Induction of *tcI 7*, a gene encoding a β-subunit of proteasome, in tobacco plants treated with elicitins, salicylic acid or hydrogen peroxide[1]

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Abstract We previously isolated, by differential display and 5′ RACE (rapid amplification of cDNA ends), cDNAs corresponding to genes activated following cryptogein treatment of tobacco cell suspensions, among them *tcI 7* (tcI for tobacco cryptogein Induced), a gene encoding a β-subunit of proteasome. Here, we report that *tcI 7* was up-regulated in tobacco plants treated with elicitins (cryptogein and parasiticein) that have been shown to induce a systemic acquired resistance (SAR). Moreover, subsequent inoculation of tobacco with the pathogen *Phytophthora parasitica* var. *nicotianae* (Ppn) was shown to induce an additional activation of *tcI 7* in tobacco plants pretreated with cryptogein. We also showed an up-regulation of *tcI 7* by salicylic acid (SA). Moreover, accumulation of *tcI 7* transcripts after treatment with cryptogein or with SA only occurred in NahG 9$^-$ tobacco plants that do not express the salicylate hydroxylase and thus are able to accumulate SA and develop a SAR. Suppressed accumulation of *tcI 7* transcripts in NahG 8$^+$ tobacco plants after cryptogein or SA treatment correlated with the loss of SAR. H_2O_2 was also shown to up-regulate *tcI 7* in tobacco plants. Using gene walking by PCR we cloned and sequenced the 5′ flanking region of *tcI 7* containing hypothetical regulatory sequences, especially myb and NF-κB boxes, that could be responsible for the regulation of *tcI 7* by salicylic acid and H_2O_2 respectively.

Key words: Elicitin; Hydrogen peroxide; Salicylic acid; Proteasome β-subunit; Systemic acquired resistance; Tobacco

1. Introduction

The hypersensitive reaction (HR) induced in many incompatible plant–pathogen interactions is associated with the activation of various defense responses such as systemic acquired resistance (SAR) [1,2]. SAR results in a coordinated activation of several families of SAR genes (especially genes encoding pathogenesis-related (PR) proteins) correlated with the development of resistance [3].

Elicitins constitute a family of small proteinaceous elicitors of defense reactions secreted by *Phytophthora* species [4]. The most studied is cryptogein (a basic elicitin of 10 323 Da), purified from a culture filtrate of *Phytophthora cryptogea*. Cryptogein causes HR-like necroses in tobacco (*Nicotiana tabacum* var. *xanthi)* plants at the site of application and on distant leaves. Tobacco plants treated with elicitins, especially cryptogein, acquire resistance to subsequent infection with the tobacco pathogen *Phytophthora parasitica* var. *nicotianae* (Ppn) as well as against other pathogens [4,5]. This elicitin-induced SAR has been correlated with the production of ethylene, phytoalexins and PR proteins in tobacco plants and/or in cultured cells [6–8] and the coordinated accumulation of transcripts from SAR genes, mainly encoding acidic and basic PR proteins [9]. Tobacco cell suspensions have been used to study the early effects of cryptogein such as alkalization of the extracellular medium [6], protein kinase activation [10], protein phosphorylation [11], calcium influx [12], oxidative burst [13], and modifications of gene expression [14]. Using a combination of differential display and 5′-RACE (rapid amplification of cDNA ends), we previously isolated cDNAs, among which *tcI 7* (tcI for tobacco cryptogein Induced), corresponding to genes early activated after treatment of tobacco cell suspensions with cryptogein [15]. Cloning and sequencing of the *tcI 7* cDNA allowed the identification of an open reading frame showing significant similarities (68%) with a β-type proteasome subunit of rat [16].

Proteasomes are multicatalytic complexes (14 α- and 14 β-subunits) involved in protein degradation in prokaryotes and eukaryotes (for review see [17]). They are also required for activation of proteins by processing of inactive precursors. The 20S core proteasome complex appears also responsible for the selective degradation of oxidatively damaged proteins in human hematopoietic cells [18]. Few data are available concerning the role of proteasomes in plants. Proteases are known to be involved in several plant cell processes such as environmental stresses (cold or high temperature, abscisic acid treatment, drought or salt stress), cell death, senescence or wounding processes (for review see [19]). Moreover, in *Arabidopsis thaliana*, the transcripts of both the α- and β-type proteasome subunits accumulated to high levels during cell proliferation simultaneously with mRNAs encoding an ubiquitin fusion protein [20].

The proteasome activity must be tightly regulated to prevent premature or unwanted proteolysis but little is known about this regulation. In animals, subunit composition of the 20S proteasome also varies among tissues and stages of development [17]. For a better understanding of the possible role of *tcI 7* in the onset of HR and/or SAR we report here a study on the regulation of *tcI 7* gene expression by elicitins

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[1] *tcI 7* accession number: Y09505.
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Abbreviations: GSP, gene-specific primer; GST, glutathione *S*-transferase; HR, hypersensitive reaction; nPCR, nested polymerase chain reaction; PAL, phenylalanine ammonia-lyase; PCR, polymerase chain reaction; Ppn, *Phytophthora parasitica* var. *nicotianae*; PR, pathogenesis-related; SA, salicylic acid; SAR, systemic acquired resistance; SDS, sodium dodecyl sulfate; SSC, sodium salt citrate; tcI, tobacco cryptogein induced

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(especially cryptogein) or salicylic acid (SA) in tobacco plants and we used gene walking by PCR for cloning and sequencing the 5′ flanking sequence of *tcI 7* allowing the localization of hypothetical regulatory sequences. All the results obtained in this work indicate that *tcI 7* could be involved early in the onset of SAR.

2. Materials and methods

2.1. Tobacco plants

Experiments were performed using *Nicotiana tabacum* cv. *xanthi* tobacco plants. Plants were grown at 24°C with a photoperiod of 16 h (light intensity of 80 μE m^{-2} s^{-1}) within 6–7 weeks. The cryptogein, SA or H_2O_2 treatments were carried out by infiltrating 10 μl of aqueous solution of cryptogein 100 nM, SA 10 mM or H_2O_2 100 mM directly into leaves through the abaxial epiderm. The infiltrated areas were spotted and harvested after various times. Control leaves were infiltrated with 10 μl of water.

In order to check SAR we used tobacco plants decapitated prior to the application of 20 μl cryptogein 5 μM (50 μg/ml) or water at the cutting sites. Necrotic lesions (HR) appeared on distant leaves within 12–24 h only in the plants pretreated with cryptogein. Biological challenge for induced resistance was performed by infiltration of Ppn (isolate 329 from the *Phytophthora* collection of INRA Antibes France: 100 μl = 100 zoospores of Ppn) versus infiltration of 100 μl water, in non-necrotic areas of leaves 24 h after the pretreatment with cryptogein or water [21]. Infiltrated areas were harvested at various times. To evaluate the level of protection, the invaded areas of each leaf were measured at different times after inoculation and compared with those of water-pretreated and Ppn-inoculated control plants.

2.2. Isolation of genomic DNA and gel electrophoresis of DNA

Total genomic DNA was isolated from control or treated leaves or leaf areas using the DNeasy Plant Mini kit (Qiagen, France) following the manufacturer's instructions. Electrophoresis was carried out on 0.8% agarose gel in TAE buffer in the presence of ethidium bromide.

2.3. Extraction of total RNA fraction

Total RNA fraction was extracted from control and treated leaves or leaf areas at various times after treatments. RNA extraction was performed using a phenol-SDS method as previously described [14] or using the Plant RNA easy minikit (Qiagen, France) following the manufacturer's instructions.

2.4. Northern blots

Northern blots were carried out according to standard protocols using 15 μg of total RNA per lane. After electrophoresis, RNA samples were transferred and UV cross-linked to Hybond N^+ filters (Amersham, France). Filters were hybridized with the specific RACE cDNAs tcI 7 [16] or with a PR-1b probe [22] as reference for the SAR gene, ^{32}P-labeled by random priming (*redi*prime, Amersham, France). Hybridizations were carried out at 42°C overnight. Filters were washed with 2×SSC, 0.1% SDS at room temperature, 4×10 min and with 0.2×SSC, 0.1% SDS at 55°C 2×10 min. Filters were analyzed with a PhosphorImager (Molecular Dynamics, France).

2.5. Gene walking by PCR

2.5.1. Preparation of the genomic DNA template for walking. The protocol was adapted from those proposed by Siebert and coworkers [23] and Devic and coworkers [24]. Tobacco genomic DNA (3.2 μg) was digested overnight at 37°C with 100 U of restriction enzyme in a 100 μl final reaction volume. Four restriction enzymes creating blunt-ended fragments were used separately: *Dra*I, *Eco*RV, *Sca*I and *Ssp*I. The reaction was stopped by cooling at 4°C and digested DNA extracted with phenol/chloroform, washed with chloroform and ethanol-precipitated. The pellet was resuspended in 20 μl of sterile water. The adapter was prepared as previously described [24]. Briefly, adapter nucleotide 1 (Eurogentec, Belgium): 5′-CTAATACGACTCACTATAGGGCTCGAGC-GGCCGCCCGGGCAGGT-3′ and adapter nucleotide 2: 5′-P-ACCTGCCC-NH_2-3′ were mixed at a final equimolar concentration of 25 μM and annealed by boiling for 1 min followed by gradual cooling to room temperature to allow formation of the duplex. Ligation of an excess of adapter (5 μM final) to 1.25 μg of digested DNA was carried out overnight at 16°C in the presence of 10 U of T4 DNA ligase (Promega, France). The ligase was heat-inactivated at 70°C for 10 min. The stock DNA template for walking was obtained by a 10 fold dilution in sterile water.

2.5.2. Primers. The primers AP1 and AP2 (corresponding to adapter) were used in combination with gene-specific primers (GSP); AP1: 5′-CCTAATACGACTCACTATAGGGCT-3′ and AP2: 5′-ACTCACTATAGGGCTCGAGCGGC-3′. Several gene-specific primers were deduced from the known part of the *tcI 7* sequence allowing high T_m and a GC content of 52–72%. MG7: 5′-GGGAGTACTTGTACACCAGCAGCAG-3′ (25-mer, GC content 56%), MG7-n: 5′-CCATTGGAACAGGGCTTGATGC-3′ (22-mer, GC content 54.5%), MG7-1: 5′-GGTGCCCATGGAGTGGGGCTGGTCC-3′ (23-mer, GC content 52%), MG7-1n: 5′-GCTGGTCCACATCGGTATTTTCC-3′ (25-mer, GC contents 72%), MG7-2: 5′-CTATGGCCAAATGGGTTAGTAGGC-3′ (25-mer, GC content 52%), MG7-2n: 5′-GCGCCAAAACTGGCTCTTAAGC-3′ (22-mer, GC content 54.5%).

2.5.3. PCR protocols. The PCR reaction mixture (total 20 μl) contained 0.4 μl of stock DNA template, 200 μM each dNTP, 0.75 U of Taq DNA polymerase (Long Expand template DNA polymerase, Boehringer, France) in buffer 3 (2.25 mM $MgCl_2$, 50 mM Tris–HCl pH 9.2, 16 mM $NH_4(SO_4)_2$, 2% DMSO and 0.1% Tween 20), 0.1 μg of Taq start antibody, 0.2 μM of each primer (AP1 and GSP). PCR reactions were performed in a MJR PTC 150 MiniCycler (MJ Research Inc., MA, USA) with the following steps: 1 cycle of denaturation at 94°C for 2 min, 30 cycles of 30 s at 94°C, 30 s at a temperature depending of the GSP (between 50 and 60°C), 4 min at 68°C followed by a final cycle of extension at 68°C for 10 min. 10 μl of the primary PCR reaction was loaded on a 1% agarose gel. The primary PCR reaction was diluted 50-fold prior the nested (n) PCR reaction done with 1 μl of the dilution in a 50 μl total volume under the same conditions using AP2 and GSP-n as primers.

2.5.4. Cloning and sequencing of GW fragments. The nPCR reaction mixtures (20 μl) were loaded on a 1% agarose gel in TBE. The bands of interest were cut from the gel and purified using Pall Biodyne membrane. The corresponding DNAs were cloned in pGEM-Teasy Vector (Promega, France) and *Escherichia coli* JM 109, by electroporation (EasyjecT Basic, Eurogentec, Belgium). The transformed colonies were screened in Xgal+IPTG-supplemented medium. DNAs were purified by mini-prep and sequenced (Genome Express Service, France). Sequence analyses were carried out mainly by GCG package and by PLACE, a database of plant *cis*-acting regulatory DNA elements (www.dna.affrc.go.jp/htdocs/PLACE).

3. Results

3.1. Accumulation of tcI 7 mRNAs mediated by elicitins in tobacco leaves

The *tcI 7* transcripts accumulated after elicitin treatment of tobacco leaves as shown in Fig. 1. The accumulation of *tcI 7* transcripts occurred slightly at 4 h and increased highly at the 6 h and 12 h time points after infiltration of cryptogein or parasiticein in tobacco leaves. A slight accumulation of *PR-1b* corresponding mRNA was detected only 12 h after the treatment with elicitins. Infiltration of tobacco leaves with water allowed us to check that infiltration alone does not induce significant activation of *tcI 7*. The accumulation of *tcI 7* transcripts in areas infiltrated with elicitins started 4 h (cryptogein) to 8 h (parasiticein) before the appearance of HR-like necrosis in these leaf areas. We previously showed an accumulation of *tcI 7* transcripts in tobacco cells that occurred within 30–60 min after the cryptogein treatment [16]. The difference between the two kinetics could be due to the slower migration of cryptogein in leaf tissues than in tobacco cell suspension cultures (observed using ^{125}I-labelled cryptogein). Moreover, the accumulation of *PR-1b* transcripts induced by cryptogein occurred within 3–4 h in tobacco cell suspensions versus 12 h in leaves.

Fig. 2a shows the activation of *tcI 7* in decapitated plants

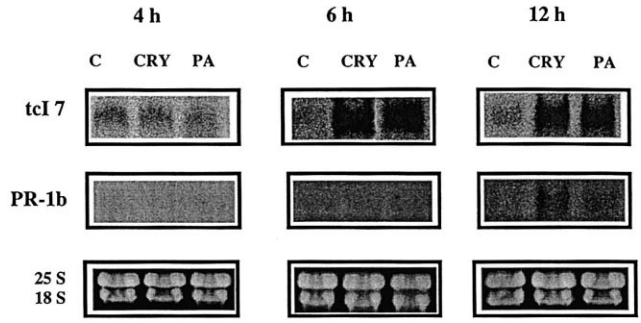

Fig. 1. Accumulation of *tcI 7* mRNA in tobacco leaves infiltrated with water or elicitins. Total RNA (15 μg per lane) was extracted from control leaves (C) or from leaves infiltrated with water or with 100 ng cryptogein (CRY) or parasiticein (PA) per leaf at indicated times. Northern blots were hybridized with the *tcI 7* full-length cDNA or with the *PR-1b* cDNA probe. Equal loading of rRNA was checked by staining with ethidium bromide.

pretreated with cryptogein: a high accumulation of transcripts occurred within 6–8 h. Protection against pathogen occurred only in plants pretreated with cryptogein (20% invasion versus 100% for control decapitated plants infiltrated with water). Fig. 2b shows the up-regulation of *tcI 7* following Ppn inoculation (higher in plants pretreated with cryptogein than in plants pretreated with water). The Ppn-inoculated plants belonged to strain 329 which does not produce an elicitin. Inoculation carried out with inactivated zoospores (by heating at 100°C) was not able to induce activation of *tcI 7*. Plants pretreated with cryptogein showed an accumulation of *PR-1b* transcripts 24 h after the treatment (time 0 for the Ppn infection). The amount of *PR-1b* transcripts decreased rapidly after the infection with Ppn as previously shown [25].

3.2. Gene walking by PCR

Gene walking allowed cloning and sequencing of the main part of the *tcI 7* gene: we confirm the sequence of an open reading frame previously obtained in our laboratory as a full-length cDNA [16]. Additionally we localized two introns (390 and 700 bp) with 5′ and 3′ splice sites that agree with the GT...AG rule. Moreover, we cloned 1319 bp localized in the 5′ flanking sequence of *tcI 7* representing the greater part of the hypothetical promoter region. The 5′ flanking sequence of the *tcI 7* gene presented in Fig. 3 shows a high degree of A+T characteristic of regulatory sequences. Sequence analysis, especially in the PLACE databank, allowed the localization of a TTATATA motif that could be a likely candidate for a TATA box, CAT boxes and of various putative regulatory elements. Specific mention is made of the mybcore (CNGTTR), mybst1 (GGATA) and ASF1 elements (TGACG) that were shown to be stimulated by salicylic acid [27] [28], a compound known to play a great role in plant defense mechanisms, especially in systemic acquired resistance. We also localized a specific sequence that could bind NF-κB (T**GGGGTCT**CC**C**) with the

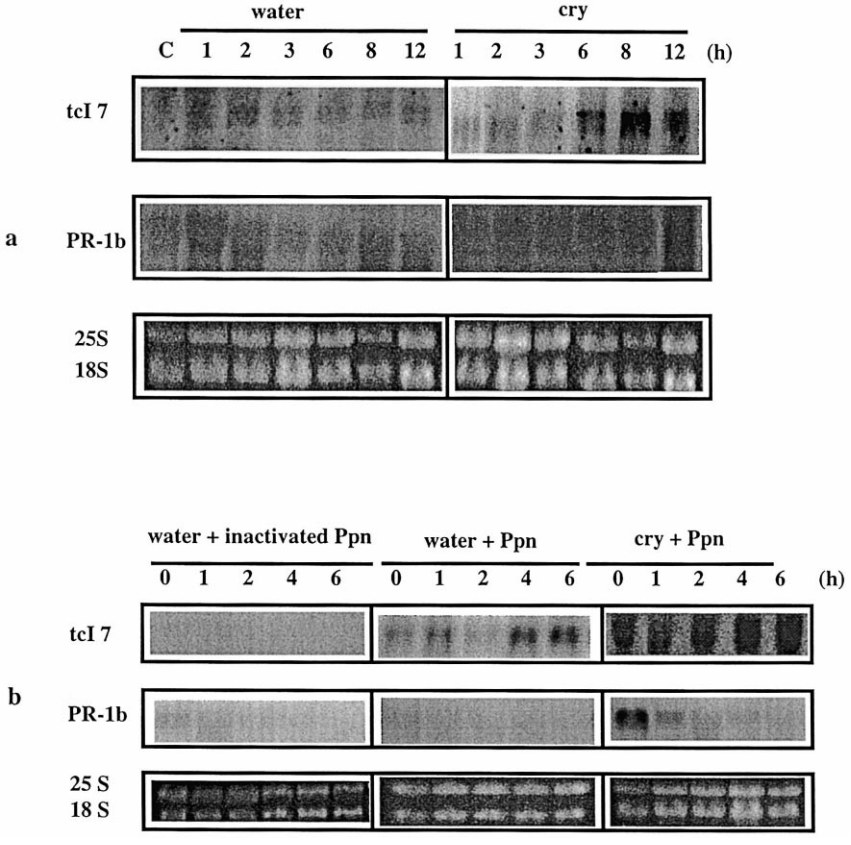

Fig. 2. Effect of the induction of HR and SAR with cryptogein on accumulation of *tcI 7* mRNA in tobacco plants. Decapitated tobacco plants were treated with water or with cryptogein (cry) at the cutting zones. The SAR induction assays were performed by infiltration of zoospores (100 zoospores per infiltration) of Ppn isolate 329 on intact leaves 48 h later. A control was carried out using inactivated zoospores (by heating at 100°C). Total RNA (15 μg per lane) was extracted before (a) or after (b) inoculation of Ppn at indicated times. Northern blots were hybridized with the *tcI 7* full-length cDNA or with the *PR-1b* cDNA probe. Equal loading of rRNA was checked by staining with ethidium bromide.

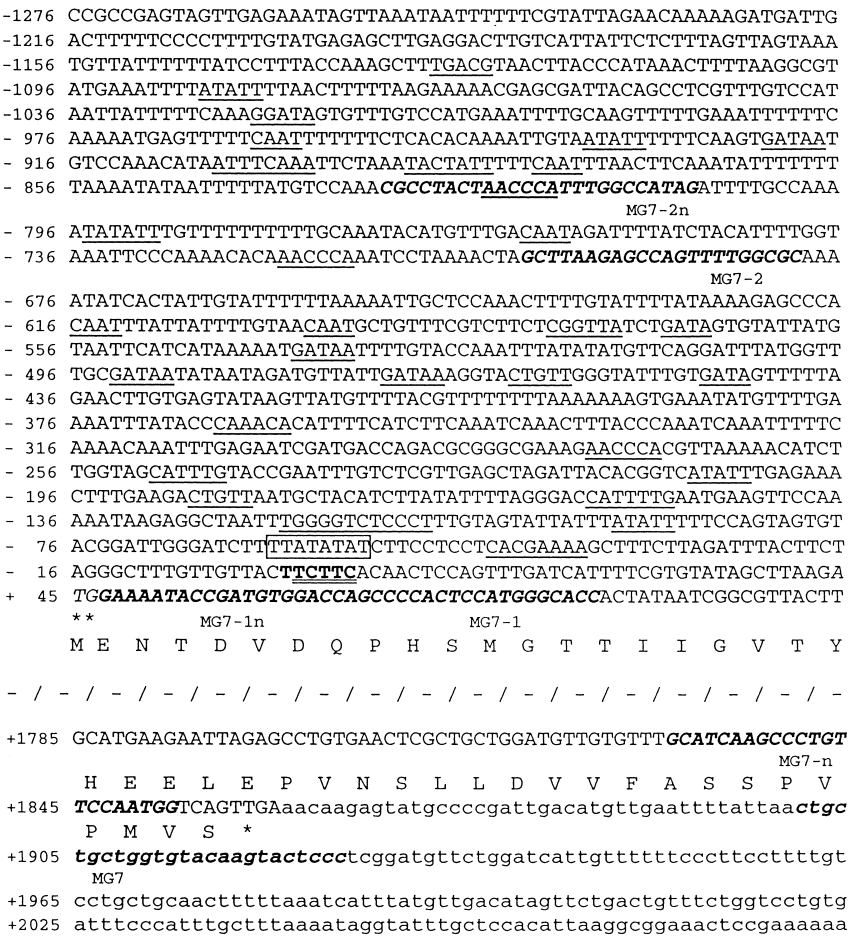

Fig. 3. Nucleotide sequence of the 5′ flanking region of *tcI 7*, obtained by PCR gene walking. The sequences of different clones obtained by PCR gene walking were close together eliminating the overlaid sequences. The TATA box is boxed. The initiation site is in bold and doubly underlined. The open reading frame starting from the *ATG* (italic) was previously described and deposited in EMBL databank (accession number Y09505). The deduced amino acid sequence is presented using the single letter amino acid code. Primers used in PCR gene walking are in bold italic. The hypothetical regulatory boxes identified by sequence analysis in the PLACE databank are underlined.

presence of a sequence of four consecutive guanine residues which appears to be critical for NF-κB binding. Such regulatory sequences exist in the promoter region of phenylalanine ammonia-lyase (PAL) and glutathione *S*-transferase (GST) genes which are regulated by H_2O_2 [36].

3.3. Accumulation of tcI 7 mRNAs mediated by salicylic acid or H_2O_2 in tobacco leaves

Results of Northern blot analysis carried out with total RNA purified from tobacco leaf areas 6 or 8 h after infiltration with SA (10 μl 10 mM) or with H_2O_2 (10 μl 100 mM) are shown in Fig. 4. SA induced an accumulation of *tcI 7* transcripts starting within 6 h and increasing after 8 h of treatment. Thus, exogenous SA alone seems able to up-regulate *tcI 7*. The very slight activation of *tcI 7* in water-treated plants could be due to the decapitation that was able to induce an accumulation of salicylic acid in the leaves below (not shown). As also shown in Fig. 4, infiltration of tobacco leaves with exogenous H_2O_2 induced an accumulation of *tcI 7* transcripts within 6 h. The accumulation increased greatly within 8 h and reached almost the same level as obtained after cryptogein treatment. *Pr-1b* corresponding mRNAs do not accumulate during the same time.

3.4. Accumulation of tcI 7 mRNAs mediated by salicylic acid in NahG transgenic tobacco plants

The Northern blots of RNA extracted from NahG 8^+ and NahG 9^- transgenic tobacco plants were a generous gift of H. Keller. Keller and coworkers [21] used lines of transgenic tobacco plants first obtained by Gaffney, NahG 8^+, express-

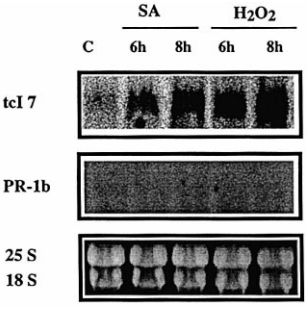

Fig. 4. Effect of SA and H_2O_2 on accumulation of *tcI 7* transcripts in tobacco leaves. Total RNA (15 μg per lane) was extracted from control leaves (C) or from leaves infiltrated with SA 10 mM or with H_2O_2 100 mM at indicated times. Northern blots were hybridized with the *tcI 7* full-length cDNA and with the *PR-1b* cDNA probe. Equal loading of rRNA was checked by staining with ethidium bromide.

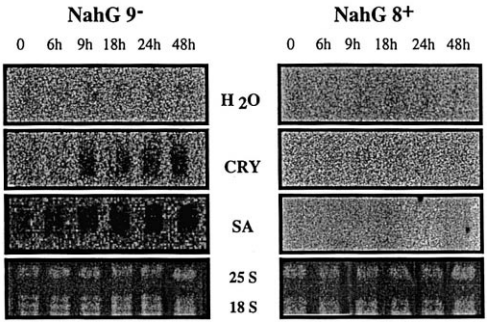

Fig. 5. Effect of salicylic depletion on the accumulation of *tcI 7* transcripts under various stimuli. Young plants of tobacco lines NahG 8^+ and NahG 9^- were treated with water, with 0.1 nmol cryptogein or with 360 nmol SA prior to RNA extraction. Total RNA (6 μg per lane) was extracted at indicated times after the onset of the treatments. Northern blots were hybridized with the *tcI 7* full-length cDNA. Equal loading of rRNA was checked by staining with ethidium bromide. Northern blots were a generous gift from H. Keller [21].

ing the *nahG* gene encoding a salicylate hydroxylase, an enzyme which catabolizes SA to catechol [26]. A transgenic line (NahG 9^-) that contains but does not express this gene was used as a control. Keller and coworkers showed that elicitin-treated tobacco plants expressing the *nahG* gene did not show SAR against infection with virulent pathogens, for example *Phytophthora parasitica* var. *nicotianae*. However, these plants responded to treatment with cryptogein by formation of necrosis. Moreover, elicitin-induced expression of genes encoding several SAR genes such as *PR-1a* and *PR-1b* was suppressed or modified in the NahG plants; the authors concluded that SA was required in elicitin-induced SAR but not in HR-like necrosis.

Fig. 5 shows that accumulation of *tcI 7* transcripts was observed 9 h after treatment with cryptogein only in NahG 9^- tobacco plants that do not express the salicylate hydroxylase and thus are able to accumulate SA after the elicitor treatment. Treatment with SA also induced an accumulation of *tcI 7* transcripts only in NahG 9^- tobacco plants within 6 h of treatment. The level of accumulation is almost the same after cryptogein and SA treatment. On the same Northern blots, Keller and coworkers [21] showed an accumulation of *PR-1b* transcripts activated within 9 h by cryptogein in NahG 8^+ and NahG 9^- but only in NahG 9^- by SA (between 24 and 48 h).

4. Discussion

The results obtained in this work led to a better understanding of the possible involvement of the *tcI 7* gene in the molecular events leading to the establishment of HR and SAR. First, the Northern blot analysis showed that *tcI 7* is strongly activated by cryptogein early before appearance of necrosis. Moreover, cryptogein induces a high activation of *tcI 7* in tobacco plants leading to SAR. The challenge inoculation with Ppn induces a high activation of *tcI 7* expression in tobacco plants pretreated with cryptogein, correlated with a severe reduction of fungal development, but Ppn alone is also able to induce *tcI 7*: at this stage we speculate that induction of *tcI 7* could be closely related to the induction of defense reactions (HR and/or SAR).

We also showed that *tcI 7* was up-regulated by infiltration of exogenous SA in tobacco leaves. Moreover, in the experiments described here, NahG 8^+ plants do not show any induction of *tcI 7* either after cryptogein treatment or after SA treatment. Nevertheless, these plants exhibit a necrosis response (HR) but no SAR after cryptogein treatment. These latter results could indicate that induction of *tcI 7*, encoding a β-subunit of proteasome, could be more involved in SAR than in HR.

A large body of evidence has accumulated suggesting a key role for SA in SAR [29–31]. Although SA is known to regulate transcription in many genes, the molecular events triggered by this molecule at the transcriptional level have to be elucidated. It has been shown that not only plant promoters but also pathogen promoters can be triggered by SA, for example the cauliflower mosaic virus CAMV 35S promoter [32]. It was also shown [33] that the binding of a tobacco cellular factor named SARP (salicylic acid response protein) correlates with the SA-induced activation of transcription. SARP is known to contain proteins immunologically related to TGA1, a transcription factor previously cloned for its ability to bind to the *as-1* element. The 5′ flanking sequence of *tcI 7* shows an *as-1*-like element and several putative binding sites for Myb proteins, redox-regulated factors found in plants and animals. Binding sites for Myb proteins are also present in the promoters of PAL genes whose expression is potentiated by SA [34] but myb-1 by itself may not be sufficient for SA inducibility of genes [28].

Moreover, we also localized a putative sequence that could bind the transcription factor NF-κB. Desikan and coworkers [36] proposed that stimulatory effects of H_2O_2 on PAL and GST mRNA accumulation suggest that there may be transcription factors sensitive to cellular oxidation status, similar to the animal transcription factor NF-κB. The ubiquitin–proteasome pathway is required for processing the NF-κB1 precursor protein and for the activation of NF-κB [37]. Moreover, the activation of NF-κB was shown to be redox-dependent [38]. Conrath and coworkers [39] reported that the transgenic tobacco plants (ubR48) expressing an inhibitor of ubiquitin-dependent protein degradation via the 26S proteasome spontaneously formed necrotic lesions and displayed altered responsiveness to TMV attack. The ubR48 transgenic plants constitutively accumulated enhanced levels of SA and/or its glucoside coinciding with a high level of PR-1. In order to study possible analogies with NF-κB activation and to understand the mechanism responsible for integrated regulation of the *tcI 7* gene, it was now necessary to analyze its putative promoter cloned in this work. First, deletion analysis of the promoter could allow the identification of the minimal region required for expression. Moreover, a strategy of site-directed mutagenesis allowed the study of the putative regulatory sequences separately and in various associations.

Based on experiments with transgenic tobacco and *Arabidopsis* plants, salicylic acid appears to act downstream of H_2O_2 rather than the reverse (for review see [35]). Regarding the results obtained in this work by Northern blot analysis and by gene walking PCR, it is necessary to study how SA and H_2O_2 could interact in activation of the *tcI 7* gene during the onset of defense reactions. Work is in progress to elucidate the role of the oxidative burst in the activation of the *tcI 7* gene after treatment with elicitins or after inoculation with Ppn.

Moreover, all these results prompted us to study more precisely the implication of proteasome in programmed cell death (apoptosis) during the HR and the establishment of the SAR. Nevertheless, the data presented in this work led us to use *tcI 7* as a new marker of SAR and that targeting the proteasome could be a viable approach to elucidate the onset of defense mechanisms.

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