9 Structure and Expression Pattern of Antifungal Protein cDNA Clone from Capsicum annuum L.

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An antifungal protein cDNA clone was isolated by screening cDNA library constructed from Capsicum annuum L. The clone, termed pCaAFP, encodes for 85 amino acids including 8 cysteine residues as a full insert size of 509 bp. The putative protein was consisted of three domains: signal peptide, chitin binding domain, and C-terminal peptide. The deduced amino acid sequence of chitin binding domain from pCaAFP showed 92% and 84% similarity with same domain from other species, Pn-AMPs and hevein. Southern blot analysis of genomic DNA showed that AFP gene existed as a single copy. Northern blot analysis revealed that CaAFP mRNA was expressed in the tissue-specific and developmentally regulated manner but the gene was not induced by some chemicals known for induction PR protein and wounding treatment. For its functional analysis, AFP gene was cloned into an E. coli expression vector, pET-30b(+), under the control of T7 promoter. Expressed fusion protein was purified by using His tag affinity column chromatography and tested on several fungal species. Purified AFP fusion protein inhibited the germination and appressorium formation of several plant pathogenic fungi. Therefore the protein encoding pCaAFP may be a hevein-type protein with fungicidal function. Localization and the functional analysis of the protein are being undertaken to elucidate its function in hot pepper.

Keywords: Capsicum annuum, AFP, hevein type, fungicidal effect