CYP76C2, an *Arabidopsis thaliana* cytochrome P450 gene expressed during hypersensitive and developmental cell death

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Abstract The characterisation of an *Arabidopsis thaliana* cytochrome P450-encoding cDNA clone, *B72*, preferentially expressed during the hypersensitive response (HR) provoked by the bacterial pathogen *Pseudomonas syringae* pathovar *maculicola*, is reported. The *B72* cDNA clone corresponded to the *CYP76C2* gene, which belongs to a small multigene family comprising four genes. HR-triggering bacteria harbouring different avirulence genes induced the accumulation of transcripts of this P450 gene. *CYP76C2* gene expression was moreover associated with various processes leading to cell death such as leaf senescence, ageing of cell cultures, wounding as well as with treatment with the necrotising heavy metal salt, lead nitrate.

Key words: Bacterial leaf spot; Defence gene; Hypersensitive response; Senescence

1. Introduction

Cytochrome P450 monooxygenases (P450) are a group of haem-containing proteins which catalyse various oxidative reactions [1,2]. In higher plants, they play important roles in the biosynthesis of cell wall constituents (lignin), signal molecules (salicylic acid) and secondary metabolites such as pigments (flavonoids), alkaloids or antimicrobial plant defence compounds (phytoalexins) [3–10], and in the catabolism of diverse substances into non-toxic (e.g. pesticide detoxification) or toxic compounds (e.g. proherbicide activation) [1]. Several P450s have been identified in various plants including *Arabidopsis thaliana* [2,7,8,11], but little is known about their induction patterns in response to environmental stimuli, especially after stress or a pathogen challenge [3,5,6,10–12]. Recently, a P450 tobacco gene, *hsr515*, was shown to be activated preferentially during the hypersensitive response (HR) [13], a programmed cell death generally associated with disease resistance to pathogens [14,15]. The so-called *hsr* (hypersensitivity-related) genes [13,16,17] were identified after inoculation of tobacco plants with an avirulent isolate of the bacterium *Ralstonia solanacearum* and are interesting molecular markers for this plant response. Homologues of the *hsr515* gene could not be detected in *A. thaliana* (unpublished results). We hypothesised that some of the *A. thaliana* P450-encoding genes were preferentially expressed during the HR and report here the characterisation of such a gene, *CYP76C2*. We show that it is activated during processes leading to various types of cell death, and thus constitutes an interesting tool for molecular and genetic analyses of the role and regulation of *hsr*-like genes during HR.

2. Materials and methods

2.1. Plant material

A. thaliana ecotype Columbia (Col-0) plants were grown as already described [18]. *A. thaliana* cell-suspension cultures (T87-C3 cells) were cultured under continuous illumination [19].

2.2. Bacterial strains, inoculation procedures and plant treatments

All *Pseudomonas syringae* strains were kindly provided by J. Dangl and grown as described [20–22].

Plant inoculations were performed using bacterial overnight cultures washed in sterile water and resuspended to an OD at 600 nm of 0.1 (5×10^7 colony-forming units per milliliter). Plants were infiltrated under vacuum for 3 min by immersing the whole plant in a bacterial suspension, and transferred to a long-day-length growth chamber.

Heat-shock treatment was carried out by transferring plants to a 30°C growth chamber. Natural plant senescence was monitored according to [19]. Wounding experiments were performed by lacerating detached leaves with a razor blade and placing them onto MS medium [23]. Lead nitrate ($Pb(NO_3)_2$) spraying was performed as described [24]. Other treatments were carried out by immersion of the stems of detached leaves in corresponding solutions buffered with NaH_2PO_4 (10 mM, pH 6): 2,4-dichlorophenoxyacetic acid (2,4-D, 10 μM), abscissic acid (ABA, 50 μM), 1-aminocyclopropane 1-carboxylic acid (ACC, 50 μM), methyl jasmonate (100 μM) and salicylic acid (250 μM).

2.3. Northern and Southern analyses

20 μg of total RNA was extracted from plant leaves [25], denatured with glyoxal and dimethyl sulphoxide [13], electrophoresed on a 1% agarose gel and transferred onto Hybond N^+ membranes (Amersham). The membranes were then hybridised overnight at 42°C with the [α-^{32}P]dCTP-labelled probe (Pharmacia Biotech) in the following hybridisation buffer: 6×SSPE, 5×Denhardt's, 50% formamide, 5% dextran sulphate, 0.5% SDS, 100 μg/ml denatured calf thymus DNA, and washed in 2×SSC, 0.1% SDS solution for 20 min at 42°C and for 20 min at 65°C before autoradiography.

1 μg of total genomic Col-0 DNA extracted from leaf tissues [26] was digested with various restriction enzymes, electrophoresed on a 0.8% agarose gel, and transferred onto Hybond N^+ membranes (Amersham). The membranes were then hybridised overnight at 65°C with the [α-^{32}P]dCTP-labelled probe in hybridisation buffer (6×SSC, 5×Denhardt's, 0.5% SDS, 100 μg/ml denatured calf thymus DNA) and washed in 2×SSC, 1% SDS at room temperature for 20 min and in 0.1×SSC, 0.1% SDS for 15 min at 42°C before autoradiography.

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Abbreviations: P450, cytochrome P450; HR, hypersensitive response; *hsr* genes, hypersensitivity-related genes; EST, expressed sequence tags; Col-0, Columbia; hpi, hours post inoculation; ABA, abscissic acid

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2.4. Nucleotide sequence analysis

Sequence compilation and analysis were performed using the Genetic Computer Group (GCG) software [27]. Searches for homologies in databases were performed using the BLAST algorithm [28].

3. Results

3.1. Identification of an A. thaliana P450 gene preferentially expressed during HR

The expression of six *A. thaliana* cDNA clones (*5C6*, *40E11*, *34C5*, *2E8*, *2B4*, *B72*) corresponding to different P450 genes, randomly isolated and characterised by partial sequencing (ESTs), was analysed. The avirulent m2 and virulent m4 isolates of *Pseudomonas syringae* pathovar *maculicola* (*P.s. maculicola*), inducing HR and disease, respectively, on *A. thaliana* Col-0 [20] were used to infiltrate plants under vacuum. Different expression patterns were observed (Fig. 1). The cDNA clones *5C6* and *40E11* corresponded to genes constitutively expressed in untreated plants as well as in plants infiltrated with the virulent or avirulent strains of the pathogen. The amount of *5C6* transcripts was much higher than that of *40E11*. Transcripts hybridising with the *34C5* probe were abundant in untreated plants as well as in mock-infiltrated plants. In leaves inoculated with bacteria, the amount of the latter transcripts decreased after 6 h. Steady-state levels of mRNAs hybridising with the cDNA clone *2B4* increased from 3 h after infiltration with $MgCl_2$ as well as after inoculation with the m2 or m4 bacterial strains. This increase in the amount of *2B4* transcripts was probably related to the stress of the inoculation procedure since it was also observed in mock-inoculated leaves. Expression of *2E8* gene(s) also appeared to be stress-inducible although higher transcript levels were detected in untreated plants. Although mRNAs corresponding to the *B72* cDNA clone were detected in control

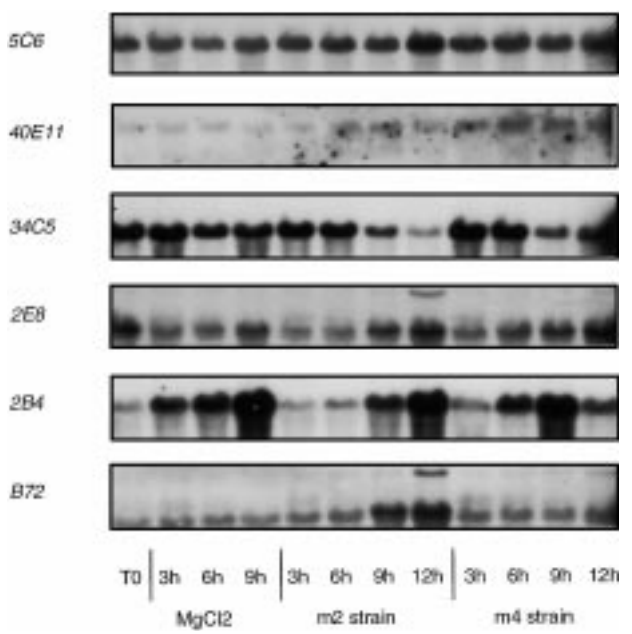

Fig. 1. Northern blot analysis of total RNA from Col-0 leaves infiltrated with buffer ($MgCl_2$), or the m2 and m4 strains of *P.s. maculicola*, hybridised with different P450-encoding ESTs. Total RNA from three different plants was extracted at 0, 3, 6, 9 and 12 hpi. The labelled inserts of the studied ESTs used as probes on the Northern blot are indicated on the left.

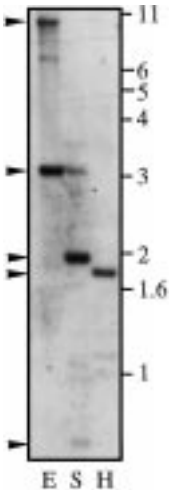

Fig. 2. Southern blot analysis of the *A. thaliana B72* gene family. 1 μg of total genomic Col-0 DNA was digested with the following restriction enzymes: E, *Eco*RI; S, *Ssp*I and H, *Hin*dIII, separated on a 0.8% agarose gel, prior to transfer and hybridisation with the labelled *B72* cDNA clone. Arrows indicate the positions of the hybridisation signals corresponding to the restriction DNA fragments containing the *CYP76C2* gene. The migration of size markers is shown on the right.

leaves, their steady-state levels increased 6 h after inoculation by the HR-inducing bacterial strain m2 and remained at lower constitutive levels in m4 inoculated plants. The accumulation of *B72* transcripts occurred before the onset of visible cell death associated with HR (12 h post inoculation, hpi). Background levels of *B72* mRNAs detected in control (T_0) and $MgCl_2$-treated plants varied from one experiment to another, probably due to the physiological state of the plants. The size of the hybridising transcripts was 1.6 kb, whatever the nature of the probe, which is in agreement with the estimated size of mRNAs corresponding to P450s. A second hybridisation signal, 2 kb large, was sometimes detected after hybridisation with two of the cDNA clones (*2E8* and *B72*), 12 h after inoculation with the m2 strain, but was not reproducibly observed.

The preferential accumulation of transcripts during the early phases of HR observed with the *B72* cDNA clone prompted us to study this P450 gene further.

3.2. The B72 cDNA clone corresponds to the CYP76C2 gene

The complete nucleotide sequence of the *B72* cDNA (1383 bp) was determined; this cDNA encodes an N-terminal truncated peptide of 421 aa including the highly conserved sequence FxxGxRxCxG, considered a fingerprint for P450 proteins [29], in its C-terminal region (data not shown). Searches for homologies in databases [27,28] indicated that the *B72* nucleotide sequence was identical to the coding region of *CYP76C2*, a genomic clone located on chromosome II and carried on a BAC clone, *F17K2* (accession number AC003680). The genomic sequence contained two introns of 109 and 342 bp and encoded a protein of 512 aa (accession number F17K2.10). Three other related genes (*CYP76C1*, *CYP76C3* and *CYP76C4*) belonging to the same P450 subfamily were found as tandem repeats on the BAC clone, separated by short intergenic regions (466, 737 and 1581 bp). The *B72* cDNA clone presented the highest level of similarity (42% aa identity) with *CYP76B1*, a *Helianthus tuberosus* gene en-

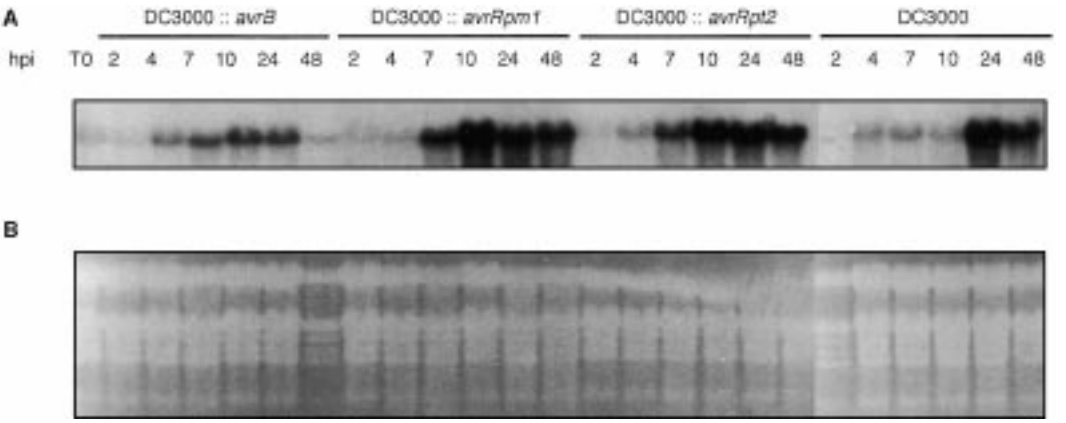

Fig. 3. A: Northern blot analysis of total RNA from Col-0 leaves infiltrated with different strains of *P. syringae* pathovar *tomato*, hybridised with the labelled *B72* cDNA clone. Total RNA from three infiltrated plants was extracted at 0, 2, 4, 7, 10, 24 and 48 hpi. B: UV light visualisation of rRNA after transfer onto the membrane used in A. On the EtBr-stained gel, the lane corresponding to the inoculation with the DC3000 strain harbouring the *avrB* gene, 48 hpi, was clearly underloaded with RNA, which explained the lower hybridisation signal in A.

coding a 7-ethoxycoumarin *O*-de-ethylase, an enzyme likely involved in xenobiotic catabolism [30].

In order to determine the size of this gene family in *A. thaliana*, Southern experiments using the insert of the *B72* cDNA clone were performed at high stringency (Fig. 2). The presence of hybridisation signals whose size is in perfect agreement with those deduced from the nucleotide sequence of the *CYP76C* genes carried by BAC clone *F17K2* was detected. For example, the *B72* probe hybridised with restriction fragments of 3.1 kb and 10.5 kb (*Eco*RI), 1.9 kb and 0.7 kb (*Ssp*I) and 1.8 kb (*Hin*dIII), corresponding to the *CYP76C2* genomic region. Under our hybridisation conditions, signals corresponding to the other three genes were much fainter (Fig. 2), as expected since *CYP76C2* presents lower levels of identity of 64.7%, 74.3% and 79% with *CYP76C3*, *CYP76C4* and *CYP76C1*, respectively.

These data suggested that CYP76C2 is encoded by a single-copy gene in *A. thaliana*. Considering the high stringency of our hybridisation conditions, mRNA accumulation observed during HR using the insert of the *B72* cDNA clone as a probe is primarily due to increased transcript levels of the *CYP76C2* gene, although the participation of the other *CYP76C* genes cannot be excluded.

3.3. Expression patterns during different plant-bacteria interactions

In order to confirm the preferential induction of the *CYP76C2* gene during HR, its expression was analysed in different plant-bacteria interactions. The HR-inducing ability of the m2 native strain of *P.s. maculicola* on *A. thaliana* Col-0 ecotype is conferred by the presence of the *avrRpm1* avirulence gene [20]. To test whether the *CYP76C2* gene was induced preferentially during the HR caused by other avirulent bacteria, the accumulation of the corresponding transcripts was estimated at different time points in Col-0 plants infiltrated with the DC3000 strain of *P. syringae* pathovar *tomato*, which causes disease on the Col-0 ecotype [21], and three different derivative strains each expressing one of the three *avr* genes *avrB*, *avrRpm1* or *avrRpt2*, all of which trigger an HR on Col-0 [22]. Northern analysis indicated that the *CYP76C2* gene was activated 2–4 h after inoculation with bacteria expressing the *avrB* gene, and slightly later (4–7 h) in plants developing a HR provoked by bacteria harbouring the *avrRpm1* or *avrRpt2* genes (Fig. 3). In all cases, the accumulation of *CYP76C2* transcripts preceded the appearance of HR (10–12 hpi). The steady-state levels of *CYP76C2* mRNAs increased and reached a plateau 10–48 h after inoculation with bacteria expressing the different *avr* genes. During the interaction leading to disease (DC3000), *CYP76C2* transcripts

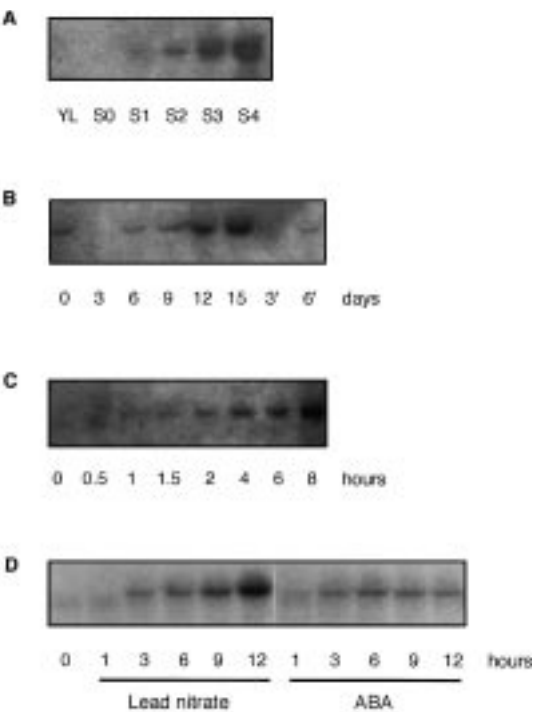

Fig. 4. Northern blot analysis of *CYP76C2* expression in various conditions. Total RNA was extracted and hybridised with the labelled *B72* cDNA clone. A: At different stages of *A. thaliana* leaf development: in young leaves (YL); in fully expanded green leaves (S0); leaves with increasing degrees of yellowing due to senescence (S1, S2, S3 and S4: 25, 50, 75 and 100% estimated, respectively). B: At different phases of an *A. thaliana* cell-suspension culture: 0-, 3-, 6-, 9-, 12-, 15-day-old cells, and the 15-day-old cells subcultured a second time and grown for 3 and 6 days (3′ and 6′ time points). C: In lacerated leaves maintained on liquid MS medium [18] for 0, 0.5, 1, 1.5, 2, 4, 6, and 8 h. D: In leaves sprayed with lead nitrate ($Pb(NO_3)_2$, 10 mM) or immersed in an ABA solution (50 μM) and frozen after 0, 1, 3, 6, 9 and 12 h.

started to accumulate at later time points (after 10 h). *CYP76C2* mRNA accumulation therefore took place during the early steps of the hypersensitive cell death provoked by different avirulence specificities although small temporal differences in the timing of transcript accumulation were observed according to the bacterial strain used.

3.4. The CYP76C2 gene is expressed during senescence, after wounding and following treatment with lead, but not in response to general stress conditions

One of the features of genes expressed during plant-pathogen interactions is their tissue-specific patterns of expression during normal plant development and in response to various stresses [31]. Several plant P450 cytochromes are developmentally regulated [32–34] and some are induced by wounding and/or during senescence [6,11,12]. Accumulation of mRNAs corresponding to *CYP76C2* gene during plant development and after different stress-inducing treatments was therefore investigated (Fig. 4). No hybridising transcripts were detected on Northern blots in healthy and mature roots, leaves, stems and flowers (data not shown), but *CYP76C2* mRNAs accumulated in senescing leaves (Fig. 4A). Since some senescence-related processes take place in *A. thaliana* cell-suspension cultures [19], *CYP76C2* transcript levels were analysed in this system. *CYP76C2* was hardly expressed in young cultures (3- and 3′-day time points) (Fig. 4B). In growing cells (3–9 days), mRNA levels remained low, but increased gradually toward the end of exponential cell growth, and was maximal in 12–15-day-old cells. Highest transcript steady-state levels of *CYP76C2* (between 12 and 15 days of culture) coincided with the decline of cell viability in this in vitro system [19]. The *B72* mRNA accumulation observed at T_0 probably corresponded to the transcripts present at 15 days in cells which were diluted with fresh medium.

After wounding, *CYP76C2* gene was activated within 1–1.5 h (Fig. 4C), and the amount of hybridising transcripts gradually increased until 8 h.

Finally, *CYP76C2* gene expression was analysed after a heat-shock treatment at 30°C and after application of various compounds such as plant hormones: 2,4-D, ABA, ACC (a precursor of ethylene), defence genes activators such as methyl jasmonate and salicylic acid, and lead nitrate, a toxic heavy metal salt known to induce necrosis on treated leaves [24]. Spraying with lead nitrate or treatment with ABA induced *CYP76C2* transcript accumulation (Fig. 4D). Hybridising signals were detected 1–3 h after application of either compound, and then increased from 3 to 12 h after lead nitrate spray, or remained at a low level until 12 h after ABA treatment.

4. Discussion

The characterisation of an *A. thaliana* P450 gene preferentially expressed during the HR caused by avirulent bacterial pathogens is reported in this study.

The different expression patterns of six different P450-encoding ESTs obtained after bacterial inoculation illustrate the great variability of expression of these genes, which reflects the wide diversity of functions of the encoded proteins [2]. Among these ESTs, *B72* presented interesting characteristics since it was preferentially expressed during the HR after bacterial inoculation. The *B72* cDNA clone corresponded to a gene, *CYP76C2*, present on a BAC clone. Interestingly, three other genes of the same P450 subfamily were also present on the same BAC clone, illustrating the clustered organisation of P450 gene families, previously described in mouse and human genomes [35] as well as in plants [32].

CYP76C2 expression was preferentially activated in the early steps of HR provoked by *P. syringae* bacteria carrying different avirulence genes, and also in tissues or cell suspensions undergoing developmental cell death.

Other *A. thaliana* genes, such as the *AIG1*, *AIG2*, and *ELI3* genes, are expressed after inoculation by avirulent *P. syringae* strains, but are highly dependent on the avirulence specificity carried by the bacterium: *AIG1* and *AIG2* genes are activated after inoculation with *P.s. maculicola* carrying the *avrRpt2* gene but not the *avrRpm1* gene; conversely, *ELI3* gene is induced in the opposite way, suggesting that different resistance gene/avirulence gene combinations can mediate distinct defence responses [36]. These observations imply that different types of genes might be involved in the establishment of HR in plants: *avr*-specific induced genes, such as *AIG* and *ELI3* genes, which might be associated with one or a limited number of avirulence specificities, and other genes, such as *CYP76C2*, which are induced more widely in the case of HR-associated resistance, supporting the notion of common steps in the downstream pathways leading to this plant response [37]. It would be interesting to analyse *CYP76C2* expression during HR triggered by viral or fungal pathogens.

CYP76C2 gene expression showed similar characteristics to the tobacco *hsr* genes: maximal induction before development of hypersensitive necrosis, very low expression in interactions leading to disease, no response to classical defence gene elicitors, and no expression during plant development [13,16,17,38]. In contrast to the latter genes, however, it was expressed during senescence. A strong correlation could also be established between the activation of *CYP76C2* gene and different treatments leading to cell death such as wounding and lead nitrate solution spray. The latter stimulus indeed induced visible necrotic lesions which resemble HR necroses caused by avirulent bacteria, and the expression of several markers associated with the HR, such as callose deposition and increase in activities of defence-related proteins [24].

Among various effectors tested, ABA, a plant hormone associated with senescence processes, induced *CYP76C2* gene expression. The expression of another metallothionein-like-encoding gene, *LSC54*, induced during senescence of *Brassica napus* and *A. thaliana* is also associated with resistance to the fungus *Peronospora parasitica* in *A. thaliana* [39]. Different physiological pathways leading to cell death (ageing, HR, wounding, treatments with necrotising factors) might thus share some common steps.

It would be interesting to test whether *CYP76C2* gene expression is altered in *A. thaliana* mutants impaired in hypersensitive cell death induction, such as the *lsd* and *acd* mutants [40,41], as this might contribute to our understanding of the role of this P450 protein in the establishment of HR. Such genes thus constitute useful tools for the development of strategies aimed at gaining a better understanding of this plant response.

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References

[1] Schuler, M.A. (1996) Crit. Rev. Plant Sci. 15, 235–284.
[2] Chapple, C. (1998) Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 311–343.
[3] Fahrendorf, T. and Dixon, R.A. (1993) Arch. Biochem. Biophys. 305, 509–515.
[4] Holton, T.A., Brugliera, F., Lester, D.R., Tanaka, Y., Hyland, C.D., Menting, G.J.T., Lu, C.Y., Farcy, E., Stevenson, T.W. and Cornish, E.C. (1993) Nature 366, 276–279.
[5] Leon, J., Yalpani, N. and Lawton, M.A. (1993) Plant Physiol. 103, 323–328.
[6] Teutsch, H.G., Hasenfratz, M.P., Lesot, A., Stoltz, C., Garnier, J.M., Jeltsch, J.M., Durst, F. and Werck-Reichhart, D. (1993) Proc. Natl. Acad. Sci. USA 90, 4102–4106.
[7] Meyer, K., Cusumano, J.C., Somerville, C. and Chapple, C.C.S. (1996) Proc. Natl. Acad. Sci. USA 93, 6869–6874.
[8] Mizutani, M., Ohta, D. and Sato, R. (1997) Plant Physiol. 113, 755–763.
[9] Pauli, H.H. and Kutchan, T.M. (1998) Plant J. 13, 793–801.
[10] Schopfer, C.R. and Ebel, J. (1998) Mol. Gen. Genet. 258, 315–322.
[11] Mizutani, M., Ward, E. and Ohta, D. (1998) Plant Mol. Biol. 37, 39–52.
[12] Frank, M.R., Deyneka, J.M. and Schuler, M.A. (1996) Plant Physiol. 110, 1035–1046.
[13] Czernic, P., Huang, H.C. and Marco, Y. (1996) Plant Mol. Biol. 31, 255–265.
[14] Klement Z. (1982) in: Phytopathogenic Prokaryotes (Mount, M.S. and Lacy, G.H., Eds.), Vol. 2, pp. 149–177, Academic Press, New York.
[15] Dangl, J.L., Dietrich, R.A. and Richberg, M.H. (1996) Plant Cell 8, 1793–1807.
[16] Marco, Y., Ragueh, F., Godiard, L. and Froissard, D. (1990) Plant Mol. Biol. 15, 145–154.
[17] Pontier, D., Godiard, L., Marco, Y. and Roby, D. (1994) Plant J. 5, 507–521.
[18] Deslandes, L., Pileur, F., Liaubet, L., Camut, S., Can, C., Williams, K., Holub, E., Beynon, J., Arlat, M. and Marco, Y. (1998) Mol. Plant-Microbe Interact. 11, 659–667.
[19] Callard, D., Axelos, M. and Mazzolini, L. (1996) Plant Physiol. 112, 705–715.
[20] Debener, T., Lehnackers, H., Arnold, M. and Dangl, J.L. (1991) Plant J. 1, 289–302.
[21] Whalen, M.C., Innes, R.W., Bent, A.F. and Staskawicz, B.J. (1991) Plant Cell 3, 49–59.
[22] Innes, R.W., Bisgrove, S.R., Smith, N.M., Bent, A.F., Staskawicz, B.J. and Liu, Y.C. (1993) Plant J. 4, 813–820.
[23] Murashige, T. and Skoog, F. (1962) Physiol. Plant 15, 473–497.
[24] Lummerzheim, M., Sandroni, M., Castresana, C., De Oliveira, D., Van Montagu, M., Roby, D. and Timmerman, B. (1995) Plant Cell Environ. 18, 499–509.
[25] Verwoerd, M.C., Dekker, B.M.M. and Hoekema, A. (1989) Nucleic Acids Res. 17, 23–62.
[26] Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W. (1984) Proc. Natl. Acad. Sci. USA 81, 8014–8018.
[27] Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395.
[28] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) J. Mol. Biol. 215, 403–410.
[29] Nelson, D.R., Koymans, L., Kamataki, T., Stegeman, J.J., Feyereisen, F., Waxman, D.J., Waterman, M.R., Gotoh, O., Coon, M.J., Estabrook, R.W., Gunsalus, I.C. and Nebert, D.W. (1996) Pharmacogenetics 6, 1–42.
[30] Batard, Y., LeRet, M., Schalk, M., Robineau, T., Durst, F. and Werck-Reichhart, D. (1998) Plant J. 14, 111–120.
[31] Bowles, D.J. (1990) Annu. Rev. Biochem. 59, 873–907.
[32] Frey, M., Kliem, R., Saedler, H. and Gierl, A. (1995) Mol. Gen. Genet. 246, 100–109.
[33] Winkler, R.G. and Helentjaris, T. (1995) Plant Cell 7, 1307–1317.
[34] Szekeres, M., Németh, K., Koncz-Kalman, Z., Mathur, J., Kauschmann, A., Altmann, T., Rédei, G.P., Nagy, F., Schell, J. and Koncz, C. (1996) Cell 85, 171–182.
[35] Gonzales, F.J. and Nebert, D.W. (1990) Trends Genet. 6, 182–186.
[36] Reuber, T.L. and Ausubel, F.M. (1996) Plant Cell 8, 241–249.
[37] Hammond-Kosack, K.E. and Jones, J.D.G. (1996) Plant Cell 8, 1773–1791.
[38] Godiard, L., Froissard, D., Fournier, J., Axelos, M. and Marco, Y. (1991) Plant Mol. Biol. 17, 409–413.
[39] Buchanan-Wollaston, V. (1997) J. Exp. Bot. 48, 181–199.
[40] Dietrich, R.A., Delaney, T.P., Uknes, S.J., Ward, E.J., Ryals, J.A. and Dangl, J.L. (1994) Cell 77, 565–578.
[41] Greenberg, J.T., Guo, A., Klessig, D.F. and Ausubel, F.M. (1994) Cell 77, 551–564.