IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH CBB RESISTANCE IN COMMON BEAN USING CDNA-AFLP

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INTRODUCTION

Common bacterial blight (CBB) of common bean (*Phaseolus vulgaris L.*), incited by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a serious seed-borne disease in both temperate and tropical bean production zones. Yield losses can exceed 40% (Miklas et al., 2006). HR45 is highly resistant to *Xap* infection on leaves and pods in the field and greenhouse. Near immunity response (NIR) to *Xap* was observed on the leaf of HR45 seven days after inoculation. In order to identify resistance genes eliciting NIR against *Xap* in HR45, the gene profiling approach was used to identify the genes that are differentially expressed in the leaves of HR45 at different hours after inoculation.

MATERIALS AND METHODS

The cDNA-amplified fragment length polymorphism (cDNA-AFLP) technology was used in this study for gene profiling. HR45 was inoculated with *Xap* using the multiple needle technique (Park and Dhanvantari, 1987). Leaf tissues were collected from mock-inoculated and inoculated plants at 0, 8, 12, 24 and 120 hours post inoculation (HPI). LI-COR AFLP Expression Analysis Kit (LI-COR Biosciences, Nebraska, Lincoln, USA) was used to perform cDNA-AFLP analysis. The differentially expressed fragments were cloned and sequenced using LI-COR IR2 sequencer (LI-COR Biosciences, Nebraska, Lincoln, USA). Cluster analysis was conducted in the TIGR Microarray Data Analysis System (www.tm4.org).

RESULTS

Thirty four different primer combinations were used in cDNA-AFLP analysis. Two thousands four hundred forty-eight transcript-derived fragments (TDF), ranging from 50 to 1,000 bp were generated. 10.6% of the TDFs had significantly altered expression level at all five time intervals post-inoculation. Majority of the TDFs were up-regulated than down-regulated upon *Xap* infection (Figure 1). Seventy-seven differentially expressed TDFs were cloned and sequenced. They were assembled into 8 contigs and 59 singletons. Thirty-two of them were linked to the TCs (Tentative Consensus sequences) which contain these ESTs in Bean Gene Index (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/est_report.pl?EST=PvXIS&gudb=p_vulgaris). The 11 clones marked with the asterisk in figure 1 had homology to genes coding for proteins involved in plant defence responses. Whereas, 6 clones marked with the plus sign were *in silico* mapped to the lower region of linkage group 6 making them putative candidate genes for a major QTL associated to CBB resistance (Liu et al, 2008).

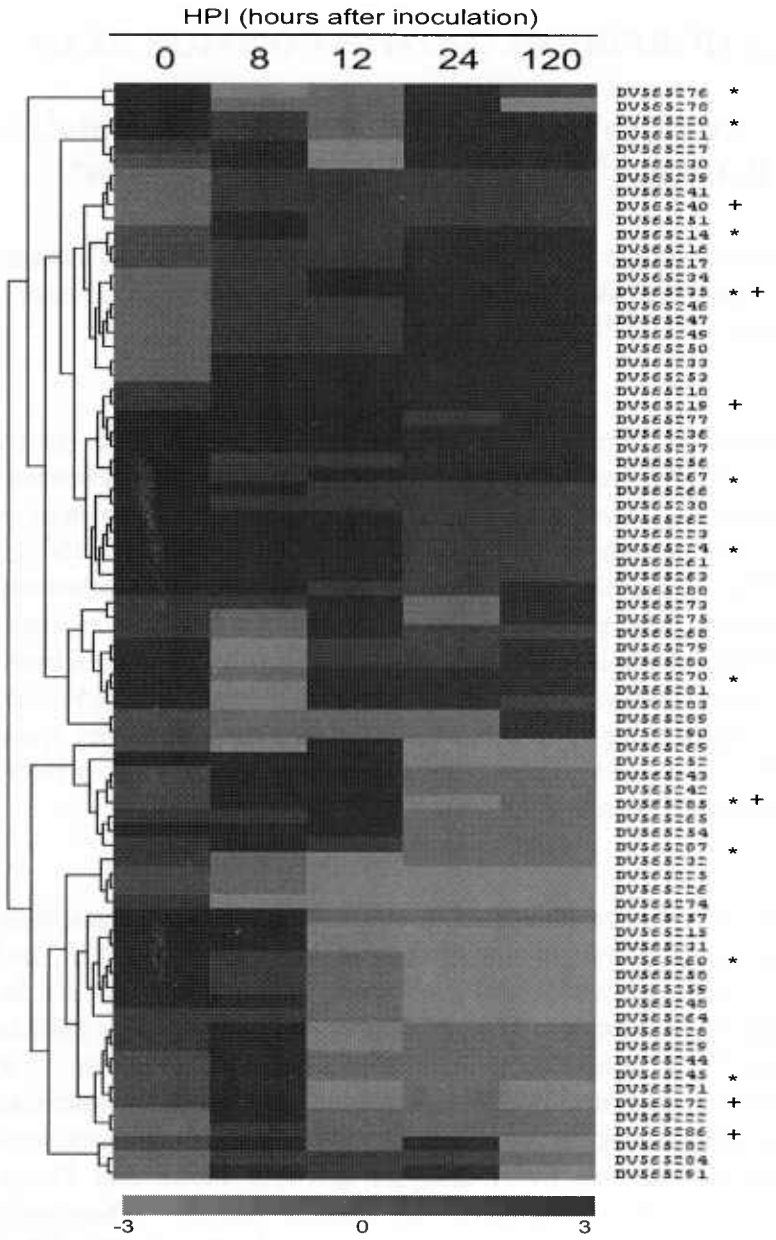

Figure1. Cluster analysis of the time-course expression profiles of 77 transcript-derived fragments. All measurements are relative to the expression level of mock-inoculated leaves at 0 hrs post-inoculation. The color saturation reflects the magnitude of the LOG2 expression ratio for each transcript. Red color marks up-regulated transcript-derived fragments after infection, whereas greens are down-regulated. The color LOG2 scale is provided at the bottom of the figure. * indicates the transcript-derived fragments homologous to proteins involved in plant defence responses. + indicates the transcript-derived fragments *in silico* mapped to the lower region of linkage group 6.

REFERENCES

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