**Development of Genomic Resources for Management of Verticillium wilt of Potato**

Final report for Northwest Potato Research Consortium

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**ACCOMPLISHMENTS:**

**PROCEDURES:**

**Sample collection:**

The potato (cv. Russet Burbank) and brown mustard (cv. ISCI 99) plants were planted in the greenhouse and inoculated with two strains (Vd-653 and Vd-111) of V. dahliae (Fig. 1A-D). Ten days after pathogen inoculation, plants were destructively sampled and flash frozen in liquid nitrogen. The collected plant samples were stored in -80C for 10 days and lyophilized for 48 hours.

**RNA Extraction and cDNA synthesis:**

Total RNA was extracted from all collected with the method described by Kumar et al. 2007. To avoid genomic DNA contamination total RNA was digested with Ambion DNAse I (RNase-free) (Invitrogen). Quantification of total RNA was completed with a QUBIT fluorometer (Life Technologies) and integrity was assessed by gel electrophoresis. The quantities of total RNA in the samples ranged from 700-900 ng/µl. RNA samples free from genomic DNA and with two clear bands representing 28S and 18S ribosomal RNA were used for the cDNA synthesis. (Fig. 1E). cDNA was synthesized from 1 µg of total RNA in 20µl reaction volume using qScriptTM cDNA SuperMIX (QuantaBio) following manufacturer’s instructions. The cDNA was diluted 1:5 in ultrapure water for potato and mustard samples and 1:2 for Verticillium dahliae samples and stored at -20C. For gene validation, primers spanning exon-exon junction, were designed for each gene with NCBI primer design software (Table). Primers with 19-23 bp size, GC content ranged between 40-60% and amplicon size of 70-180 bp were used.

**Quantitative real-time PCR:**

RT-qPCR experiments were performed in a QuantStudio3 (Applied Biosytems) using SYBRTM Select Master Mix (2X) (Applied Biosystems). 10µl reaction volume consisted of 5µl SYBR Select mastermix, 0.5µl of each forward and reverse primers (10µM), 1µl diluted cDNA and 3µl sterile water. Cycling conditions were polymerase activation at 95C for 10 minutes and 40 cycles of denaturation at 95C for 15s and annealing at 60C for 1 min. Melting curve analysis was performed to check single product amplified by the primers from 60C to 95C with fluorescence reading acquired at 0.05C increment per cycle. Three biological and three technical replications were used for all genes. No template control was included in each reaction as a negative control. Efficiency of primers and Ct values were calculated using LinRegPCR software. Differential gene expression was calculated using method. The elongation factor (elfa), actin (ACT-2) and elfa genes were used for normalization of the qpcr reactions for S. tuberosum, B. juncea and Verticillium dahliae respectively. A t-test at significance of difference at p<0.05 was performed.

Both qpcr and Rna seq showed similar direction of gene expression level changes (both upregulated or downregulated)

Fold changes

Correlation coefficients, RMSD and kappa statistics satisfied

**RESULTS/DISCUSSION:**

***Publications*:**

None to date.

***Presentations and Reports:***

None to date.

***Acknowledgements***

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**REFERENCES:**