学 位 論 文 要 旨

# Regulation of plant defense response via heat shock transcription factors in cucumber

# キュウリの熱ショック転写因子による植物防御応答の調節

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Environmental stress affects physiological and metabolic changes in plants, adversely affecting plant growth and development. Plant adaptation in response to stress is an important stage for survival under abiotic and biotic stress. Heat stress may potentially induce a plant defense response against pathogen infection. The increased plant resistance to pathogens under heat stress is known as heat shock-induced resistance (HSIR). The HSIR mechanism is associated with systemic acquired resistance (SAR) in inducing plant resistance via the accumulation of salicylic acid (SA), detected after heat shock treatment (HST). Additionally, an alternative pathway is suggested because some defense genes were upregulated through heat shock response (HSR) after HST. In HSR, heat shock transcription factors (HSFs) play a vital role in inducing heat-related genes by binding to the heat shock element (HSE) motifs in the promoter region of their genes. HSE motifs are also found in the upstream region of defense-related genes, indicating the possible role of HSFs in triggering those genes and leading to increased plant resistance. This study focused on investigating the contribution of HSFs as proposed pathways in the HSIR mechanism by observing cucumber resistance against gray mold after HST and a heat shock protein 90 inhibitor (HSP90 inhibitor; geldanamycin [GDA]) treatment and by examining the gene expression of HSFs and pathogenesis-related (PR) proteins that either contain or are devoid of HSE motifs after treatments. The different SAR and HSF pathways in HSIR are explained to certify and validate the role of HSFs in activating plant resistance under HST.

HST at 50 °C for 20 s reduced the gray mold lesion area in cucumber plants at seven days post-inoculation; moreover, it was also observed in both treated and untreated leaves at seven days after partial HST, which is consistent with the expression of peroxidase (*POX)* and pathogenesis-related protein 1 (*PR1*) *C1* in both treated and untreated leaves 24 h after the same treatment. These results suggest that HST induced systemic resistance in cucumber, confirming the involvement of SAR in the HSIR mechanism.

Gene expression of PR proteins was investigated as a gene marker for the defense system, including genes of PR proteins with and without the HSE motifs in their upstream region. HSE motifs are essential as a recognition site of HSFs to trigger the expression of target genes. *HSFA2* and *HSFB2* upregulated at 12 h after HST, which is earlier than the upregulation of *POX* and *PR1* at 24 and 48 h after treatment, respectively. This indicates possible HSF involvement in the gene expression of PR proteins by binding to the HSE motifs in their upstream region, particularly *POX* with HSE motifs. Its motif was also found upstream of *PR1.CI*, *POX73*, and *POX43*. All PR protein genes with the HSE motif were upregulated 24 h after HST, while almost all genes of PR proteins without HSE (*POX2*, *POX21*, *POX42*, and *PR1*) were not upregulated, except for *POX21*. Upregulation of genes encoding PR proteins suggests that HSFs could trigger their gene expression by binding the HSF-HSE motif, while SAR may still be involved in the gene expression of PR proteins after HST due to the upregulation of gene encoding PR proteins without the HSE motif (*POX21*) and systemic resistance after partial HST. Therefore, SAR and the proposed HSFs pathways are linked to the induction of plant resistance after HST.

GDA application is expected to activate HSF-mediated pathway without inducing SAR pathway; thus, the enhanced defense response via HSFs could be distinguished from SAR. GDA treatment maintained the upregulation of gene encoding PR proteins, thereby reducing gray mold lesions. Conversely, all genes of PR proteins lacking the HSE motif did not upregulate after the same treatment. Under partial GDA treatment, the lowest reduction of gray mold lesion and upregulation of genes encoding PR proteins were only observed in GDA-treated leaves, indicating that GDA treatment induced local resistance, in contrast to systemic induced resistance in HST. The failure of systemic resistance suggests possible inoperative SAR in GDA-treated plants. These results show that the HSFs seemingly play a critical role in the gene expression of PR proteins, resulting in increased resistance against *B. cinerea* in cucumber after GDA treatment.

Our study reports that the HSIR mechanism involves SAR-mediated pathway via the accumulation of SA, resulting in enhanced disease resistance in cucumber. It also involves an HSF-mediated pathway that is associated with locally induced resistance and concealed by the SAR. This investigation of additional pathway via HSFs improves the comprehensive information of the molecular mechanisms of HSIR in activating defense responses against *B. cinerea* infection in cucumber plants. A more understanding of the mode of action of heat shock on plant defense is needed to understand the potential of HST in inducing resistance and to implement HST in plant disease management programs.