BIOLOGIA PLANTARUM 45 (3): 321-326, 2002

The expression of a cytosolic cyclophilin promoter from periwinkle in transgenic tobacco plants

A.-M. DROUAL*, J. CRECHE**, F. ANDREU**, J.-C. CHENIEUX**, M. RIDEAU**[1] and S. HAMDI***

*Cropdesign NV, Technologiepark 3, B-9052 Zwijnaarde, Belgium**
*Faculty of Pharmacy, EA2106, 31 Avenue Monge, F-37200 Tours, France***
*Faculty of Sciences, UMR 619, BCMDP, Avenue des Facultés, F-33405 Talence, France****

Abstract

The cloning of a 465 bp fragment from the 5'-flanking region of the gene encoding a cytosolic cyclophilin from periwinkle was achieved through inverse polymerase chain reaction. The DNA fragment was fused to a *gus*A-intron marker then introduced into tobacco by *Agrobacterium tumefaciens*-mediated transformation. Histochemical analysis of the transgenic shoot cultures demonstrated that the construct was able to drive GUS expression in stomata guard cells, but not in mesophyll cells when shoots were still attached to the callus from which they were initiated. In separated transgenic shoots and in seedlings, GUS was expressed in external and internal phloem and root hairs, respectively. GUS activity in transgenic tobacco seedlings was also investigated by fluorimetric assays. Treatments with NaCl or ABA decreased promoter activity whereas treatment with yeast extracts increased it.

Additional key words: *Catharanthus roseus*, GUS expression, promoter analysis, stress wounding.

Introduction

Cyclophilins (CyPs) are peptidyl-prolyl *cis-trans* isomerases found in all living organisms (Chou and Gasser 1997). Most of them are 18 - 23 kD proteins located in cytosol, endoplasmic reticulum, mitochondria, nuclei and (in plants) chloroplasts. In addition, high molecular mass proteins containing a CyP domain have been characterised in animals, fungi and plants (for example, Ferreira *et al.* 1996, Duina *et al.* 1996, Fulgosi *et al.* 1998).

Low molecular mass cytosolic CyPs form stable complexes with the polyprotein Gag of the human immunodeficiency virus-1 (Franke *et al.* 1994), the *Agrobacterium tumefaciens* protein VirD2 (Deng *et al.* 1998), and the mammalian transcription factor YY1 (Yang *et al.* 1995). They participate to the regulation of signal transduction in animal and plant cells (Luan *et al.* 1993, Jackson and Soll 1999, Jin *et al.* 2000) and may also enter the nucleus, bind to DNA, and then modify transcription processes, or damage the genome during apoptosis (Krummrei *et al.* 1995, Montague *et al.* 1997). In animals, cytosolic CyPs protect the cells against peroxide-induced death (Doyle *et al.* 1999) and their concentrations were found to increase after oxygen stress (Santos *et al.* 2001). In yeasts, enhanced accumulation of cytosolic CyPs is concomitant with enhanced sensitivity to heat (Sykes *et al.* 1993). In plants, *CyP* transcripts are frequently accumulated during biotic or abiotic stresses (Marivet *et al.* 1992, 1994, 1995, Droual *et al.* 1997, Kullertz *et al.* 1999, Godoy *et al.* 2000). However, the exact physiological functions of the cytosolic CyPs remain largely unknown.

We previously isolated a cDNA (here designated *CrCyP1*) corresponding to a gene encoding a cytosolic CyP in periwinkle (Clastre *et al.* 1995). We found changes in transcript accumulation in the whole plant during development and in cell cultures after treatments with various plant growth regulators or environmental

Received 25 June 2001, *accepted* 22 November 2001.
Abbreviations: ABA - abscisic acid; BAP - benzylaminopurine; *CrCyP1* - gene encoding the cytosolic cyclophilin; CrCyP1 - cyclophilin protein; *CyP* - cyclophilin gene; CyP - cyclophilin; GUS - β-glucuronidase; IPCR - inverse polymerase chain reaction; MS - Murashige and Skoog culture medium; NAA - α-naphtaleneacetic acid; RACE - rapid amplification of cDNA ends.
Acknowledgements: This work was supported by funds from the Ministère de l'Education Nationale, de la Recherche et de la Technologie, France. We wish to thank Prof. Marc Van Montagu for his generous gift of the pgiBin19 vector. The nucleotide sequence reported in this paper has been submitted to the GenBank, EMBL and DDBJ nucleotide sequence data bases under accession number AJ277875.
[1]Corresponding author; fax (+33) 247 276660, e-mail: rideau@univ-tours.fr

factors (Droual *et al.* 1997). In the present paper, we investigate the expression of a short promoter fragment of the periwinkle *CyP* gene in transgenic tobacco plants. The approach used is to study the expression of the *Gus* reporter gene placed under the control of the *CyP* promoter in the transgenic plants.

Materials and methods

Plants: Normal and transgenic tobacco plants (*Nicotiana tabacum* L., cv. Petit Havana SR1) were grown either *in vitro* in 500 cm^3 flasks containing 200 cm^3 hormone-free Murashige and Skoog (MS) agar medium at 24 °C under 16-h photoperiod [*Sylvania Grolux* tubes, Revimex, Tours, 18 µmol(photons) m^{-2} s^{-1}] or in the greenhouse in pots containing a mixture of soil and vermiculite at day/night temperature of 25/22 °C, and 12-h photoperiod (*Sylvania Grolux* tubes and Hg vapour lamps, irradiance 45 µmol m^{-2} s^{-1}).

Promoter isolation by IPCR: Two µg of genomic DNA from periwinkle were digested for 3 h with *Bgl*II and *Bam*HI (10 units each), then circularised at 12 °C overnight at a concentration of 50 ng DNA per mm^3 in 1X ligation buffer (10 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM ATP, 30 mM Tris pH 7.8) and 1.5 unit of T4 DNA ligase. The DNA was then extracted with phenol/chloroform, precipitated with ethanol/sodium acetate (-20 °C, overnight), dried, and re-suspended in sterile distilled water. The reaction medium (0.05 cm^3) for IPCR contained 100 ng of circularised DNA, 1.5 mM $MgCl_2$, 0.2 M of each dNTP, 1 µM of primers P1 and P2 (based on the *CrCyP1* sequence: Clastre *et al.* 1995; EMBL access number no X85185) and 2.5 units of *pfu* Taq DNA polymerase (*Stratagene*). P1 (= 5'-GGTTAGGCTGGTAGAGGATAAG-3') and P2 (= 5'-GAGTTTTCTTCGATATGAGCGTCG-3') hybridised to the cDNA sense and antisense strands in such a way that their 3' termini faced in opposite directions and were separated from each other by 3 bp. DNA amplification was carried out through 35 cycles of 45 s denaturation at 94 °C, 1 min-annealing at 60 °C, and 90 s extension at 72 °C, completed with a final extension at 72 °C for 10 min. An aliquot of PCR products was analysed on agarose gels. The longest IPCR product was re-amplified with P1 and P2 and cloned into the PCR-Script Amp SK(+)vector.

To determine the transcription start, total RNA isolated from 4-day-old periwinkle suspension cells was used as a template for 5'-RACE with a Gibco-BRL kit. Two primers specific to *CrCyP1*, *ie* P3 = 5'-CACGGGGAAGGAGAAT-3' (for reverse trans-cription) and P4 = 5'-TACCGCTCCTTCCGGTGCCTTTTTC-3' (for PCR) were used as 3' primers. The RACE products were cloned in pGEM-T-Easy vector, then sequenced on both strands.

Construction of the promoter-*gus* fusions and tobacco transformation: A 465 bp *CrCyP1* promoter was constructed by PCR amplification with the longest IPCR product and primers P5 (= 5'-GCCCGGTTAGGATCCGTAGAGGATAA-3') and P6 (= 5'-CCAACTCTCTTAAAGCTTACGAGTT-3'). P5 contained a *Bam*HI site and was used as antisense primer; P6 contained a *Hind*III site and was used as sense primer. The PCR product digested with *Bam*HI and *Hind*III was cloned into the pBlueScript vector, sequenced to confirm that no nucleotide change had been introduced by the PCR method, then fused to a *gusA* intron open reading frame in pGiBin19 (a pBin19-derivated binary vector obtained from Prof. M. Van Montagu) and digested with *Bam*HI and *Hind*III). The promoter-*gus* construct (designated *pCrCyP1-gus*) was mobilized into the *Agrobacterium tumefaciens* strain C58C1 (containing the plasmid pGV 2260: Deblaere *et al.* 1985). Leaf discs were transformed according to Horsch *et al.* (1985) and grown on MS agar medium containing 50 mg dm^{-3} kanamycin monosulphate, 500 mg dm^{-3} claforan, 60 g dm^{-3} sucrose, 0.1 mg dm^{-3} NAA and 1 mg dm^{-3} BAP. Transformed calli that appeared on the edges of the leaf discs were transferred on a medium of same composition, except higher concentration in BAP (2 mg dm^{-3}) to favour shoot development. After 3 weeks, transgenic shoots were individually transferred on phytohormone-free medium and maintained as plants *in vitro*, or transferred into the greenhouse and allowed to undergo self-pollination. To verify the number of copies of the introduced gene, DNA was extracted from transgenic plants according to Droual *et al.* (1997). The 1958 bp *Sac*I/*Bam*HI coding sequence of the *gus* gene was labelled to high specific activity, then 10 µg DNA were cleaved with appropriate restriction enzymes and electrophoresed in 0.8 % neutral agarose gel. Hybridizations were performed for 18 h at 65 °C in 5 × SSC, 5 × Denhardt's, and 100 µg of salmon sperm DNA per cm^3. Only plants containing one copy of the gene were chosen for further experiments.

Histochemical localisation of β-glucuronidase activity: Histochemical analysis of GUS activity was carried out on Ro plants and F1 seedlings. Root tips, manual cross-sections through stems and roots, entire leaves and seedlings were pre-incubated in 90 % acetone for 45 min at -20 °C, washed two times in 0.1 M phosphate buffer (pH 7), and submersed in a staining solution containing 0.5 mg of 5-bromo-4-chloro-3-indolyl-D-glucuronide (X-gluc dissolved in N,N-dimethylsulfoxide) per cm^3 of phosphate buffer, in the presence of 1 mM each of ferricyanide and ferrocyanide. After staining, plant materials were fixed in 2.5 % glutaraldehyde (in

phosphate buffer). Green tissues were rinsed 3 times in phosphate buffer and incubated (30 min each) in successive bathes of 50, 75 and 95 % aqueous ethanol to remove chlorophylls. Whole seedlings were cleared in lactophenol.

β-glucuronidase activity in treated transgenic tobacco seedlings: Ro and F1 seeds were germinated aseptically at 24 °C in 10 mM MES buffer (pH 7.0) under continuous white light [18 μmol m^{-2} s^{-1}]. Three days after germination, various filter-sterilised chemicals were added to the germination medium. The seedlings were harvested at different times, washed with cold distilled water, freeze-dried and ground (4 °C) with 0.5 cm^3 extraction buffer (50 mM Na-phosphate, 10 mM ethylenediamine tetracetic acid, 10 mM β-mercapto-ethanol, 0.1 % triton X-100, pH 7.0). After centrifugation (10 min, 4 °C, 17 300 *g*), supernatants were transferred in ice-cold tubes. Aliquots were used for protein quantification (Bradford 1976). For fluorimetric assays, 10 - 20 μg proteins were added to 1 cm^3 of extraction buffer containing 1 mM X-gluc and incubated at 37 °C. After 3 h, 0.2 cm^3 of the reaction mixture were mixed with 1.8 cm^3 0.2 M Na_2CO_3. Fluorescence was measured (λ_{exc} 365 nm, λ_{em} 455 nm) and converted to nmol 4-methyl-umbelliferone (MU). GUS activity was expressed in mmol(MU) g^{-1}(protein) min^{-1}.

Results and discussion

Characterization of a promoter fragment of the cytosolic cyclophilin gene from periwinkle: The 5' domain (up to a *Bgl*II site) comprised 24 bp belonging to primer P2 and 480 bp matching the *CrCyP1* nucleotide sequence that encodes the amino acids 6 to 160 of the protein. The 3' domain comprised 465 bp with a 52-bp region (including the 23 bp of primer P1) matching perfectly the 5' end of *CrCyP1*. The probable transcription start of *CrCyP1* was determined by 5' RACE. Sequencing of several amplified RACE products suggested the presence of 70 transcripted bases upstream of the ATG codon.

Several putative *cis*-acting regulatory elements were found using http://www.dna.affrc.go.jp/htdocs/PLACE and http://sphinxrug.ac.be:8080/plantCARE databases, or by direct comparison with literature data (Fig. 1). The comparison with the promoter of the cyclophilin gene from maize (Marivet *et al.* 1995) showed commune regulatory elements: the A/T-rich enhancer domain, at least a G-box sequence, and the GAAGATTCC heat shock motif. Two G-box motives are also present in the promoters of the cytosolic *CyP* genes *AtCyP1* and *AtCyP2* from *Arabidopsis* (Saito *et al.* 1999). Metal regulatory sequences occurred in both periwinkle and maize CyP promoters, but with different motives (TGCACACC in maize versus ACACAAG in periwinkle). Similarly, two different gibberellic acid-responsive elements (CCTTTT and GCAACG) were

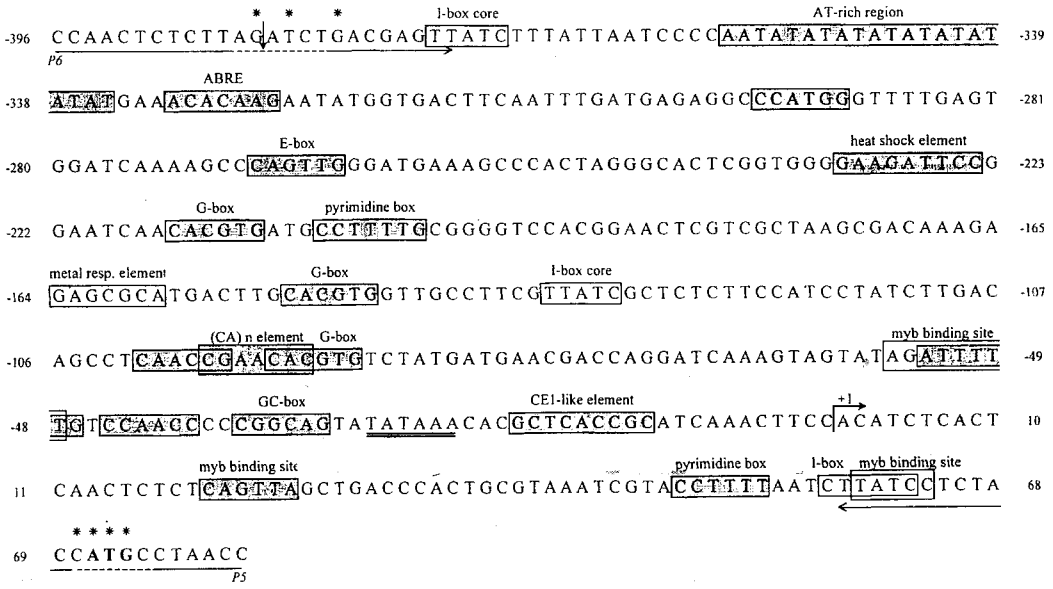

Fig. 1. Nucleotide sequence of the 5' upstream sequences of *Cr-CyP1*. These sequences extend from -380 (relative to the initiation start +1) to +80. The ATG codon is in bold and the TATA box is underlined twice. Some important motifs for plant gene regulation are boxed (those on the +DNA strand are in grey). The direction and nucleotide sequences of primers P5 and P6 used for the construction of *pCyP465-gus* and the *Bam*HI and the *Hind*III restriction enzyme sites are also indicated. Stars indicated the nucleotides modified in the primers to create these sites.

present in periwinkle and maize *CyP* promoters, respectively. The presence of the heat shock motif and G-box motif (*i.e.* the core of several ABA response elements) agrees with previous experiments in which we found that transferring periwinkle suspension cells from 27 to 4 °C, or treating the cells with NaCl or ABA changed *CrCyP1* transcript accumulation (Droual *et al.* 1997).

Detection of the cyclophilin expression. To get further information on the regulation of expression of *CrCyP1*, we fused the 5' upstream region to the coding region of a *gus*A-intron gene and transformed tobacco leaf discs with this construct. Firstly, we analysed histochemically GUS activity in 15 independent transgenic shoots (about 1 cm high) still attached on the kanamycin-resistant calli from which they were initiated. All these transformants contained one copy of the construct. They exhibited intense GUS staining of the guard cells of leaf stomata, but epidermis and mesophyll cells remained unstained (Fig. 2*A,B*). Secondly, we separated transgenic shoots from the calli and transferred them onto hormone-free MS medium. A positive GUS reaction appeared in the mesophyll cells of the youngest leaves. Similar observations were found when the separated transgenic shoots were grown on media containing 0.1 mg dm^{-3} NAA and/or 2 mg dm^{-3} BAP. This shows that the appearance of GUS expression in mesophyll cells was not caused by the deletion of hormones from the medium, but rather by the suppression of the callus at the basis of the stem. A question arises concerning the messengers building the communication between the callus and the mesophyll cells. One can hypothesise that specific proteins or mRNAs are transported from the callus to the leaf cells, but this remains to be tested.

When the shoots cultured on hormone-free medium grew taller, GUS expression progressively disappeared from the leaf mesophyll cells. By contrast, the phloem cells of the stems and the tips of the roots that developed at the basis of the shoots within 2 weeks of culture

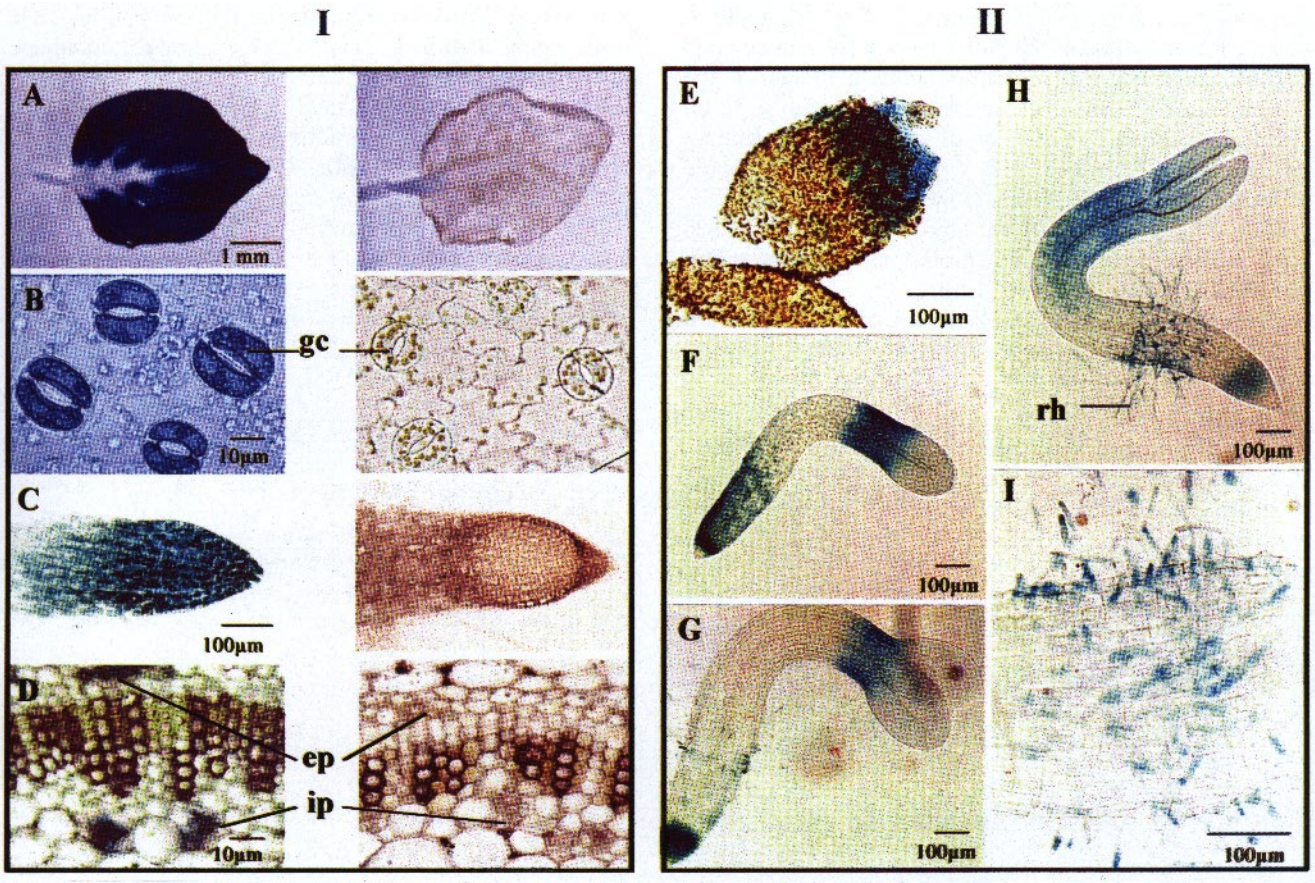

Fig. 2. Pattern of expression of the *Cr-CyP1* promoter-*gus* intron gene fusion in transgenic tobacco plants: staining *in situ* for GUS activity.
Panel I - shoots grown *in vitro* (*left* and *right columns* correspond to transformed and control shoots, respectively). *A* - young leaves from shoots after their separation from the calli and their cultivation on MS agar medium; *B* - epidermis of leaves removed from shoots still attached on the callus; *C* - apex of roots developed at the basis of the stem; *D* - hand-cut cross-section of shoots cultured *in vitro* on phytohormone-free MS agar medium, showing the staining of both internal and external phloem cells (ip - internal phloem, ep - external phloem, gc - guard cells).
Panel II - F1 transgenic tobacco seedlings. *E* - seedling at the moment of radicle emergence showing the GUS staining of the seed coat; *F* - 3-d-old seedling; *G* - 7-d-old seedling; *H* - 10-d-old seedling (rh - root hairs); *I* - magnification of root hairs showing GUS expression.

showed a positive staining (Fig. 2*C,D*). Since these events occurred in several independent transgenic shoots, changes in GUS staining are probably not due to the instability of the transgenic tissues. GUS activity in the phloem agrees with results obtained in similar experiments with *pCyP-gus* transgenic seedlings of *Arabidopsis* (Saito *et al.* 1999).

GUS expression was also determined in F1 seedlings. X-gluc staining was positive in the internal seed coat around the emerging root of 1-day-old seedlings (Fig. 2*E*). In 2-d-old seedlings, GUS was strongly expressed in the root meristem, the elongation zone that preceeds it, and the basis of cotyledons (Fig. 2*F*).

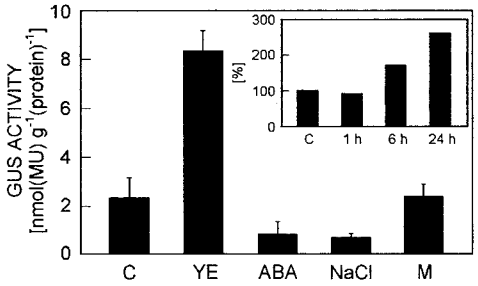

Fig. 3. Effect of various treatments on the GUS activity after 24 h of treatment of 3-d-old F1 seedlings. C- non transformed (control) plant, YE - 1.5 % yeast extract, ABA - 1 mM abscisic acid, NaCl - 170 mM sodium chloride, M - 1 % macerozyme. The *bars* represent standard error of the mean ($n = 4$). The inset gives the timing of the effect of yeast extract on GUS activity: data are given in percentage of the control after 1, 6 and 24 h of treatment.

Thereafter, staining disappeared from the elongation zone of the root. It was retained in the meristems and appeared in the root hairs (Fig. 2*G-I*).

Finally, GUS activity was determined fluorimetrically with tissue homogenates from three-day-old seedlings treated for 24 h with various chemicals (Fig. 3). Different concentrations in salicylic acid, BAP and ethylene (through ethefon degradation) had no clear effect on GUS activity (not shown). Macerozyme as well had no effect. 1.5 % yeast extracts clearly enhanced GUS activity whereas 1 mM ABA or 170 mM NaCl decreased it (the NaCl effect may be due to enhanced endogenous ABA contents). Responses to ABA, NaCl and yeast extract differed slightly among independent transgenic clones but were similar from one clone to another.

In conclusion, our results show that the 465-bp *CrCyP1* promoter is sufficient to drive GUS expression in transgenic tobacco plants. Therefore, this promoter can be used to search cis-regulatory domains. Changes in glucuronidase activity confirm other histochemical analysis (Saito *et al.* 1999) and Northern blottings (Marivet *et al.* 1992, Gasser *et al.* 1990, Marty *et al.* 1993, Scholze *et al.* 1999, Godoy *et al.* 2000) showing organ/cell specific expression and growth- and stress-dependent expression of the cytosolic cyclophilin genes. Indeed, we cannot rule out the existence of *cis*-acting elements outside of the proximal region of *CrCyP* which might change the expression patterns. This may explain why GUS activity was decreased after treating the transgenic tobacco seedlings with NaCl, whereas plant CyP genes are usually up-regulated by NaCl.

References

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Chou, I.T., Gasser, C.S.: Characterization of the cyclophilin gene family of *Arabidopsis thaliana* and phylogenetic analysis of known cyclophilin proteins. - Plant mol. Biol. **35**: 873-892, 1997.

Clastre, M., Maaroufi, H., Andreu, F., Chénieux, J.C., Rideau, M., Hamdi, S.: Isolation of a full-length cDNA encoding a cytosolic cyclophilin from periwinkle (*Catharanthus roseus*). - Plant Physiol. **109**: 1497, 1995.

Deblaere, R., Bytebier, B., De Greve, H., Deboeck, F., Schell, J., Van Montagu, M., Leemans, J.: Efficient octopine Ti plasmid-derived vectors for *Agrobacterium*-mediated gene transfer to plants. - Nucl. Acids Res. **13**: 4777-4788, 1985.

Deng, W., Chen, L., Wood, D.W., Metcalfe, T., Liang, X., Gordon, M.P., Comai, L., Nester, E.W.: *Agrobacterium* VirD2 protein interacts with plant host cyclophilins. - Proc. nat. Acad. Sci. USA **95**: 7040-7045, 1998.

Doyle, V., Virji, S., Crompton, M.: Evidence that cyclophilin-A protects cells against oxidative stress. - Biochem. J. **341**: 127-132, 1999.

Droual, A.M., Maaroufi, H., Crèche, J., Chénieux, J.C., Rideau, M., Hamdi, S.: Changes in the accumulation of cytosolic cyclophilin transcripts in cultured periwinkle cells following hormonal and stress treatments. - J. Plant Physiol. **151**: 142-150, 1997.

Duina, A.A., Marsh, J.A., Gaber, R.F.: Identification of two CyP-40-like cyclophilins in *Saccharomyces cerevisiae*, one of which is required for normal growth. - Yeast **12**: 943-952, 1996.

Ferreira, P.A., Nakayama, T.A., Pak, W.L., Travis, G.H.: Cyclophilin-related protein RanBP2 acts as chaperonne for red/green opsin. - Nature **383**: 637-640, 1996.

Franke, E.K., Yuan, H.E., Luban, J.: Specific incorporation of cyclophilin A into HIV-1 virions. - Nature **372**: 359-362, 1994.

Fulgosi, H., Vener, A.V., Altschmied, L., Herrmann, R.G., Andersson, B.: A novel multi-functional chloroplast protein: identification of a 40 kDa immunophilin-like protein located in the thylakoid lumen. - EMBO J. **17**: 1577-1587, 1998.

Gasser, C.S., Gunning, D.A., Budelier, K.A., Brown, S.M.: Structure and expression of cytosolic cyclophilin/peptidyl-prolyl *cis-trans* isomerase of higher plants and production of active tomato cyclophilin in *Escherichia coli*. - Proc. nat. Acad. Sci. USA **87**: 9519-9523, 1990.

Godoy, A.V., Lazzaro, A.S., Casalongué, C.A., San Segundo, B.: Expression of a *Solanum tuberosum* cyclophilin gene is regulated by fungal infection and abiotic stress conditions. - Plant Sci. **152**: 123-134, 2000.

Horsch, R.B., Rogers, S.G., Fraley, R.T.: Transgenic plants. - Cold Spring Harb. Symp. quant. Biol. **50**: 433-437, 1985.

Jackson, K., Soll, D.: Mutations in a new *Arabidopsis* cyclophilin disrupt its interaction with protein phosphatase 2A. - Mol. gen. Genet. **262**: 830-838, 1999.

Jin, Z.G., Melaragno, M.G., Liao, D.F., Yan, C., Haendeler, J., Suh, Y.A., Lambeth, J.D., Berk, B.C.: Cyclophilin A is a secreted growth factor induced by oxidative stress. - Circ. Res. **87**: 789-796, 2000.

Krummrei, U., Bang, R., Schmidtchen, R., Brune, K., Bang, H.: Cyclophilin-A is a zinc-dependent DNA binding protein in macrophages. - FEBS Lett. **371**: 47-54, 1995.

Kullertz, G., Liebau, A., Rucknagel, P., Schierhorn, A., Diettrich, B., Fischer, G ., Luckner, M.: Stress-induced expression of cyclophilins in proembryonic masses of *Digitalis lanata* does not protect against freezing/thawing stress. - Planta **208**: 599-605, 1999.

Luan, S., Li, W., Rusnak, F., Assmann, S.M., Schreiber, S.L.: Immunosuppressants implicate protein phosphatase regulation of K^+ channels in guard cells. - Proc. nat. Acad. Sci. USA **90**: 2202-2206, 1993.

Marivet, J., Frendo, P., Burkard, G.: Effects of abiotic stresses on cyclophilin gene expression in maize and bean, and sequence analysis of bean cyclophilin cDNA. - Plant Sci. **84**: 171-178, 1992.

Marivet, J., Frendo, P., Burkard, G.: DNA sequence analysis of a cyclophilin gene from maize: developmental expression and regulation by salicylic acid. - Mol. gen. Genet. **247**: 222-228, 1995.

Marivet, J., Margis-Pinheiro, M., Frendo, P., Burkard, G.: Bean cyclophilin gene expression during plant development and stress conditions. - Plant mol. Biol. **26**: 1181-1189, 1994.

Marty, I., Brudigou, C., Chartier, Y., Meyer, Y.: Growth-related gene expression in *Nicotiana tabacum* mesophyll protoplasts. - Plant J. **4**: 265-278, 1993.

Montague, J.W., Hughes, F.M., Cidlowski, J.A.: Native recombinant cyclophilins A, B, and C degrade DNA independently of peptidylprolyl *cis-trans*-isomerase activity. Potential roles of cyclophilins in apoptosis. - J. biol. Chem. **272**: 6677-6684, 1997.

Saito, T., Tadakuma, K., Takahashi, N., Ashida, H., Tanaka, K., Kawamukai, M., Matsuda, H., Nakagawa, T.: Two cytosolic cyclophilin genes of *Arabidopsis thaliana* differently regulated in temporal- and organ-specific expression. - Biosci. Biotechnol. Biochem. **63**: 632-637, 1999.

Santos, A.N., Korber, S., Kullertz, G., Fischer, G., Fischer, B.: Oxygen stress increases prolyl *cis/trans* isomerase activity and expression of cyclophilin 18 in rabbit blastocysts. - Biol. Reprod. **62**: 1-7, 2001.

Scholze, C., Peterson, A., Diettrich, B., Luckner, M.: Cyclophilin isoforms from *Digitalis lanata*. Sequences and expression during embryogenesis and stress. - J. Plant Physiol. **15**: 212-219, 1999.

Sykes, K., Gething, M.J., Sambrook, J.: Proline isomerases function during heat shock. - Proc. nat. Acad. Sci. USA **90**: 5853-5857, 1993.

Yang, W.M., Inouye, C.J., Seto, E.: Cyclophilin A and FKBP12 interact with YY1 and alter its transcriptional activity. - J. biol. Chem. **270**: 15187-15193, 1995.