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Molecular cloning and functional characterization of a cystatin from *Brassica rapa* L.

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Objectives

We have tried to isolate cysteine protease inhibitor from *Brassica rapa* L and conduct molecular analysis using its gene

Materials and Methods

1. Materials: Flower tissue of *Brassica rapa* L(cv. Osome)
2. Methods: Sequence, RT-PCR analysis, Over-expressed in *Escherichia coli*, Reverse zymogram assay

Results and Discussion

A cDNA fragment encoding the cysteine protease inhibitor, cystatin, was cloned from cabbage (*Brassica rapa* L.) flower tissue. This full length cDNA fragment was comprised 638 nucleotides, consisting of a 67-nucleotide 5' untranslated region, open reading frame of 408 nucleotides, and a 163-nucleotide 3' untranslated region. Its deduced protein sequence compared 135 amino acid residues without any cysteine with a predicted molecular mass of 15.5kDa and a calculated isoelectric point (pI) 4.6, and it shared 50-70% identity to the reported phytocystatins. Also this protein contains besides a C-terminal extension, several motifs conserved in all members of the phytocystatin (PhyCys) superfamily: (i) a GG and LGRYCV-like motifs towards the n-terminal part of the protein; (ii) the reactive site QVVAG, and (iii) a conserved PW, downstream of the reactive site. RT-PCR analyses indicated that this gene was expressed in fully expanded leaves, in flower tissues, but not in the early development stages. This gene was constructed in a fusion vector and was easily over-expressed in *Escherichia coli*; satisfactory over-expression of nonfusion cystatin was achieved after an additional start codon was inserted prior to its coding sequence. A reverse zymographic assay showed that both recombinant proteins were functionally active as phytocystatins and were resistant to papain digestion in a gel. However, no inhibitory activity against bromelain was apparent by the reverse zymogram assay. This recombinant protein was a good inhibitor of the in vitro growth of phytopathogenic fungi *Botrytis cinerea* and *Fusarium oxysporum*.

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