cDNA-AFLP analysis reveals that maize resistance to *Bipolaris maydis* is associated with the induction of multiple defense-related genes

GAO Zhihuan[1,2], XUE Yongbiao[2] & DAI Jingrui[1]

1. Department of Plant Genetics and Breeding, China Agricultural University, Beijing 100094, China;
2. Laboratory of Plant Genetics and Developmental Biology, Institute of Developmental Biology, Chinese Academy of Sciences, Beijing 100080, China

Correspondence should be addressed to Xue Yongbiao and Dai Jingrui (e-mail: ybxue@public3.bta.net.cn, daimin@public3. bta.net.cn)

Abstract **The fungal pathogen *Bipolaris maydis* invades by direct penetration into maize leaf veins. In order to understand the resistance mechanism of maize to *B. maydis* strain 523, cDNA-AFLP (amplified fragment length polymorphism) analysis was conducted to compare the changes in mRNA transcripts in response to *B. maydis* infection between a highly disease-resistant (HDR) line and a susceptible (S) line. 13 cDNA fragments derived from the genes showing enhanced expression after fungal infection, named *HDR* genes, were isolated from the HDR line. Northern blot analysis showed that 5 *HDR* genes were induced by fungal infection in the HDR, but not the S lines. The 5 *HDR* genes showed homology to previously characterized genes involved in disease resistance. A full-length *HDR10* cDNA was isolated. It had a capacity to encode a protein of 284 amino acids. The deduced amino acid sequence of the *HDR10* gene was homologous to a fungal infection-induced protein from *Cicer arietinum* and a hypersensitive response protein from maize, respectively. These results suggest that maize resistance to *B. maydis* infection in the HDR line may be mediated by the induction of multiple defense-related genes.**

Keywords: maize, *Bipolaris maydis*, cDNA-AFLP, plant defense, gene expression.

Bipolaris maydis, a fungal pathogen, produces a mycotoxin involved in the induction of maize leaf blight[1]. Compared with the mycotoxins produced by other fungal pathogens[2], the toxin from *B. maydis* strain 523 was a host nonselective type[3]. To prevent potential yield loss caused by *B. maydis* infection, the knowledge of maize and *B. maydis* interaction on the molecular basis is required.

Disease resistance in plants often involves specific recognition of invading pathogens followed by activation of a defense response. Rapid production of active oxygen species (AOS) is one of the earliest defense responses[4]. In addition to contributing directly to plant defense, AOS plays a role in signaling, leading to the induction of defense genes[5,6]. Plants that resist pathogen attack frequently develop a hypersensitive response (HR), in which a rapid and localized cell death occurs at the infection site. The formation of the necrotic lesions is associated with the restriction of pathogen multiplication and spread. Consequently, the resistant plants develop no further symptoms, whereas the pathogen-susceptible lines usually become systemically infected. Several days after HR induction, the systemic acquired resistance (SAR) develops throughout the plant. SAR confers plants an enhanced resistance not only to a secondary challenge by the initial infecting pathogen, but also to a broad range of unrelated pathogens[7]. Therefore, the activation of disease resistance is concomitant with increased expression of a large number of defense-associated genes in the inoculated leaf, including those encoding peroxidases, cell wall proteins, hydrolytic enzymes, proteinase inhibitors and enzymes involved in the phenylpropanoid biosynthetic pathway[7,8]. Although in different plant-pathogen interactions the specific resistance mechanisms may differ from each other, it is now clear that resistance response in different plant species often involves the activation of common sets of defense related genes.

In this note, we investigated gene expression changes following *B. maydis* infection of both resistant and susceptible maize lines using the cDNA-AFLP (amplified fragment length polymorphism) approach. Our results provide evidence for the involvement of multiple defense related genes in maize resistance to *B. maydis* infection.

1 Materials and methods

(i) Fungal strains, plants and growth conditions. *B. maydis* strain 523 was a single spore isolate provided by the Institute of Physiology and Biochemistry, Hebei Academy of Agricultural and Forestry Sciences. It was grown at 25℃ as described previously[3]. 10 maize alloplasmic cytoplasmic male sterile (CMS) inbred lines were gifts from the Molecular Genetics Group, Department of Crop Science, China Agriculture University.

(ii) Inoculation of *B. maydis*. Spores of *B. maydis* were collected from potato dextrose agar plates and were suspended in sterile distilled water with a small amount of Tween-20 (Sigma) at a concentration of 10^6 spores per mL. Inoculations were performed by spraying the spore suspension onto the surface of leaves detached from maize seedlings at the 5-leaf stage. Inoculated leaves were allowed to dry for 30 min, followed by incubation at 25℃ under a high humidity condition. Leaf samples were taken at different time points, frozen immediately in liquid nitrogen and stored at −80℃.

(iii) RNA extraction and cDNA synthesis. Total RNAs were extracted from healthy and infected maize

(C)1994-2021 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

leaves using the RNeasy Plant Mini Kit (QIAGEN, Germany). The resultant RNA was converted into cDNA using Superscript II reverse transcriptase (Gibco BRL, USA) and SMART™ cDNA Library Construction Kit (CLONTECH, USA) according to the manufacturer's recommendations.

(iv) cDNA-AFLP analysis. Template for cDNA-AFLP was prepared according to Bachem et al.[9] using *Ase* I and *Taq* I. The sequences of primers and adapters employed for AFLP reactions were as follows: *Ase* I adapter top strand, 5′-CTCGTAGACTGCGTA- CC- 3′; *Ase* I adapter bottom strand, 5′-TAGGTACGC-AGTC-3′; *Taq* I adapter top strand, 5′-GACGATGAG-TCCTGAC-3′; *Taq* I adapter bottom strand, 5′-CGGT-CAGGACTCAT-3′; *Ase* I pre-amplification primer, 5′-CTCGTAGACTGCGTACCTAAT-3′; *Ase* I selective amplification primers, 5′-GACTGCGTACCTAATNN-3′ (N = G, A, T or C); *Taq* I pre-amplification primer, 5′-GACGATGAGTCCTGACCGA-3′; *Taq* I selective amplification primers 5′-GATGAGTCCTGACCGANN-3′ (N = G, A, T or C). AFLP reactions were performed according to Bachem et al.[9] except that a mixture of Taq and Pfu DNA polymerases (160 : 1 unit) was used for polymerase chain reaction (PCR) in a final volume of 10 μL. Selective amplified products were separated in a 6% polyacrylamide gel that was run at 80 W until bromophenol blue reached the bottom of the gel. The gel was subject to silver staining involving the following steps: soak gel in 10% acetic acid for 40 min; wash gel in de-ionized water 3 times, 3 min each; stain gel using the $AgNO_3$ solution ($AgNO_3$ 1.0 g/L, 37% formaldehyde 1.5 ml/L) for 30 min; wash gel in de-ionized water for 5 s; soak gel in the developing solution (Na_2CO_3 30 g/L, 10 mg/mL NaS_2O_3 200 μL/L, 37% formaldehyde 1.5 mL/L) until the appearance of clear bands (Note: the staining background could be decreased by pre-cooling the Na_2CO_3 solution to 0℃); stop band development in 10% acetic acid for 5 min; wash gel in de-ionized water for 5 s and allow the gel to dry in air.

(v) Isolation and sequencing of cDNA-AFLP fragments. The desired bands displayed by cDNA-AFLP experiments were recovered the gel with the aid of a razor blade. The cDNA was eluted by incubating individual gel slices in 50 μL of SDW overnight at 37℃. The eluted fragments were re-amplified by PCR under the same conditions as the pre-amplification step of the cDNA-AFLP analysis, followed by cloning into the pGEM-T vector (Promega, USA). DNA sequence in selected clones was determined using the automatic sequencer ABI 377. Nucleotide and derived amino acid sequences were compared to those deposited in the EMBL database as well as the EST databases using the BLAST program[10].

(vi) Construction and screening of cDNA Library. Total RNA was extracted from maize leaves 24 h after inoculation with the spores of *B. maydis*. Double stranded cDNA was synthesized and inserted into the λTriplEx2 vector (Clontech, USA). After packaging (Packagene, Promega), the library was plated at a density of 10000 plaques per 90-mm plate. Plaque lifts (BioTrace NT nitrocellulose membrane, Gelman Sciences, USA) were screened with radioactive probes prepared with reamplified AFLP fragments using an oligo-labeling kit (Promega). Hybridization and post hybridization washing were performed following the guidelines specified by Gelman Sciences.

(vii) Northern and Southern blot analyses. Aliquots (20 μg) of total RNA were separated in a denaturing 1.4% agarose gel followed by blotting onto nylon membrane (Hybond-N+; Amersham, UK) using 7.5 mmol/L NaOH as the transferring solution. Genomic DNA samples (10 μg in each) were digested with appropriate restriction enzymes, separated in agarose gels and blotted onto nylon membrane according to Sambrook et al.[11]. Hybridization and post hybridization washes was conducted as detailed in ref. [11].

2 Results

(i) cDNA-AFLP analysis of mRNAs from HDR and S maize leaves after *B. maydis* infection. Light microscopy showed that strain 523 (C523) of *B. maydis* invaded maize leaf veins by direct penetration (data not shown). 3 d after inoculation, conspicuous spots developed on the maize leaf surface. A total of ten inbred CMS lines were tested and showed differential responses to the pathogen. Relatively large and wilting spots were formed on the leaves of line 888, indicating that this line was susceptible and was therefore designated the S line in this work. In contrast, tiny and necrotic lesions were formed on the leaves of line 882, suggesting that this line was highly resistant and was consequently designated the HDR line in subsequent investigations. In order to reveal potential changes in the gene expression in response to C523 infection, temporal mRNA transcript profiles were compared between the healthy and infected leaves of the two lines using cDNA-AFLP. In total, 35 primer set combinations were used per cDNA sample for selective amplification. A typical example of the obtained cDNA-AFLP gel is shown in fig. 1. In the case of the HDR line, 787 fragments over 200 bp were amplified for the healthy leaves whereas 488 fragments were recorded for the infected leaves. In the S line, 766 and 523 fragments were amplified for the healthy and infected leaves, respectively. Assuming that each amplified fragment represented a single gene, these results suggest that, in both the HDR and S lines, the expression of a considerable proportion of maize genes was attenuated or eliminated following *B. maidis* infection.

(C)1994-2021 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

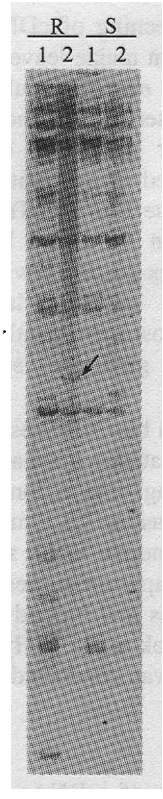

Fig. 1. cDNA-AFLP analysis of mRNA from the HDR and S maize leaves. PAGE gel was stained by $AgNO_3$. 1, Health leaves; 2, leaves inoculated by fungi after 24 h. R, Highly disease-resistant maize; S, disease-susceptible maize.

In addition to the above changes in the number of displayed fragments, cDNA fragments showing enhanced or induced patterns after inoculation were also found. 13 cDNA fragments with this pattern were isolated from the HDR line, and were named *HDR* genes (see fig. 1 for example). Importantly, they were induced by the fungal infection in the HDR line, but not the S line, suggesting that their function may be related to the highly disease-resistant response observed in the HDR line. The cDNA fragments were cloned and sequenced. After nucleotide and amino acid sequence comparison, six induced sequences, namely, *HDR1, 4, 5, 6, 7* and *10* were found to possess homology to the previously characterized genes (table 1).

(ii) Expression patterns of *HDR* genes. The differential expression pattern of five *HDR* genes (*HDR1, 4, 6, 7* and *10*) was examined in the leaves during a 72-h time period after inoculation by Northern analysis. All five *HDR* genes were induced evidently by fungal infection in the HDR but not the S line, confirming that they were involved in the development of the resistance resp- onse in the HDR line (fig. 2). The expression patterns of the five *HDR* genes could be further divided into three types (fig. 3). First, *HDR1* and *HDR4* expression increased initially and decreased later with the maximum expression occurring at 24 h post inoculation. Second, *HDR6* and *HDR7* expression increased continuously during the 72-h period. *HDR6* was induced over 10 times by 72 h whereas *HDR7* induced about twofold during the same period. Third, *HDR10* exhibited a fluctuating expression pattern. It was induced 12 h post inoculation followed by a decrease at 24 h and a further induction and decrease cycle between 48 and 72 h. These results demonstrated that *HDR* gene induction varied in strength and timing in the HDR line, suggesting that multiple gene regulatory pathways may be involved in the formation of the resistant state.

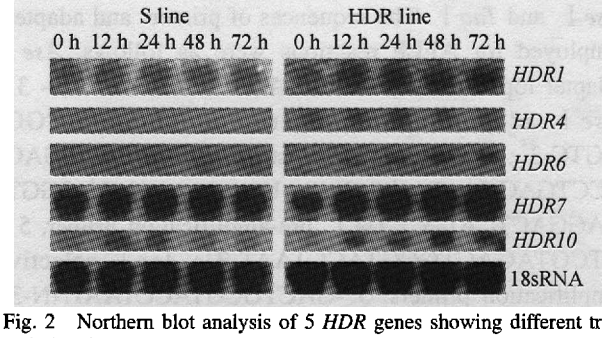

Fig. 2 Northern blot analysis of 5 *HDR* genes showing different transcriptional modes.

(iii) Isolation of a full length cDNA for *HDR10*. In order to isolate a full-length cDNA for the *HDR10* gene, a radioactive probe prepared from its corresponding AFLP fragment was used to screen the cDNA library constructed from the inoculated HDR line. Two positive clones were isolated with different 5′ ends. The longer one was 1380 bp and was capable of encoding a polypeptide of 284 aa (fig. 4). Hydropathy plot analysis indicated that, within the predicted protein sequence, several potential hydrophobic regions showed a feature of membrane-anchored proteins (data not shown). The deduced HDR10 protein possessed a 98% identity to a hypersensitive-induced response protein (see table 1) from maize, a hypothetical protein (accession number: CAA10289) induced by fungal infection in *Cicer arietinum* and several other membrane proteins, such as the stomatin-like protein (CAB50468) and GNA1220 (AAF42660). Southern hybridization showed that *HDR10* was a single copy gene in the maize genome (data not shown).

3 Discussion

In the previous studies, plant gene expression changes in response to pathogen infection were often analyzed using differential display RT-PCR and cDNA-AFLP. This approach led to the identification of many disease-resistance related genes[12—14]. By adopting this technique in our work, we identified multiple disease-related genes associated with the resistance response of the maize HDR line to *B. maydis* infection.

Recent molecular and genetic studies have shown that disease resistance is developed through a complex interaction between the pathogen and its host[15, 16]. For example, several different signaling and biochemical pathways are involved in the disease resistance mediated by R (resistance) genes[8, 14]. In the HDR line, disease re-

(C)1994-2021 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

Table 1 *HDR* sequences and their homology to previously characterized genes

Name	AFLP fragment	Length/bp	Homology[a)]	Blast score[b)]
HDR1	9	300	putative acyl-coA dehydrogenase from *Arabidopsis thaliana* (AF049236)	2e-14
HDR2	14	410	no	
HDR3	29	440	no	
HDR4	39	290	Casein kinase II, alpha chain (CK II) from *Z. mays* (P28523)	3e-25
HDR5	47	460	*Z. mays* complete chloroplast genome (X86563)	e-132[b)]
HDR6	48	480	*Pisum sativum* mRNA for second sucrose synthase (AJ001071)	4e-22[b)]
HDR7	53	470	translation elongation factor eEF-1 alpha chain from maize (S66338)	1e-23
HDR8	55	240	no	
HDR9	58	270	no	
HDR10	63	360	hypersensitive-induced response protein from *Zmays* (AAF68389)	1e-32
HDR11	68	300	no	
HDR12	69	260	no	
HDR13	70	330	no	

a) GenBank accession numbers of sequences homologous to AFLP fragment are in parentheses; b) BLASTN scores.

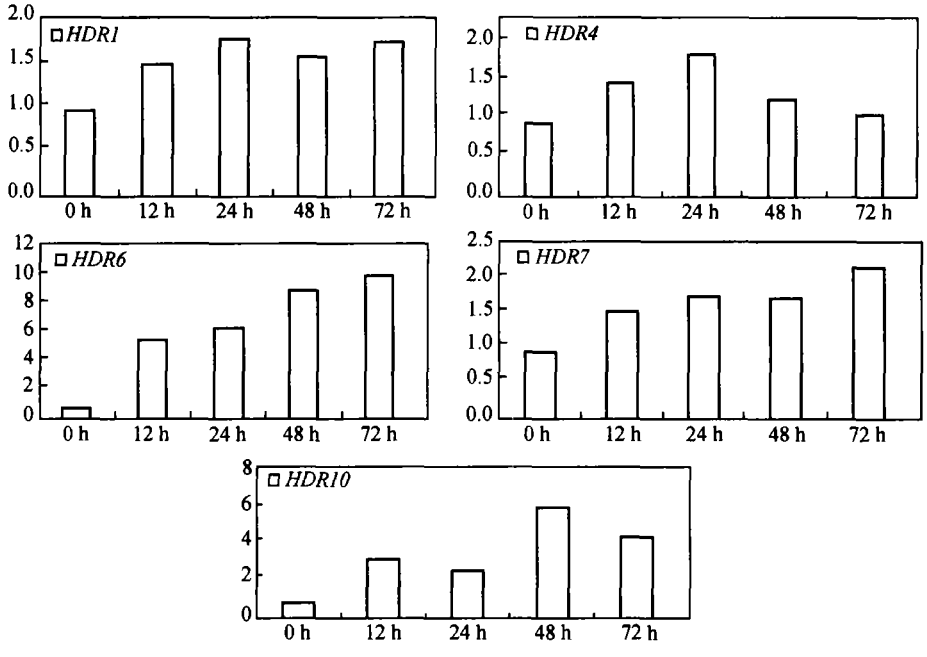

Fig. 3. Comparison of transcriptional modes among 5 *HDR* genes in HDR line. 0 h, Gene expression in healthy leaves; 12—72 h, gene expression in infected leaves at different time points post-inoculation. Expression amount in 0 h was regarded as 1 when compared with gene expression in other time points.

sistance is phenotypically different from the one mediated by the R gene. The identification of the genes induced in this type of infection may provide clues to the mechanism underlying this kind of resistance. HDR1 is similar to Arobidopsis acyl-coA dehydrogenase, which is the first key enzyme in the degradation process of fatty acid. HDR4 is homologous to maize casein kinase (CK II). CK II is a kinase responsible for the activation of GL-binding lipoxygenase (gbLox)[17]. Increased Lox activity has been shown to contribute to disease resistance in a number of ways[18, 19]. One conceivable consequence of the increased Lox activity might be the arrest of fungal hyphae development.

HDR6 and *HDR7* likely encode a sucrose synthase and translation elongation factor eEF-1 alpha chain, respectively. Sucrose synthase gene expression has been shown to be up-regulated by a decrease in leaf osmotic potential, implying a role of this enzyme in cell dehydration protection[20]. Because the leaves treated with the C523 toxin produced wilting spots, it is possible that the toxin treatment could produce a stress like dehydration to maize leaf-cells leading to the induction of the sucrose synthase gene. Translation elongation factor eEF1 plays an important role in regulating gene expression. Although there has been no report so far on a relationship between eEF1 and plant disease resistance, several studies showed that the induction of eEF1 could be enhanced by the environmental stresses, such as low temperature[21] and salt

(C)1994-2021 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

stress, heat shock and drought[22]. Intriguingly, although *HDR7* was highly expressed in the S line in the absence of fungal infection, no induction of this gene was observed in the S line following pathogen inoculation. Therefore, we deduce that the induction of genes like *HDR6* and *HDR7* would alleviate the stress induced by the fungal toxin and would form a part of the disease resistance in the HDR maize line.

```
GGGCAAAGACGGTGTATCGAAGGGTCGACAATGGTTACGACGATCGGTAGTCCATAGCAG   60
CGGCGGGCGCAACTTCGACCCGGTTTTCTTCCCATATATCTGTCAAATCCTTCTCCGGTT  120
ACCCTCCATCGGCAGCAATTTCCGCCATAGATACCGGCGATCGAGGGCCTCGGACAACCC  180
AATCGACGGAGCAGCGCGGCGGCAGGAGAGGGCAAACCCTTTTCTTCTCAGTACTCTACT  240
TGTCTGCGCCTGGATCCCCTCCGCAGGAAGATAAGACATGGGTCAAGCACTCGGCTTGAT  300
                                  M  G  Q  A  L  G  L  I
TCAAGTGGATCAATCGACAGTAGCCATCAAGGAAACTTTTGGGAAGTTTGACGAGGTCCT  360
  Q  V  D  Q  S  T  V  A  I  K  E  T  F  G  K  F  D  E  V  L
AGAGCCTGGATGCCACTTCTTGCCATGGTGCATAGGGAAGCAAATTGCTGGGTATCTGTC  420
  E  P  G  C  H  F  L  P  W  C  I  J  K  Q  I  A  J  Y  L  S
ACTGCGTGTGCAGCAGCTTGATGTCCGCTGCGAAACAAAGACAAAGGATAATGTCTTCGT  480
  L  R  V  Q  Q  L  D  V  R  C  E  T  K  T  K  D  N  V  F  V
CAATGTTGTGGCATCTGTGCAATATCGTGCCCTTGCCGATAAGGCATCTGATGCATTTTA  540
  N  V  V  A  S  V  Q  Y  R  A  L  A  D  K  A  S  D  A  F  Y
CAGGCTTAGTAACACCAGGGAGCAAATCCAGTCATATGTTTTTGATGTCATCAGGGCAAG  600
  R  L  S  N  T  R  E  Q  I  Q  S  Y  V  F  D  V  I  R  A  S
TGTTCCGAAGATGAACTTGGATGATGCCTTTGAGCAGAAGAATGAAATCGCCAAAGCTGT  660
  V  P  K  M  N  L  D  D  A  F  E  Q  K  N  E  I  A  K  A  V
AGAAAATGAACTTGAAAAGGCAATGTCTATGTATGGATACGAGATTGTGCAGACTCTGAT  720
  E  N  E  L  E  K  A  M  S  M  Y  G  Y  E  I  V  Q  T  L  I
TGTTGATATTGAGCCTGATGAGCATGTGAAGAGAGCTATGAATGAGATCAATGCAGCTGC  780
  V  D  I  E  P  D  E  H  V  K  R  A  M  N  E  I  N  A  A  A
TAGGCTGAGGTTGGCTGCCAGCGAGAAAGCTGAGGCAGAGAAGATACTGCAGATCAAGAG  840
  R  L  R  L  A  A  S  E  K  A  E  A  E  K  I  L  Q  I  K  R
AGCCGAAGGTGATGCAGAATCCAAGTACTTGGCGGGTCTGGGTATCGCAAGACAGCGCCA  900
  A  E  J  D  A  E  S  K  Y  L  A  G  L  G  I  A  R  Q  R  Q
GGCTATAGTGGATGGGCTCAGAGACAGTGTTCTTGCTTTCTCTGAGAATGTGCCTGGGAC  960
  A  I  V  D  G  L  R  D  S  V  L  A  F  S  E  N  V  P  G  T
CTCTGCAAAGGATGTCATGGACATGGTTCTGGTTACCCAGTACTTCGACACCATGGAGGA 1020
  S  A  K  D  V  M  D  M  V  L  V  T  Q  Y  F  D  T  M  E  E
GATTGGGGCCCCGTCCAAGTCCCCGTCGGTTTTCACCCCCCATGGACCAGGTGCTGTCAG 1080
  I  J  A  P  S  K  S  P  S  V  F  T  P  H  J  P  J  A  V  R
AGACATCGCAGCGCAGATAAGGGATGGTCAGCCCCAGGCCAGTCTGCTGTGAGCACGTTG 1140
  D  I  A  A  Q  I  R  D  J  Q  P  Q  A  S  L  L  .
GAAAAGGCATGCTTTTGACATTCCCATCAGTAAAGAAGTACATATACATCATGAGGAGTA  1200
AAAAAAAACTTACTAGCGATAATGTGAGTACAGCTTGTTCGTTCTATCTATATGGTGCAT  1260
GTGATTATTATTATGGAGATTAATGTTAAATAGGTTCGCATGTATTGTGCCAGAACAAGG  1320
CTAAGGACCAGTTTCCGTAAAAAAATGTATGTTCGGAACTTATCCAAAAAAAAAAAAAAA  1380
```

Fig. 4. The nucleotide sequence and deduced protein sequence of *HDR10* (EMBL accession number:AJ292360).

In addition to its homology with a maize protein involved in the HR, *HDR10* was highly similar to a hypothetical protein (accession number: CAA10289) induced by fungal infection in *Cicer arietinum*. *HDR10* and its homologs are likely to be membrane proteins because of the existence of hydrophobic domains in their predicted amino acid sequences. One possibility is that the membrane bound HDR10 may be a plant receptor protein, which could interact with pathogen-derived signal molecule(s). The fluctuating induction pattern of *HDR10* may be an indication that the signal transduction process during disease resistance development involves a cascade of amplifications. The initial perception of pathogen attack would generate more than one signal molecules[8], which would be followed by a second wave of signal transduction events leading to a multitude of defense responses involving the function of many different types of defense related genes.

References

1. Wei, J. K., Liu, K. M., Pathological and physiological identification of race C of Bipolaris maydis in China, Phytopathology, 1988, 78(5): 550.
2. Walton, J. D., Host-selective toxins: agents of compatibility, Plant Cell, 1996, 8: 1723.
3. Gao, Z. H., Xue, Y. B., Dai, J. R., The pathogenic site of the C-toxin derived from *Bipolaris maydis* race c in maize (*Zea mays*), Chinese Science Bulletin, 2000, 45(19): 1787.
4. Lamb, C., Dixon, R. A., The oxidative burst in plant disease resistance, Annu. Rev. Plant Physiol. Plant Mol. Biol, 1997, 48: 251.
5. Jabs, T., Tschope, M., Colling, C. et al., Elicitor-stimulated ion fluxes and O_2^- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley, Proc. Natl. Acad. Sci. USA, 1997, 94: 4800.
6. Yang, Y. O., Shah, J., Klessig, D. F., Signal perception and transduction in defense responses, Genes Dev., 1997, 11: 1621.
7. Guo, A., Salih, G., Klessig, D. F., Activation of a diverse set of genes during the tobacco resistance response to TMV is independent of salicylic acid, induction of a subset is also ethylene independent, Plant J., 2000, 21: 409.
8. Hammond-Kosack, K. E., Jones, J. D. G., Resistance gene-dependent plant defense responses, Plant Cell, 1996, 8: 1773.
9. Bachem, C. W. B., van der Hoeven, R. S., de Bruijn, S. M. et al., Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: Analysis of gene expression during potato tuber development, Plant J., 1996, 9: 745.
10. Altschul, S. F., Madden, T. L., Schaffer, A. A. et al., Gapped BLAST and PSI-BLAST: A new generation of protein database search programs, Nucleic Acids Res., 1997, 25: 3389.
11. Sambrook, J., Rritsch, E. F., Maniatis, F., Molecular Cloning: A Laboratory Manual, 2nd ed., New York: Cold Spring Harbor Laboratory Press, 1989.
12. Benito, E. P., Prins, T., van Kan, J. A. L., Application of differential display RT-PCR to the analysis of gene expression in a plant-fungus interacton, Plant Mol. Biol., 1996, 32: 947.
13. Truesdell, G. M., Dickman, M. B., Isolation of pathogen/stress-inducible cDNAs from alfalfa by mRNA differential display, Plant Mol. Biol., 1997, 33: 737.
14. Durrant, W. E., Rowland, O., Piedras, P. et al., cDNA-AFLP reveals a striking overlap in race-specific resistance and wound response gene expression profiles, Plant Cell, 2000, 12: 963.
15. Heath, M. C., Reaction of nonsuscepts to fungal pathogens, Annu. Rev. Phytopathol., 1980, 18: 211.
16. Morel, J. B., Dangl, J. L., The hypersensitive response and the induction of cell death in plants, Cell Death Differ., 1997, 4: 671.
17. Shimoyama, Y., Ohtaka, H., Nagata, N. et al., Physiological correlation between glycyrrhizin, glycyrrhizin-binding lipoxygenase and casein kinase II, FEBS Lett., 1996, 391(3): 238.
18. Keppler, L. D., Novacky, A., Involvement of membrane lipid peroxidation in the development of a bacterially induced hypersensitive reactoion, Phytopathology, 1986, 76: 104.
19. Croft, K. P. C., Juttner, F., Slusarenku, A. J., Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* pv *phaseolicola*, Plant Physiol., 1993, 101: 13.
20. Dejardin, A., Sokolov, L. N., Kleczkowski, L. A., Sugar/osmoticum levels modulate differential abscisic acid-independent expression of two stress-responsive sucrose synthase genes in Arabidopsis, Biochem J., 1999, 1(344): Pt 2, 503.
21. Berberich, T., Sugawara, K., Harada, M. et al., Molecular cloning, characterization and expression of an elongation factor 1 alpha gene in maize, Plant Mol. Biol., 1995, 29(3): 611.
22. Li, Z. Y., Chen, S. Y., Inducible expression of translation elongation factor 1A gene in rice seedlings in response to environmental stresses, Acta Botanica Sinica., 1999, 41(8): 800.

(Received January 2, 2001; accepted April 5, 2001)

(C)1994-2021 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net