Eur J Plant Pathol (2010) 127:149–156
DOI 10.1007/s10658-010-9581-5

BAK1 and BKK1 in *Arabidopsis thaliana* confer reduced susceptibility to *turnip crinkle virus*

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Accepted: 7 January 2010 / Published online: 4 February 2010

Abstract BAK1 and BKK1 are receptor-like protein kinases (RLKs) involved in brassinosteroid signal transduction and plant resistance to bacteria and fungi. Here we report that loss-of-function mutants of *BAK1* or *BKK1* in Col-0 showed enhanced susceptibility to *Turnip crinkle virus* (TCV) infection. Cell death and chlorosis occurred much earlier in *bak1-4* and *bkk1-1* mutants than in wild-type plants, suggesting an important role of BAK1 and BKK1 in controlling cell death associated with TCV infection. The *bak1-4* and *bkk1-1* mutants showed the elevated transcription levels of pathogenesis genes, such as *PR1*, *PR2*, *PR5*, *PDF1.2*. Enhanced expression of these genes, however, failed to resist the transport of TCV virus. At 9 days postinoculation, TCV levels were the highest in the systemic leaves of *bak1-4*, but were undetected in *BAK1* or *BKK1* overexpressing plants. These results signify that both BAK1 and BKK1 are important components in controlling TCV infection in Arabidopsis plants.

Keywords *Turnip crinkle virus* (TCV) · Susceptible · Cell death · BAK1 · BKK1

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Introduction

Plants use two modes of recognition to sense a pathogen and to activate an array of responses to protect themselves from pathogen attack. First, the plant resistance (R) gene products may perceive a corresponding pathogen-encoded avirulence protein (Avr). Recognition by resistance-gene products efficiently activates defense responses to infection by an invading pathogen via intricate pathways requiring multiple signalling molecules, including salicylic acid (SA), ethylene (ET), and jasmonate (JA) (Feys and Parker 2000), and other phytohormones such as brassinosteroids (Nakashita et al. 2003). This response is induced within hours, and generally accompanied by a form of localized programmed cell death (PCD), termed hypersensitive response (HR), to restrict the pathogen at the primary infection site (Heath 2000). Second, plants may also use pattern-recognition receptors (PRRs) which are generally localized at the cell surface to recognize microbial associated molecules patterns (MAMPs) and activate the first line of innate-immune responses (Zipfel et al.

2006; Chisholm et al. 2006). For example, a receptor kinase BAK1 is involved in signaling by the FLS2 and EFR receptor kinases upon perception of the conserved pathogen associated molecular patterns (PAMPs) flagellin and elf18 molecules respectively, through formation of an elicitor-dependent protein complex (Chinchilla et al. 2007; Heese et al. 2007). However, it was previously described that BAK1 associates with the plant hormone receptor BRI1 to regulate development signalling (Li et al. 2002; Nam and Li 2002). Therefore, BAK1 seems to act as a co-receptor which can interact and activate a number of ligand-binding receptor kinases at the plasma membrane to initiate signalling cascades upon perception of the appropriate stimuli. Just as in the recognition of distinct MAMP by specific PRRs, it usually activates convergent immune responses (Shan et al. 2008). If the recognition fails, due to the absence of the avirulence gene in the pathogen or the resistance gene in the plant or the absence of MAMP responses, the plants cannot activate immunity response. For example, BAK1 null mutant plants display enhanced susceptibility to a virulent *Pto*DC3000 (Kemmerling et al. 2007). Previous studies have confirmed the role of BAK1 in responses to bacterial and fungal attack. It would be interesting to know whether BAK1 also plays a role in regulating plant resistance to virus infection.

BAK1 belongs to the *SOMATIC EMBRYOGENSIS RECEPTOR KINASE (SERK)* gene family which contains five-members. A close analogue of *BAK1/SERK3* is *BKK1*/*SERK4* (Hecht et al. 2001; He et al. 2007). BAK1 null mutant plants show enhanced cell death upon infection by certain pathogens (Kemmerling et al. 2007). BKK1 plays a redundant role with BAK1 in mediating cell death control (He et al. 2007). Due to high sequence identity and functionally redundant roles between BAK1 and BKK1, we would like to know whether BKK1 is also involved in the immunity response to pathogens. To test the role of BAK1 and BKK1 in virus infection, we inoculated *Turnip crinkle virus* (TCV) on both loss-of-function null mutants and gain-of-function transgenic plants of *BAK1* and *BKK1* to analyze the responses.

The virion of TCV is morphologically icosahedral, composed of 180 coat protein subunits and one molecule of linear positive-sense single-stranded RNA (Carrington et al. 1989). *Arabidopsis thaliana* is the host of TCV and capable of supporting TCV replication and spreading. But different Arabidopsis ecotypes may develop different responses when infected by the same pathogen. For example, Arabidopsis ecotype Col-0 develops systemic symptoms after TCV invasion, but ecotype Di-17 is resistant to TCV infection. In Di-17, the resistant gene, *HRT* (*hypersensitive response to TCV*) which belongs to the CC-NBS-LRR class of R genes in *A.thaliana*, can recognize TCV coat protein and confer resistance to TCV. But Col-0, lacking HRT, is susceptible to TCV and can be systemically infected, TCV is then termed virulent to Col-0, and the interaction between Col-0 and TCV is compatible (Cooley et al. 2000). In this study, to elucidate the role of BAK1 and BKK1 in response to TCV infection, only Col-0 plants were used in all experiments to eliminate the effect of HRT conferring resistance.

Materials and methods

Plant materials and growth conditions

A. thaliana ecotype Col-0 seedlings were grown in a growth chamber with 22°C temperature, and 16 h light/8 h dark condition. The *BAK1* and *BKK1* single T-DNA knockout lines were *bak1-4* (SALK_116202) and *bkk1-1* (SALK_057955), respectively. *BAK1* and *BKK1* transgenic plants were obtained by cloning these two genes into the binary vector *pBIB-BASTA-35S-FLAG* and transforming into Col-0 (He et al. 2007).

Virus infection

TCV inoculum was prepared by grinding symptomatic tissues in potassium phosphate buffer (0.03 M, pH 7.0) at a 1:10 (wt/vol) tissue/buffer ratio. Rosette leaves of 4- to 5-week-old *Arabidopsis thaliana* plants were inoculated by rubbing (Wang et al. 2002).

RT-PCR analysis

Three micrograms of total RNA were reverse transcribed in a 20-ul volume with MMLV reverse transcriptase (TaKaRa), then diluted to 60 microlitres. Two microlitres of first-strand cDNA were used for PCR amplification with GoTaq (Promega). The PCR

cycles used were *BAK1* 27 and 30, *BKK1* 27 and 30, *PR1* 30, *PR2* 27, *PR5* 27, *PDF1.2* 32, and *EF1α* 22, respectively. The primers for *BAK1* and *BKK1* were the same as those used for cloning (He et al. 2007). The primers for defence-related genes and *EF1α* were the same as previously reported (He et al. 2007; Kemmerling et al. 2007).

Western-blot analysis

Western blot analysis was performed by using TCV coat protein specific antiserum as previously described (Xi et al. 2007).

Tissue staining

Trypan blue solution was prepared by mixing Phenol-10 g, Lactic Acid-10 ml, Glycerin-10 ml, H_2O-10 mlvtrypan blue-20 mg and two parts of 95% Ethanol. After staining, the tissues were cleared by chloral hydrate (1 ml H_2O per 2.5 g chloral hydrate). The used protocols were the same as previously reported (Lam 2004).

Results

Expression levels of *BAK1* and *BKK1* are up-regulated by TCV infection

The expression levels of *BAK1* and *BKK1* during infection by TCV in systemic leaves were analyzed by RT-PCR. In Col-0, the expression levels of *BAK1* and *BKK1* were induced after TCV inoculation. At six days postinoculation (dpi), the transcription level of *BAK1* was increased noticeably compared to that of control (Fig. 1). Up-regulation of *BAK1* and *BKK1* by TCV inoculation suggests that these two receptor-like kinase genes are involved in defensive response of Arabidopsis plants to TCV infection.

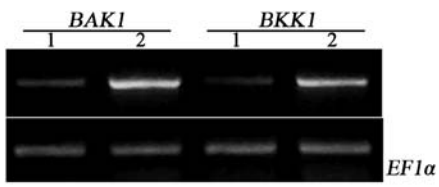

Fig. 1 Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis of *BAK1*, *BKK1* transcripts on systemic leaves in wild-type Col-0 infected with TCV (27cycles), EF1α transcripts were used as loading controls (1: mock-inoculation as control, 2: inoculation with TCV at 6 dpi)

Loss-of-function mutants of *BAK1* or *BKK1* show enhanced disease symptoms caused by TCV infection

Two homozygous T-DNA inserted knock-out mutants of *BAK1* and *BKK1* (*bak1-4* and *bkk1-1*, He et al. 2007) lacking *BAK1* and *BKK1* transcripts were used to analyze the effect of BAK1 and BKK1 in regulating TCV defensive response in Arabidopsis. Previous studies indicated that *bak1-4* mutant plants are more susceptible to virulent *Pto*DC3000 and necrotrophic fungi (Kemmerling et al. 2007). It is interesting to test whether *bak1-4* is also more susceptible to TCV infection compared to wild-type plants. *bak1-4* and *bkk1-1* null mutants were inoculated with TCV, and mock-inoculated plants were also used as a control. The inoculated plants were checked for the development of disease symptoms. Generally, TCV infected plants showed systemic disease symptoms, including leaf distortion and crinkling, chlorotic lesions, and even dwarfism. The chlorosis phenomenon of the inoculated leaves in *bak1-4* and *bkk1-1* mutants became visible at 9 dpi (Fig. 2a). The leaves of the infected plants gradually withered after 12 days. *bak1-4* mutants showed the most severe necrotic lesions and the entire inoculated leaves became wilted later.

TCV was also inoculated to transgenic plants overexpressing *BAK1* and *BKK1* to test whether the disease symptoms were correlated with the expression levels of *BAK1* and *BKK1*. At the same time point the transgenic plants displayed milder disease symptoms, which is similar to that of Col-0 (Fig. 2a). At 15 dpi we recorded the numbers of wilted leaves of whole plants. We found more wilted leaves in *bak1-4* or *bkk1-1* mutants than in other lines (Fig. 2c). After infection, for a long time, all the inoculated plants, including Col-0, *bak1-4*, *bkk1-1*, and overexpression lines, developed chlorosis symptoms. Viruses are able to move from inoculated leaves to un-inoculated leaves, causing disease symptoms there. Therefore, it could be observed that loss-of-function mutants of *BAK1* or *BKK1* exhibited more rapid and severe disease symptoms following TCV infection.

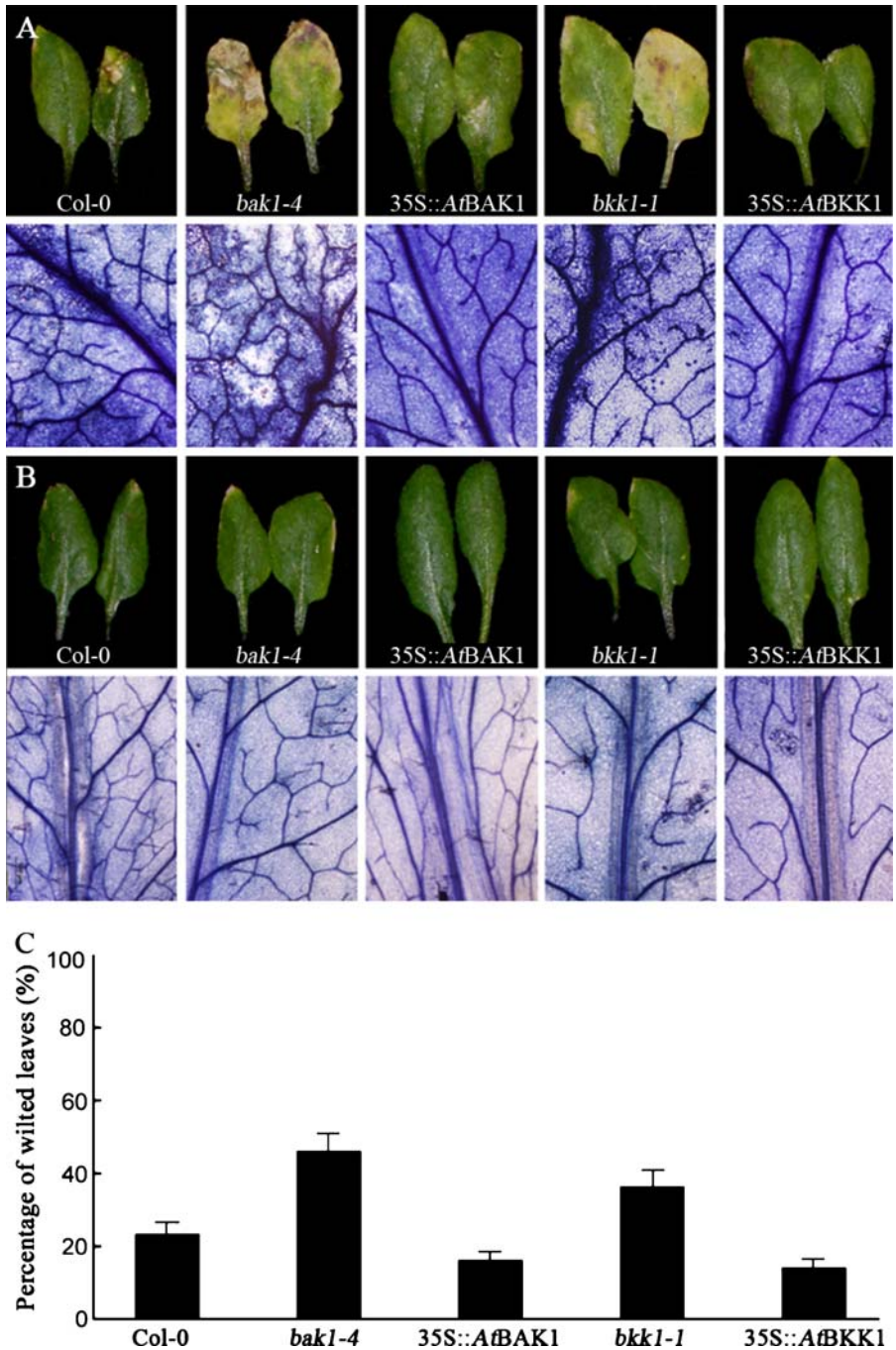

Fig. 2 Different expression of *BAK1* or *BKK1* in plants results in altered susceptibility to infection by TCV. **a** The disease symptoms and micrograph images on TCV inoculated leaves at 9 dpi, **b** The symptoms and micrograph images on mock-inoculated leaves as control, the inoculated leaves were stained with trypan blue, **c** The percentage of wilted leaves in whole plant determined at 15 dpi, Error bars show standard deviations (n=3)

bak1-4 and *bkk1-1* leaves show enhanced cell death symptoms following TCV infection

To test whether the necrosis was associated with cell death, the inoculated leaves of Col-0, *bak1-4*, *bkk1-1*, and *BAK1*or *BKK1* overexpressing plants were stained with trypan blue. Microscopic examination revealed that the leaves of *bak1-4* mutants exhibiting severe disease symptoms showed intensely stained areas. The dead cells were mainly seen around veins. In *bkk1-1* mutants, dead cells were mainly observed in the areas close to major veins. It was also observed

in Col-0 at the same point in time. *BAK1* and *BKK1* overexpressing plants, however, showed significantly reduced cell death compared to *bak1-4* and *bkk1-1* mutants at 9 dpi (Fig. 2a). The mock-inoculated leaves did not show obvious cell death based on trypan blue staining results (Fig. 2b).

Spreading of cell death surrounding veins would lead to the depletion of nutrients of leaves, causing the entire leaf to wilt. Therefore, the inoculated leaves of *bak1-4* mutant infected with TCV first developed chlorotic lesions and wilt. The staining results were consistent with the exhibited disease symptoms.

Defence-related genes are significantly up-regulated in *bkk1-1* after TCV infection

Since the wild type and transgenic plants exhibited opposite disease symptoms relative to the mutants, we set to determine whether the reduced susceptibility is associated with the elevated transcription levels of defence-related genes. We took systemic leaves from the 6 dpi TCV infected and mock-inoculated plants. The transcription levels of selected defence response genes were examined by RT-PCR: *PR1*, *PR2* and *PR5* for the SA signalling pathway (Uknes et al. 1992) and *PDF1.2* for the JA/ET signalling pathway (Penninckx et al. 1996). It was found that *PR1*, *PR2*, *PR5*, *PDF1.2* transcription levels increased in *bkk1-1* mutant after infection with TCV at 6 dpi. In *bak1-4* mutant the expression also could be induced slightly, but was not obvious compared to the *bkk1-1* mutant. The expression levels of these four genes in transgenic and Col-0 plants were not up-regulated compared to controls at this time point (Fig. 3).

Higher expression levels of *BAK1* and *BKK1* are associated with a delay of the TCV systemic infection

To examine whether the enhanced expression of defence-related genes is associated with the increased resistance against accumulation of virulent virus, we checked the accumulation of TCV coat protein in systemic leaves at various time points by western blotting. When the disease symptom appeared, TCV coat protein was easily detected in un-inoculated leaves, which indicated that TCV had already spread and replicated largely in systemic leaves. The accumulation level of viruses was much higher in *bak1-4* mutants than that in other lines at 9 dpi. No apparent differences between *bkk1-1* mutant and Col-0 were observed. Conversely, in *BAK1* or *BKK1* overexpressing lines, TCV transport was possibly blocked, because a TCP coat protein signal was not detected in the systemic leaves at 9 dpi (Fig. 4). Although *bak1-4* and *bkk1-1* mutants showed elevated expression levels of multiple defence genes, the accumulation of TCV was not suppressed. It is consistent with previous observations that constitutive expression of PR genes is not sufficient to confer resistance to TCV in Col-0 (Kachroo et al. 2000).

Discussion

Our results confirm that BAK1 and BKK1 are associated with disease resistance. Loss-of-function mutants of *BAK1* or *BKK1* showed enhanced progression of disease symptoms. Microscopic examina-

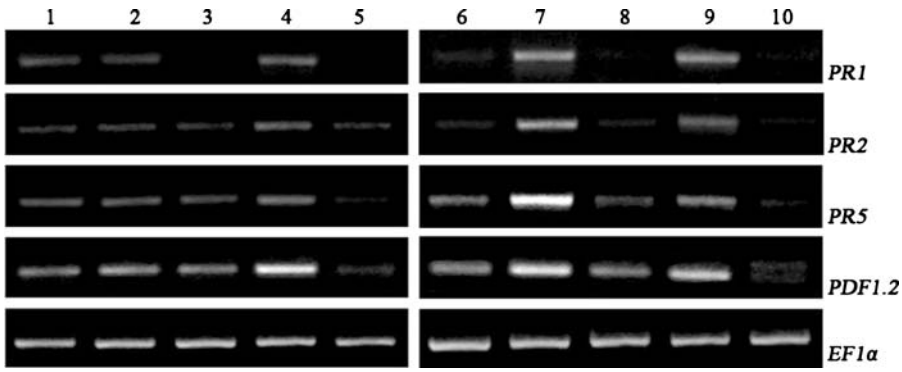

Fig. 3 Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis of pathogen-related genes transcript accumulation in different genotypes (line1~5: mock-inoculation as control, line6~10: inoculation with TCV at 6 dpi. line1, 6: Col-0, line2, 7: *bkk1-1* mutant, line3, 8: 35S::*At*BKK1, line4, 9: *bak1-4* mutant, line5, 10: 35S::*At*BAK1)

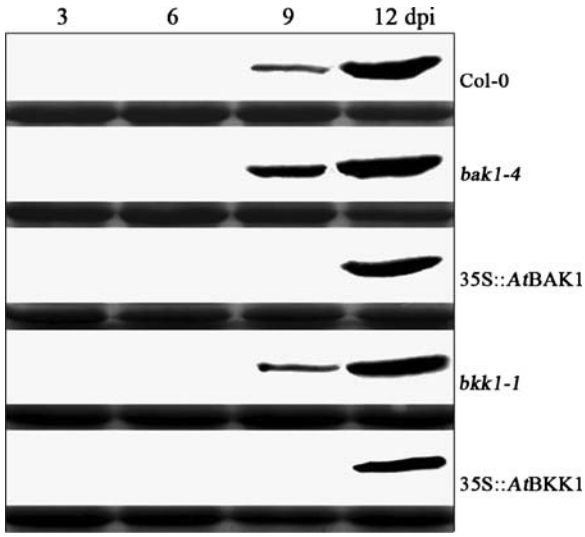

Fig. 4 Detection of the TCV coat protein in systemic leaves from Col-0, *bak1-4*, *bkk1-1* and transgenic plants by specific antibody with western blot in different days

tion also indicated that the mutants also showed elevated cell death. These results suggest that both genes are important regulators of cell death associated with disease susceptibility. Our data are consistent with recent findings by other groups. For example, when challenged with the virulent *Pseudomonas syringae* strain, *bak1-4* mutants generated leaf chlorosis followed by spreading necrosis. However, infection of Col-0 plants resulted in lesions that were restricted within the inoculated sites. A complete decay of *bak1-4* plants was observed after infected with the fungus *Botrytis cinerea* (Kemmerling et al. 2007). The authors demonstrated that the loss-of-function mutant of *BAK1* exhibited an enhanced susceptibility to several pathogens, including bacteria and necrotrophic fungi. BAK1 constitutes a novel negative control element of microbial-infection-induced PCD in plants (Heese et al. 2007; Kemmerling et al. 2007), which is a common host response in plant-pathogen interactions and mediates both disease resistance and susceptibility (Greenberg and Yao 2004). Our results indicate BAK1 deficient plants also exhibit enhanced susceptibility to virulent TCV infection. BKK1, a close analogue of BAK1, plays a functionally redundant role with BAK1.

bak1-4 bkk1-1 double mutants display spontaneous cell death, leaf chlorosis and constitutive defence responses (He et al. 2007). *PR1, PR2, PR5* genes which are associated with SA-regulated defence pathway constitutively express at very high levels in *bak1-4 bkk1-1*. Overexpression of bacterial *NahG* partially rescues its seedling lethality phenotype (He et al. 2007), suggesting that the seedling lethality of *bak1-4 bkk1-1* double mutants is associated with the SA-regulated signalling pathway. In this report, although the *bak1-4* and *bkk1-1* single knock out mutants do not exhibit spontaneous cell death, they show an enhanced leaf necrosis upon TCV infection. After detecting the expression of marker genes of pathogen related signal pathways in *bak1-4* and *bkk1-1* single mutants, it was found that *PR1, PR2, PR5* were constitutively expressed in *bak1-4* mutant, and the transcription levels were higher than Col-0 and could be induced slightly upon TCV infection during the initial infection period, however, in *bkk1-1* the elevated expression levels were obvious. This result illustrates that loss of *BAK1* or *BKK1* function enhances the expression of pathogen-related genes, which may be associated with disease development upon TCV infection. Previous studies also indicated that SA, ethylene and JA signal pathways played roles in disease development (Greenberg et al. 2000; Pilloff et al. 2002).

We also found that *BAK1* and *BKK1* expression affects the spread of the virus. Virus usually can spread from the infected cells through plasmodesmata or through damaged walls of infected cells (Lucas 1995). To achieve long-distance movement plant viruses may enter the vascular system (phloem and/or xylem), move within, and exit the vasculature at some distal points (Opalka et al. 1998). Therefore, cell death around veins may affect the virus's ability to spread to systemic leaves. In generally, three putative inducers to cell death after pathogen invasion have been identified. One is HR triggered response in host cells *via* perception of an avirulence product, as described in various incompatible plant-microbe interactions, which can restrict the spread of virus (Yao et al. 2002; Heath 1998). Another PCD initiator is phytotoxins secreted by compatible pathogens. The third is fatal damage of the cell caused by pathogen infection, including physical wounding, deficit of nutrients and malfunction of the cellular metabolism (Yao et al. 2002). Cell death induced by compatible TCV may belong to the third category, because TCV does not provoke HR or produce known toxins. Yao et al. (2002) found that many viral virions accumulated in the adjacent alive region of compatible

Ryegrass mottle virus-inducing cell death. This is clearly different from the report on N gene-mediated programmed cell death induced by *tobacco mosaic virus* (TMV) in tobacco plants, in which the virus elicited HR in the inoculated cells, restricting movement of the virus to uninoculated leaves (Whitham et al. 1994). It indicates that cell death plays a different role between incompatible and compatible interactions. Therefore, a local lesion can not restrict the TCV spread in compatible interaction; by contrast, it may benefit the movement of TCV during a certain stage.

The detailed mechanisms of BAK1 and BKK1 in controlling virus related resistance need additional studies.

Acknowledgments This work was supported by the National Key Basic Research"973" Program of China (2009CB118500), National Natural Science Foundation of China (30970214, 30670166) and Project of Chinese Ministry of Education (108110). We thank Anne Simon for providing TCV antibody.

References

Carrington, J. C., Heaton, L. A., Zuidema, D., Hillman, B. I., & Morris, T. J. (1989). The genome structure of Turnip crinkle virus. *Virology, 170*, 219–226.

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J. D., et al. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature, 448*, 497–500. doi:10.1038/nature05999.

Chisholm, S. T., Coaker, G., Day, B., & Staskawicz, B. J. (2006). Host-microbe interactions: shaping the evolution of the plant immune response. *Cell, 124*, 803–814. doi:10.1016/j.cell.2006.02.008.

Cooley, M. B., Pathirana, S., Wu, H. J., Kachroo, P., & Klessig, D. F. (2000). Members of the Arabidopsis HRT/RPP8 family of resistance genes confer resistance to both viral and oomycete pathogens. *Plant Cell, 12*, 663–676. doi:10.1105/tpc.12.5.663.

Feys, B. J., & Parker, J. E. (2000). Interplay of signalling pathways in plant disease resistance. *Trends in Genetics, 16*, 449–455. doi:10.1016/S0168-9525(00)02107-7.

Greenberg, J. T., & Yao, N. (2004). The role and regulation of programmed cell death in plant-pathogen interactions. *Cellular Microbiology, 6*, 201–211. doi:10.1111/j.1462-5822.2004.00361.x.

Greenberg, J. T., Silverman, F. P., & Liang, H. (2000). Uncoupling salicylic acid-dependent cell death and defense-related responses from disease resistance in the Arabidopsis mutant acd5. *Genetics, 156*, 341–350.

He, K., Gou, X., Yuan, T., Lin, H., Asami, T., Yoshida, S., et al. (2007). BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways. *Current Biology, 17*, 1109–1115. doi:10.1016/j.cub.2007.05.036.

Heath, M. C. (1998). Apoptosis, programmed cell death and the hypersensitive response. *European Journal of Plant Pathology, 104*, 117–124. doi:10.1023/A:1008645520976.

Heath, M. C. (2000). Hypersensitive response-related death. *Plant Molecular Biology, 44*, 321–334. doi:10.1023/A:1026592509060.

Hecht, V., Vielle-Calzada, J. P., Hartog, M. V., Schmidt, E. D., Boutilier, K., Grossniklaus, U., et al. (2001). The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. *Plant Physiology, 127*, 803–816. doi:10.1104/pp.010324.

Heese, A., Hann, D. R., Gimenez-Ibanez, S., Jones, A. M., He, K., Li, J., et al. (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 12217–12222. doi:10.1073/pnas.0705306104.

Kachroo, P., Yoshioka, K., Shah, J., Dooner, H. K., & Klessig, D. F. (2000). Resistance to Turnip crinkle virus in Arabidopsis is regulated by two host genes and is salicylic acid dependent but *NPR1*, ethylene, and jasmonate independent. *Plant Cell, 12*, 677–690. doi:10.1105/tpc.12.5.677.

Kemmerling, B., Schwedt, A., Rodriguez, P., Mazzotta, S., Frank, M., Qamar, S. A., et al. (2007). The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. *Current Biology, 17*, 1116–1122. doi:10.1016/j.cub.2007.05.046.

Lam, E. (2004). Controlled cell death, plant survival and development. *Nature Reviews. Molecular Cell Biology, 5*, 305–315. doi:10.1038/nrm1358.

Li, J., Wen, J., Lease, K. A., Doke, J. T., Tax, F. E., & Walker, J. C. (2002). BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell, 110*, 213–222. doi:10.1016/S0092-8674(02)00812-7.

Lucas, W. J. (1995). Plasmodesmata: Intercellular channels for macromolecular transport in plants. *Current Opinion in Cell Biology, 7*, 673–680. doi:10.1016/0955-0674(95)80109-X.

Nakashita, H., Yasuda, M., Nitta, T., Asami, T., Fujioka, S., Arai, Y., et al. (2003). Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. *Plant Journal, 33*, 887–898. doi:10.1046/j.1365-313X.2003.01675.x.

Nam, K. H., & Li, J. (2002). BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell, 110*, 203–212. doi:10.1016/S0092-8674(02)00814-0.

Opalka, N., Brugidou, C., Bonneau, C., Nicole, M., Beachy, R. N., Yeager, M., et al. (1998). Movement of rice yellow mottle virus between xylem cells through pit membranes. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 3323–3328.

Penninckx, I. A., Eggermont, K., Terras, F. R., Thomma, B. P., Samblanx, G. W., Buchala, A., et al. (1996). Pathogen-induced systemic activation of a plant defensin gene in Arabidopsis follows a salicylic acid-independent pathway. *Plant Cell, 8*, 2309–2323.

Pilloff, R. K., Devadas, S. K., Enyedi, A., & Raina, R. (2002). The Arabidopsis gain-of-function mutant dll1 spontane-

ously develops lesions mimicking cell death associated with disease. *Plant Journal, 30*, 61–70. doi:10.1046/j.1365-313X.2002.01265.x.

Shan, L., He, P., Li, J., Heese, A., Peck, S. C., Nurnberger, T., et al. (2008). Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. *Cell Host & Microbe, 4*, 17–27. doi:10.1016/j.chom.2008.05.017.

Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., et al. (1992). Acquired resistance in Arabidopsis. *Plant Cell, 4*, 645–656.

Wang, Y., Gaba, V., Yang, J., Palukaitis, P., & Gal-On, A. (2002). Characterization of Synergy Between *Cucumber mosaic virus* and Potyviruses in Cucurbit Hosts. *Phytopathology, 92*, 51–58. doi:10.1094/PHYTO.2002.92.1.51.

Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C., & Baker, B. (1994). The product of the tobacco mosaic virus resistance gene N: similarity to toll and the interleukin-1 receptor. *Cell, 78*, 1101–1115. doi:10.1016/0092-8674(94)90283-6.

Xi, D., Feng, H., Lan, L., Du, J., Wang, J., Zhang, Z., et al. (2007). Characterization of Synergy between *Cucumber mosaic virus* and *Tobacco necrosis virus* in *Nicotiana benthamiana*. *Journal of Phytopathology, 155*, 570–573. doi:10.1111/j.1439-0434.2007.01279.x.

Yao, N., Imai, S., Tada, Y., Nakayashiki, H., Tosa, Y., Park, P., et al. (2002). Apoptotic Cell Death is a Common Response to pathogen Attack in Oats. *Molecular Plant-Microbe Interactions, 15*, 1000–1007. doi:10.1094/MPMI.2002.15.10.1000.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D., Boller, T., et al. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. *Cell, 125*, 749–760. doi:10.1016/j.cell.2006.03.037.