Reduction of lesion growth rate of late blight plant disease in transgenic potato expressing harpin protein*

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Abstract Using harpin protein gene from apple fire blight pathogen Erwinia amylovora and potato prp1-1 promoter as main DNA elements, the feasibility of using pathogen infection-induced hypersensitive response was explored as a new strategy of engineering fungal disease resistance. Three plant transformation vectors were constructed and 68 transgenic potato plants were produced through Agrobacterium mediated transformation method. Southern, Northern and Western blot analysis demonstrated the insertion, transcription and protein expression of harpin protein gene in transgenic plants. Disease resistance test using a complex race of Phytophthora infestans as challenging pathogen showed that both constitutive and pathogen infection-induced expression of harpin protein gene in transgenic potato reduced the lesion growth rate of fungus. Among plants where harpin protein gene expression was induced only by fungus infection, two plants were found to be highly resistant to P. infestans infection. Fungal hyphae were not produced on total of 30 inoculated leaves from the two resistant plants and necrotic lesion was limited to inoculation area. The results highlighted that engineered hypersensitive response in plants was a very promising approach to produce fungal disease resistant genotype.

Keywords: fungal disease, hypersensitive response, harpin, potato late blight.

The hypersensitive response produced by plants to pathogen infection was one of active defensive reactions and was thought as the most powerful defensive way found in nature. It was an attempt to explore new approaches of engineering hypersensitive response to pathogen infection[1]. Based on the "gene for gene" relationship explaining interactions between plants and pathogens, de Wit proposed a new approach of controlling plant disease focusing on the engineered hypersensitive response, called two components system of non-specific resistance[2]. Recent experimental evidence indicated that recognition in the same cell of products of plant resistance gene (R) and corresponding pathogen avirulence gene (avr) resulted in cell hypersensitive necrosis[3,4], however, pathogen infection-induced cell death can not only keep whole plant healthy, but also limit the development of pathogen in inoculated leaves[5].

Harpin, a membrane associated protein, was encoded by hrpN, a member of hrp (hypersensitive response and pathogenecity) gene family in plant pathogen bacterium[6] and isolated from apple fire blight bacterium Erwinia amylovora. Purified harpin protein, when being penetrated into intercellular space of leaves of solanaceae plants such as tobacco and tomato, induced cell hypersensitive necrosis around the penetrating area[6]. Therefore, it is worthwhile to demonstrate if harpin protein gene driven by pathogen infection induced promoter could be used to form an effective disease resistant system in transgenic plant. Targeting on potato late blight caused by P. infestans, the authors provide evidence to indicate that engineered hypersensitive response is a very useful approach to develop disease resistant genotype.

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1 Materials and methods

1.1 Materials

The plant materials used in our experiments included potato cultivar "Desiree" provided by Dr. Qu Dongyu in Vegetables and Flowers Institute of Chinese Academy of Agricultural Sciences. Genomic DNA of *Nicotiana tabacum* cultivar "Xanthi-nc" was provided by Dr. Pang Shengzhi in Cornell University. Plasmid pRLGEMB2 containing harpin protein gene coding region was constructed by the authors[7]. pBIS25 containing gene expression cassette composed of double CaMV 35S promoter, Ω sequence from AMV, and NOS terminator was purchased from PBI Company of Canada. T-DNA vector pBINPLUS[8], potato diploid line LineV and R2, and *P. infestans* isolate 90128 (race 1, 3, 4, 6, 7, 8, 10, 11) were provided by Dr. Andy Pereira and Dr. Vivianne Vleeshouwers in CPRO-DLO, the Netherlands. Other plasmids were maintained by our laboratory.

1.2 Methods

1.2.1 PCR and molecular cloning. Methods for PCR and molecular cloning referred to that described by Sambrook et al. [9].

1.2.2 Potato transformation. Methods for PCR and molecular cloning referred to that described by Visser et al. [10].

1.2.3 Molecular detection of transgenic plants. The methods for extraction of DNA, RNA, Southern and Northern hybridization followed that described by Sambrook et al. with some modification[9]. For detection of protein expression using dot blot-ELISA, 100 mg leave tissue was collected and ground in 100 μL 1 × PBS buffer containing 140 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na₂HPO₄, 1.8 mmol/L KH₂PO₄, pH 7.3. The supernatant of centrifuged samples was diluted 10 times for use. The rabbit antibody against harpin protein was produced by the authors. The second antibody was goat anti-rabbit antibody labeled by alkaline phosphatase purchased from Sigma Company. NBT/BCIP system was used to indicate the reaction. For Western blot analysis, total protein, intercellular protein and intracellular protein were isolated respectively according to the methods described by de Wit and Spikman[11]. SDS-PAGE and Western blot hybridization followed the methods described by Sambrook et al. [9].

1.2.4 Disease resistance test. Totally 55 morphologically normal and healthy transgenic plants were used for disease resistance test. Both plants regenerated directly from stem and transformants containing empty vector pBINPLUS were used as negative control. Late blight susceptible cultivar "Bintje" was used to test the pathogenicity of *P. infestans* isolate.

The methods for preparation of culture medium for *P. infestans* growth and induction of zoospores referred to that described by Caten and Jinks[12]. The concentration of zoospores was 50 000 per mL. Biological assay was done using detached leaves from plants of 8-week old in a greenhouse. From top of each plant the third, fourth, and fifth of fully expanded leaves were collected. There are 5 compound leaves in each potato leaf. Only one site for each compound leaf on back surface was inoculated. There are a total of 15 inoculation sites for each transgenic plant with 10 μL of inoculation (total of 500 zoospores). The temperature in culture room for infection and growth of inoculated *P. infestans* was 18°C at light for 16 h and 15°C at dark for 8 h. Relative