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ARTICLE

Plant protein degradation affects transcription of genes associated with bacterium-induced basal resistance

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ABSTRACT Basal reistance (BR), being the first line of all active defense forms in plants, is induced rapidly after a microbial invasion of the plant tissues. There is an endeavor to discover the processes taking place in the plant cell during BR. It is already known that upon a bacterial attack certain plant genes show transcriptional changes. Our present work is an excerpt of studies made on the effect of protein degradation systems on the expression of BR associated genes. Tobacco plants were infected with a non-HR inducing bacterium (*Pseudomonas syringae* pv. *syringes hrcC*) to trigger BR. Parallel treatments were carried out with protein degradation inhibitors. Alterations in gene expression were measured with real-time PCR and microarray methods. Our results support that protein degradation may affect the BR-related genes through the decomposition of signalling proteins and/or transcription factors that play role in the development of defense responses.
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KEY WORDS

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protein degradation
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It is crucial for plants to give rapid response to a microbial attack. Therefore, basal resistance (BR) – the first line of active defense – is triggered shortly after the plant cell has sensed the presence of any microbial intruder (Klement at al. 2003). In case of a bacterial infection BR is primarily induced by common bacterial cell surface molecules, such as flagellin (protein of the flagella) or lipopolysaccharide. Thus, non-pathogenic bacteria are also capable for BR induction. There are plant receptors recognizing the conservative regions of such molecules and activating signalling cascade(s). This process will lead to the activation of defense-related genes and finally come down to resistance responses (Bittel and Robatzek 2007).

The regulation of BR processes and the way BR mechanisms act on microbial activity and proliferation are yet poorly understood. However, there have been some promising discoveries done in the field. For example, different plant cell wall components accumulate at bacterial sites (Bestwick et al. 1995) and various antimicrobial enzymes – e.g. chitinase(s) – appear in the intercellular spaces of leaves (Ott et al. 2006). Previously we could identify hundreds of genes in tobacco leaves that were activated during the bacterium-induced BR. The functional classification of these genes shows that a broad range of plant processes are involved in BR development (Szatmári et al. 2006; Szatmári and Bozsó unpublished). One of these functional classes consists of the genes of protein degradation. There are series of evidence showing that protein degradation – either by proteinases or the proteosome system – plays an important role in plant defense systems ranging from signal production to regulation (van der Hoom and Jones 2004).

In our present work we studied the effects of the proteosome system inhibition on BR associated gene transcription in tobacco leaves.

Materials and Methods

Plant material

Tobacco plants (*Nicotiana tabacum* L. cv. Samsun) were planted in soil and grown under normal greenhouse conditions (18-23°C). Two days before inoculation 6-8-week-old tobacco plants were placed in a growth chamber set to 16/8 hour light/dark period at 20°C. After bacterial inoculation the plants were returned to the same growth chamber and lit continuously.

Bacterial infection and protease inhibitor treatment

Pseudomonas syringae pv. *syringae hrcC* (61-1530B strain, provided by Alan Collmer of Cornell University, Ithaca, USA), a mutant that lacks the ability to induce HR was used for inoculation. Bacteria were cultured overnight at 27°C on King's B medium completed with 50 μg ml^{-1} of kanamycin. Bacterium suspension in distilled water (10^{-8} ml^{-1}) was injected in the leaf's intercellular spaces using hypodermic syringe fitted with needle (Klement 1990). Some leaves were

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treated with a mixture of bacterium suspension and protease inhibitor. The same infiltration method was used for distilled water and pure protease inhibitor control.

Real-time PCR analysis of plant gene expression

100 mg of sample were taken 0, 3, 6, 12, 24, and 48 hours after inoculation. Samples were snap-frozen in liquid nitrogen and stored at -70°C until further procession. 2.5 µg total RNA was used for cDNA synthesis. 2.5 µl of a 10-fold dilution of the cDNA stock were used in 15 µl reactions. Primer concentrations were 3 µM. PCR was carried out using the iQ SYBR Green 2x Supermix (Biorad, USA) on the DNA Engine Opticon2 (MJ Research, USA). Cycling parameters were the same for all primers: initial 95°C for 6 min, followed by 40 cycles of 95°C for 30 sec, 60°C for 1 min and plate read step. Measured C(T) values were normalized against actin (internal control) values. Standard deviation was calculated from at least two repetitive measurements of each sample. Values of the untreated controls were given the arbitrary value of 1.

Microarray experiments

Leaf samples were collected 6 hours after inoculation. cDNA labeling and hybridizations were carried out by TIGR Potato Functional Genomics Project using TIGR Potato 10K cDNA Array. The significantly activated or repressed genes from normalized data were selected by Rank Products analysis (Breitling et al. 2004). The genes that were above the selection limit (5% FDR) were chosen as transcriptionally altered genes. Experiments were repeated three times.

Results and Discussion

Microarray studies were performed with TIGR Potato 10K cDNA array to investigate how the proteosome degradation system affects the gene expression during the bacterium-induced BR. The high rate of genetic homology between potato and tobacco allowed us to hybridize our tobacco samples on potato chip. Six hours after inoculation there were 500 genes that were activated or repressed during BR in the samples treated with BR-inducing *P. syringae hrcC* mutant compared to the water infiltrated controls.

To block the proteosome system MG115 (a specific inhibitor of the chymotrypsin-like activity of the proteasome; 50 µM) was mixed with the bacterium suspension. The control treatment in this case was the infiltration of leaves with bacterium suspension not containing the inhibitor. There were twenty-three genes found to be up- or down-regulated during BR and their expression to be influenced significantly by the inhibitor. The inhibitor had a diverse effect on BR: in several cases it blocked the induction of BR-activated genes while in some instances the BR-induced gene expression was enhanced even further. There were two cases when the inhibitor reversed the BR-triggered gene repression. The function of the affected genes varied from cell rescue (glutathione S-transferase) through e.g. signaling (phospholipase A), transcription regulation (constant-like b zinc finger protein transcription factor) or defense (polygalacturonase inhibitor protein). The effect of the proteosome degradation inhibitor overlapped with other signal pathway inhibitors we investigated parallelly (Bozsó et al. unpublished).

To verify the microarray results and to extend the study to the effects of blocking the proteosome degradation, time course experiments were carried out. The changes in expression of some BR-activated genes were measured using real-time PCR method with gene specific primers. The results confirmed that blocking the proteosome system can attenuate or completely inhibit the expression of several early BR-associated cell rescue and signaling genes.

In summary our results show that the proteosome degradation system may be involved in the regulation of the bacterium-induced BR-associated gene expression, thus the resistance response. The protein degradation may affect the BR-related genes through the decomposition of signalling proteins and/or transcription factors that are positive or negative regulators of defense responses. In some instances it was proved that this type of protein degradation plays an essential role in the development and in the regulation of plant defense responses. For example, jasmonic acid signalling and salicylic acid independent defense responses are regulated this way (Xia et al. 1998; Kim et al. 2002). The importance of this degradation system in plant defense shows that pathogenic bacteria can overcome the plant defense system by manipulating it (Abramovitch et al. 2006).

To find new members of plant proteases that are involved in BR, both other types of inhibitors and the function of the activated protease genes should be investigated in our future studies.

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