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Arabidopsis thaliana defense-related protein ELI3 is an aromatic alcohol:NADP$^+$ oxidoreductase

(benzyl alcohol dehydrogenase/disease resistance/fungal elicitor)

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ABSTRACT **We expressed a cDNA encoding the *Arabidopsis thaliana* defense-related protein ELI3-2 in *Escherichia coli* to determine its biochemical function. Based on a protein database search, this protein was recently predicted to be a mannitol dehydrogenase [Williamson, J. D., Stoop, J. M. H., Massel, M. O., Conkling, M. A. & Pharr, D. M. (1995) *Proc. Natl. Acad. Sci. USA* 92, 7148–7152]. Studies on the substrate specificity now revealed that ELI3-2 is an aromatic alcohol: NADP$^+$ oxidoreductase (benzyl alcohol dehydrogenase). The enzyme showed a strong preference for various aromatic aldehydes as opposed to the corresponding alcohols. Highest substrate affinities were observed for 2-methoxybenzaldehyde, 3-methoxybenzaldehyde, salicylaldehyde, and benzaldehyde, in this order, whereas mannitol dehydrogenase activity could not be detected. These and previous results support the notion that ELI3-2 has an important role in resistance-related aromatic acid-derived metabolism.**

Plant defense toward potential pathogens encompasses a wide array of mechanisms, some leading to the rapid reinforcement of preexisting structural barriers, others to the *de novo* synthesis of a large diversity of defense-related compounds via transcriptional activation of the corresponding genes. In recent years, numerous plant genes potentially involved in the pathogen defense response have been isolated. However, many of them were detected by various differential screening approaches solely on the basis of enhanced expression levels without knowledge of the biochemical functions of the encoded proteins (1–3). In several cases, functional identification was subsequently achieved, for example, by expression in *Escherichia coli* or yeast, by the use of specific antibodies or by genetic complementation studies. Often, however, inference of function was merely based on deduced amino acid sequence similarity to known proteins, a valuable but not unequivocal means of identification.

The *eli3* gene was originally identified as part of the defense response in parsley [*Petroselinum crispum* (*Pc*)] (4). Expression of this gene was shown to be rapidly and transiently stimulated in cultured parsley cells upon treatment with a cell wall preparation (elicitor) from the phytopathogenic fungus *Phytophthora sojae*, and histochemical studies revealed local and rapid accumulation of ELI3 mRNA around fungal infection sites in parsley leaves (5). The presence of this gene in other plant species, such as potato (*Solanum tuberosum*), alfalfa (*Medicago sativa*), and *Arabidopsis thaliana* (*At*) was also demonstrated (6). Subsequently, two sequence-related counterparts of *eli3* in parsley were isolated from *A. thaliana* (*Ateli3-1* and *Ateli3-2*), and their expression was shown to be induced by fungal elicitor in cultured *A. thaliana* cells (6). Additional evidence for an important role of the *eli3* gene product in plant disease resistance came from genetic studies demonstrating that *eli3* expression was dependent on the presence of the *RPMI* resistance gene in *A. thaliana* (7). The *RPM1* locus confers resistance to *Pseudomonas syringae* strains carrying the corresponding avirulence (*avr*) gene *avrRpm1* (8).

The ELI3 cDNAs from parsley and *A. thaliana* share 67% nucleotide and 70% deduced amino acid sequence identity (7). At the time of their isolation, no related sequences were found in the various data bases. Meanwhile, several plant cinnamyl-alcohol dehydrogenases (CAD) with similarity to the deduced ELI3 proteins have been reported (9). Based on this similarity, it was possible that the *eli3* gene encodes a CAD. Recently, however, Williamson *et al.* (10) suggested that ELI3 is a mannitol dehydrogenase (MTD). Again, this was based on sequence similarity to an MTD from celery [*Apium graveolens* (*Ag*), *Ag*MTD]. This proposal prompted speculation as to the functional relevance of *eli3* gene expression (10), particularly in an accompanying commentary (11), which, in view of our present data, is no longer tenable. We now provide evidence that ELI3-2 from *A. thaliana* is a novel type of aromatic (preferably benzyl) alcohol dehydrogenase with substrate specificity distinct from both CAD and MTD.

MATERIALS AND METHODS

Materials. *Actinobacillus* mannitol dehydrogenase was purchased from Sigma; NAD$^+$, NADH, NADP$^+$, and NADPH were from Boehringer Mannheim. The following aldehydes and alcohols were obtained from Aldrich: cinnamaldehyde, cinnamyl alcohol, sinapaldehyde, coniferaldehyde, coniferyl alcohol, 3,5-dimethoxy-4-hydroxybenzaldehyde, 2-methoxybenzyl alcohol, 3-methoxybenzyl alcohol, salicylaldehyde β-*O*-glucoside, and 4-coumaryl alcohol. Acetaldehyde, D-mannitol, benzaldehyde, benzyl alcohol, salicylaldehyde, 2-hydroxybenzyl alcohol, 2-methoxybenzaldehyde, 3-methoxybenzaldehyde, 4-methoxybenzaldehyde, 4-hydroxybenzaldehyde, 3,4-dimethoxybenzaldehyde, and vanillin were from Merck, and capronaldehyde was from Fluka. 4-Coumaraldehyde was a kind gift from J. Grima-Pettenati (Toulouse, France) and W. Heller (Neuherberg, Germany). All substrates were dissolved in methoxyethanol.

Expression in *E. coli*. One of two established, closely related *A. thaliana* ELI3 cDNAs, *At*ELI3-2, containing the entire coding region (7), was cloned into the expression vector pQE50 (Qiagen, Düsseldorf, Germany), and the resulting construct, pQE50-ELI3-2, was introduced into the *E. coli* host strain SURE. The transformed bacterial cells were grown at 37°C in Luria–Bertani medium containing 1 mM $ZnCl_2$ and 50 μg/ml

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Abbreviations: *Pc*, *Petroselinum crispum*; *At*, *Arabidopsis thaliana*; *Ag*, *Apium graveolens*; CAD, cinnamyl-alcohol dehydrogenase; BAD, benzyl alcohol dehydrogenase; *Eu*, *Eucalyptus gunnii*; MTD, mannitol dehydrogenase; IPTG, isopropyl β-D-thiogalactoside.

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ampicillin until they reached an OD_{600} of 0.8–1.0. The cells were then cooled to 15°C, and 1 mM isopropyl β-D-thiogalactoside (IPTG) was added to induce ELI3-2 protein synthesis. The bacteria were grown for another 16 h and then harvested by centrifugation. As controls, *E. coli* SURE cells containing the pQE50 expression vector and *E. coli* BL21 cells expressing a CAD cDNA (*Eu*CAD2) from *Eucalyptus gunnii* (*Eu*; ref. 12) were treated and processed in the same manner. Preparation of crude extracts from *E. coli* transformants was performed according to Lauvergeat *et al.* (13). Briefly, the harvested cells were suspended in lysis buffer (20 mM Tris·HCl, pH 7.5/10% glycerol/5 mM DTT/0.1% Nonidet P-40/1 mM phenylmethylsulfonyl fluoride/1 mM EDTA/5 μg/ml leupeptin). Lysozyme was added to a final concentration of 2 mg/ml, and the cells were incubated at 4°C until lysis occurred. Nuclease (Benzonase, Eurogentec, Brussels, Belgium) was added to a final concentration of 50 units/ml, and the mixture was incubated at 4°C for about 15 min. Cell debris was pelleted by centrifugation, and the supernatant was tested for enzyme activity. For the analysis of total bacterial proteins, aliquots were pelleted in a microcentrifuge, boiled in SDS-lysis buffer [0.1 M Tris·HCl, pH 6.8/1.6% (vol/vol) glycerol/0.008% bromphenol blue/4 mM EDTA/10 mM DTT/3% (wt/vol) SDS] for 3 min at 95°C and loaded onto a 12% SDS-polyacrylamide gel. Proteins were visualized by Coomassie blue staining. Protein concentrations were determined spectrophotometrically by the Bradford assay (14).

Assay for MTD Activity. MTD activity was measured by monitoring the reduction of NAD^+ spectrophotometrically at 340 nm according to Stoop *et al.* (15). The assay mixture contained 100 mM 1,3-bis[tris(hydroxymethyl)methylamino]propane, pH 9.0, 2 mM NAD^+, 150 mM D-mannitol, and pQE50-ELI3 bacterial extract in a total volume of 1 ml.

Assay for Aromatic Alcohol Dehydrogenase Activity. Recombinant bacterial extracts were assayed spectrophotometrically for aromatic alcohol dehydrogenase activity by both the oxidation of the aromatic alcohols and the reduction of the corresponding aldehydes, as described for CAD activity (16). The assay was carried out at 30°C in 1 ml of reaction mixture containing, for the aldehyde substrates, 200 μM KH_2PO_4/Na_2HPO_4, pH 6.5, 34 μM aldehyde substrate, 200 μM NADPH or NADH, and 1–60 μl of protein extract, and for the alcohol substrates, 100 mM Tris·HCl (pH 8.8), 100 μM alcohol substrate, 200 μM $NADP^+$ or NAD^+, and 1–100 μl of protein extract. The molar extinction coefficients (ε_{340}) used were $18.5 \times 10^3\ M^{-1}\ cm^{-1}$ at pH 8.8 for coniferylaldehyde, $15.8 \times 10^3\ M^{-1}\ cm^{-1}$ at pH 6.5 for sinapaldehyde, $23.5 \times 10^3\ M^{-1}\ cm^{-1}$ at pH 6.5 for 4-coumaraldehyde, and $6.3 \times 10^3\ M^{-1}\ cm^{-1}$ at pH 6.5 for NADH and NADPH. For detailed kinetic analyses, only the aldehyde substrates were used, because oxidation of the respective alcohol substrates was 50–100 times slower.

RESULTS

Expression of *At*ELI3-2 in *E. coli*. IPTG-induced expression of the *At*ELI3-2 cDNA in *E. coli* at various temperatures ranging from 25 to 37°C resulted in the exclusive accumulation of ELI3-2 protein in inclusion bodies, as analyzed by SDS/PAGE. This problem was partly overcome by reducing the temperature to 15°C before the addition of IPTG, as described by Lauvergeat *et al.* (13). An appreciable amount of the 45-kDa ELI3-2 protein remained under these conditions in the soluble fraction. This fraction was used for all subsequent experiments, in parallel with identically treated control extracts derived from bacteria containing either the pQE50 vector or expressing the *Eu*CAD2 cDNA.

Assay for MTD Activity. Considering the recently reported sequence similarity of *Ag*MTD with *At*ELI3 and *Pc*ELI3 (10), we first tested whether the recombinant *At*ELI3-2 protein showed MTD activity. Fig. 1 illustrates that no NAD^+-dependent MTD activity was observed with the ELI3-2-containing bacterial extracts. This applies to various substrate concentrations analyzed. Use of 0.2 units of a commercially available MTD demonstrated the functionality of the assay. Furthermore, addition of authentic MTD to the crude *At*ELI3-2 preparation confirmed the NAD^+ dependency of the reaction and excluded the presence of inhibitory factors in the enzyme assay. In all cases, $NADP^+$ could not substitute for NAD^+ as cofactor (data not shown).

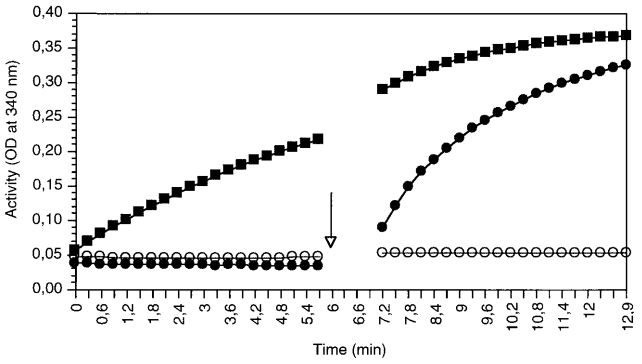

FIG. 1. Spectrophotometric assay for MTD activity in protein extracts from ELI3-2-expressing *E. coli* cells. Crude extract (242 μg of protein) was incubated in MTD assay buffer with (circles) or without (open circles) the cofactor NAD^+. The arrow indicates the time point of addition of 0.2 units of authentic *Actinobacillus* MTD (solid squares).

Assay for CAD Activity. Sequence similarity of ELI3 to various plant CADs has also been observed (9). To address this point, the *At*ELI3-2 preparation was tested for CAD activity using various cinnamyl aldehydes (bottom line in Fig. 2) and alcohols. As illustrated in Fig. 3*A*, low but measurable activity

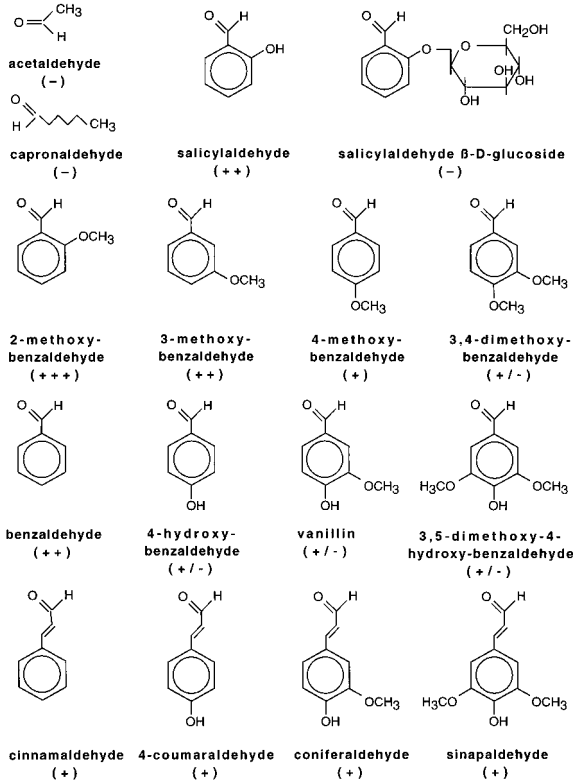

FIG. 2. Chemical structures of various aldehydes tested as potential substrates of *At*ELI3-2. Symbols indicate high (+++), intermediate (++), low (+ or +/−), and undetectable (−) rates of conversion.

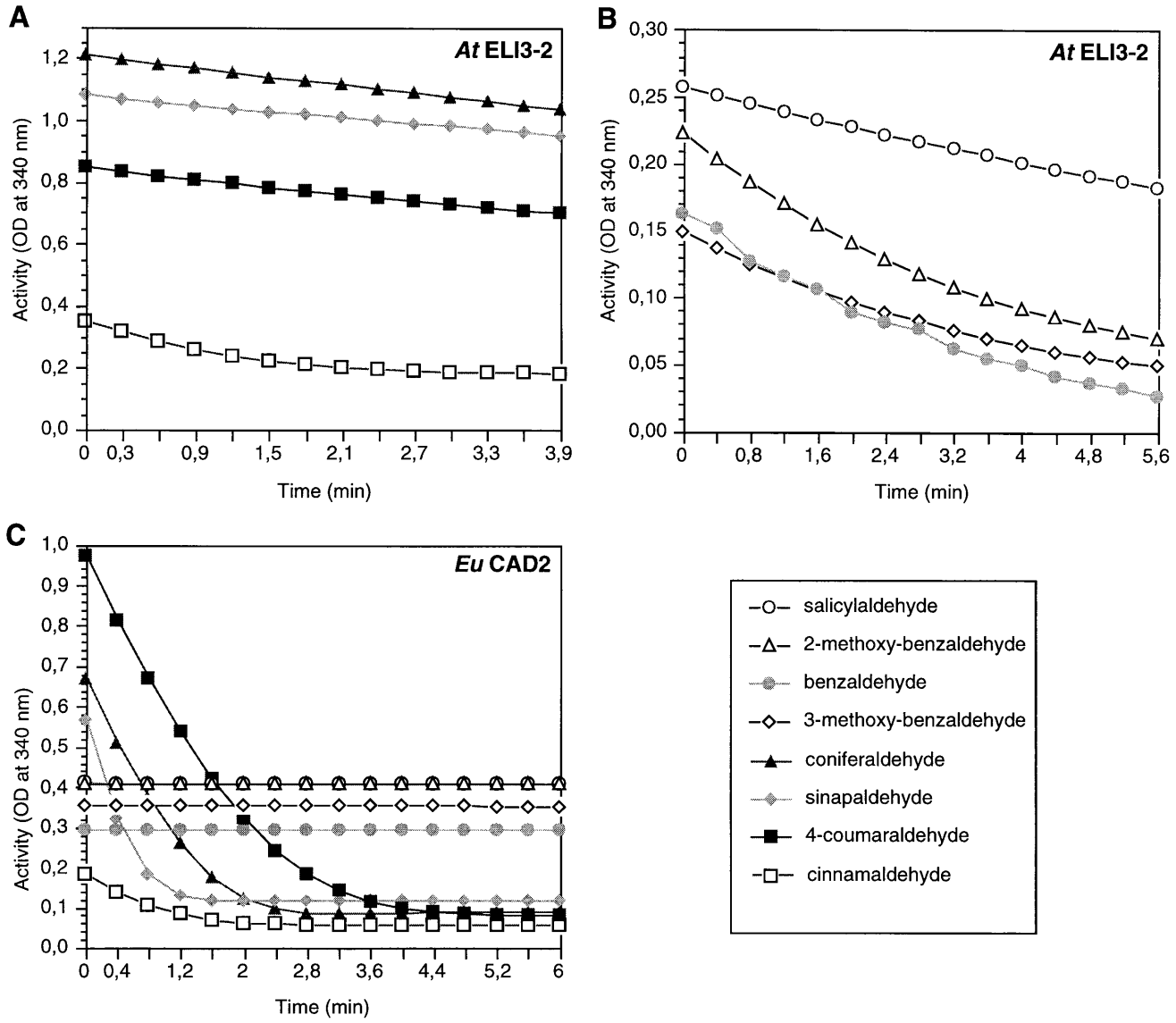

FIG. 3. Relative conversion rates of various aldehydes using 60 μg (*A*) or 5 μg (*B*) of a crude bacterial *At*ELI3-2 extract, or 3.4 μg (*C*) of an analogous, crude bacterial *Eu*CAD2 extract. Symbols are explained in *D*.

was detected for cinnamaldehyde, 4-coumaraldehyde, coniferaldehyde, and sinapaldehyde. However, large amounts (60 μg) of protein extract were required to detect this activity. Therefore, additional aldehydes (Fig. 2) were tested as possible substrates. These assays revealed that several benzaldehyde derivatives, including 2-methoxybenzaldehyde, 3-methoxybenzaldehyde, salicylaldehyde, and benzaldehyde, were used more efficiently than the corresponding cinnamaldehyde derivatives. Fig. 3*B* depicts the activities observed with these substrates using only 5 μg of the bacterial protein extract.

Various other aromatic aldehydes, such as 4-hydroxybenzaldehyde, 4-methoxybenzaldehyde, vanillin (3-methoxy-4-hydroxybenzaldehyde), 3,4-dimethoxybenzaldehyde, and 3,5-dimethoxy-4-hydroxybenzaldehyde, were converted even less efficiently than the cinnamaldehyde derivatives, whereas salicylaldehyde β-*O*-glucoside and two aliphatic aldehydes, acetaldehyde and capronaldehyde, were not converted at all (Fig. 2). Oxidation of the corresponding alcohols was also observed, albeit at 50- to 100-fold lower rates. In contrast to *At*ELI3-2, authentic *Eu*CAD2 from analogous bacterial extracts showed a strong preference for cinnamyl aldehydes/alcohols, with no conversion of the four major *At*ELI3-2 substrates detectable even at high protein concentrations (Fig. 3*C*). Extracts from IPTG-stimulated bacteria harboring the pQE50-vector construct showed no detectable aromatic aldehyde reductase activity.

Thus, biochemical analysis of the recombinant *At*ELI3-2 protein revealed its function as an aromatic aldehyde reductase. The enzyme had an absolute requirement for NADPH as cofactor; NADH could not substitute for NADPH. Quantitative analysis by high performance liquid chromatography

Table 1. Affinity of *At*ELI3-2 for selected substrates (for structures, see Fig. 2)

Substrate	K_m, μM	V_{max}, nkat·mg^{-1}	V_{max}/K_m, nkat·mg^{-1}·μM^{-1}
2-Methoxybenzaldehyde	1.3	24.8	19.1
3-Methoxybenzaldehyde	6	17.5	2.9
Salicylaldehyde	12.5	13.8	1.1
Benzaldehyde	25	26.2	1.1
Cinnamaldehyde	24	17.5	0.7
4-Coumaraldehyde	35	1.9	0.05

The values given were determined from Lineweaver–Burk and Hanes plots using 5–18 μg protein from the transformed *E. coli* strain pQE50-ELI3 and varying concentrations of the indicated substrates.

confirmed the stoichiometric conversion of 2-methoxybenzaldehyde to 2-methoxybenzyl alcohol.

Kinetic Parameters. To estimate the relative affinities for some of the most efficient aldehyde substrates, the apparent K_m and V_{max} values were calculated from Lineweaver–Burk and Hanes plots (Table 1) using the recombinant enzyme in crude bacterial extracts. The results demonstrate that (*i*) 2-methoxybenzaldehyde is the most efficient of all *At*ELI3-2 substrates tested; (*ii*) various unsubstituted and substituted benzaldehydes are more efficient substrates than unsubstituted and substituted cinnamaldehydes; and (*iii*) the substrate specificity of *At*ELI3-2 differs greatly from that of an authentic CAD. We determined the apparent K_m of *Eu*CAD2 for coniferyl aldehyde under our conditions (5 μM) and found it to be in good agreement with the reported value of 4.5 μM (12). The apparent K_m values for NADPH using the four most efficient benzaldehyde derivatives (Table 1) as substrates for *At*ELI3-2 were in the range of 9–32 μM.

DISCUSSION

These results demonstrate that the *eli3-2* gene from *A. thaliana* encodes an aromatic alcohol:$NADP^+$ oxidoreductase and not, as recently predicted, an MTD. Although the precise function of the enzyme *in vivo* is not entirely clear from our present data, it is most likely involved in the generation of benzyl alcohol derivatives. In any case, the apparent substrate specificity is distinct from that of any known NAD^+- or $NADP^+$-dependent oxidoreductase, including MTD and CAD. Considering the strong preference for various benzaldehydes among the substrates tested, as well as the analogy to CAD, which preferentially accepts cinnamyl aldehyde derivatives as substrates, we propose the general name BAD for *At*ELI3-2. Functionally related enzymes, likewise exhibiting higher specificity for benzyl alcohols compared with coniferyl alcohol, may have been detected previously in *E. gunnii* (17) and *Phaseolus vulgaris* (18) but have not been further characterized.

Although biochemical evidence of the functions of *At*ELI3-1 (7) and *Pc*ELI3 (4) is not yet available, sequence comparison with *At*ELI3-2 (85 and 70% amino acid identity, respectively) suggests that they too may be BADs. Moreover, the established tandem location of the *eli3-1* and *eli3-2* genes on chromosome 4 of *A. thaliana*, separated by only $\approx$2 kb (7), suggests, apart from functional similarity, a common evolutionary origin for these two *At*ELI3 isoforms. Even more equivocal is the function of two *A. thaliana* proteins (deduced from GenBank accessions *At*P42734 and *At*Z31715), which were recently proposed to be CADs solely on the basis of their sequence similarity with the authentic enzyme. Although *At*Z31715 may indeed be a CAD (76% identity with *Eu*CAD2), *At*P42734 shares greater similarity with *At*ELI3-2 (63% identity compared with 52% identity with *Eu*CAD2) and thus may rather be a BAD. However, from the obvious functional dissimilarity of *At*ELI3-2 and *Ag*MTD, despite their large sequence similarity (68% identity), we conclude that one has to be extremely cautious with such functional assignments, even though numerous successful cases exist.

The functional assignment of ELI3 is particularly interesting in view of its close association with both local defense gene expression at pathogen infection sites (5) and genetically determined disease resistance (7, 19), even though the genetic link was not always observed (20, 21). It is possible that the putative involvement of BAD in the conversion of benzaldehyde derivatives to the corresponding benzyl alcohols is associated either with the incorporation of phenolic defense materials into the cell wall or with the metabolism of soluble compounds, or both. Although, to our knowledge the accumulation of wall-bound benzyl alcohols has not been described in relation to pathogen defense, two benzaldehydes, 4-hydroxybenzaldehyde and vanillin (Fig. 2), along with unidentified wall components, have been isolated from fungal elicitor-treated, cultured *P. crispum* (22) and *S. tuberosum* cells (23), as well as from fungus-infected *S. tuberosum* leaves (23), and syringaldehyde was found to accumulate in fungus-infected lettuce leaves (24). Further analysis in this direction may reveal additional, structurally related compounds, possibly including the products of BAD activity.

If BAD generates soluble benzyl alcohols *in vivo,* these may have a role in the signaling process during infection, as suggested by the close structural relationship of the most efficient substrates of *At*ELI3-2 with salicylic acid, an established signal molecule in plants (25) that occurs in various structural variants (26). However, similar to the occurrence of multiple CAD (9, 27) and other alcohol dehydrogenase (28) isoforms with distinct substrate specificities, functionally distinct BADs may exist to fulfill different physiological roles. In this regard, surprising observations may lie ahead, analogous to the recent finding that the gene *TASSELSEED2* encodes an alcohol dehydrogenase required for sex determination in *Zea mays* (29).

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1. Collinge, D. B., Gregersen, P. L. & Thordal-Christensen, H. (1994) in *The Induction of Gene Expression in Response to Pathogenic Microbes*, ed. Basra, A. S. (Dekker, New York), pp. 391–433.
2. Dixon, R. A. & Harrison, M. J. (1990) *Adv. Genet.* **28,** 165–234.
3. Kombrink, E. & Somssich, I. E. (1995) *Adv. Bot. Res.* **21,** 1–34.
4. Somssich, I. E., Bollmann, J., Hahlbrock, K., Kombrink, E. & Schulz, W. (1989) *Plant Mol. Biol.* **12,** 227–234.
5. Schmelzer, E., Krüger-Lebus, S. & Hahlbrock, K. (1989) *Plant Cell* **1,** 993–1001.
6. Trezzini, G. F., Horrichs, A. & Somssich, I. E. (1993) *Plant Mol. Biol.* **21,** 385–389.
7. Kiedrowski, S., Kawalleck, P., Hahlbrock, K., Somssich, I. E. & Dangl, J. L. (1992) *EMBO J.* **11,** 4677–4684.
8. Debener, T., Lehnackers, H., Arnold, H. & Dangl, J. L. (1991) *Plant J.* **1,** 289–302.
9. Boudet, A. M., Lapierre, C. & Grima-Pettenati, J. (1995) *New Phytol.* **129,** 203–236.
10. Williamson, J. D., Stoop, J. M. H., Massel, M. O., Conkling, M. A. & Pharr, D. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7148–7152.
11. Dong, X. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7137–7139.
12. Grima-Pettenati, J., Feuillet, C., Goffner, D., Bordereis, G. & Boudet, A. M. (1993) *Plant Mol. Biol.* **21,** 1085–1095.
13. Lauvergeat, V., Kennedy, K., Feuillet, C., McKie, J. H., Gorrichon, L., Baltas, M., Boudet, A. M., Grima-Pettenati, J. & Douglas, K. T. (1995) *Biochemistry* **34,** 12426–12434.
14. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
15. Stoop, J. M. H., Williamson, J. D., Conkling, M. A. & Pharr, D. M. (1995) *Plant Physiol.* **108,** 1219–1225.
16. Wyrambik, D. & Grisebach, H. (1975) *Eur. J. Biochem.* **59,** 9–15.
17. Hawkins, S. W. & Boudet, A. M. (1994) *Plant Physiol.* **104,** 75–84.
18. Grima-Pettenati, J., Campargue, C., Boudet, A. & Boudet, A. M. (1994) *Phytochemistry* **37,** 941–947.
19. Mittal, S. & Davis, K. R. (1995) *Mol. Plant-Microbe Interact.* **8,** 165–171.
20. Reuber, T. L. & Ausubel, F. M. (1996) *Plant Cell* **8,** 241–249.
21. Ritter, C. & Dangl, J. L. (1996) *Plant Cell* **8,** 251–257.
22. Kauss, H., Franke, R., Krause, K., Conrath, U., Jeblick, W., Grimmig, B. & Matern, U. (1993) *Plant Physiol.* **102,** 459–466.
23. Keller, H., Hohlfeld, H., Wray, V., Hahlbrock, K., Scheel, D. & Strack, D., (1996) *Phytochemistry* **42,** 389–396.
24. Bennett, M., Gallagher, M., Fagg, J., Bestwick, C., Paul, T., Beale, M. & Mansfield, J. (1996) *Plant J.* **9,** 101–115.

25. Klessig, D. F. & Malamy, J. (1994) *Plant Mol. Biol.* **26,** 1439–1458.
26. Pierpoint, W. S. (1994) *Adv. Bot. Res.* **20,** 163–235.
27. Campbell, M. M. & Sederoff, R. R. (1996) *Plant Physiol.* **110,** 3–13.
28. Jörnvall, H., Persson, B. & Jeffery, J. (1987) *Eur. J. Biochem.* **167,** 195–201.
29. DeLong, A., Calderon-Urrea, A. & Dellaporta, S. L. (1993) *Cell* **74,** 757–768.