGTGTTTGGACAAGTTGTTGAAGGCTTGGATGTGATTAAGAAGGCCGAGGCTGTTGGATCTAGTTCT 468
BF153634  347 GGGAAGCACGTCGTGTTTGGACAAGTTGTTGAAGGCTTGGATGTGATTAAGAAGGCCGAGGCTGTTGGATCTAGCTCT 424
BI176350  355 GGGAAGCACGTCGTGTTTGGACAAGTTGTTGAAGGCTTGGATGTGATTAAGAAGGCCGAGGCTGTTGGATCTAGCTCT 432

AY972080  319 GGAAGGTGCTCCAAGCCTGTTGTGGTTGCTGACTGCGGTCAACTCTAGATCTGATGATGATGTTTTGAACGTTGATGA 396
AF126551  469 GGAAGGTGCTCCAAGCCTGTTGTGGTTGCTGACTGCGGTCAACTCTAGATCTGATGATGATGTTTTGAACGTTGATGA 546
BF153634  425 GGAAGGTGCTCCAAGCCTGTGGTGGTTGCTGACTGCGGTCAACTCTAGATCTGATGATGATGTTATAAACGTTGATGA 502
BI176350  433 GGAAGGTGCTCCAAGCCTGTGGTGGTTGCTGACTGCGGTCAACTCTAGATCTGATGATGATGTTATAAACGTTGATGA 510

AY972080  397 TGATCTAGTTTTTATCAGTCTTTTCTATATTTGAGTCGCCGTTTTAGGCTTTGTTTTTAATTTCAACCTATCTT--CT 472
AF126551  547 TGATCTAGTTTTTATCAGTCTTTTCTATATTTGAGTCGCCGTTTTAGGCTTTGTTTTTAATTTCAACCTATCTTTCCT 624
BF153634  503 TGATCTAGTTTTTATCAGTCTTTTCTATATTTGAGTCGCCGTTTTAGGCTTTGTTTTTAATTTCAACCTATCTT---T 577
BI176350  511 TGATCTAGTTTTTATCAGTCTTTTCTATATTTGAGTCGCCGTTTTAGGCTTTGTTTTTAATTTCAACCTATCTT---T 585

AY972080  473 GCTTGTGTCTTGTTGGGTCGG-TCGG-TCAAGT-CTAGG--TA--CGTAATTGGTGTGTG                  525
AF126551  625 GCTTGTGTCTTGTTGGGTCGGGTCGGGTCAAGTTCTAGGGTTAACCGTAATTGGTTGTTGTGTTGCTTCTACCGGTTT 702
BF153634  578 ACTTG---C---TTGGATCTGGTCGGGTCGAGTTCTAGGGTTAACCGTAATTGGTTGTGGTG                 633
BI176350  586 ACTTG---C---TTGGATCTGGTCCGGTCGAGTTCTAGGTTAAACCGTA-TTG                       631
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Fig. 2. Multiple sequence alignment of *Solanum tuberosum* cyclophilin genes. GenBank AY972080 cultivar BP1 this study; BI76350 cv. Kennebeck; BF153634 cv. Kuras and AF126551 cv. Spunta. Identical residues are indicated in *grey*, the ATG start codon is indicated with an *asterix* and the stop codons are *boxed*.

leaves and also non-treated, upper leaves to test for a possible systemic response. Northern analysis of RNA isolated at 0, 6, 9, 12 and 24 h post infection indicates the enhanced CyP gene expression in infected leaves with a time frame similar to that obtained with cultured cells (Fig. 4).

CyP is known to be a stress-responsive protein, with the intensity and time frame of the response depending on the plant species; *e.g*. during heat stress, CyP mRNA exhibited a prominent accumulation in maize plants, but was down-regulated in bean. Differences in the induction kinetics in response to different stresses are also observed, depending on the plant investigated (Marivet *et al.* 1994, Godoy *et al.* 2000). Upon infection of bean with alfalfa mosaic virus, systemic accumulation of CyP mRNA was

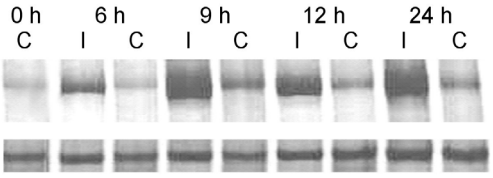

Fig. 3. Induction kinetics of *S. tuberosum* CyP cDNA derived from mRNA in control and elicitor treated *S. tuberosum* cell suspensions. *Top*: constitutive expression in control cells at 0 h is visible. Differences in CyP amplicons in water-treated cells (C) and cells treated with 50 μg cm^{-3} *P. infestans* elicitor for 6, 9, 12 and 24 h (I) are shown. *Bottom*: DDRT-PCR of a constitutive amplicon that is not responsive to *P. infestans* elicitor.

observed in noninfected leaves (Marivet *et al.* 1994). In addition, following localized chemical treatment with ethephon and salicylic acid, CyP mRNA accumulation was reported in untreated, aerial parts of the plants, but only at 72 and 96 h following treatment. Results from this study show a slow, weak systemic response in the upper, non-treated leaves, from 12 to 24 h, indicating that, in addition to pathogen infection, other signalling agents such as wounding or salicylic acid are needed for a strong systemic response (Fig. 4).

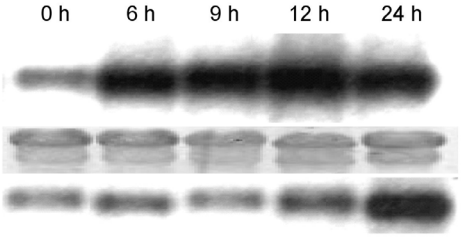

Fig. 4. Pathogen inducible expression of *S. tuberosum* CyP in local, treated leaves (*upper panel*). RNA was isolated from potato leaves sprayed with zoospores of *P. infestans* at the time points as indicated. mRNA accumulation reached a maximum at 12 h. Equal amounts of RNA was separated on agarose gels (rRNA, *middle panel*), blotted and hybridized with a labelled probe derived from the CyP cDNA clone. The expression pattern in non-treated systemic leaves (*lower panel*) only show a comparative slow, weak response.

Results obtained in this study differ from that reported for cyclophilin induction in potato tubers where *Fusarium eumartii* elicitors and salicylic acid had no effect on induction (Godoy *et al.* 2000). These differences may reflect the existence of different CyP genes which are differentially regulated under stress conditions in cells and tissues of the potato plant. As yet, no systematic investigation into the organization of potato cyclophilin genes and the characteristics of their promoters has been performed.

Cyclophilins, which catalyses protein folding and other transconformational reactions in the cell, might be needed in greater amounts to accelerate protein maturation during stress conditions. Alterations in gene expression are not only related to developmental conditions, but are also induced in response to endogenous signals in various stress responses (Marivet *et al.* 1992, 1995). In infected potato plants CyP might function as a chaperone-like molecule in order to decrease the risk of proteolytic degradation or to avoid aggregation, reactions that take place during the stress condition. In plants launching an active defense, higher amounts of CyP might also be needed to process mRNA (Romano *et al.* 2004), accelerate the protein folding steps and therefore the maturation process of newly synthesized pathogenesis related (PR)-proteins and others known to be induced during defense and believed to play protective functions. In this regard it is interesting that in potato cell suspension cultures CyP is induced by salicylic acid, a signalling molecule in the induction of defense responses, specifically those associated with infection.

Cyclophilins are thought to function as ATP-independent chaperone proteins, often in cooperation and association with heat shock protein (Hsp) 70, Hsp60 (Rassow *et al.* 1995) and Hsp90 (Hoffmann *et al.* 1995). Certain CyPs are heat shock responsive (Luan *et al.* 1994) and indeed, putative heat shock elements occur in the promoter region of the cyclophilin gene from maize (Marivet *et al.* 1995). The trigger event leading to enhanced *CyP* gene expression might be oxygen stress and reactive oxygen species (Navarette Santos *et al.* 2000), a condition also found during heat stress that leads to the induction of heat shock proteins (Vayssier and Polla 1998) and during pathogenic activity in plant tissues accompanied by the oxidative burst (Kombrink and Schmelzer 2001).

The obtained results on the accumulation of potato CyP mRNA in response to salicylic acid, *P. infestans* elicitor and *P. infestans* infection, together with multiple other known external stimuli, further suggest that CyPs play an important and general role, although not yet well defined, in plant stress responses.

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Within the last four decades plant *in vitro* cultures have developed from a laboratory curiosity to a real industry. Its use in horticulture, agriculture and forestry is currently expanding worldwide. New areas of plant biotechnology continually add new terms and definitions. Therefore this comprehensive (more than 1500 items) dictionary will be certainly welcome not only by scientists and university teachers but also by students and practitioners.

Entries, including abbreviations and acronyms, are arranged in alphabetical order. The book provides the reader clear and concise explanation of individual terms. Most of them include reference citations which point mainly to general textbooks in plant anatomy, biochemistry, histochemistry, developmental biology, genetics, microbiology, plant pathology and of course plant tissue culture. Some of items are accompanied by self-explanatory flow charts, tables and illustrations. Entries are cross-referenced within the text where appropriate.

It is possible to suppose that this user-friendly dictionary will be at hand on shelves of reference libraries in laboratories all over the world.

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