7. Role of Host Cellular Factors and Pepper SAR8.2 in the Establishment of Systemic Acquired Resistance in *Capsicum annuum*

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Introduction

Plants possess inducible defense systems to withstand attack by pathogens. The induction of systemic acquired resistance (SAR) in plants results in the development of a long-lasting, broad spectrum immunity in non-infected tissues against a broad range of plant pathogens, including viruses, bacteria and fungi (Ross 1961; Ryals et al.1996). The level of protection may vary depending on the organism used for the primary inoculation and particularly on the extent of the necrosis. Although the parameters that are critical for the induction of resistance are still not understood, a common feature of most inducing treatments appears to be the local development of necrosis. SAR is associated with a rapid increase in the oxidative burst, such as the generation of superoxide (O_2^-) and subsequent accumulation of hydrogen peroxide (H_2O_2) in plant tissues (Lamb and Dixon 1997; Alvarez et al. 1998). The reactive oxygen species (ROS) induce the coordinate expression of a set of so-called SAR genes. In tomato plants, H_2O_2 has been demonstrated to function as a second messenger mediating the systemic expression of various defense-related genes (Orozco-Cardenas et al., 2001). In addition, the oxidative burst in incompatible pathogen-challenged *Arabidopsis* leaves was found to activate a secondary systemic burst in distal parts of the plants, leading to establishment of systemic immunity via the expression of defense-related genes (Alvarez et al., 1998). However, whether or not H_2O_2 is the primary signal that induces defense-related genes or their interactions with other signal intermediates such as salicylic acid (SA), jasmonic aicd (JA) or ethylene in the establishment of SAR is not yet known.

Furthermore, SAR is associated with the coordinate expression of a set of so-called SAR genes (Ward et al. 1991). These SAR genes include genes encoding pathogenesis-related (PR) proteins. The PR proteins include enzymes that not only modify the cell wall (chitinase and -1,3-glucanase) (Schroder et al. 1992), but also have antimicrobial activity thaumatin group (Woloshuk et al. 1991)). Another groups of

defense-related proteins are thionins (Bohlmann and Apel 1987, Bohlmann 1994), defensins (Broekaert et al. 1995) and SAR8.2 (Ward et al. 1991). SAR8.2 comprises a small gene family that is induced by all of the resistance-inducing stimuli. SAR8.2 was found to be induced to a lesser extent but faster in tobacco than the other SAR-related genes (Ward et al. 1991).

Induction of systemic acquired resistance in pepper plants

Plants infected by pathogens undergo reprogramming during initiation of both local defense and systemic acquired resistance (SAR), accompanied by the molecular and biochemical events, including expression of defense-related genes, oxidative burst and structural barriers. The inoculation of primary pepper leaves with an avirulent strain of *X. campestris* pv. *vesicatoria* induced systemic acquired resistance (SAR) in the non-inoculated, secondary leaves. This SAR response was accompanied by the systemic expression of the defense-related genes, a systemic microoxidative burst generating H_2O_2, and the systemic induction of both ion-leakage and callose deposition in the non-inoculated, secondary leaves. Some defense-related genes encoding PR-1, chitinase, osmotin, peroxidase, PR10, thionin, and SAR8.2 were markedly induced in the systemic leaves. The conspicuous systemic accumulation of H_2O_2 and the strong increase in peroxidase activity in the pepper leaves was suggested to play a role in the cell death process in the systemic micro-hypersensitive responses (HR), leading to the induction of SAR. Treatment of the primary leaves with diphenylene iodinium (DPI), an inhibitor of oxidative burst, substantially reduced the induction of some of the defense-related genes, and lowered the activation of the oxidative bursts in the systemic leaves distant from the site of the avirulent pathogen inoculation and subsequently SAR. Overall, these results suggest that the induction of some defense-related genes as well as a rapid increase in oxidative burst is essential for establishing SAR in pepper plants.

Expression of pepper SAR8.2 gene

Pepper SAR8.2 genes, designated *CASAR82A*, *B* and *C* that are induced by all the biotic and abiotic stresses were isolated from a pepper cDNA library constructed with the mRNAs from pepper plants infected with *Xanthomonas campestris* pv. *vesicatoria*. The pepper *CASAR82A*, *B* and *C* gene products are very similar to each other in amino acid sequences having 43-50% homology with those of tobacco SAR8.2 genes. The *CASAR8.2* genes were not constitutively expressed in all the organs of healthy pepper plants. In contrast, the *CASAR82A* gene was locally or systemically

induced in pepper plants infected by either *X. campestris* pv. *vesicatoria*, *Colletotrichum coccodes* or *Phytophthora capsici*. Significantly strong induction of the *CASAR82A* gene also was found in pepper leaves treated with either ethylene, methyl jasmonate, indole-3-acetic acid, abscisic acid, salicylic acid, benzothiadiazole, DL--amino-n-butyric acid or hydrogen peroxide. Interestingly, the transcription of the *CASAR82A* gene was rapidly triggered by high salinity, drought or low temperature stresses, but not by mechanical wounding. *In situ* hybridization results revealed the *CASAR82A* mRNAs were localized in phloem and epidermal cells of pepper leaf and stem tissues infected by *C. coccodes* and *P. capsici*, or treated with salicylic acid. These results thus suggest that pepper SAR8.2 genes may be valuable as a molecular marker for the detection of various pathogen infection, abiotic elicitors and environmental stresses.

Overexpression of a pathogen-inducible pepper SAR8.2 gene

The *Arabidopsis* transgenic lines overexpressing the pepper *CASAR82A* gene were generated to evaluate the physiological and molecular functions of the pepper SAR8.2 protein in plant defense. The *CASAR82A* gene was integrated into the genome of the transgenic *Arabidopsis* plants. The transgenic lines grew faster than the wild type plants, indicating that CASAR82A may be involved in plant development. The ectopic expression of CASAR82A in *Arabidopsis* accompanied the expression of the *Arabidopsis* PR-genes including PR-1, PR-2, PR-4 and PR-5. Overexpression of the *CASAR82A* The enhanced the resistance against infections by Pseudomonas syringae pv. tomato, *Fusarium oxysporum* f.sp. *matthiolae* or *Botrytis cinerea*. The purified recombinant CASAR82A protein did not show antifungal activity against *F. oxysporum*. The transgenic plants also exhibited increased tolerance to NaCl and drought at all plant growth stages. Morevoer, the transgenic plants were highly tolerant to oxidative stress by methyl viologen. Together, the enhanced resistance to pathogen infection and environmental stresses in the CASAR82A transgenic lines correlated with the enhanced expression of the *CASAR82A* gene.

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