Plant defense signaling network study by reverse genetics and protein-protein interaction

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Incompatible plant-pathogen interactions result in the rapid cell death response known as hypersensitive response (HR) and activation of host defense-related genes. To understand the molecular and cellular mechanism controlling defense response better, several approaches including isolation and characterization of novel genes, promoter analysis of those genes, protein-protein interaction analysis and reverse genetic approach etc. By using the yeast two-hybrid system a clone named *Tsip1*, *Ts*i1-*i*nteracting *p*rotein 1, was isolated whose translation product apparently interacted with Tsi1, an EREBP/AP2 type DNA binding protein. RNA gel blot analysis showed that the expression of *Tsip1* was increased by treatment with NaCl, ethylene, salicylic acid, or gibberellic acid. Transient expression analysis using a *Tsip1::smGFP* fusion gene in *Arabidopsis* protoplasts indicated that the Tsip1 protein was targeted to the outer surface of chloroplasts. The targeted Tsip1::smGFP proteins were diffused to the cytoplasm of protoplasts in the presence of salicylic acid (SA). The PEG-mediated co-transfection analysis showed that Tsip1 could interact with Tsi1 in the nucleus. These results suggest that Tsip1-Tsi1 interaction might serve to regulate defense-related gene expression. Basically the useful promoters are valuable tools for effective control of gene expression related to various developmental and environmental condition. In practical terms, the promoter itself is a very valuable asset as a patent and can be used for development of pathogen and harsh environment resistant crop plants and/or increasing the crop yield in a favorable environmental manner. Genome Walker PCR experiment for isolation of promoters of mainly already characterized genes in the hot pepper and northern blot analysis for characterization of new genes were paralleled to obtain the ambitious quantitative result. For the functional analysis of promoters, promoter deletion study with GUS reporter system was carried out for dissection of useful domain search. In addition, *in vivo* functional study was done by introducing these promoters into model plant, tobacco plant. Proteomic approach combined with important domain binding method was also launched to analyze specifc factors including transcription factors which could bind to a specific domain. Eventually the thorough functional analysis of the useful promoters will contribute to analysis of genome and development of useful crops.

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