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The identification of candidate genes associated to CBB resistance in common bean (*Phaseolus vulgaris* L.) using cDNA-AFLP

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Introduction

➢Common bacterial blight (CBB), incited by *Xanthomonas axonopodis pv. Phaseoli* (*Xap*), is a serious seed-borne disease of common bean (*Phaseolus valgaris* L.) in both temperate and tropical production zones (Liu et al., 2008).

➢The lines HR45 is highly resistant to *Xap* infection on leaves and pods in the field and greenhouse (Park et al., 1995). A major QTL was identified and mapped to the lower region of Linkage Group B6 (LG B6; Chromosome 6) (Liu et al., 2008). 64% of the phenotypic variation associated with CBB resistance could be explained by this major QTL (Liu et al., 2008).

➢In the toolbox of current expression studies, the cDNA-amplified fragment length polymorphism (AFLP) technique facilitated the choice of candidate genes, especially for un-sequenced genomes (Meyers et al., 2004).

Materials and methods

➢Common bean line HR45 was grown in a growth chamber under 8/16 h of light/dark at 25/23°C and a humidity of 75%.

➢ The plants were inoculated at the unifoliolate leaf stage using the multiple-needle technique. The fully expanded primary two leaflets were inoculated with *Xap* culture (108cfu/ml) (Park et al., 1995).

➢cDNA-AFLP was conducted using the AFLP expression analysis kit (LI-COR, USA) according to manufacturer's instructions. PCR products were separated and displayed on an IR2 DNA analyzer (LI-COR, USA).

➢The bands of interest were excised from the gel and sequenced. All sequences were submitted to the National Centre for Biotechnology Information (NCBI) database with accession numbers from DV565214 to DV565291.

➢Sequences were subjected to BlastX similarity search analysis against NCBI protein database and annotated according to their homology with known protein sequences.

➢Sequences were aligned with the soybean genome (http://soybase.org/gbrowse/cgi-bin/gbrowse/gmax1.01/) using 80% query coverage and 80% identity cutoffs. The syntenic regions of common bean were identified according to the bean/soybean synteny map (http://www.css.msu.edu/bic/PDF/Bean_Genomics_Status_2008.pdf).

➢Real-time RT-PCR was carried out using iQ SYBR Green supermix (Bio-Rad, USA) in a CFX96 Real-Time PCR Detection System (Bio-Rad, USA). Data were analyzed using CFX manager (Bio-Rad, USA). Fold change in RNA expression was estimated using threshold cycles, by the ΔΔCT method.

Results and discussion

➢In order to understand the molecular mechanisms underlying CBB resistance in HR45, cDNA-AFLP technique was used to identify the genes that are differentially expressed in the leaves of HR45 at different time-periods after inoculation. Selective amplifications with 34 primer combinations allowed the visualization of about 2,448 transcript-derived fragments (TDFs) in infected leaves; 10.6% of them were differentially expressed (Figure 1).

➢77 differentially expressed TDFs were cloned and sequenced. 50.6% (39 of 77) of the TDFs representing modulated bean transcripts were not previously reported in any EST database.

➢The expression patterns of 10 representative TDFs were further confirmed by Real-time RT-PCR (Figure 2).

➢BLAST analysis suggested that 40% (31 of 77) of the TDFs were homologous to the genes related to metabolism, photosynthesis, and cellular transport, whereas 28% (22 of 77) of the TDFs showed homology to the genes involved in defence response, response to stimulus, enzyme regulation, and transcription regulation.

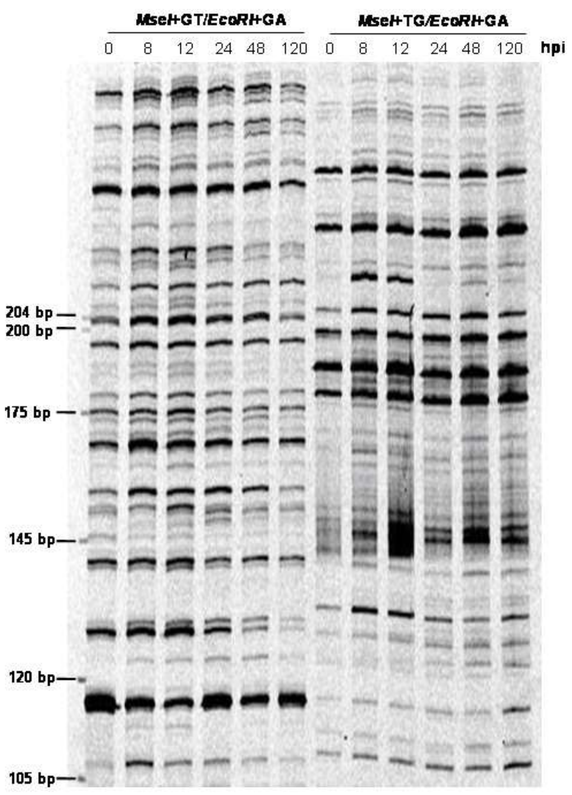

Figure 1. cDNA-AFLP analysis of HR45 leaves after inoculation with *Xap*.

An example shows selective transcript amplifications from inoculated leaves collected at 0, 8, 12, 24 and 120 hours post inoculation (hpi) with two different primer combinations MseI+GT/EcoRI+GA and MseI+TG/EcoRI+GA.

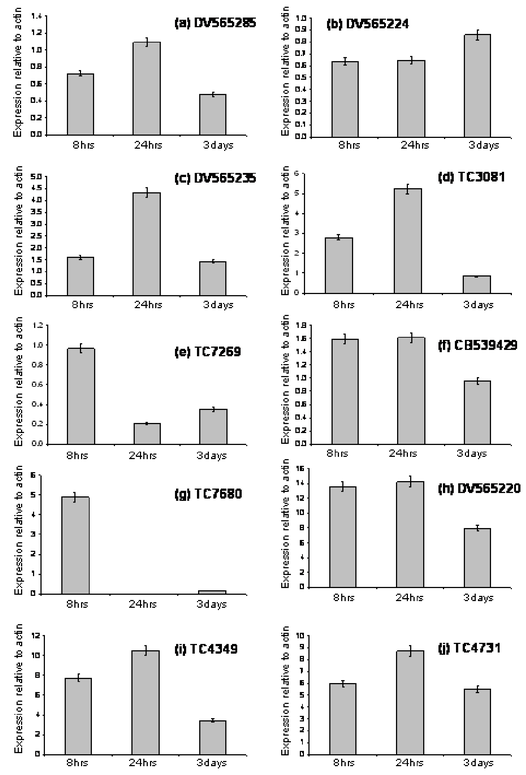

Figure 2. Real-time RT-PCR analysis of expression levels of ten selected TDFs in infected HR45 leaves.

Samples were taken at time points 8 hours, 24 hours, and 3 days post inoculation (dpi). Expression was normalized according to reference gene, *actin*. Experiments were done in quadruplicate (n=4) and error bars indicate standard deviation among the quadruplicate samples.

➢The 22 pathogenesis-related TDFs were selected as functional candidate genes in association with CBB resistance.

➢In addition, another 6 TDFs were *in silico* mapped in the lower region of linkage group B6 (the genomic location of the previously identified CBB resistance QTL in HR45) and chosen as positional candidate genes using comparative mapping.

Conclusions

➢The cDNA-AFLP technique was applied to identify 77 TDFs that are differentially expressed in the leaves of HR45 at different time-periods after inoculation. This study provides the first global catalogue of bean genes expressed upon *Xap* infection. 22 functional and 6 positional TDFs were selected as the candidate genes associated with CBB resistance. This information will help to elucidate the molecular basis of the resistance response process and identify the genes underlying the major CBB-resistance QTLs.

References

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