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Molecular Characterization of Cinnamate 4-Hydroxylase Gene Family in Red Hot Pepper (*Capsicum annuum* L.)

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Objectives

Cloning of Cinnamate 4-hydroxylase genes involved in the capsaicin biosynthetic pathway of red hot pepper and characterization of the expression pattern.

Materials and Methods

1. Materials

Plant – *Capsicum annuum* L.

2. Methods:

Genomic southern and northern blot hybridization were carried out as described by Sambrook and Russell (2001). C4H cDNA fragments were isolated from the cDNA libraries constructed with total RNA of root tissues infected by a typical fungal pathogen, *Phythophthora capsici*. The probe fragment was obtained by PCR with a degenerated primer set and total cDNA fractions as a template. Tissue- and wound-specific expression pattern was examined by RT-PCR technique.

Results and Discussion

Genomic southern blot hybridization indicates that there are at least more than three different isoforms of C4H in red hot pepper. We initially isolated three different isoforms of C4H cDNA fragments that are highly homologous (over 95.8%) each other and designated as PC4H-1, PC4H-2, and PC4H-3, respectively. They are also highly homologous to most of other plant's known C4H sequences (over 84% identity) but share only 59 to 61% amino acids identity with C4H1 of *Citrus sinensis* and *Phaseolus vulgaris*, respectively. There are a long stretch of hydrophobic amino acids (30 a.a), followed by the (P/I)PGPX(G/P)XP consensus sequence which is highly conserved amongst plant P450s. The result of northern blot hybridization with the 3'-UTR region of PC4H-2 as a probe indicates that the transcripts of PC4H-2 are highly abundant in the seeds of pepper fruit but none or only basal levels are detected in other tissues. In addition, the results of RT-PCR with gene-specific primer sets suggested that the transcript levels of PC4H-1 and PC4H-2 are highly induced in fruit and root after 6 hrs after wounding whereas no such a dramatic induction pattern is observed in leaves and stem tissues. In case of PC4H-3, however, almost none or very low basal levels of transcripts are examined and does not respond at all to the mechanical wounding.

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