Research Note

Evidence for Different Signaling Pathways Activated by Inducers of Acquired Resistance in Wheat

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Acquired resistance (AR) of wheat (*Triticum aestivum*) to the powdery mildew fungus *Erysiphe graminis* f. sp. *tritici* can be induced either by inoculation with the nonhost pathogen *E. graminis* f. sp. *hordei* or by treatment with chemical substances such as benzo(1,2,3)thiodiazole-7-carbothioic acid *S*-methyl ester (BTH). In the dicotyledonous plants tobacco and *Arabidopsis*, induction of AR by pathogens and BTH is accompanied by the expression of a characteristic set of genes. Here we report that in wheat, BTH treatment failed to activate genes whose transcripts accumulate after AR induction by nonhost pathogens, whereas BTH-inducible genes were not activated by an appropriate pathogen inoculation. This suggests that at least two different pathways exist for the induction of AR in monocots.

Acquired resistance (AR) is an inducible defense mechanism exhibited by many plants that provides protection against a broad range of pathogens. AR, which was first observed by Chester (1933) and later described by Ross (1961), can be induced by incompatible pathogens, by pathogen-derived elicitors (Schaffrath et al. 1995; Keller et al. 1996), and by certain chemicals, including salicylic acid (SA) (White 1979), 2,6-dichloroisonicotinic acid (INA) (Métraux et al. 1991), and benzo(1,2,3)thiodiazole-7-carbothioic acid *S*-methyl ester (BTH) (Görlach et al. 1996). After induction, protection against a later infection can be restricted to the leaf treated with an inducer (local acquired resistance, LAR) or can encompass tissue of the plant that has not been treated (systemic acquired resistance, SAR) (Ross 1961). SAR has been most extensively characterized in cucumber (Kuč et al. 1975; Kuč and Richmond 1977), tobacco (Kuč 1982; Vernooij et al. 1995), and more recently in *Arabidopsis* (Mauch-Mani and Slusarenko 1994; Lawton et al. 1995). Related sets of gene families (SAR genes) have been characterized in tobacco (Ward et al. 1991) and *Arabidopsis* (Uknes et al. 1992) that are coordinately activated during the SAR response. These genes encode pathogenesis-related (PR) proteins and are likely to play an important role during the onset and maintenance of SAR, since constitutive expression of such genes in transgenic plants can enhance disease resistance (Broglie et al.

1991; Alexander et al. 1993; Liu et al. 1994; Logemann et al. 1994; Jach et al. 1995).

In both *Arabidopsis* and tobacco, the same set of SAR genes and the same spectrum of resistance specificities were activated by the chemical inducers SA, INA, and BTH and the biological inducing agents (Ward et al. 1991; Uknes et al. 1992, 1993; Vernooij et al. 1995; Lawton et al. 1995, 1996; Friedrich et al. 1996). SA appears to be an important component of the signal transduction pathway leading to SAR. Not only can SA induce SAR when applied exogenously, but it was shown to accumulate during the onset of SAR in cucumber (Métraux et al. 1990), tobacco (Malamy et al. 1990), and *Arabidopsis* (Uknes et al. 1993). Furthermore, genetically engineered tobacco and *Arabidopsis* plants unable to accumulate SA failed to exhibit SAR after induction with pathogens (Gaffney et al. 1993; Vernooij et al. 1994). However, application of INA and BTH still induced SAR and SAR gene activity in such plants (Delaney et al. 1994; Vernooij et al. 1994; Friedrich et al. 1996; Lawton et al. 1996). This, together with the fact that SAR induction by SA, INA, and BTH is blocked in the SAR-defective *Arabidopsis* mutant *nim1*, indicates that these chemicals act through a common SAR signal transduction pathway (Delaney et al. 1995; Lawton et al. 1996).

AR in monocots is less well studied. After induction with biological agents, LAR has been demonstrated in barley (Ouchi et al. 1974), rice (Horino 1976), and wheat (Schweizer et al. 1989). SAR was reported in rice after preinoculation with *Pseudomonas syringae* pv. *syringae* (Smith and Métraux 1991). As in dicotyledonous plants, AR can also be triggered by application of chemicals, e.g., by probenazole in rice (Watanabe et al. 1979), by INA in barley (Kogel et al. 1994), and by SA, INA, and BTH in wheat (Görlach et al. 1996).

LAR in wheat against powdery mildew induced by inoculation with the nonhost pathogen *Erysiphe graminis* f. sp. *hordei* (which causes powdery mildew of barley) is accompanied by the accumulation of mRNA species, including the ones represented by the WIR (for wheat induced resistance) cDNA clones (Schweizer et al. 1989; Kmecl et al. 1995). WIR1 and its homolog WIR4 encode a putative cell wall protein (Bull et al. 1992); WIR2, a thaumatinlike protein (Rebmann et al. 1991b); and WIR3, a peroxidase (Rebmann et al. 1991a). Similarly, a set of transcripts that accumulate after chemical resistance induction have been cloned as cDNAs (cWCI clones, for wheat chemical induction) (Görlach et al. 1996). cWCI-2 encodes a lipooxygenase; cWCI-4 encodes a

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cysteine proteinase; and cWCI-1, cWCI-3, and cWCI-5 encode proteins of no known function (Görlach et al. 1996). On the basis of their primary sequences, the sets of available WIR and cWCI clones do not overlap.

We wanted to determine whether the accumulation of WIR and WCI transcripts can be induced by both biotic and chemical resistance activators, i.e., whether the corresponding genes exhibit an expression pattern analogous to the SAR genes of tobacco and *Arabidopsis*. To this end, 14-day-old wheat plants (*Triticum aestivum* cv. Kanzler) grown under a 16-h photo-period were either inoculated with *E. graminis* f. sp. *hordei* spores or sprayed with a 1 mM BTH solution as described by Kmecl et al. (1995) and Görlach et al. (1996), respectively. RNA was extracted at various time points after the treatments and subjected to gel blot analysis according to standard procedures (Maniatis et al. 1982) with WIR and WCI cDNAs as probes. The results are depicted in Figure 1. As expected, inoculation with the nonhost pathogen *E. graminis* f. sp. *hordei* resulted in the transient accumulation of WIR transcripts (Fig. 1A). In contrast, *E. graminis* f. sp. *hordei* inoculation had no effect on cWCI transcript levels (Fig. 1B). Similarly, cWCI-specific transcripts accumulated after BTH treatment (Fig. 2A), as was reported by Görlach et al. (1996). However, WIR transcript levels were identical in BTH- and mock-treated plants (Fig. 2B). These results show that BTH is not capable of inducing genes typically activated in biologically induced wheat plants and that WCI genes are not inducible by a treatment with a biological inducer. Additional experiments with SA and INA yielded results identical to the ones obtained with BTH (data not shown). WCI transcript accumulation was reported to occur 9 to 12 days after infec-

tion of wheat with a compatible race of wheat powdery mildew (Görlach et al. 1996). However, by that time, infected leaves are already heavily overgrown by the fungus, and thus this late accumulation may be a secondary effect rather than a direct result of the host-parasite interaction.

The fact that in wheat the biological and chemical AR inducers result in the accumulation of nonidentical sets of transcripts indicates the existence of different signal transduction pathways. SA, INA, and BTH interfere with one of these, while the other one is independent of these chemicals but is activated by the biological inducer *E. graminis* f. sp. *hordei*. Equivalent pathways may also exist in rice. AR in rice induced by *P. syringae* pv. *syringae* (Smith and Métraux 1991) results in the accumulation of transcripts homologous to the wheat WIR1, WIR2, and WIR3 cDNA clones PIR1 (*unpublished results*), PIR2 (Reimann and Dudler 1993), and PIR3 (Reimann et al. 1992). BTH has been reported to be a potent inducer of AR in rice as well (Görlach et al. 1996). However, BTH does not lead to the accumulation of PIR transcripts in rice (*unpublished results*). Since clones representing chemically induced rice transcripts were not available and we were unable to detect transcript accumulation in BTH-treated or *P. syringae* pv. *syringae*-inoculated rice plants with the heterologous WCI cDNAs as probes, the reciprocal experiment could not be performed.

Evidence for different pathways leading to defense responses has recently also been reported for *Arabidopsis* and tobacco. Whereas classical SAR in these plants requires the SA signaling pathway, systemic resistance induced in *Arabidopsis* by certain root-colonizing bacteria was reported to be independent of SA accumulation and SAR gene expression

Fig. 1. Time course of mRNA accumulation in wheat in response to *Erysiphe graminis* f. sp. *hordei* (Egh) inoculation or mock treatment. Total RNA was extracted at the time points indicated and subjected to gel blot analysis. Identical blots containing 10 µg of RNA per lane were hybridized to radiolabeled inserts of WIR (A) and WCI (B) cDNAs. The autoradiograms of RNA blots and one representative corresponding gel stained with ethidium bromide (Et Br) are shown. The lane in B labeled C contained control RNA extracted from BTH-treated plants 3 days after spraying.

(Pieterse et al. 1996). The pathogen-induced systemic activation of a defensin gene in *Arabidopsis* has also been found to be SA independent (Penninckx et al 1996). Furthermore, Malamy et al. (1996) reported evidence for the occurrence of an SA-independent pathway leading to resistance to tobacco mosaic tobamovirus and expression of a *SAR* gene in tobacco.

Our results demonstrate that *WIR* and *WCI* genes are activated by different signaling pathways. Thus, the behavior of these genes is different from that of the *SAR* genes characterized in the dicotyledonous species tobacco and *Arabidopsis*. It has been postulated that a chemical inducer of true *SAR* should elicit the same responses as biological inducers (Kessmann et al. 1994). Because a biological model of *SAR* in wheat has not been described, it is not clear at present whether BTH induces *SAR* in wheat, and presently none of the *WIR* and *WCI* genes qualifies as a *SAR* gene as defined in dicot model systems.

One of the two different pathways is activated by inoculation with *E. graminis* f. sp. *hordei* and the other by foliar application of BTH, and both treatments lead to the establishment of a physiological state of increased disease resistance. It is not known at present how the underlying mechanisms of disease resistance are related in the two cases. Extreme possi-

bilities are that they are identical, i.e., that common molecular processes causing AR are activated, or that they are unconnected and different molecular events underlying phenotypically similar AR phenomena are activated. If the former were the case, one would have to conclude that the activation of the genes corresponding to the *WIR* and *WCI* cDNA clones is not causally connected to the establishment of the resistant state. In contrast, a causal function would be compatible, although by no means proved, with the latter possibility. Of course, intermediate situations between the two extremes are also possible. One could speculate that biological and chemical inducers activate overlapping sets of genes and that the products of specific subsets of these genes are causing increased resistance toward specific pathogens. Experiments with transgenic plants that exhibit altered expression characteristics of such genes may help to determine their function.

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Fig. 2. Time course of mRNA accumulation in response to BTH treatment. Wheat seedlings were sprayed with a 1 mM suspension of BTH in distilled water, and mock-treated plants were sprayed with distilled water. Total RNA was extracted at different time points and loaded on gels (10 µg per lane). Identical blots were probed with radiolabeled inserts of *WCI* (A) and *WIR* (B) cDNAs. Loading of equal amounts of RNA was confirmed by ethidium bromide (Et Br) staining. A representative gel is shown. The lane in B labeled C contained RNA extracted from plants 24 h after inoculation with *Erysiphe graminis* f. sp. *hordei*.

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