OB8. Identification and expression of novel PR-protein shows induced *Erysiphe graminis* in wheat (*Triticun aestivum* L.)

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Objectives

In this paper, we present the results of the first large-scale, systematic attempt to characterize the powdery mildew proteome. Beta-1,3-glucanases are important components of plant defense in response to attack by pathogens. In addition RT-PCR analysis showed the mRNA encoding powdery mildew was also revealed under fungus stress conditions.

Materials and Methods

Inoculation temperature regime of 20/18℃ and a photoperiod of 16 h light (500 uE/m2/sec) were used for each treatment and experimental replicate. Two-dimensional electrophoresis, *N*-terminal amino acid sequence analysis, internal amino acid analysis, Reversed-phase (RP)-high-pressue liquid chromatography (HPLC), homology search of amino acid sequence and identification of cDNA encording the sequenced protein were used for this analysis.

Total RNA were extracted from leaves and spikes following the procedure of (Higa et al. 2003). The RT-PCR was carried out according to RNA PCR kit protocol using the total RNA (approximately 100 mg) from pathogen inoculated and control plants.

Results and Discussion

Separation of the first dimension was performed using isoelectric focusing across two pH range; pH 3.5-10.0 and pH 4.0-6.5. Proteins were blotted to PVDF excised and characterized using conventional *N*-terminal Edman degradation microsequencing. Sequences were submitted to NCBI, SWISS-PROT and TrEMBL databases via FASTA algorithum. Two proteins, which found in abundance under infection conditions, was expression and identified as β-1,3-glucanase and glucan endo-1,3-β-D-glucosidase, a pathogenesis-related (PR-2) protein involved in protein degradation. RT-PCR of RNA isolated from leaf of powdery mildew showed an increase in expression of β-1,3-glucanase reflecting either increased transcription or stability of the mRNA in wheat, supporting the proteome evidence. Thus in infected powdery mildew of wheat an increased in β-1,3-glucanse and glucan endo-1,3-β-D- glucosidase may play a part in the loss of proteins.

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