8. Functional analyses of pepper pathogenesis-related protein 1 and chitinase in disease resistance and abiotic stress tolerance of *Capsicum annuum* and *Arabidopsis thaliana*

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Pathogenesis-related (PR) genes are induced and accumulated in plant tissues infected by pathogens. The PR proteins are characterized by several biochemical properties, i.e., low molecular weight, stability at low pH, resistance to proteases. Their functional roles during pathogenesis and defense response have been suggested in a variety of plant species (Van Loon and Van Strien, 1999). The PR proteins are classified into 14 groups (PR-1 to PR-14) based on their structural homologies within the groups (van Loon and van Strien, 1999). Most PR proteins exhibit induced systemic expression in the uninoculated plant tissues around the infection site and their direct antimicrobial activities *in vitro*. However, the biological functions of these PR proteins during the defense reactions are not yet clearly understood.

Numerous pathogenesis-related genes were differentially expressed in pepper leaf and stem tissues during the compatible and incompatible interactions with *Xanthomonas campestris* pv. *vesicatoria* and *Phytophthora capsici*. It was shown previously that the *CABPR1* and *CAChi2* genes encoding a basic PR-1 protein and class II basic chitinase, respectively, are differentially expressed in pepper leaf and stem tissues during the compatible and incompatible interactions with *Xanthomonas campestris* pv. *vesicatoria* and *Phytophthora capsici* (Hong et al.2000; Kim and Hwang, 2000). The rapid and strong induction of the *CABPR1* and *CAChi2* expression in the incompatible interactions indicated that CABPR1 and CAChi2 might play a critical role in the active defense responses to pathogen infection. The *CABPR1* and *CAChi2* genes also were ethylene-inducible in pepper leaf tissues. The *CABPR1* and *CAChi2* transcripts were predominantly expressed in the phloem cells of the stem tissues infected by the *Phytophthora capsici*(Lee et al., 2000a; Lee et al., 2000b). The PR-1 proteins were predominantly located in the intercellular spaces of the pepper stem tissues infected by *P. capsici*. Chitinase specifically targeted the cell wall of virulent and avirulent isolates of *Phytophthora capsici* in the pepper stem tissues.

Activation of pepper basic PR-1 gene promoter during defense signaling to pathogen, abiotic and environmental stresses

CABPR1 promoter region was isolated and functionally characterized in tobacco leaves to identify the cis-acting regulatory sequences that are involved in *CABPR1* gene expression. Constructs harboring the 5'-serially deleted *CABPR1* promoter, which was fused to the β-glucuronidase (GUS) gene, were evaluated for their promoter activity in the tobacco leaves. The *CABPR1* promoter of 1670 bp in size was locally or systemically induced during a compatible interaction with *Pseudomonas syringae* pv. *tabaci*. The *CABPR1* promoter also was differentially activated by treatment with ethylene, salicylic acid, nitric oxide, high salinity, drought and low temperature. The expression of the pepper transcription factors, CAZFP1 and CARAV1, activated the *CABPR1* promoter. Analyses of a series of 5'-deletions of the *CABPR1* promoter indicated that novel cis-acting elements are essential for induction by pathogen and abiotic elicitors are localized in the region between -1670 bp and -1466 bp upstream from the translation start site. These results suggest that *CABPR1* promoter activation is essential for regulating *CABPR1* gene expression in response to pathogen, abiotic and environmental stresses, possibly by transactivating the CAZFP1 and CARAV1 transcription factors. The cis-acting elements essential for the differential response to various stresses may exist in the different *CABPR1* promoter regions.

Induction of enhanced disease resistance and oxidative stress tolerance by overexpression of pepper basic PR-1 gene in *Arabidopsis*

The pathogen- and ethylene-inducible pepper basic PR-1 gene, *CABPR1*, was strongly expressed in pepper leaves by osmotic and oxidative stresses. The pepper *CABPR1* was introduced into the *Arabidopsis* plants under the control of the cauliflower mosaic virus 35S promoter. PCR-amplification with the *Arabidopsis* genomic DNA and Northern blot analyses confirmed that the pepper *CABPR1* gene was integrated into the *Arabidopsis* genome, where it was overexpressed in the transgenic *Arabidopsis* plants under normal growth conditions. The constitutive overexpression of *CABPR1* induced the expression of the *Arabidopsis* PR-genes including *PR-4*, *PR-5* and *PDF1.2*. Enhanced resistance to phytopathogenic bacteria, *Pseudomonas syringae* pv. *tomato* DC3000, was also observed in the transgenic *Arabidopsis* plants. *CABPR1* overexpression in the transgenic *Arabidopsis* caused enhanced seed germination under NaCl (ionic) and mannitol (non-ionic) osmotic stresses. High salinity and dehydration stresses during seed germination of the transgenic plants were not found at the early seedling stage. The

transgenic *Arabidopsis* plants exhibited a higher tolerance to oxidative stress by methyl viologen at the seed germination, seedling and adult plant stages. These results suggest that the *CABPR1* gene may function in the enhanced disease resistance and oxidative stress tolerance of transgenic *Arabidopsis* plants.

Induction by pathogen, salt and drought of a basic class II chitinase mRNA and its in situ localization in pepper (*Capsicum annuum*)

Northern blot and *in situ* hybridization analyses revealed that a pepper basic class II chitinase gene (*CAChi2*) is constitutively expressed in floral organs and root endodermis, but not in leaf, stem and fruit of pepper. Resistance of pepper leaves to *Colletotrichum coccodes* infection at a late growth stage was correlated with induction of β-1,3-glucanase and PR-1 mRNA, but not of chitinase (*CAChi2*) mRNA. Transcriptional activation of the *CAChi2* gene in pepper leaves occurred during anthracnose development. The *CAChi2* transcripts were mainly localized in phloem cells of vascular tissues of pepper leaves infected with *C. coccodes*. The *CAChi2* gene was also differentially induced in leaf and stem tissues by treatment with abscisic acid (ABA), sodium chloride or drought. Strong accumulation of the *CAChi2* transcripts occurred in pepper stem tissues due to high salt and drought and also due to treatment with ABA. Theses results suggest involvement of the chitinase gene in protection of pepper plants against the pathogen, but also document cross-talk with stress signals mediated by ABA, high salinity and drought.

Promoter activation of pepper class II basic chitinase gene, *CAChi2*, and enhanced bacterial disease resistance and osmotic stress tolerance in the *CAChi2*-overexpressing *Arabidopsis*

The *CAChi2* promoter activation by bacterial infection and osmotic stresses was examined using the Agrobacterium-mediated transient expression assay. Several stress-related cis-acting elements were revealed within the upstream genomic sequence of the *CAChi2* gene. In tobacco leaf tissues transiently transformed with the *CAChi2* promoter--glucuronidase (GUS) gene, the *CAChi2* promoter was up-regulated by *Pseudomonas syringae* pv. *tabaci* infection. The *CAChi2*-GUS activation was closely related to osmotic stresses such as mannitol and NaCl treatments. The -378 *CAChi2* promoter was sufficient for the *CAChi2* gene induction by salicylic acid treatment. The *CAChi2* overexpression in the transgenic *Arabidopsis* plants enhanced bacterial disease resistance against *Pseudomonas syringae* pv. *tomato* infection. The *CAChi2* overexpressing-

Arabidopsis plants also exhibited increased tolerance to NaCl and mannitol-induced osmotic stress during seed germination and seedling growth. The *CAChi2* overexpression induced expression of the NaCl stress-responsive gene *RD29A* in the absence of NaCl stress. The CAChi2-overexpressing transgenic plants exhibited increased sensitivity to abscisic acid during seed germination.

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