生物工程学报
journals.im.ac.cn
cjb@im.ac.cn

Chin J Biotech 2008, October 25; 24(10): 1707-1713
Chinese Journal of Biotechnology ISSN 1000-3061

研究报告

杆状病毒 *p35* 基因诱导烟草产生广谱抗病机理分析

王志华[1,2], 宋建华[1,2], 张勇[1,2], 杨宝玉[1], 王瑶[1], 陈士云[1]

1 中国科学院武汉病毒研究所, 武汉 430071
2 中国科学院研究生院, 北京 100049

摘 要: 来源于昆虫病毒和动物的抗细胞凋亡基因能够诱导植物对生物或者非生物胁迫产生抗性, 但其抗性机理有不同甚至相反的报道。本研究将来源于苜蓿银纹夜蛾核多角体病毒的 *p35* 基因转化烟草, T1 代转化烟草 Western blotting 检测 P35 蛋白的表达, 转化烟草接种烟草花叶病毒(Tobacco mosaic virus, TMV)抗病效果增强。进一步的抗病机理研究表明, 转化和野生型烟草感染 TMV 后诱导过氧化氢积累无明显区别, 野生型烟草感染 24 h 后出现 DNA Laddering 而转化烟草则没有; Western blotting 结果显示 PR-1 蛋白表达没有显著差异。但接种另外一种病原真菌核盘菌(*Sclerotinia sclerotiorum*)后的 RT-PCR 分析结果表明, 表达 P35 蛋白的烟草可增强感染核盘菌后 PR-1 基因的转录, 而且表达时间提前。以上结果说明 *p35* 基因介导的广谱抗病反应的机理与接种的不同病原有关, 对不同病原物的抗病机理存在差异, 除抑制细胞凋亡外, 还可能通过激活 PR 基因的表达提高对病原物的抗病能力。

关键词: 细胞凋亡, 杆状病毒, P35, 病程相关蛋白

Mechanism Analysis of Broad-spectrum Disease Resistance Induced by Expression of Anti-apoptotic *p35* Gene in Tobacco

Zhihua Wang[1,2], Jianhua Song[1,2], Yong Zhang[1,2], Baoyu Yang[1], Yao Wang[1], and Shiyun Chen[1]

1 *State Key Laboratory of Virology*, *Wuhan Institute of Virology*, *Chinese Academy of Sciences*, *Wuhan* 430071, *China*
2 *Graduate School of the Chinese Academy of Sciences*, *Beijing* 100049, *China*

Abstract: Studies have shown that transgenic plants expressing antiapoptotic genes from baculovirus and animals increase resistance to biotic and abiotic stress. However, the mechanism under these resistances is conjectural, or in some cases even controversy. In the present study, the *p35* gene from baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) was expressed in tobacco, and for the first time P35 protein was detected in transgenic plants by Western blotting. Inoculation of T1 transgenic tobacco leaves with tobacco mosaic virus (TMV) showed enhanced resistance, and DNA laddering was observed after TMV infection in control but not in transgenic plants. DAB staining showed that TMV infection did not affect peroxide induction of transgenic plants, Western blotting analysis of PR1 protein also showed no difference of control and transgenic plants. Inoculation of fungus (*Sclerotinia sclerotiorum*) using a detached leaf assay showed enhanced resistance of transgenic leave tissue. RT-PCR analysis demonstrated that *p35* gene expression induced earlier expression of PR1 gene after *S. sclerotiorum* infection. Taken together, our results suggest that the mechanism under enhanced disease resistance by P35 protein is possibly related to the activation of PR-related proteins in addition to the inhibition of programmed cell death, depending on the pathogens challenged.

Keywords: antiapoptotic, baculovirus, P35, pathogenesis-related protein

Received: January 9, 2008; **Accepted:** March 24, 2008
Supported by: the National High-Tech Project (863 Program) (No. 2003AA214050), and the National Natural Science Foundation of China (No. 30370133).
Corresponding author: Shiyun Chen. Tel: +86-27-87199354; Fax: +86-27-87199354; E-mail: sychen@wh.iov.cn
国家高技术研究发展项目(863 计划) (No. 2003AA214050), 国家自然科学基金(No. 30370133)资助。

Introduction

Plant defense mechanisms against invading pathogens often include rapid cell death, known as the hypersensitive response (HR). HR is in association with rapid and localized cell death at the infected sites of host tissues and plays a role in preventing growth and spread of pathogens into healthy tissues[1]. HR is a form of programmed cell death (PCD), the genetically controlled suicide of cells. Apoptosis is a type of PCD with specific biochemical characteristics[2]. The tight regulation of this program is essential to ensure that it is only activated in the required cells at the proper moment. In addition to these local responses, the uninfected portions of the plant usually develop systemic acquired resistance (SAR), which is manifested as enhanced resistance to a subsequent challenge by the initial or even unrelated pathogens, and is often associated with pathogenesis- related (PR) gene expression[3].

In plants, apoptosis occurs morphologically similar to that of animals, and induces characteristic morphological and biochemical alterations in cells[4,5]. Plant proteases that are functionally equivalent to animal caspases are also reported[6–9]. Expression of antiapoptotic genes in plants has demonstrated to induce broad-spectrum resistance to biotic and abiotic stress, e.g. plant pathogens, wounding, salt, cold, UV-B, and herbicide treatments[8–13]. In one study, plants expressing human *Bcl-2* and *Bcl-xl*, nematode *CED-9*, or baculovirus *IAP* genes all conferred heritable resistance to three necrotrophic fungal pathogens and a virus, and DNA laddering occurs in susceptible but not resistant transgenic tobacco tissue during the normal course of infection by a fungal pathogen *Sclerotinia sclerotiorum*, suggesting that disease development required host–cell death pathways[12]. In another study, expression of *bcl-xL* and *ced-9* in tomato enhances tolerance to both virus-induced biotic stress and cold chilling-induced abiotic stress[9]. Using chloroplast-directed herbicides to treat tobacco plants, wild-type plants died with features associated with apoptosis, while transgenic plants expressing *bcl* genes survived and did not show any apoptotic-like characteristics, suggesting that chloroplast serve as a location for these animal anti-apoptotic proteins in addition to the established mitochondrial location[10]. All these studies suggest that the function of antiapoptotic genes is to inhibit cell death and thus increase resistance to biotic and abiotic stress.

Like antiapoptotic genes from animals, the *p35* gene from baculovirus has also been shown to inhibit caspases in host cells and suppresses apoptosis[14–16]. In vivo expression of *p35* in transgenic systems results in resistant to diverse types of induced apoptosis and delay of cell death caused by both developmental and pathological signals in nematodes[17], drosophila[18], and mammals[19,20]. These studies suggest that *p35* gene-induced apoptotic regulation is conserved across the kingdoms. Early work in insect cells showed that P35 functions directly as an antioxidant by mopping out free radicals and consequently prevents cell death by acting at an upstream step in the reactive oxygen species-mediated cell death pathway[21]. However, the action mechanism of these antiapoptotic genes in plants is conjectural. Expression of *p35* gene in tomato plants has demonstrated to suppress cell death caused by either a fungal toxin or infection of certain bacterial and fungal pathogens[8]. Infection of *p35*-expressing tobacco plants with tobacco mosaic virus (TMV) disrupts N-mediated disease resistance, causing systemic spreading of the virus within a resistant background, while plants expressing mutant variants of the P35 protein do not show inhibition of HR cell death or enhanced virus systemic movement, suggesting that *p35* gene expression in tobacco delays HR induced cell death, which provides evidence for the participation of caspase-like proteases during HR response[22].

In this report, we have shown that expression of baculovirus *p35* gene in tobacco lead to the protection against diseases caused by a plant virus and a fungus. We also provide evidence that the mechanism under disease resistance is depended on the pathogens challenged.

1 Materials and methods

1.1 Binary vector construction and plant transformation

The coding region of the *p35* gene was cloned by PCR with the wild-type *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) DNA as template. The following primers were used: 5′-ATTA GATCTCGTTGCTTGTATTAAGTGAGAGC-3′ and 5′-GGCGGTCACCTGATCGTGCATTACAAGTAGA-3′. The PCR product was sequenced and subsequently cloned into the pGEMT-easy vector, and the *uidA* gene in the binary vector pCAMBIA1305 (purchased from CAMBIA, Australia) was replaced by the *p35* gene by restriction with *Bgl* II and *Bst*E II. The *p35* gene was then under the control of the cauliflower mosaic virus *35S* subunit and the nopaline synthase terminator. *Agrobacterium tumefaciens* strain EHA105 containing the binary construct was used to transform leaf discs of tobacco (*Nicotiana tabacum* cv. Xanthi) following the standard protocol[23]. Primary transgenic plants were selected on MS medium containing 30 mg/L

hygromycin. Hygromycin-resistant plants were planted in the greenhouse and set seeds. T1 transgenic tobacco plants with segregation against hygromycin as well as the empty vector control plants were grown at 25°C with 16 h light periods in a growth chamber and used for subsequent studies.

1.2 Confirmation of transgenic plants

Integration of the *p35* gene into tobacco genome was confirmed by PCR. Briefly, genomic DNA was isolated from leaves of 8-week-old greenhouse-grown T1 tobacco plants, and the presence of the *p35* gene was amplified by PCR using primers used for *p35* gene cloning.

Expression of P35 protein in transgenic tobacco plants was analyzed by Western blotting. T1 tobacco fully expanded leaves were homogenized in liquid nitrogen by grinding with small plastic pestles in extraction buffer[24], and resuspended in cold extraction buffer (25 mmol/L Tris pH 7.0, 50 mmol/L NaCl, 2 mmol/L β-mercaptoethanol, and 1 mmol/L phenyl- methylsulfonyl fluoride, 2 g/mL aprotinin, 2 g/mL pepstain A and 2 g/mL leupeptin). Protein concentration was measured according to the method of Bradford using the Bio-Rad reagent with BSA (Sigma) as a standard. For immunoblot analysis, samples were boiled for 10 min in sample buffer, and proteins were separated by 10% SDS-PAGE and subsequently blotted onto PVDF (polyvinylidene difluoride) membrane (Millipore) by semidry electroblotting. After blocked for 1 h in Tris-buffered saline (20 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, and 0.1% Tween 20) with 5% nonfat dried milk at room temperature, the membrane was then incubated for 1 h with a 1:500 dilution (*V*/*V*) of a polyclonal rabbit anti-p35 primary antibody (purchased from BD Biosciences Pharmingen, PA, USA), and then with goat-antirabbit alkaline phosphatase conjugated secondary antibody. Alkaline phosphatase was detected using the alkaline phosphatase conjugate substrate kit (Bio-Rad) following the manufacturer′s instructions.

1.3 Pathogens inoculation

T1 transgenic as well as the control tobacco plants were either inoculated by rubbing fully expanded leaves with carborundum plus TMV in 50 mmol/L phosphate buffer, pH 7.0 or buffer with carborundum only (mock inoculation). After infection, plants were maintained at 22°C in a growth chamber. The infected leaves were collected at different times, quickly frozen in liquid nitrogen, and stored at −80°C until analysis.

For fungal inoculation, a detached-leaf assay was used as described[12]. A minimum of three leaves per plant from a minimum of three independently transformed plant lines were inoculated by placing 5-mm-diameter agar plugs containing actively growing hyphal tips from 3-day-old colonies of either *S. sclerotiorum* grown on potato dextrose agar. Leaves were placed on moistened sterile filter paper in glass Petri dishes and incubated in the dark at 25°C. All experiments were repeated at least three times. The inoculated leaves were collected at different times and quickly frozen in liquid nitrogen, stored at −80°C until analysis.

1.4 H_2O_2 detection after TMV infection

H_2O_2 detection was carried out as described by Talarczyk *et al*[25]. Leaves from control and transgenic tobacco plants infected with TMV were taken 24 h post infection, placed in 1 mg/mL DAB (3, 3′-Diaminobenzidine)-HCl, pH 3.8, and incubated in the growth chamber for 6 h. Leaves were cleared in boiling ethanol (96%, *V*/*V*) for 10 min to remove chlorophyll and H_2O_2 in the tissue was examined.

1.5 Western blotting of PR1 protein after TMV inoculation

At different times after TMV inoculation as stated above, leaf tissues were collected and frozen immediately in liquid nitrogen. Total proteins were extracted and Western blotting of PR1 was carried out as mentioned above except the PR1 polyclonal antibody (raised against rabbit in our lab) was used instead of the P35 antibody.

1.6 RT-PCR analysis of *PR1* gene expression after fungal inoculation

At 6 h, 12 h, 24 h, 48 h and 72 h after inoculation with *S. sclerotiorum*, leaf tissues were frozen immediately in liquid nitrogen. Total RNA was isolated by RNA-Solv reagent (Omega Biotek Inc., Product No. R6830-01) according to the manufacturer's instructions, and then treated by RQ1 RNase–Free DNase (Promega) to avoid DNA contamination. RT-PCR analysis of *PR1* gene expression was carried out as described with actin as an internal control[26]. The following primers were used: actin sense: 5′-ATGGCAGACGGTGAGG ATATTCA-3′, and antisense: 5′-GCCTTTGCAATCC ACATCTGTTG-3′, *PR-1a* sense: 5′-TAGTCATGGGA TTTGTTCTC-3′, and antisense: 5′-TCAGATCATAC ATCAAGCTG-3′. The PCR program was as follows: 94°C for 2 min, then 25 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1.5 min, and a final extension step was carried out at 72°C for 10 min.

2 Results

2.1 Confirmation of *p35* gene expression in transgenic tobacco lines

Hygromycin-resistant T0 transgenic tobacco plants harboring the *p35* gene were selected and planted in

the greenhouse, and the integration of the *p35* transgene was confirmed by PCR analysis using T1 transgenic lines (Fig. 1A). Western blotting analysis further confirmed the existence of the P35 protein in transgenic tobacco lines (Fig. 1B). To our knowledge, this is the first evidence of P35 protein existence in transgenic plants. All these results confirmed the integration and expression of the *p35* gene in tobacco.

2.2 Resistance to TMV is due to inhibition of apoptosis

Detached-leaf assay on plant pathogens are experimentally convenient, and have been confirmed to be responded similarly to inoculate whole plants under greenhouse conditions[12]. We therefore adopted this assay in our experiments. Transgenic plants expressing *p35* gene were challenged with TMV. The virus caused large, spreading necrotic lesions on inoculated control leaves (Fig. 2A). Necrotic symptoms were greatly reduced or absent on tobacco leaves expressing *p35* gene after inoculation with TMV (Fig. 2B).

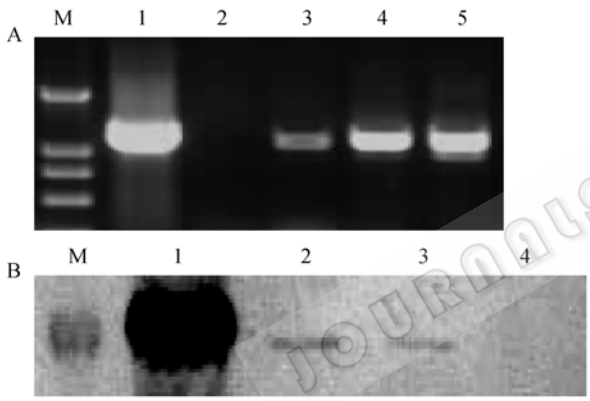

Fig. 1 Confirmation of *p35* gene expression in tobacco
(A) PCR analysis; 1: DL2000 marker; 2: plasmid DNA positive control; 3: tobacco containing vector alone served as negative control; 4–6: three independent transgenic lines. (B) Western blotting of P35 protein; 1: protein marker; 2: positive control from baculovirus P35 protein; 3, 4: two independent T1 transgenic lines; 5: empty vector transgenic tobacco control

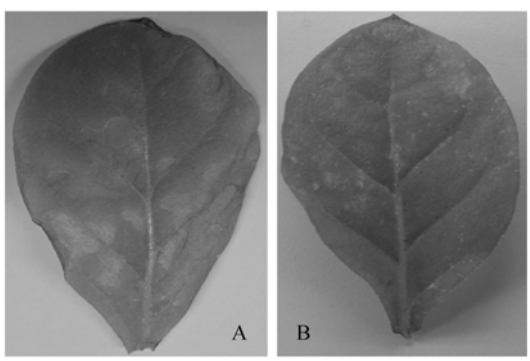

Fig. 2 Hypersensitive response of empty vector transgenic tobacco control (A) and *p35* transgenic (B) tobacco leaves at 48 h after TMV infection

One classical biochemical method for demonstrating apoptosis is the presence of DNA ladder. Genomic DNA was isolated from tobacco leaves after inoculation with TMV. As shown in Fig. 3, TMV was inducing DNA fragmentation, the isolated DNA was fragmented and formed characteristic ladders when control leaves were inoculated, as shown in lanes 1, 3, 5 of 6 h, 12 h and 24 h after inoculation. Transgenic tobacco harboring *p35* not only was resistant to TMV (Fig. 2B), but DNA laddering did not occur during the same time period (Fig. 3, lanes 2, 4 and 6). These observations suggest that TMV induces PCD during infection, and *p35* gene inhibits cell death when challenged with plant virus.

H_2O_2 was visible as a brown precipitate in the tissue examined, but no differences exist compared the transgenic and control after TMV infection (Fig. 4). To further confirm if TMV infection triggers earlier PR gene expression of transgenic plant, Western blotting was carried out on control and transgenic plants at different times after TMV infection. As shown in Fig. 5, no difference was observed.

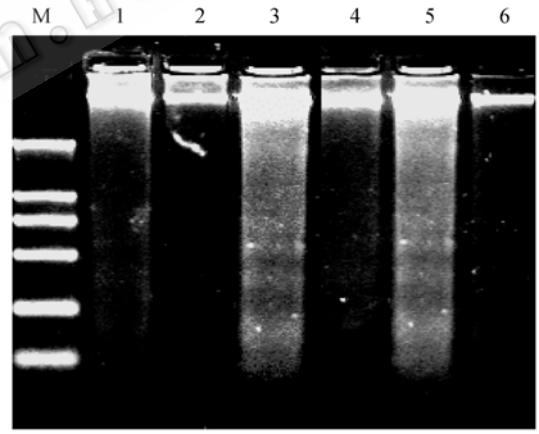

Fig. 3 Analysis of DNA Laddering after inoculation with TMV
Ethidium bromide-stained agarose gel of DNA extracted after challenge with TMV. M: DL2000 marker; 1, 3, 5: DNA was extracted from empty vector transgenic tobacco control at 6, 12 and 24 h post inoculation, respectively; 2, 4, 6: DNA was extracted from transgenic line expressing *p35* at 6, 12 and 24 h post inoculation, respectively

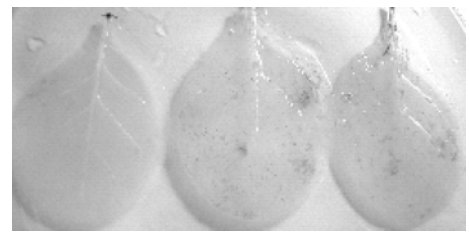

Fig. 4 Visualization of H_2O_2 accumulation by DAB staining in tobacco leaf tissues at 6 h after inoculation with TMV
From left to right: empty vector control without TMV inoculation, empty vector control with TMV inoculation and *p35*-expression tobacco leaf after TMV inoculation

2.3 Fungal infection triggers early PR gene expression

When transgenic tobacco leaves were inoculated with *S. sclerotiorum*, the fungus grew along the leaf surfaces of plants expressing the antiapoptotic genes and failed to colonize and infect host tissue. In contrast, the control plants were highly susceptible with macroscopic symptoms at 8 h after inoculation. By 24 h post inoculation, symptoms of disease were clearly visible (Fig. 6).

To further analysis if P35 protein expression triggers known defense-signaling pathways, we used RT-PCR to detect *PR1* gene expression at different time after *S. sclerotiorum* inoculation. As shown in Fig. 7, at different times after infection, *p35* gene expression triggered early and elevated expression of defense-related genes early in infection than vector control plants. This result was reproduced in three independent experiments, and the *PR1* gene expression all showed the same pattern. This result provides evidence for the first time that, depending on the pathogens challenged, antiapoptotic gene expression in plant induces disease resistance has different mechanism in addition to inhibit apoptosis.

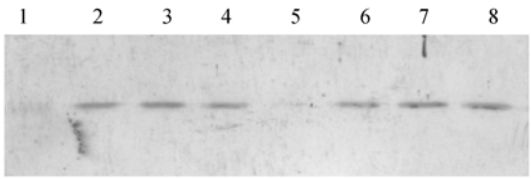

Fig. 5 Western blotting of PR-1 protein at different times after TMV infection

1~4: *p35* transgenic tobacco at 6, 12, 24, 48 h after TMV infection; 5~8: empty vector control tobacco at the same corresponding time

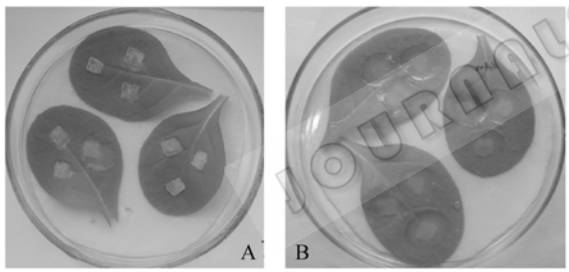

Fig. 6 Disease symptoms of *p35* transgenic (A) and empty vector control (B) tobacco leaf tissues 24 h after inoculated with *S. sclerotiorum*

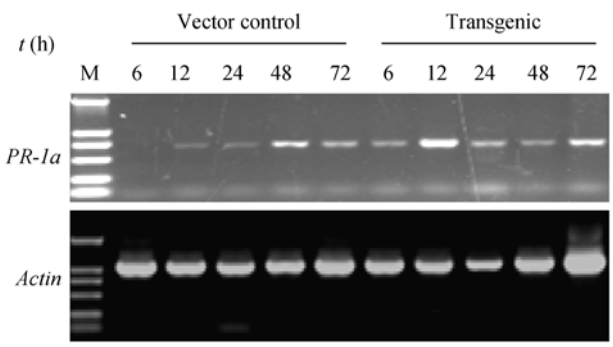

Fig. 7 Expression of *PR1* gene in vector control and *p35* transgenic line in response to *S. sclerotiorum* infection

Total RNA was extracted from 8-week-old plant leaves at 6, 12, 24, 48 and 72 h after *S. sclerotiorum* infection. Numbers along the top of the panel indicate time (h) after inoculation. Actin was served as an internal standard control. Results were reproduced in three independent experiments

3 Discussion

Studies have implicated that modification of pathways regulating PCD in plants is a potential strategy for engineering broad-spectrum disease resistant plants. Although animal anti-apoptotic proteins were detected in transgenic plants[10,12], but P35 protein was not detected in transgenic plants[8,22]. It was inferred by the authors that it was correlated to the low accumulation of the protein and its stability in the plant cell. To verify that P35 protein expression was the cause of disease resistance, plants expressing high levels of *p35* mRNA were tested for the presence of the P35 protein. Although the biological effects of the *p35* transgene and the accumulation of *p35* mRNA are unequivocal, neither Western analysis nor caspase inhibition assays were able to detect P35 protein in extracts of *p35* transgenic plants[22]. To demonstrate that active P35 protein could be made from transgenic construct, protein extracts of the *A. tumefaciens* strain that was used for plant transformation were tested, and the result showed that both P35 protein and P35 activity were present, but not in control *A. tumefaciens* extracts[8]. Northern blots with RNA isolated from independent transgenic tobacco lines confirmed high level expression of the *p35* transcript, but P35 protein accumulation assayed by Western blotting was extremely low. The possible explanations proposed by the authors were poor translation of the *p35* transgene due to codon bias, and also the stability of P35 protein in plant cells[22].

In our study, the integration and expression of *p35* gene was confirmed by PCR and Western blotting (Fig. 1). P35 protein was not detected at first, after modification of protein extraction method, the expected result was obtained (Fig. 1B). This indicates that P35 was unstable in transgenic plants and was easy to degrade during protein extraction process. Some regulatory mechanisms that underlie PCD are thought to be conserved in animals and plants, and many studies have provided evidence that PCD in both

shares components that include caspase activity[27]. However, there is only indirect evidence that caspases are present in plants. The identification of a protease exhibiting caspase activity is essential in elucidating the molecular mechanism that operates PCD in plants[28]. In recent years, it was found that the caspases-like protease existed in course of plant PCD[27]. P35 protein was detected in our transgenic tobacco plants, it was necessary to be further studied that whether the P35-caspases complex could be detected or not.

In mammalian systems, it is not entirely clear how antiapoptotic genes promote cell survival. Several mechanisms have been proposed to explain how Bcl-2 prevents apoptosis[29,30], including prevention of cytochrome c release from mitochondria, prevention of reactive oxygen-induced cell death, dimerization with and inactivation of death-promoting apoptotic proteins such as Bax, and sequestration of caspase-activating proteins. In plants, the mechanism of action of these antiapoptotic genes is conjectural[8,12], other mechanisms have been proposed in addition to prevent PCD. Evidence has shown that antiapoptotic genes like animal Bcl-xL is thought to suppress plant cell death and enhance the viability in stressful environments by contributing to the maintenance of the homeostasis of organelle[13,31]. Induction of systemic acquired resistance (SAR) response by antiapoptotic genes, as indicated by induction of PR gene expression, is also proposed for enhanced disease resistance[12]. In their preliminary experiments, there was no consistent pattern of induction of defense genes (e. g. PR proteins) across independently generated transgenic lines, but the authors admitted that they cannot rule out that these genes may, in some cases, trigger known defense-signaling pathways[12]. In another study, no evidence was found to support the proposal that the expression of *p35* triggers SAR, as indicated by the lack of *PR1* gene expression in the *p35* transformed tomato plants[8]. Transgenic *p35* tobacco plants showed delayed HR cell death upon challenge with TMV, suggesting that timely activation of cell death is necessary for effective TMV containment within the primary infection site[22]. Our present results provide evidence that *PR1* gene expression was triggered during early fungal infection but not TMV infection, suggesting that the mechanisms under disease resistance induced by *p35* gene are dependent on pathogens challenged. Further work is needed to confirm this by generating transgenic tobacco with a truncated variant of *p35* mutant unable to suppress HR cell death, as reported by del Pozo and Lam[22].

Acknowledgements

We thank Dr. Shengyi Liu of Oil Crops Institute, Chinese Academy of Agricultural Sciences for *Sclerotinia sclerotiorum* strain. We thank Dr. Zhihong Hu and Dr. Xiulian Sun for supports during the research of this study.

REFERENCES

[1] Heath MC. Hypersensitive response-related death. *Plant Mol Biol*, 2000, **44**: 321–334.

[2] Morel JB, Dangl JL. The hypersensitive response and the induction of cell death in plants. *Cell Death Differ*, 1997, **4**: 671–683.

[3] Uknes S, Dincher S, Friedrich L, *et al*. Regulation of pathogenesis-related protein-1a gene expression in tobacco. *Plant Cell*, 1993, **5**: 159–169.

[4] Ryerson DE, Heath MC. Cleavage of nuclear DNA into oligonucleosomal fragments during cell death induced by fungal infection or by abiotic treatments. *Plant Cell*, 1996, **8**: 393–402.

[5] Wang H, Li J, Bostock RM, *et al*. Apoptosis: A functional paradigm for programmed plant cell death induced by a host-selective phytotoxin and invoked during development. *Plant Cell*, 1996, **8**: 375–391.

[6] Chichkova NV, Kim SH, Titova ES, *et al*. A plant caspase-like protease activated during the hypersensitive response. *Plant Cell*, 2004, **16**: 157–171.

[7] Hansen G. Evidence for Agrobacterium-induced apoptosis in maize cells. *Mol Plant Microbe Interact*, 2000, **13**: 649–657.

[8] Lincoln JE, Richael C, Overduin B, *et al*. Expression of the antiapoptotic baculovirus p35 gene in tomato blocks programmed cell death and provides broad-spectrum resistance to disease. *Proc Natl Acad Sci USA*, 2002, **99**: 15217–15221.

[9] Xu P, Rogers SJ, Roossinck MJ. Expression of antiapoptotic genes bcl-xL and ced-9 in tomato enhances tolerance to viral-induced necrosis and abiotic stress. *Proc Natl Acad Sci USA*, 2004, **101**: 15805–15810.

[10] Chen S, Dickman MB. Bcl-2 family members localize to tobacco chloroplasts and inhibit programmed cell death induced by chloroplast-targeted herbicides. *J Exp Bot*, 2004, **55**: 2617–2623.

[11] de Freitas DS, Coelho MC, Souza MT, *et al*. Introduction of the anti-apoptotic baculovirus p35 gene in passion fruit induces herbicide tolerance, reduced bacterial lesions, but does not inhibits passion fruit woodiness disease progress induced by cowpea aphid-borne mosaic virus (CABMV). *Biotechnol Lett*, 2007, **29**: 79–87.

[12] Dickman MB, Park YK, Oltersdorf T, *et al*. Abrogation of disease development in plants expressing animal antiapoptotic genes. *Proc Natl Acad Sci USA*, 2001, **98**: 6957–6962.

[13] Qiao J, Mitsuhara I, Yazaki Y, *et al*. Enhanced resistance to salt, cold and wound stresses by overproduction of animal cell death suppressors Bcl-xL and Ced-9 in tobacco cells-their possible contribution through improved function of organella. *Plant Cell Physiol*, 2002, **43**: 992–1005.

[14] Clem RJ, Fechheimer M, Miller LK. Prevention of apoptosis by a baculovirus gene during infection of insect cells. *Science*, 1991, **254**: 1388–1390.

[15] Danon A, Rotari VI, Gordon A, *et al*. Ultraviolet-C overexposure induces programmed cell death in *Arabidopsis*, which is mediated by caspase-like activities and which can be suppressed by caspase inhibitors, p35 and defender against apoptotic death. *J Biol Chem*, 2004, **279**: 779–787.

[16] Zhou Q, Krebs JF, Snipas SJ, *et al*. Interaction of the baculovirus anti-apoptotic protein p35 with caspases. Specificity, kinetics, and characterization of the caspase/p35 complex. *Biochemistry*, 1998, **37**: 10757–10765.

[17] Sugimoto A, Friesen PD, Rothman JH. Baculovirus p35 prevents developmentally programmed cell death and rescues a ced-9 mutant in the nematode *Caenorhabditis elegans*. *Embo J*, 1994, **13**: 2023–2028.

[18] Davidson FF, Steller H. Blocking apoptosis prevents blindness in Drosophila retinal degeneration mutants. *Nature*, 1998, **391**: 587–591.

[19] Hisahara S, Araki T, Sugiyama F, *et al*. Targeted expression of baculovirus p35 caspase inhibitor in oligodendrocytes protects mice against autoimmune-mediated demyelination. *Embo J*, 2000, **19**: 341–348.

[20] Viswanath V, Wu Z, Fonck C, *et al*. Transgenic mice neuronally expressing baculoviral p35 are resistant to diverse types of induced apoptosis, including seizure-associated neurodegeneration. *Proc Natl Acad Sci USA*, 2000, **97**: 2270–2275.

[21] Sah NK, Taneja TK, Pathak N, *et al*. The baculovirus antiapoptotic p35 gene also functions via an oxidant-dependent pathway. *Proc Natl Acad Sci USA*, 1999, **96**: 4838–4843.

[22] del Pozo O, Lam E. Expression of the baculovirus p35 protein in tobacco affects cell death progression and compromises N gene-mediated disease resistance response to tobacco mosaic virus. *Mol Plant Microbe Interact*, 2003, **16**: 485–494.

[23] Horsch RB, Fry JE, Hoffmann NL, *et al*. A simple and general method for transferring genes into plants. *Science*, 1985, **227**: 1229–1231.

[24] Zhang S, Du H, Klessig DF. Activation of the tobacco SIP kinase by both a cell wall-derived carbohydrate elicitor and purified proteinaceous elicitins from *Phytophthora* spp.. *Plant Cell*, 1998, **10**: 435–450.

[25] Talarczyk A, Krzymowska M, Borucki W, *et al*. Effect of yeast CTA1 gene expression on response of tobacco plants to tobacco mosaic virus infection. *Plant Physiol*, 2002, **129**: 1032–1044.

[26] Bouarab K, Melton R, Peart J, *et al*. A saponin-detoxifying enzyme mediates suppression of plant defences. Nature, 2002, **418**: 889–892.

[27] Sanmartin M, Jaroszewski L, Raikhel NV, *et al*. Regulating death since the origin of life. *Plant Physiol*, 2005, **137**: 841–847.

[28] Hatsugai N, Kuroyanagi M, Yamada K, *et al*. A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. *Science*, 2004, **305**: 855–858.

[29] Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. *Science*, 1998, **281**: 1322–1326.

[30] Adams JM, Cory S. Bcl-2-regulated apoptosis: mechanism and therapeutic potential. *Curr Opin Immunol*, 2007, **19**: 488–496.

[31] Mitsuhara I, Malik KA, Miura M, *et al*. Animal cell-death suppressors Bcl-x(L) and Ced-9 inhibit cell death in tobacco plants. *Curr Biol*, 1999, **9**: 775–778.