**Final report for Northwest Potato Research Consortium**

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

**PERSONNEL:**

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**REPORTING PERIOD**: Year Initiated: 2020; Terminating Year: 2021.

**ACCOMPLISHMENTS:**

The goal of this project is to provide genomic resources of potato, brown mustard, and *Verticillium dahliae* for potato scientists and stakeholders. More specifically, this project aims to validate the differentially expressed genes (DEGs) in *Solanum tuberosum*, *Brassica juncea* and *Verticillium dahliae* upon infection with two strains of *V. dahliae*. The Quantitative Real Time-PCR (qRT-PCR) method was used to analyze the expression changes of the DEGs to validate the DEGs obtained from RNA sequencing. The genes with highest fold change values or pathogenicity/virulence/defense response genes in transcriptome study were selected for this validation project. Total 12, 12, and four comparisons were validated for potato, mustard and *V. dahliae* genes respectively. Gene expression changes were in same direction those validated genes. The correlation coefficient between fold changes obtained from RNA seq and qPCR methods were 0.91, 0.85, and 0.96 for *S. tuberosum*, *V. dahliae* and *B. juncea* respectively. Some interesting pathogenicity and defense response genes like PR-genes of host and virulence related genes like peptidase, in pathogen has been validated. This study confirms the transcriptional changes in potato, brown mustard and *V. dahliae* upon *V. dahliae* infection and shed lights molecular mechanisms and pathways governing these changes.

**PROCEDURE:**

**Sample collection:**

The potato (cv. Russet Burbank) and brown mustard (cv. ISCI 99) plants were planted in Turface® (Profile Products LLC, Buffalo Grove, IL) in 3.5” pots in the greenhouse and fertigated with 20-10-20 NPK daily. After emergence, plants were drenched with two strains of Verticillium dahliae, Vd-653 and Vd-111. Vd-653 and Vd-111 are highly aggressive and less aggressive in potato, respectively (Dung et al 2013). Both isolates can colonize brown mustard but do not show symptoms (Wheeler and Johnson 2016). 100 ml of each isolate with a concentration of 1 x 106 conidia/ml was inoculated, and a sterile distilled water was used a control (Fig. 1A-D). The greenhouse experiment was conducted in a randomized complete block design with a 2×3 factorial arrangement with ten replications. Ten days after *V. dahliae* inoculation, plants were destructively sampled, and flash-frozen in liquid nitrogen. The collected plant samples were stored in -80°C for ten days and lyophilized for 48 hours.

**RNA Extraction and cDNA synthesis:**

Total RNA was extracted from all collected samples following the method described by Kumar et al. 2007 with some modification. 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 72-2000 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 and qRT-PCR validation. (Fig. 1E). cDNA was synthesized from 1 µg of total RNA in 20µl reaction volume using qScriptTM cDNA SuperMIX (QuantaBio) following the manufacturer’s instructions. The cDNA was diluted to 1:5 in DNAase and RNAase free sterile water for S. tuberosum and B. juncea gene validation and 1:3 for V. dahliae gene validation and stored at -20°C. For gene validation, primers were designed spanning exon-exon junction (if applicable) for each gene with NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast). The primer sequence and amplicon length are presented in Table 1. The differentially expressed genes (DEGs) with the highest fold change values or putative pathogenicity or virulence-related genes were selected for each host. Primers with 19-23 bp size, 40-60% GC content, amplicon size of 70-180 bp with no self-annealing and primer dimer formation were used.

**Quantitative real-time PCR:**

qRT-PCR experiments were performed in a QuantStudioTM Real-Time PCR System (Applied Biosystems) using SYBRTM Select Master Mix (2X) (Applied Biosystems) in 10µl reaction volume. The reaction mixture consisted of 5µl SYBR Select Master Mix(2X), 0.5µl of each forward and reverse primers (10µM), 1µl diluted cDNA and 3µl sterile water. The cycling conditions for qRT-PCR were 95°C for 10 minutes, followed by 40 cycles of 95°C for 15s and 60°C for 1 min. Melting curve analysis was performed from 60°C to 95°C with fluorescence reading acquired at 0.5°C increments per cycle for the presence of a single amplified product. Three biological and three technical replications were used for all genes. No template control was included in each reaction as a negative control. The efficiency of primers and Cq values were calculated using LinRegPCR software and (Ruijter et al. 2009). The Cq value for each biological replicate was calculated by taking the average Cq values of three technical replicates and Log2 fold change value was derived using the delta-delta Ct method for each comparison (Livak and Schmittgen 2001). Solanum tuberosum elongation factor 1-α (EF1α), Brassica juncea actin gene (ACT-2) and Verticillium dahliae elongation factor 1-α (EF1α) genes were used for normalization of the DEGs for respective hosts. The fold change values obtained from qRT-PCR were compared with fold changes obtained from RNA sequencing to check whether the gene expression level changes are in the same direction or not (i.e. upregulation and downregulation). The correlation coefficient was calculated between fold change obtained from these two methods was calculated for each host.

**RESULTS/DISCUSSION:**

The most commonly used method, quantitative Real Time-PCR (qRT-PCR) was used to analyze and validate the gene expression changes obtained from transcriptome study (Taylor et al 2010). The expression changes of the nine, six and four differentially expressed genes (DEGs) were confirmed in potato, brown mustard and *V. dahliae* respectively. For potato and brown mustard genes, three different comparisons were made namely, Vd-653 vs control, Vd-111 vs control and Vd-111 vs Vd-653. Total 12 and 11 different comparisons had gene expression changes in same direction for potato and brown mustard respectively. Similarly, four DEGs of *V. dahliae* showed fold changes in same direction as RNA seq results (Figure 2). The correlation coefficient between gene expression changes of validated DEGs from qRT-PCR and obtained results from RNA seq were 0.91, 0.85, and 0.96 for potato, brown mustard, and *V. dahliae* respectively (Figure 3). Since, RNA-Seq and qRT-PCR use different normalization process for data analysis, correlation was performed over other statistical analysis to compare fold change between RNA-Seq and qRT-PCR. To be more specific, RNA-Seq data was normalized using DESeq2 size factor (Love et al. 2014) however for qRT-PCR reference genes of respective host was used for the normalization.

Two genes of potato, *PRS2\_SOLTU* and *IER1\_SOLLC* did not show same direction of expression changes as RNA-Seq results. Similarly, *UVB31\_ARATH*, *LUC7L3*, Cluster-15354.91015 genes of brown mustard also did not show similar pattern of fold change as RNA-Seq data (data not shown). Different sensitivity of two methods, use of different template RNA for quantification and inconsistent expression of transcripts in some treatments could be potential reasons for such differences in results (Wang et al.2016). For Brassica gene, *UVB31\_ARATH*, there was either no amplification of gene or very poor amplification in certain treatments. This inconsistency can be explained by no expression of gene in certain treatments throughout all biological replications in RNA-Seq count data.

At the brighter side, some interesting pathogenicity and defense response genes in potato and mustard like PR- genes and virulence related genes like peptidase in Verticillium has been validated.

This study provides the valuable information about global transcriptional changes in virulence genes of *V. dahliae* upon interaction with its host, potato and endophyte, mustard. Similarly, transcriptional changes in defense/pathogenicity or other pathways in different hosts upon infection with both highly aggressive and less aggressive *V. dahliae* isolates.

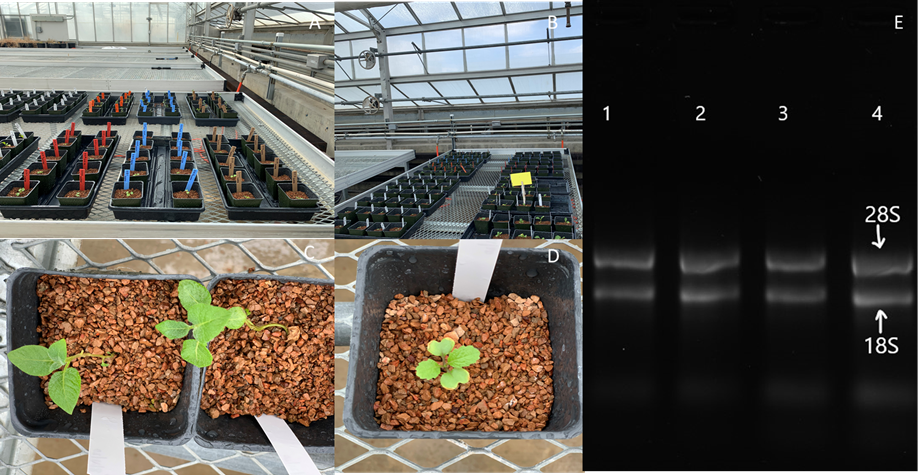


Figure 1. A, B) Greenhouse experiment, C) Potato plantlets, D) Mustard plants, and E) Total RNA integrity test on 1% agarose gel. Lane 1,2,3 and 4 indicates RNA samples with two 28S and 18S ribosomal RNA.

**Table 1:** List of primer sequence of differentially expressed genes (DGEs) used for the qRT-PCR validation

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Forward sequence (5’ to 3’) | Reverse sequence (5’ to 3’) | Amplicon size (bp) |
| St\_*PR04\_SOLLC* | GCCGTGCAATTGTGGGTGTC | CGCACACTTTTCCACTAGCAC | 76 |
| St\_*ABAH1\_ARATH* | CCACTTCCTCCTGGTACTTTAGG | AACTTGTTTAGCTGCCTCTGG | 177 |
| St\_PGSC0003DMG400024310 | GAGAAGGAAGATTGGTGGGACA | CTACCCATCCCTCCTCCACA | 105 |
| St\_*LOX12\_SOLTU* | ATTAGCTCTGTTCAAGGTGATCC | TCTCCAAGTAGGCTGGATTGC | 70 |
| St\_*PRR1\_TOBAC* | TGTCTTTTGCCCTTGAAGGCT | GACAACGTCTCACCAGCTCT | 115 |
| St\_*CHSB\_SOLTU* | GAGCTCAAGGAGAAATTTAAGCG | ACAACAACTATGTCTTGCCTTGC | 149 |
| St\_*EDL3\_ARATH* | AATGGTCGGATCGGAGGAGA | TCGGATTACACCCGCAACAG | 70 |
| St\_*WRK40\_ARATH* | AGACAACCCATCTCCAAGAGC | TCGATTGGTCTTCCACGCTT | 95 |
| St\_*TIF5A\_ARATH* | ATGTCCGAGCCTTCATCACC | GGAGCAACTAGTGATGGTATGGT | 130 |
| St\_ *EF1α* | ATTGGAAACGGATATGCTCCA | TCCTTACCTGAACGCCTGTCA | 101 |
| Vd\_*AOX\_PODAS* | GCTGCGTGGAAGTTTGTGC | TTCTTGTCAACCTGCTGCTCA | 83 |
| Vd\_*YDDQ\_BACSU* | AAGATTGTGCTCGTCGGGTA | TCTCAGCCAGAGCAACCTTC | 163 |
| Vd\_mRNA\_1341 | GCTGTCCGCATCTGACTTGT | GGTGACGTTGAACTTTGCCA | 97 |
| Vd\_*AYG1\_ASPFU* | GATTCGGCTGACCCAGACAG | ACCTTGCCCATATCGAACCG | 89 |
| Vd\_*EF1α* | CTTCCAACGTCATCACCT | CTCAGAGCGAACTCATACT |  |
| Bj\_Cluster-15354.86688 | ATTCACACTGCTCCACGCTA | GGCTGAAGGGTGAGAATGGG | 78 |
| Bj\_*NUP1\_ARATH* | CCATCCTTGCTTGGATTGCC | ATGCAGGAGGCTAAGGTTGG | 110 |
| Bj\_*PDRP2\_ARATH* | TATAAAGCAGGCAGCGAAGC | GAGAGCACTCCCCAACGAT | 105 |
| Bj\_*SCL1\_ARATH* | AACTGCTGAAAAGGATGACAAGT | TGCTCTTGCTGCTTTCCGTT | 84 |
| Bj\_Cluster-15354.44072 | TGCGTTCCTCAGAACCAGAG | AGCTTCTTCTCCACTGCTGAC | 106 |
| Bj\_Cluster-15354.125147 | GCGAGAGCAAGACACAAAAGG | TGTTTGCTGAGGGGAGTTCG | 77 |
| Bj\_*ACT-2* | TGGGTTTGCTGGTGACGAT | TGCCTAGGACGACCAACAATACT | 290 |



**Figure 2:** Validation of relative expression changes of selected DEGs of *S. tuberosum*, *V. dahliae*, and *B. juncea* with the qRT-PCR method. The x-axis represents the name of the genes, and the y-axis represents gene fold change value for the given comparison (shown in legend).



**Figure 3:** Scatter plots showing the linear relationship between qRT-PCR and RNA-Seq gene expression changes in *S. tuberosum*, *V. dahliae*, and *B. juncea*. R-value represents the correlation coefficient for the respective host.

***Publications*:**

Manuscript in preparation

***Presentations and Reports:***

None to date.

***Acknowledgements***

This project was funded by the Northwest Potato Consortium.

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