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A W-box is required for full expression of the SA-responsive gene *SFR2*

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Abstract

Transcripts of *SFR2*, a member of the *S* family of receptor kinase genes, accumulate rapidly in *Brassica oleracea* leaves in response to wounding, bacterial infection and following treatment with salicylic acid (SA). Expression of a chimeric gene consisting of the *SFR2* 5′ flanking sequence fused to the *gusA* reporter gene is also induced in wounded and SA-treated *Arabidopsis* plants indicating that the observed response is conferred by the *SFR2* promoter. We show here that, in *Arabidopsis* plants carrying the salicylate hydroxylase (*NahG*) transgene, wound induction of the *SFR2* promoter–*gusA* reporter fusion was abolished, indicating that, as has previously been shown for the response to bacterial infection, SA is required for the response to wounding. Deletion analysis of the *SFR2* promoter identified a region necessary for full expression following SA treatment. This region, which includes two putative W-boxes, is conserved in the promoter of the *Arabidopsis SFR2* homologue, *ARK3*. Deletion of a 12 bp region containing the two W-box motifs reduced the response to SA treatment. Tandem repeats of the W-box-containing element fused upstream of a CaMV *35S* minimal promoter enhanced reporter gene expression in transgenic *Arabidopsis* both in the absence and presence of SA. Gel-mobility shift assays showed that *Arabidopsis* leaf extracts contained factors that bound to a fragment of the promoter spanning the putative W-boxes and that a fragment in which these motifs were mutated was unable to compete for binding. In summary, induction of the *SFR2* promoter in response to bacterial infection and wounding requires SA, and full expression of the induced gene requires the presence of a functional element containing W-box motifs in the *SFR2* promoter. The involvement of two W-boxes indicates that transcription factors of the WRKY family may play a key role in mediating these responses.
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Keywords: *Arabidopsis thaliana*; *Brassica oleracea*; Plant defence; WRKY

1. Introduction

Salicylic acid (SA), a product of plant secondary metabolism derived from the phenylalanine ammonium lyase and isochorismate synthase pathways, plays a key role in plant defence responses to pathogens (Vernooij et al., 1994). When invaded by pathogens, resistant plants induce defence mechanisms both locally and in distant, non-infected tissues. Local defences are usually associated with a rapid, programmed cell death at the site of infection termed the hypersensitive response (HR). The various molecular responses accompanying HR restrict growth of the pathogen and its spread throughout the plant. Exposure to pathogens that cause necrosis can also induce systemic acquired resistance (SAR), which confers sustained immun-

Abbreviations: as-1, activation sequence-1; *ARK1/2/3*, Arabidopsis receptor kinase 1/2/3; BTH, benzothiadiazole; CaMV, cauliflower mosaic virus; Col-0, Columbia-0; GUS, β-glucuronidase; *gusA*, β-glucuronidase gene; HR, hypersensitive response; INA, 2,6 dichloroisonic acid; ISR, induced systemic resistance; JA, jasmonic acid; KCl, potassium chloride; 4-MUG, 4-methylumbelliferyl-D-glucuronide; 4-MU, 4-methylumbelliferyl; *NahG*, salicylic hydroxylase; PR, pathogenesis related; PRK, plant receptor kinase; *RLK1*, receptor-like kinase 1; *RKS1*, receptor kinase of the S domain class 1; SA, salicylic acid; SAR, systemic acquired resistance; *SFR1/2/3*, S family receptor 1/2/3; SLG, S locus glycoprotein; SRK, S locus receptor kinase; WAK1, wall associated kinase 1.

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ity to further infection by a broad spectrum of pathogens (Hunt et al., 1996). In many plant–pathogen interactions, HR and SAR are accompanied by the accumulation of a large group of pathogenesis-related (PR) proteins.

In both *Arabidopsis* and tobacco, SA is generally necessary and sufficient for the induction of HR and SAR. Application of exogenous SA triggers synthesis of PR proteins and confers resistance to pathogens (Yang et al., 1997). These same two phenomena, PR protein production and induced resistance, were observed in transgenic tobacco plants that produced SA constitutively (Verberne et al., 2000). Increases in the levels of endogenous SA and its conjugates in pathogen-infected wild-type plants correlate with the expression of PR genes and the development of disease resistance. Furthermore, HR, PR gene expression and disease resistance are compromised in *Arabidopsis* plants carrying mutations that prevent SA accumulation such as *eds1* and *pad4* (Shah, 2003). Similar observations have been made with transgenic tobacco and *Arabidopsis* plants, where SA accumulation is prevented by the constitutive expression of the *NahG* transgene (Gaffney et al., 1993). *NahG* encodes a bacterial salicylate hydroxylase which converts SA to biologically inactive catechol, but does not metabolise two synthetic functional analogs of SA, 2,6 dichloroisonicotinic acid (INA) and benzothiadiazole (BTH). These compounds can restore disease resistance in SA-depleted *NahG* tobacco and *Arabidopsis* plants. Although these different lines of evidence argue for an essential role for SA in the onset of SAR, graft experiments demonstrate that SA is neither the translocated signal nor is it required for generation of the translocated signal. However, SA is required to mediate the translocated signal, the nature of which remains unknown (reviewed in Vernooij et al., 1994).

Transmembrane sensors play an essential role in perceiving external stimuli from the environment as well as stimuli communicated through the plant body by endogenous signals. In *Arabidopsis*, plant receptor kinases (PRKs; Cock et al., 2002) constitute the largest recognizable class of transmembrane sensors; a superfamily of 417 PRK genes has been described in this species (Shiu and Bleecker, 2001). The PRK superfamily is monophyletic with respect to the kinase domain but can be subdivided into at least 21 classes based on the sequence of the putative extracellular domain. Plant PRKs play important roles in various development processes and in defence responses to both biotic (e.g., disease) and abiotic stresses (e.g., wounding). Thus, the polypeptide systemin which mediates systemic wound signalling in tomato binds to the PRK SR160 (Scheer and Ryan, 2002), *Arabidopsis* FLS2 is involved in pathogen recognition mechanisms via perception of flagellin, a bacterial elicitor, and subsequent phosphorylation of intracellular target proteins (Gómez-Gómez and Boller, 2000) and, in monocots, the rice PRK Xa21 mediates resistance to the bacterial pathogen *Xanthomonas oryzae oryzae* (Song et al., 1995). The fact that expression of several PRKs has been shown to be induced following SA treatment also argues that these molecules play a general role in the defence response (Ohtake et al., 2000; Du and Chen, 2000; Pastuglia et al., 1997, 2002).

The first PRK gene shown to be induced during the defence response was *S gene family receptor 2* (*SFR2*) from *Brassica oleracea* (Pastuglia et al., 1997). The *SFR2* gene was isolated in a screen for vegetatively expressed genes related to the self-incompatibility locus genes *S locus glycoprotein* (*SLG*) and *S locus receptor kinase* (*SRK*; Stein et al., 1991). Additional, expressed members of the S domain class of PRK genes have been described in *B. oleracea* (*SFR1* and *SFR3*; Pastuglia et al., 2002) and *Arabidopsis* (e.g., *RLK1, RKS1, ARK1*, *ARK2* and *ARK3*; RLK for receptor-like kinase, RKS for receptor kinase of the S domain class, *ARK* for *Arabidopsis* receptor kinase; Tobias et al., 1992; Dwyer et al., 1994; Walker, 1993; Ohtake et al., 2000).

In contrast to *SRK*, *SFR2* is expressed in vegetative tissues, mainly in leaves. *SFR2* was shown to be rapidly induced in leaves of *B. oleracea* by wounding, bacterial infection and salicylic acid, indicating a role in the defence response. Experiments in transgenic tobacco have demonstrated that the 1516 bp upstream of the *SFR2* coding region is sufficient to induce expression of a fused *gusA* reporter gene in response to SA treatment and bacterial infiltration (Pastuglia et al., 1997). Similarly, the 1516 bp promoter of *SFR2* is activated upon bacterial infection in *Arabidopsis* (Pastuglia et al., 2002).

Here, we show that induction of *SFR2* by wounding requires SA, as has been previously observed for the induction of this gene following bacterial infection. Analysis of the *SFR2* promoter identified a *cis*-acting element that plays a major role in mediating the SA response.

2. Materials and methods

2.1. Plant material and growth conditions

The *Arabidopsis thaliana* ecotype used was Columbia-0 (Col-0). Seed from transgenic *Arabidopsis* Col-0 plants expressing the *NahG* gene under the control of the enhanced CaMV 35S promoter from CaMV was obtained from John Ryals.

Plants were grown on soil in a growth chamber with a 12 h light/dark period at 20 °C (light)/14 °C (dark). Treatments were performed after 8–10 weeks of growth (prior to bolting).

2.2. Promoter constructs

Cloning of the 1516 bp *SFR2* promoter region upstream of the *gusA* reporter gene has been described by Pastuglia et al. (1997). Deletions from the 5′ end of the 1516 bp *SFR2* promoter fragment (Fig. 2) were generated by PCR using specific primers with 5′ extensions containing *Hin*dIII and *Xba*I restriction sites and cloned into pBI101.

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