MPMI Vol. 10, No. 8, 1997, pp. 1028–1030. Publication no. M-1997-0916-01O.

For the Record

A Novel Class of Elicitin-like Genes from *Phytophthora infestans*

Sophien Kamoun, Hannele Lindqvist, and Francine Govers

Department of Phytopathology, Wageningen Agricultural University, Wageningen, and Graduate School of Experimental Plant Sciences, The Netherlands

Accepted 4 August 1997.

Elicitins are a family of structurally related proteins that induce hypersensitive response in specific plant species. Two *Phytophthora infestans* cDNAs, *inf2A* and *inf2B*, potentially encoding novel elicitin-like proteins, were isolated from a cDNA library made from infected potato tissue. Multiple sequence alignments and phylogenetic analyses of 19 elicitins and elicitin-like proteins from nine *Phytophthora* spp. and from *Pythium vexans* suggest that there are at least five distinct classes within the elicitin family.

Additional keywords: glycoprotein, serine-threonine rich.

Elicitins are a family of structurally related proteins that induce hypersensitive response in particular plant species, specifically *Nicotiana* spp. in the Solanaceae and *Raphanus* and *Brassica* spp. in the Cruciferae (Bonnet et al. 1996; Kamoun et al. 1993b). Elicitins are secreted by all tested *Phytophthora* and *Pythium* spp. and are thought to be major determinants of the resistance response of *Nicotiana* against most *Phytophthora* spp. (Grant et al. 1996; Yu 1995). Several elicitins were purified from culture filtrates and sequenced (Huet et al. 1995; Pernollet et al. 1993; Ricci et al. 1989). Additionally, genes encoding elicitins were cloned by a combination of polymerase chain reaction amplifications and hybridization methods (Kamoun et al. 1993a, 1997; Mao and Tyler 1996; Panabières et al. 1995). We recently reported the cloning of an elicitin cDNA, *inf1*, selected from a library constructed from RNA isolated from leaves of potato 3 days after inoculation with *P. infestans* 88069 (Kamoun et al. 1997). In addition to cDNA clones containing *inf1* sequences, hybridization of the library with a probe internal to the *parA1* gene of *P. parasitica* (Kamoun et al. 1993a) yielded a total of six weakly hybridizing clones. DNA sequence analysis revealed that these clones contain cDNA sequences of two novel, elicitin-like genes named *inf2A* and *inf2B*. Open reading frames of 558 and 570 bp, corresponding to predicted proteins of 185 and 189 amino acids, were found for *inf2A* and *inf2B*, respectively. Multiple alignments of 19 elicitin and elicitin-like sequences (including INF2A and INF2B) from nine *Phytophthora* spp. and from *Pythium vexans* indicate a high degree of homology between the different proteins (Fig. 1). Similar to elicitins, INF2A and INF2B contain a putative hydrophobic signal peptide of 20 amino acids, followed by a conserved 98-amino-acid elicitin domain (residues 21 to 118). However, in contrast to elicitins, INF2A and INF2B bear additional C-terminal domains of 67 and 71 amino acids, respectively. Remarkably, in both proteins, these C-terminal domains are rich in serine (15 and 16% of the INF2A and INF2B C-terminal domain), threonine (36 and 31%), alanine (27 and 25%), and proline (8 and 9%). This amino acid composition and the distribution of the four residues suggest the presence of clusters of O-linked glycosylation sites (Wilson et al. 1991). Interestingly, similar to INF2, numerous surface and cell-wall-associated proteins consist of a signal peptide and a functional extracellular domain followed by a serine-threonine rich O-glycosylated domain (Jentoft 1990). Such proteins were shown to have a "lollipop on a stick" structure in which the O-glycosylated domain forms an extended rod that anchors the protein to the cell wall, leaving the extracellular N-terminal domain exposed on the cell surface (Jentoft 1990). Future experiments with immunological techniques will help determine whether INF2A and INF2B are indeed surface and/or cell-wall-associated glycoproteins in *P. infestans*.

To further investigate the relationship between INF2 and elicitins, we reconstructed the phylogeny of the elicitin family by the neighbor joining method (Fig. 2). This was done with the programs SEQBOOT (for bootstrap resampling), PROTDIST (for computing distance measures between proteins), NEIGHBOR (for applying the neighbor joining method), and CONSENSE (for computing consensus trees) of the PHYLIP 3.5c software package (J. Felsenstein, University of Washington, Seattle). A total of 1,000 bootstrap replications were conducted to determine the statistical significance of the obtained branches. Even though only the conserved elicitin domains (amino acids 21 to 118 in Figure 1) were compared, the results confirmed the classification expected from the different sizes of the proteins. Based on the phylogenetic analysis and the overall structure of the proteins, we propose to divide elicitins and elicitin-like proteins into five classes. Previously, the major, secreted, 98-amino-acid elicitins of *Phytophthora* were divided into acidic (here named class IA) and basic elicitins (class IB) (Kamoun et al. 1993b; Pernollet et al. 1993; Ricci et al.

Corresponding author: S. Kamoun;
E-mail: Sophien.Kamoun@medew.fyto.wau.nl

Nucleotide sequence data are to be found at GenBank as accession nos. AF004951 for *inf2A* and AF004952 for *inf2B*.

1989). Proteins belonging to these two classes appear on separate clusters in the tree, confirming this division. The acidic elicitins of *P. vexans* (class Py) and the highly acidic elicitin-like proteins of *P. cryptogea,* which possess a short hydrophilic C-terminal tail (class II, Fig. 1), all appear on significantly distinct branches. Similarly, the novel INF2A and INF2B (class III) also appear on a distinct branch and differ significantly from other elicitins and elicitin-like proteins, as illustrated by the 100% stability and the length of the branch. Despite the relatively high amino acid sequence divergence (52 to 56% identity) between the elicitin domain of INF2 and elicitins, the conserved motifs of elicitins, mainly the six cysteine residues and the antiparallel β-sheets, are present in INF2. Therefore, it will be interesting to determine whether INF2 can elicit defense responses in plants similar to elicitins and whether it is related to previously described glycoprotein elicitors from *Phytophthora* (Baillieul et al. 1996).

Another notable feature of the phylogenetic tree is the occurrence of elicitins and elicitin-like proteins from different classes in the same isolate of *P. cryptogea, P. megasperma,* and *P. infestans*. It remains to be determined whether the distribution of the different classes within *Phytophthora* reflects taxonomic associations and/or specific morphological, physio-

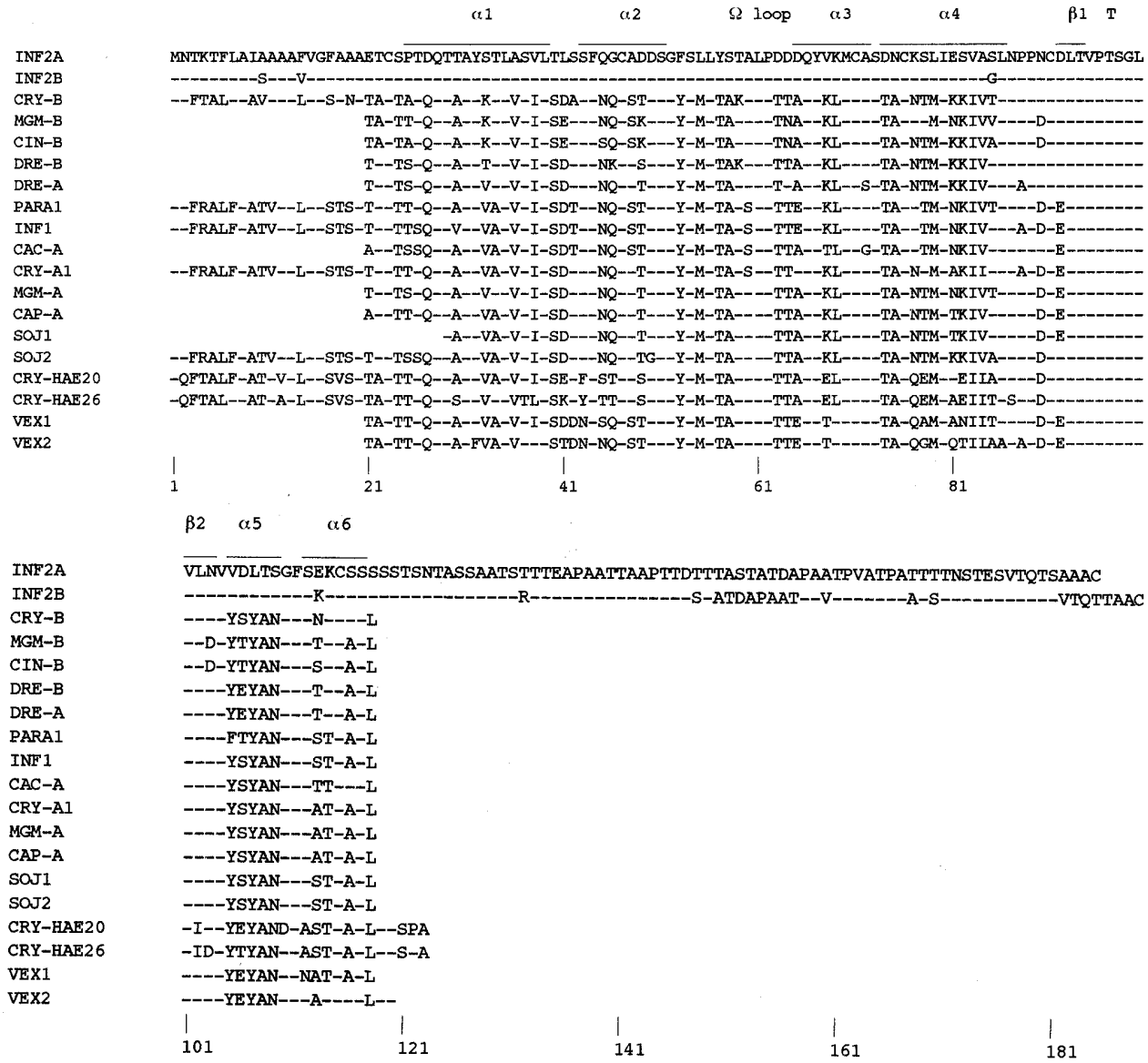

Fig. 1. Multiple alignments of members of the elicitin family from *Phytophthora* spp. and from *Pythium vexans*. Multiple alignments of 19 elicitin and elicitin-like sequences from *Phytophthora infestans* (INF2A, INF2B, and INF1), *P. cryptogea* (CRY-B, CRY-A1, CRY-HAE20, and CRY-HAE26), *P. megasperma* (MGM-B and MGM-A), *P. cinnamomi* (CIN-B), *P. drechsleri* (DRE-B and DRE-A), *P. parasitica* (PARA1), *P. cactorum* (CAC-A), *P. capsici* (CAP-A), *P. sojae* (SOJ1 and SOJ2), and *Pythium vexans* (VEX1 and VEX2) (Huet et al. 1995; Kamoun et al. 1993a, 1997; Mao and Tyler 1996; Panabières et al. 1995; Pernollet et al. 1993; Ricci et al. 1989) were conducted with the program CLUSTAL-W (J. D. Thompson et al., EMBL, Heidelberg, Germany). The secondary structure elements indicated above the sequences correspond to CRY-B as described in Boissy et al. (1996). Residue numbers are indicated under the sequences. The signal peptide sequences (residues 1 to 20) of some elicitins are unknown since only mature proteins were sequenced. The first eight residues of SOJ1 were not determined (Mao and Tyler 1996).

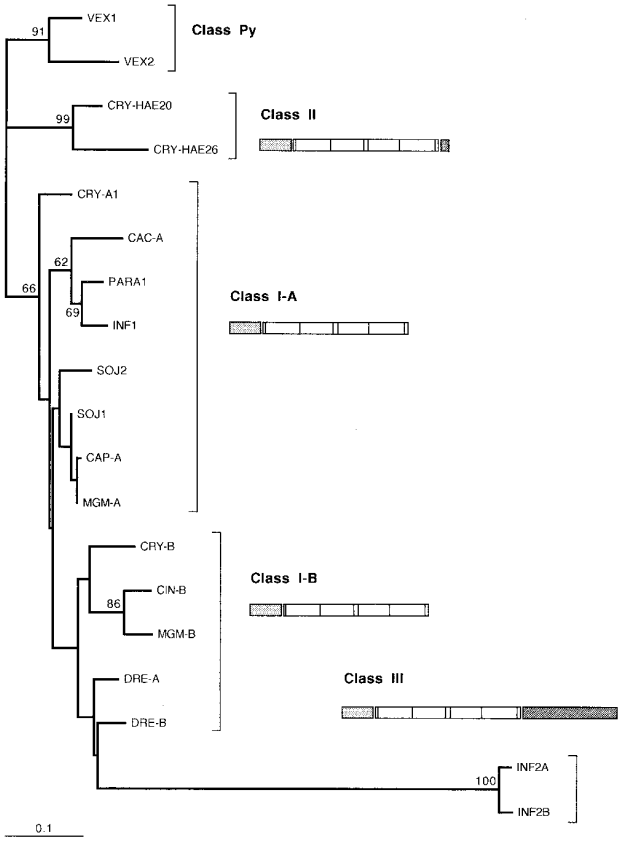

Fig. 2. Phylogeny reconstruction of the elicitin family from *Phytophthora* spp. and *Pythium vexans*. The phylogenetic tree was constructed by the neighbor-joining method based on residues 21 to 118 (elicitin domain) of the multiple alignment of elicitin sequences shown in Figure 1. Bootstrap values above 50% from 1,000 replications are indicated at the nodes. The length of the branches reflect weighted amino-acid substitutions, and the scale bar represents 10% weighted sequence divergence. VEX1 and VEX2 were used as outgroups. The five classes representing main clusters of the tree are indicated and schematic structures of the proteins are shown alongside. The signal peptide (shaded boxes), the elicitin domain (open boxes), and the C-terminal domain (black boxes) are indicated. Vertical bars in the elicitin domain represent conserved cysteine residues.

logical, or virulence traits. This may help unravel the function of this complex family of proteins in *Phytophthora* and *Pythium*.

ACKNOWLEDGMENTS

We thank Tony van Kampen for technical assistance. This work was supported by the Netherlands Technology Foundation (STW) and was coordinated by the Life Sciences Foundation (SLW) (project 805-22-767).

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