DOCTORAL THESIS

Mechanism of WRKY transcription factors-mediated defense and heterosis in Arabidopsis polyploids

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Abstract

WRKY transcription factors (TFs) belong to a large family of regulatory proteins in plants that modulate many plant processes. Extensive studies have been conducted on WRKY-mediated defense response in *Arabidopsis thaliana* and many crop species. This study aims to investigate the potential roles and contributions of WRKY TFs regulation in improving defense response in the resynthesized *Arabidopsis* allotetraploids (*Arabidopsis suecica*) from two related autotetraploid progenitors, *Arabidopsis thaliana* (At4) and *Arabidopsis arenosa* (Aa). Upon infection by *Pseudomonas syringae* (*Pst*), the allotetraploids has showed enhanced resistance against the pathogen when compared to the parents. Rapid induction of *WRKY18, WRKY40, WRKY38, WRKY53, WRKY6;* MAP kinase pathway related genes, *WRKY33, PAD3*; SA-pathway related genes, *ICS1, EDS1, PBS3, MYB31*; was evident in response to *Pst* and salicylic acid treatment in the allotetraploids. Cleaved amplified polymorphic sequences analysis further revealed that the *AtWRKY18, AaWRKY40, AtWRKY33,* and *AtWRKY60* alleles expressed at higher levels when compared to their respective homoeologs in the allotetraploids, suggesting potential altered protein-protein interaction networks in the hybrids. Therefore, a split-luciferase complementation assay was used to characterize and quantify protein-protein interaction among these homoeologous WRKYs in the allotetraploids. Results showed that preferential protein-protein interactions exist for the *cis*-interacting AtWRKY18/AtWRKY18 homodimer or *trans*-interacting AtWRKY18/AaWRKY40 heterodimer when compared to the respective

interacting complexes. In addition, differential affinities of WRKY18 and WRKY40 homo- and hetero- dimers toward the W-boxes at the *WRKY60* promoter were observed. In the allotetraploids, *PR1* expression was repressed under basal state when compared to the progenitors. Although *PR1* is expressed at a higher level in *A. thaliana*, its expression fold change was higher and faster in the allotetraploids upon salicylic acid treatment. Transient expression of WRKY18 or WRKY40 homodimer in various combinations induced differential expression of *PR1* gene in their respective *wrky18* and *wrky40 Arabidopsis thaliana* mutants. In contrast, similar *PR1* induction by homodimer in various combinations was observed when they were transiently expressed in the allotetraploids. In addition, transgenic *AtWRKY18* overexpression plant displayed enhanced disease resistance against *Pst* when compared to *AaWRKY18* overexpression lines. Such enhanced disease resistance was found to associate with the higher expression of *PR1* and *PR2* in *AtWRKY18* transgenic lines. Moreover, differential *Pst*-induced expression of the direct targets (*ICS1*, *EDS1* and *PBS3*) of WRKY18 in the *Arabidopsis* AtWRKY18 and AaWRKY18 overexpressors supported a biological difference between the At and Aa homodimers in mediating the targets regulation, thus contributing to the difference in disease responses. Overall, our findings suggested that the rapid differential alleles expression and altered protein-protein or protein-DNA interactions of WRKY transcription factors could contribute to the improved defense in the allotetraploids, providing a molecular basis of for heterotic phenotype development in hybrids.

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