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Isolation and functional analysis of defense-related genes in pepper (*Capsicum annuum*)

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Plants defend themselves against pathogen attack by activating a multicomponent defense response. The activation of the plant defense response requires recognition of the pathogen and initiation of signal transduction processes that finally result in a spatially and temporally regulated expression of individual defense reactions. One of the most-studied plant defense responses is the synthesis of defense-related proteins. These include resistance genes involved in gene-for-gene interactions leading to hypersensitive cell death, genes encoding pathogenesis-related (PR) proteins, enzymes involved in the generation of phytoalexins, the enzymes of oxidative stress protection, lignification and others. Many of these genes are upregulated when the plant is attacked by pathogens. Just how many inducible defense genes exist in plant genomes is difficult to estimated, because it is likely that some, if not many, of these genes have dual or multiple functions.

We are studying the molecular basis of pathogen recognition, signal transduction and gene expression changes involved in disease resistance of pepper. Towards this goal, we use various approaches including molecular biology, biochemistry and more recently functional genomics to examine the interaction between pepper (*Capsicum annuum*) and *Xanthomonas campestris* pv. *vesicatoria*, *Phytophthora capsici*, *Colletotrichum coccodes* or *C. gloeosporioides*. Previous work demonstrated that some PR proteins such as chitinases and ß-1, 3-glucanases are greatly induced and accumulated in pepper leaves or stem infected by *X. campestris* pv. *vesicatoria* or *P. capsici*. These hydrolases purified from pepper leaves or stem tissues have antifungal activity *in vitro* against some fungal pathogens. To date, we are isolating specific defense-related genes, recently isolated Capsicum annuum-induced (CAI) genes encoding PR-1, chitinase, ß-1, 3-glucanase, thionin, lipid transfer protein, leucines-rich-repeat (LRR) protein, stellacyanin, cyclophilin, chitin-binding protein, SAR8.2, ascorbic peroxidase, thioredoxin peroxidase, peroxidase, etc. were identified. *In situ* hybridization revealed that some PR genes encoding PR-1, chitinase and thionins are expressed in the phloem areas of vascular bundles in pepper stem tissues infected by *P. capsici*, but especially strongly in the incompatible interaction. Many of the inducible, defense-related genes of pepper appears to be regulated by signal pathways involving one or more of

regulators ethylene, Jasmonic acid (JA) and salicylic acid (SA). There is much cross-talk between signal pathways leading to inducible defense gene expression. The regulators JA, SA and ethylene control and potentiate activities of each other. In particular, pepper leucines-rich-repeat (LRR) protein gene bas been demonstrated to mediate recognition and interaction in the pepper extracellular matrix as a component of a signal transduction pathway during the pathogen infection. It has also been suggested that pepper ascorbate peroxidase and thioredoxin peroxidase genes may function as regulators of H2O2 and peroxidase activity during the HR to incompatible pathogen interaction of pepper plant. Further screens and functional analyses of other inducible defense-related genes in pepper are in progress.

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