

RESEARCH PAPER

Cross activity of orthologous WRKY transcription factors in wheat and *Arabidopsis*

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Abstract

WRKY proteins are transcription factors involved in many plant processes including plant responses to pathogens. Here, the cross activity of TaWRKY78 from the monocot wheat and AtWRKY20 from the dicot *Arabidopsis* on the cognate promoters of the orthologous PR4-type genes *wPR4e* and *AtHEL* of wheat and *Arabidopsis*, respectively, was investigated. *In vitro* analysis showed the ability of TaWRKY78 to bind a –17/+80 region of the *wPR4e* promoter, containing one *cis*-acting W-box. Moreover, transient expression analysis performed on both TaWRKY78 and AtWRKY20 showed their ability to recognize the cognate *cis*-acting elements present in the *wPR4e* and *AtHEL* promoters, respectively. Finally, this paper provides evidence that both transcription factors are able to cross-regulate the orthologous PR4 genes with an efficiency slightly lower than that exerted on the cognate promoters. The observation that orthologous genes are subjected to similar transcriptional control by orthologous transcription factors demonstrates that the terminal stages of signal transduction pathways leading to defence are conserved and suggests a fundamental role of PR4 genes in plant defence. Moreover, these results corroborate the hypothesis that gene orthology imply similar gene function and that diversification between monocot and dicot has most likely occurred after the specialization of WRKY function.

Key words: *Arabidopsis thaliana*, gene expression, gene orthology, PR4 genes, transient expression analysis, transcription, regulation, *Triticum aestivum*.

Introduction

Several investigations suggest that pathogenesis related (PR) proteins participate in plant defence mechanisms as many of them are endowed with antimicrobial activity against plant pathogens. Different types of PR proteins have been recognized and classified into 17 families on the basis of structural–functional characteristics and serological

relatedness (Van Loon *et al.*, 2006a). Most genes and related proteins are induced by different stress stimuli, such as infection by viruses, bacteria, and fungi and treatment with the defence-related phytohormones salicylic acid (SA), jasmonic acid (JA) or ethylene (ET). PR proteins and genes have recently been extensively reviewed (van Loon *et al.*,

Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane carboxylic acid; CaMV, cauliflower mosaic virus; CD-WRKY, C-terminal WRKY domain; EMSA, electrophoretic mobility shift assay; ERFs, ethylene-responsive element binding factors; ET, ethylene; GST, glutathione-S-transferase; INA, 2,6-dichloroisonicotinic acid; IPTG, isopropyl β-D-1-thiogalactopyranoside; JA, jasmonic acid; LUC, luciferase; Me-JA, methyl-jasmonate; PDB, potato dextrose broth; PEG, polyethylene glycol; PR, pathogenesis related; qPCR, quantitative PCR; SA, salicylic acid; TCV, turnip crinkle virus; TFs, transcription factors; UTR, 5'-untranslated region.
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