3. HRT-mediated *Turnip Crinkle Virus* Resistance in *Arabidopsis*: Identification of HRT-mediated cell death signaling components using VIGS in *N. benthamiana*

Jeong Mee Park

Plant Genomics Laboratory, KRIBB, Daejeon 305-600, Korea

Turnip crinkle virus (TCV) inoculation onto resistant *Arabidopsis* ecotype Dijon (Di-17) leads to a hypersensitive response (HR) on the inoculated leaves. A dominant gene, *HRT*, which confers an HR to TCV, has been cloned from Di-17 plants by map-based cloning. *HRT* is a LZ-NBS-LRR class resistance gene and it belongs to a small gene family that includes *RPP8*, which confers resistance to *Peronospora parasitica* Emco5. Outside of the LRR region, HRT and RPP8 proteins share 98% amino acid identity while their LRR regions are less conserved (87% identity). *HRT*-transformed *Arabidopsis* plants developed an HR but generally remained susceptible to TCV, due to a dominant *RRT* allele, which is not compatible with resistance. However, several transgenic plants that overexpressed *HRT* at much higher than Di-17 showed micro-HR or no HR when inoculated with TCV and resistant to infection. Both the HR and resistance are dependent on salicylic acid but independent of NPR1, ethylene or jasmonic acid. *Arabidopsis* plants containing both TCV coat protein gene and HRT developed massive necrosis and death in seedlings, indicating that the TCV coat protein is an avirulence factor detected by the HRT.

To investigate structure-function relationships of HRT, 11 chimeras have been made by swapping of domains between the HRT and its paralog, RPP8, which confer resistance to *Peronospora parasitica* Emco5. The ability of these chimeric proteins to induce an Avr-dependent HR to TCV was then assessed using an Agrobacterium-mediated transient expression assay. Co-expression of the HRT and its elicitor, the TCV coat protein (CP), results in rapid cell death. Following infiltration into *N. bentahamiana* leaves, three constructs elicited an HR (HRds1, HRds3, and HRds10). The chimeric construct HRds10, in which LRRs 8-14 of RPP8 were exchanged with the corresponding sequences from HRT, induces cell death in the absence of viral coat protein. We also generated transgenic Col-0 (TCV-susceptible) plants expressing the 11 chimeric constructs under control of CaMV 35S promoter and

then checked defense responses against to TCV and *Peronospora parasitica* Emco5 infections, respectively.

To study the role of known defense signaling genes and also to isolate novel signaling components in the HRT-mediated hypersensitive response pathway, we have used a tobacco rattle virus (TRV) based virus induced gene silencing (VIGS) system as a genomics tool. Previously, we identified a collection of genes expressed during the incompatible interactions between pepper and pathogens using microarray analyses. To access the requirement of these genes in the hypersensitive response (HR) induced by HRT/CP, the full-length or partial cDNAs described above were subcloned into a TRV silencing vector. Using this approach we have isolated preliminary about fifty candidate genes that when silenced compromised the HRT-mediated HR.

E-mail: jmpark@kribb.re.kr