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

Final report for Northwest Potato Research Consortium

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**ACCOMPLISHMENTS:**

**METHODOLOGY:**

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 a subset of differentially expressed genes in potato, mustard, and Verticillium dahliae upon infection with two strains of V. dahliae.

**Sample collection:**

The greenhouse experiment was conducted with 2\*3 factorial design arranged in randomized complete block design with 10 replications. The potato (cv. Russet Burbank) and brown mustard (cv. ISCI 99) 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 100 ml of two strains (Vd-653 and Vd-111) with concentration of 1 x 106 *V. dahliae* conidia/ml and a sterile distilled water control as control (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 -80°C for 10 days and lyophilized for 48 hours.

**RNA Extraction and cDNA synthesis:**

Total RNA was extracted from all collected samples 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 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 manufacturer’s instructions. The cDNA was diluted to 1:5 in DNAase and RNAase free sterile water for potato and mustard’s genes validation and 1:2.5 for Verticillium dahliae’s genes validation and stored at -20C. For gene validation, primers were designed for each gene with NCBI Primer-BLAST (Table 1). The differentially expressed genes (DEGs) with highest fold change values or putative pathogenicity or virulence related genes were selected for each host. In potato and mustard the ge Primers with 19-23 bp size, 40-60% GC content, and amplicon size of 70-180 bp were used.

**Quantitative real-time PCR:**

qRT-PCR experiments were performed in a QuantStudio3 (Applied Biosystems) using SYBRTM Select Master Mix (2X) (Applied Biosystems) with 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 polymerase activation at 95C for 10 minutes followed by 40 cycles of denaturation at 95C for 15s and annealing at 60C for 1 min. Melting curve analysis was performed for the presence of 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 (Ruijter et al 2009). Log2 fold changes values were calculated using method for each comparison (Livak et al 2001). *Solanum tuberosum* elongation factor (elfa) gene, *Brassica juncea* actin gene (ACT-2) and *Verticillium dahliae* elfa genes were used for normalization of the DEGs. The fold change values were assessed to check whether the gene expression level changes are in same direction or not. In addition, correlation coefficient between fold change obtained from qRT-PCR and RNA-Seq was calculated for each host.

**RESULTS/DISCUSSION:**

1. **Identification of the molecular mechanisms upon pathogen infection to identify novel target for the disease management**
2. **Identify global transcriptional changes in host and pathogen (with different level of susceptibility) during pathogenesis**
3. **Identify major pathways responsible for susceptibility in host and virulence in pathogen or resistance in endophyte.**
4. **Functional analysis of the DEGs obtained from this study to decipher host-pathogen interaction mechanism**

**Some validated susceptibility/defense related genes:**

**Potato:**

***PR04\_SOLLC* (DEFENSE RESPONSE AGAINST PATHOGEN, RESPONSE TO BIOTIC STIMULUS)**

***ABAH1\_ARATH* (Oxidoreductase activity, stress response)**

***PRR1\_TOBAC* (DEFENSE RESPONSE, RESPONSE TO BIOTIC STIMULUS)**

***IER1\_SOLLC* (response to wounding) defense mechanism, PCD**

***WRK40\_ARATH* (defense response to fungus/bacteria/chitin/wounding)**

***TIF5A\_ARATH* (defense response)**

**Verticillium:** YDDQ\_BACSU (hydrolase/ catalytic activity//molecular\_function)

AYG1\_ASPFU: Pathogenecity related (peptidase, hydrolase)(Decreases the protection against the host's immune defenses )

AOX\_PODAS(Oxidoreductase)

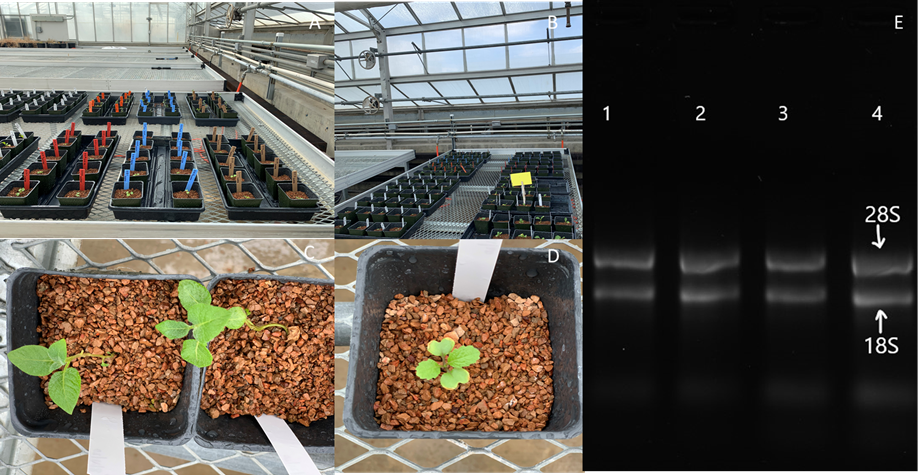


Figure 1. A, B) Greenhouse experiment, C) Potato plantlets, D) Mustard plants, and E) Total RNA integrity test on 1% agarose gel. Lane 1,2,3 and 4 indicates RNA samples with two 28S and 18S ribosomal RNA.

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

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Forward sequence (5’ to 3’) | Reverse sequence (5’ to 3’) | Product length(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\_ef1alpha | 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\_ef-1 | 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 potato, mustard, and Verticillium with qRT-PCR method. The x-axis represents the name of the genes and y-axis represents the normalized expression level of the transcript for the given comparison (shown in legend). Standard deviation for biological replicates (n=3) is indicated by error bars.



**Figure 3:** Scatter plots showing the linear relationship of gene expression levels between qRT-PCR and RNA-Seq in potato, Verticillium, and mustard. R value represents the correlation coefficient for each host.

***Publications*:**

Manuscript in preparation

***Presentations and Reports:***

None to date.

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