

Functional characterization of chili pepper transcription factors associated with disease resistant responses

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During the long process of host-pathogen co-evolution, plants have developed various elaborated mechanisms to destroy pathogen attack. Upon pathogen infection, plants activate a number of early responses that lead to the activation of genetically determined mechanisms. One of the most efficient resistance responses raised by pathogen attack is the hypersensitive response (HR), which is characterized by rapid and localized cell death at the site of pathogen invasion. The HR is often associated with the activation of defense response that leads to the development of systemic acquired resistance (SAR). SAR develops in the uninfected parts of locally infected plants, which provides pre-formed resistance against further infection by a broad spectrum of pathogens.

The initial perception and early signal transduction events may be distinct for various pathogen elicitors. However, the molecular events that follow pathogen recognition are not completely understood, but it is generally accepted that plants activate a large number of defense-related genes that are not usually expressed under normal growth conditions. Furthermore, many defense signals are possibly integrated into a few terminal pathways that lead to the transcriptional activation of defense genes in cells. In some cases, pathogen-induced or salicylic acid, ethylene/jasmonic acid-activated transcription factors may play an important role in controlling defense gene expression and resistance responses. The recently identified four major families of plant transcription factors associated with plant defense responses are AP2/EREBP, bZIP, Myb, and WRKY transcription factors. Ectopic expression of some of these transcription factors improves the resistance to pathogen attack, suggesting that these factors participate in defense mechanisms. However, very few transcription factors that respond specifically and directly to pathogens have been characterized in plants.

We focused on the isolation and functional analysis of transcription factors that are involved in plant defense against pathogen attacks. Toward the understanding of regulatory mechanisms in defense response of plant-pathogen interactions, we have performed experiments for isolation of a mass of genes expressed during plant defense

responses in interaction of between hot pepper plant (*Capsicum annuum* cv. *Bugang*) and nonhost pathogens (*Xanthomonas axonopodis* pv. *glycines* Xag 8ra). My laboratory generated 9,000 ESTs from hot pepper, extensively analyzed their sequences using Arabidopsis database (<http://mips.gsf.de/desc/thal>), and applied various sequence analysis tools and methods. A number of transcription factors were selected from the EST sequence database (<http://plant.pdrc.re.kr/>) for study the expression profiles.

First, we have identified the TFIIIA-type Cys2/His2 zinc finger transcription factor (CaPIF1) as a first time in our attempt to defense mechanism against pathogen attack in plant. The first TFIIIA-type zinc-finger protein in plants, EPF1, was identified in *Petunia*. EPF1-type genes encode DNA-binding proteins containing the Cys2/His2 zinc finger (CX2CX3FX3QALGGHX3H). In plants, the zinc fingers in these proteins bear a conserved QALGGH motif that is believed to function in floral organ-specific transcriptional regulation. Interestingly, this conserved sequence motif has not been reported to occur in any organisms other than plants. In several plants, many of EPF-type of zinc finger proteins has been also identified in the regulation of important biological processes that are unique, such as flower development, nitrogen fixation, and responses to environmental signals. So far, the roles of EPF-type zinc finger proteins during pathogen infection have not been determined. We isolated a novel pathogen-responsive transcription factor from hot pepper plant that we called CaPIF1. This gene identified a putative nuclear protein localized in the nucleus. Interestingly, transgenic tobacco plants overexpressing CaPIF1 showed increased resistance to challenge with bacterial pathogens. These plants also showed constitutive upregulation of multiple defense-related genes. Moreover, virus-induced gene silencing (VIGS) of the CaPIF1 ortholog in *Nicotiana benthamiana* enhanced susceptibility to same bacterial pathogens. To date, this is the strong evidence showing that EPF-type zinc-finger proteins play an essential role in defense and the expression of PR- and defense-related genes in plants.

Second, we have reported on the isolation and characterization of a *Capsicum annuum* NAC domain gene, CaNAC1, which is induced by the infection of bacterial and viral pathogens during resistant interactions. The NAC domain-proteins, including NO APICAL MERISTEM (NAM), ATAF1, 2 and CUP-SHAPED COTYLEDON (CUC2), is widely distributed to a large multigene family with very diverse functions in plants but so far, has not been found in other eukaryotes. Recently, the NAC proteins has been demonstrated that it confer regulation of the plant specific biological process that are unique, such as development of a shoot apical and floral meristem, lateral root formation in auxin signaling, leaf senescence, geminivirus DNA replication, pathogen and wound. However, the roles of NAC domain transcription factors during pathogen infection are largely unknown. We report the isolation and characterization of a

CaNAC1 gene that is preferentially induced by infection with avirulent bacterial or viral pathogens. Moreover, this gene was also induced by signaling molecules, such as salicylic acid and ethylene that accumulate in plants during pathogen infection. We propose that the *CaNAC1* gene is involved in signal transduction pathways leading to defense responses of pepper plants against pathogen infection.

In summary, we aim to identify and characterize plant transcription factor for biological processes involved in activation of plant defenses after pathogen attack. We have already demonstrated that 1) a plant EPF-type zinc-finger protein, CaPIF1, involves in defense against pathogens 2) expression of a novel NAC domain-containing transcription factor (*CaNAC1*) is preferentially associated with incompatible interactions between chili pepper and pathogens. The roles of pathogen-responsive two transcription factors in the plant defense response against pathogen will be discussed further.

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