Cloning and Characterization of the Cysteine Proteinase Inhibitor (CPI) Gene Family in Tobacco (*Nicotiana tabacum* L.)

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Abstract

In plants, cysteine proteinase inhibitors (CPI or cystatin) are implicated in biotic and/or abiotic stress responses, and developmental regulation. Using the techniques of RT-PCR and SMART RACE, full-length cDNAs of four *CPI* genes were cloned for the first time from *Nicotiana tabacum* L. cv. K326, respectively, named *NtCPI1*, *NtCPI2*, *NtCPI3* and *NtCPI4*. Their sequences had been deposited in GenBank, with accession Nos KF057988, KF057989, KF057990 and KF057991. Genomic DNA sequences analysis showed that *NtCPI1* and *NtCPI2* each had a single intron, while others had no intron. The four genes were predicted products of 98, 98, 120 and 123 amino acid residues, respectively. In addition to the typical inhibitory motifs, namely the central signature motif QXVXG, a GG doublet in the N-terminal region, and A/PW residues in the C-terminal part, these deduced amino acid sequences contained the PhyCys-specific LARFAV-like motif in the N-terminal region, of which a N-terminal signal peptide of 27 residues was found in both NtCPI3 and NtCPI4. Meanwhile, the transcripts of the four genes were found in roots, stems, leaves and buds by real-time quantitative PCR, which indicated that they were broadly expressed in tobacco. This study had laid the foundation for further exploring the physiological functions of these cysteine proteinase inhibitor genes in plants.

Introduction

Plant cystatins are called phytocystatins (PhyCys) and have been described as plant inhibitors of papain-like cysteine proteinases. The cystatin inhibitory mechanism is produced by a tight and reversible interaction with their target enzymes. Two possible roles have been proposed for these plant inhibitors: (i) to act as regulators of proteolysis during seed development and germination, organogenesis, programmed cell death, fruit development; and (ii) to contribute to plant defense by inhibiting exogenous proteases, such as those from insect pests and nematodes, and furthermore provide defense against phytopathogenic fungi and bacteria.

Despite the important roles played by PhyCys proteins in diverse biological processes, to our knowledge, there has been no report related to the cloning of tobacco cys genes. Here we report the cloning of cDNA sequences of four tobacco cys genes, the bioinformatics analysis of these sequences and the expression detection of cys genes in in roots, stems, leaves and buds.

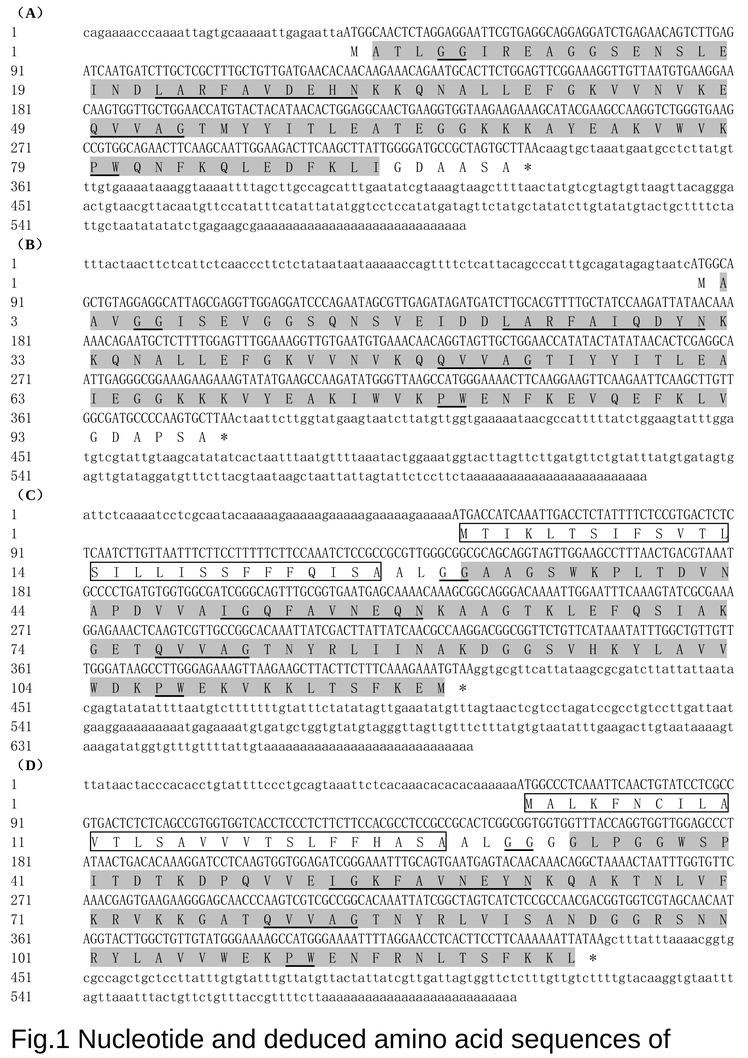

Fig.1 Nucleotide and deduced amino acid sequences of *NtCPI1* (A), *NtCPI2* (B), *NtCPI3* (C) and *NtCPI4* (D)

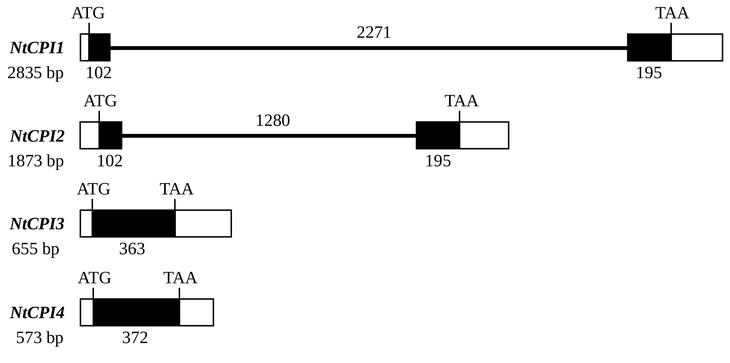

Fig.2 Comparison of the genomic organization of CIP genes

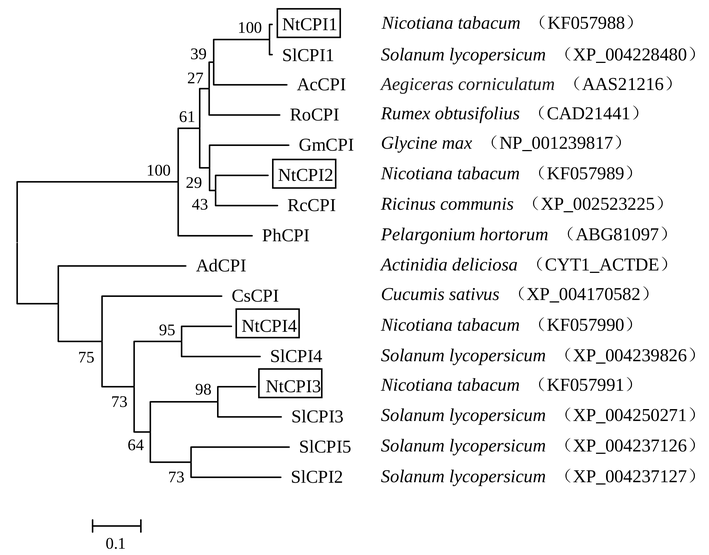

Fig.3 Phylogenetic analysis of amino acid sequences of CPIs from tobacco and other species

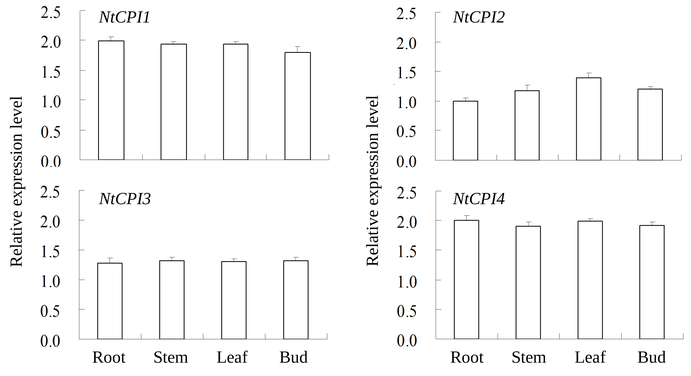

Fig.4 Expression characteristics of four CIP genes in different tissues of tobacco

Conclusions

1. Four *CPI* genes were cloned for the first time from *Nicotiana tabacum* L. cv. K326, respectively, named *NtCPI1*, *NtCPI2*, *NtCPI3* and *NtCPI4*.
2. The four genes were predicted products of 98, 98, 120 and 123 amino acid residues, respectively, with the typical inhibitory motifs of phytocystatin superfamily, namely the central signature motif QXVXG, a GG doublet and LARFAV-like motifs in the N-terminal part, and conserved A/PW residues in the C-terminal region.
3. The four *NtCPI* genes were found to be broadly expressed in different organs including roots, stems, leaves and buds.