Promoter Studies of Chemically Induced *BCI*-Genes in the Pathosystem Barley – Powdery Mildew

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Abstract

Chemical resistance inducers like BTH (S-methyl benzo (1,2,3)-thiadiazole-7-carbothiate) and DCINA (2,6-dichloro isonicotinic acid) activate resistance in barley against powdery mildew (*Blumeria graminis* f.sp. *hordei*). Nine BTH induced genes (*Bci*, barley chemically induced) have been identified in barley (BESSER *et al.* 2000) which are not responsive to pathogens in contrast to PR-proteins. From two *Bci*-genes (*Bci3*: similar to *vsp*, *Bci4*: Ca^{2+}-binding EF-hand protein), the promoters were isolated. In transient transformation assays using promoter::GFP and promoter::GUS-constructs the functionality of these chemically induced promoters were studied. To identify the minimal promoter and regions with regulatory elements 5'-deletion constructs were used. Additionally, gel mobility shift assays were performed.

Keywords: barley; promoter; *Bci*; BTH; SAR

INTRODUCTION

Chemical induced resistance (CIR) has been demonstrated in cereals against *Blumeria graminis* using biological extracts and synthetic compounds. The most effective chemicals, such as DCINA and BTH (the active ingredient of Bion®), are thought to mimic salicylic acid (SA). Another natural inducer might be jasmonic acid (JA), one product of the octadecanoid pathway. This phytohormone is implicated in dicots in resistance to insects and pathogens as well as in wound resistance.

MATERIAL AND METHODS

Chemical treatment. Before transformation leaves were chemically treated. For induction leave segments were floated on 45 μM JA or H_2O for 24 h, on 1M sorbitol or H_2O for 1 h. BTH or WP (wettable powder) as control was applied as a soil drench with 60 ppm for 48 h.

Transient transformation. Transient transformation by particle bombardment was conducted according to SCHWEIZER *et al.* (1999) of the barley *Bci3* promoter (–1700) and its deletion derivatives (–1200, –900, –600, –300) as well as the 35S-promoter as control. The promoters were fused with a green fluorescence protein (GFP) on a pUC18 vector. Amount of GFP cells was determined 24 h after transformation.

Transient transformation by *Agrobacterium*-mediated gene transfer: *Bci3* and *Bci4* promoters were fused with an intron containing β-glucuronidase (GUS) reporter gene on a modified binary vector pCAMBIA1391X. Four-days-old barley seedlings were vacuum infiltrated with *Agrobacterium tumefaciens* strain LBA4404 containing the barley *Bci3* and *Bci4* promoter::GUS (pCAMBIA1391X) and 35S promoter::GUS as control (pCAMBIA1301). After two days the activity of the GUS reporter gene was determined by staining or fluorimetric GUS assay (JEFFERSON 1987).

RESULT AND DISCUSSION

Nine BTH-induced genes have been identified in barley (BESSER *et al.* 2000). From two of the *Bci*-genes (*Bci3*: similar to *vsp*, *Bci4*: Ca^{2+}-binding EF-hand protein) the promoters were isolated using iPCR and BAC library screening. Table 1 shows expression of these genes following different treatments (BESSER *et al.* 2000). In Figure 1 putative responsive elements are marked which were identified *in silico* (LESCOT *et al.* 2002).

Table 1. Expression analysis after different treatments (BESSER *et al.* 2000)

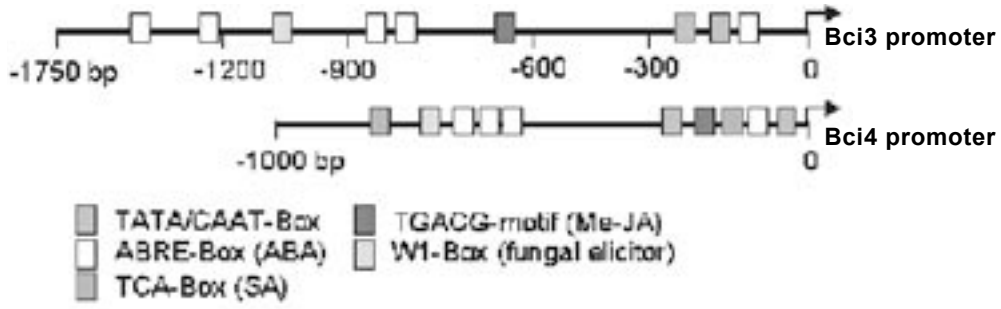
	Bci3	*Bci4*
BTH	+	+
JA	+	0
Sorbitol	–	0

+ = up regulation, – = down regulation, 0 = not differential

Functional characterization of barley *Bci3* promoter was carried out by a modified transient biolistic transformation assay (SCHWEIZER *et al.* 1999). A promoter::GFP construct and its deletion derivatives were used in chemically treated barley leaves. The *Bci3* promoter showed induction in response to JA (Figures 1 and 2). Deletion analysis showed that all derivatives have similar levels of activity and inducibility. Sorbitol increases the endogenous JA-level in leaves due to osmotic stress. After sorbitol induction a reduction in the activity of *Bci3* promoter and its deletions were found (Figure 2). Interestingly, the –1200 construct exhibited in both treatments significantly lower levels of activity and responsibility, suggesting that negative regulatory sequence(s) may be present in the region between –1200 and –900.

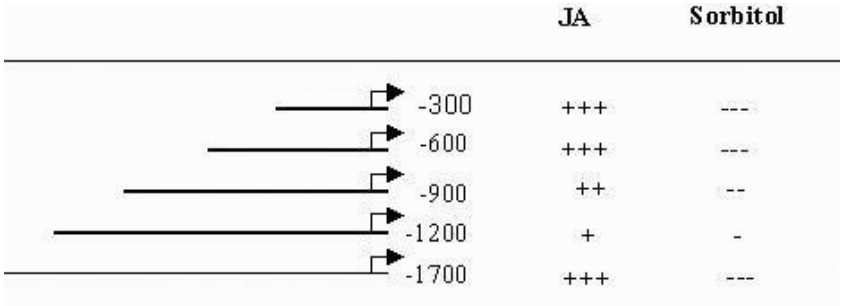
With biolistic transformation methods only epidermal cells are transformed. *Bci3* and *Bci4* are both strongly expressed in the mesophyll. Therefore, an *Agrobacterium*-mediated method for transient monocot-transformation was established modifying dicot systems (KAPILA *et al.* 1997; YANG *et al.* 2000). With this system only mesophyll cells were transformed (data not shown).

The BTH-inducible *Bci3* and *Bci4* promoters were fused with an intron containing β-glucuronidase (GUS) reporter gene on a modified binary vector (pCAMBIA1391X). After the resulting constructs were introduced into *A. tumefaciens* strain LBA4404, transient transformation was carried out by agroinfiltration in combination with BTH treatment. Both *Bci* promoters were induced in the mesophyll by BTH whereas the 35S promoter showed no induction (data not shown).



Barley *Bci3* and *Bci4* promoters were analysed *in silico* using plantCARE, a database of plant cis-acting elements (LESCOT *et al.* 2002)

Figure 1. Putative responsive elements in the barley *Bci3* and *Bci4* promoters



Transient transformation was conducted by particle bombardment of *Bci3* promoter::GFP constructs and its deletion derivatives in JA- and sorbitol-induced leaves. Amount of GFP cell was determined 24 h after transformation
+ = induction, – = repression in activity compared to the control. Different levels in promoter activity is indicated with more than one sign

Figure 2. Deletion analysis of barley *Bci3* promoter

In this study, we successfully applied a transient *Agrobacterium*-mediated transformation assay for barley (and wheat). From the results we conclude that both isolated barley *Bci* promoters are functional and chemically inducible.

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