Cloning and functional analysis of type I and type II metacaspases during flower senescence in *Petunia x hybrida* cv. Mitchell Diploid

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Abstract
Senescence is a highly regulated process and the final stage of plant development, which ultimately results in the programmed death of cells, organs, tissues or whole plants. Caspases are key regulators of the cell death program in animals, but to date no homologs of caspases have been found in plant genome databases. While caspase-like activity has recently been demonstrated in various plant cell death models, the corresponding genes for these activities have never been identified. While a caspase related family of proteases (metacaspases) has been identified in plant and fungal genomes using iterative PSI-BLAST, the function of metacaspases in plants is still largely unknown. As a first step to understanding the role of metacaspases, a type I (*PhMCA1*) and a type II (*PhMCA2*) metacaspase have been cloned from *Petunia x hybrida* cv. Mitchell Diploid. The expression of *PhMCA1* and *PhMCA2* has been investigated in various petunia tissues using real-time RT-PCR.

Results

**Fig. 1.** Phylogenetic tree of the metacaspases of Norway spruce, tomato, Arabidopsis and petunia

The phylogenetic tree was constructed by MEGA3 software. Numbers in parentheses correspond to loci for Arabidopsis and Genbank accession numbers for Norway spruce (mcII-Pa) and tomato metacaspases (LeMCA1) indicated by *.

**PhMCA1** is a type I metacaspase and **PhMCA2** is a type II metacaspase.

**Figure 4.** Relative expression of *PhMCA1* and *PhMCA2* in *Petunia x hybrida* cv. Mitchell Diploid (MD) and *etr1-1* during corolla senescence
metacaspases during flower senescence, a type I and a type II metacaspase were cloned from petunia and their expression in various organs, under biotic stress (Botrytis cinerea infection) and by ethylene was investigated.

Materials and Methods

Plant materials

_Petunia x hybrida_ cv. Mitchell diploid and ethylene insensitive transgenic petunias (35S::etr1-1, line 44568) were grown in the greenhouse and used for all real time RT-PCR analysis.

Cloning of PhMCA1 and PhMCA2

EST sequences of PhMCA1 and PhMCA2 were obtained from Computational Biology and Functional Genomics laboratory (http://combio.dfc.i.cornell.edu/tgi/) and SOL genomics network (http://www.sgn.cornell.edu) websites. 5‘ and 3’ RACE (Random Amplification of Complementary Ends) was performed to clone full length cDNAs using primers designed from the EST sequences. Sequencing was performed at the MCI (Molecular Cellular Imaging Center) at OARDC.

Real time RT-PCR

Total RNA treated with RNase-free DNase (Promega) isolated from _Petunia x hybrida_ cv. Mitchell Diploid corollas, anthers, ovaries, roots, leaves and stems was used to synthesize cDNA by reverse transcription reaction. The relative expression levels were analyzed and normalized to the petunia actin gene (Phactin).

Botrytis cinerea infection

_B. cinerea_ was cultured and inoculated as described by Benito et al. (1998) onto petunia leaves. Conidia were harvested from sporulating plates by washing with sterile water and conidial suspension was washed three times by centrifugation (8 min, 114 g) and resuspended in Gamborg’s B5 basal medium, including vitamins, glucose (10 mM), and sodium phosphate buffer (10 mM; pH 6.0). 8 week old leaves were removed from plants and petioles were inserted in microcentrifuge tubes filled with water. Tubes containing leaves were kept in a plastic box with high humidity. Leaves were inoculated with conidial suspensions, then leaves were dried at room temperature for 30 min. The plastic container was closed and incubated at 20°C with a 16 h photoperiod.

Relative expression levels of _PhMCA1_ and _PhMCA2_ were measured by real time RT-PCR as described in Materials and Methods. The data shown are means and standard errors. Corolla development stages and number of days after flower opening are indicated as shown. ND = not determined

**Expression of PhMCA1 was increased during corolla senescence in MD, but was low throughout corolla development in ethylene insensitive petunia (etr1-1). PhMCA2 is expressed at the early stage of corolla development in both MD and etr1-1.**

Conclusions

-A type I and a type II metacaspases were cloned and sequenced from _Petunia x hybrida_ cv. Mitchell Diploid.
-Both metacaspases (PhMCA1 and PhMCA2) contain the catalytic residues (His and Cys) that are highly conserved among caspases.
-The transcript abundance of the type I metacaspase (PhMCA1) increases during flower senescence, while the type II metacaspase (PhMCA2) was upregulated following _Botrytis cinerea_ infection.
-Ethylene appeared to be required for the expression of PhMCA1 and PhMCA2 expression may be regulated by ethylene and additional signals during corolla senescence.

Literature Cited


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