Analysis of Gene Expression of Switchgrass (*Panicum virgatum*) Inoculated with Anthracnose (*Collectotrichum navitas*)

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ABSTRACT

Switchgrass (*Panicum virgatum* L.) is a warm-season, perennial grass, native to North America. It has a potential of being used as an alternative source for biofuel and it plays a vital role in our ecosystem. Anthracnose disease, caused by the fungi *Colletotrichum navitas*, affects switchgrass. Our research focuses on the switchgrass cultivar "Alamo." Switchgrass plants grown for 8 weeks were inoculated with two isolates of *C. navitas*. Simultaneously, control plants were also grown under similar conditions, however inoculated with distilled water only. After 10 days, the leaves from the inoculated plants showing disease symptoms were collected and the pathogen was re-isolated into a PDA plate and incubated at 24° C with 65% RH to complete the Koch's postulates. Molecular analysis involved DNA and RNA isolations from the inoculated and control plants. Polymerase chain reaction (PCR) was performed using genomic DNA from the control plants to verify primer integrity. Then RNA was isolated from each inoculated and control plants. The extracted RNA was synthesized to cDNA, which we used in Reverse Transcriptase PCR, to check for presence and differential expressions of the selected disease resistance genes. The selected genes were designed from differential expression between switchgrass cultivars "Carthage" and "BN-309-69" that have been challenged with anthracnose in the field. However, our results demonstrated that the primer sets did not amplify in the cDNAs isolated from our greenhouse inoculations. We postulate that these primers may have been designed from sequences including intronic regions which are not present in cDNA. Thus, amplification will not occur. Future work should include the redesigning of primers from the coding sequence only.

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a warm-season, perennial grass that grows on the North American landscape and serves as a potential source of renewable energy (Crouch *et al*., 2009). Characteristics of switchgrass that makes it a potential biomass energy crop includes limited agricultural inputs, and the consistently high yield in a wide range of environments (McLaughlin and Kzsos, 2005). An expanding biofuel industry in the United States (U.S.) led to an increase of switchgrass monoculture, which results to production problems caused by pest and diseases (Crouch *et al*., 2009). In the U.S., one fungal disease that has proven to be a problem for switchgrass production is anthracnose. Switchgrass-producing states such as Iowa, Tennessee, North Carolina, Pennsylvania, New Jersey and New York have experienced incidents of anthracnose disease outbreaks over the last decade (Gravert and Munkvold, 2002). In 2008, anthracnose disease symptoms were observed in several cultivars of switchgrass plants grown in Freehold, New Jersey. Upon microscopic observations of leaf lesions, the presence of masses of black setae were observed in long rows, running parallel to the leaf veins. Molecular phylogenetics and morphological characters indicated *Colletotrichum navitas* as the causal pathogen (Crouch *et al.,* 2009). Anthracnose is virulent on susceptible varieties of switchgrass. Resistance genes are activated when exposed to biotic stresses such as anthracnose. Gene expression analysis differentiates functional genes expressed between treated and non-treated samples. In this study, mRNA serves as a key role to understand gene expression. Primers sets of BZIP, WRKY2, RPMI, and several well-known disease resistance genes were amplified in genomic DNA in switchgrass cultivar "Alamo". The significance in the presence of disease resistance genes in genomic DNA can be used for further molecular analysis of gene expression.

OBJECTIVES

- Inoculate switchgrass plants with anthracnose isolates.
- Confirm that *C. navitas* was the pathogen associated with the anthracnose symptoms observed in the inoculated plants.
- Validate PCR primers with genomic DNA isolated from switchgrass.
- Identify differential gene expression among inoculated and non inoculated switchgrass plants.

MATERIALS AND METHODS

Plant Materials. Switchgrass cultivar Alamo seeds were planted in pots and grown in the greenhouse for eight weeks. Each treatment was replicated 3 times. There was a total of 9 pots overall.

Fungal Isolates. Two anthracnose isolates, Cn100NY09 and Cn101NY09 obtained from Cornell University, were used for this research. The cultures were grown on potato dextrose agar (PDA) plates maintained at 24° C with 65% RH for 2 weeks (Figure 1).

Figure 1. A 2-week old culture of *C. navitas* isolate growing on a PDA plate.

Inoculation Method. Spores from each isolate were harvested from PDA plates. Spore suspensions for each isolate were adjusted to 5 x10^4 conidia/ml and sprayed onto the switchgrass plants. For the control switchgrass plants, autoclaved distilled water was also sprayed. The plants were kept in a humid chamber made of plastic cover and sealed with autoclave bags at the bottom (Figure 2).

MATERIALS AND METHODS (Cont.)

Figure 2. Humidity chamber set-up of the inoculated and control switchgrass plants.

- **DNA extraction** DNA was extracted from the control switchgrass plants for PCR verification of the selected disease resistant genes.
- **PCR**. The 10 primers used were *6KG, 6KGF2, 2NG, 2NG1, 7NG, 7NG1, 1KG, 1KG1, 2KG* and *2KG*1. Gel electrophoresis was done on 1% agarose gel.
- **RNA extraction** RNA was extracted from all three groups of the switchgrass samples (Control, Cn101NY09, and Cn101NY09).
- **cDNA Synthesis** The extracted RNA was then synthesized to cDNA using PhotoScript II used in RT-PCR.
- **Reverse Transcriptase PCR.** RT-PCR was conducted using cDNA to check for differential expressions of the selected disease resistance genes.

RESULTS

- We completed a successful inoculation of switchgrass with anthracnose. Both isolates, Cn100NY09 and Cn101NY09, produced symptoms on switchgrass (Figure 3A and B), while our control plant showed no symptoms or signs of the disease (Figure 3C).

Figure 3. Isolate Cn100NY09 (A) was inoculated on the switchgrass leaf and showed symptoms of anthracnose disease. Isolate Cn101NY09 (B) also showed symptoms of the disease. The control switchgrass (C) did not show any symptom.

- We verified 8 of the 10 PCR primer sets amplifications associated with disease resistance and transcription factors in genomic DNA isolated from the control plant (Figure 4).

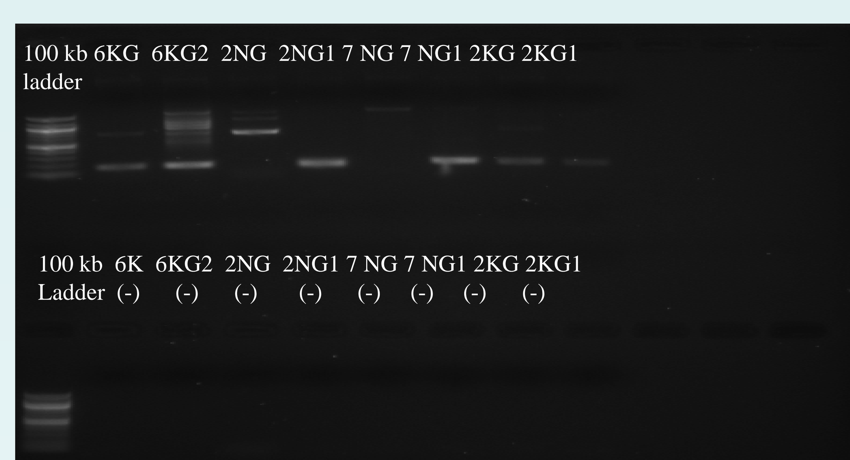

Figure 4. Gel picture showing results for PCR with genomic DNA using 8 disease resistant primers.

RESULTS (Cont.)

- To analyze gene expression, primer sets associated with disease resistance in cDNA was used in PCR. Every primer set designed was not expressed, except for the control housekeeping gene named HSP90 (Figure 5).

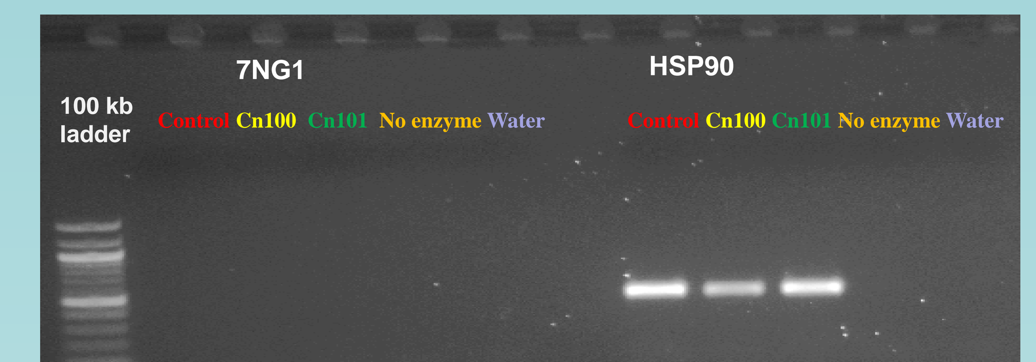

Figure 5. Gel picture showing results for reverse transcriptase (RT) PCR with cDNA using 7NG1 and Hsp90 primers. All 8 primer sets including 7NG1 were not present or expressed in the inoculated and control plants.

CONCLUSIONS and DISCUSSIONS

Anthracnose Inoculation

The *C. navitas* cultures were obtained and used to inoculate healthy switchgrass plants. Successful inoculations were done. The anthracnose observed from greenhouse inoculations were characteristic of the disease, which showed lesions that are tan colored and elongate, with sharply tapered ends and reddish brown borders. The switchgrass plants that showed symptoms of the anthracnose disease were re-isolated and appeared to share similarities with the original culture.

Molecular Analysis

Disease resistance genes were present in the genomic DNA. However, only the housekeeping gene HSP90 amplified in the cDNA of both control and inoculated switchgrass samples. The control and inoculated switchgrass plants did not show gene expression possibly due to the location of the designed genes when converted into cDNA. The selected genes were designed from differential expression between switchgrass cultivars "Carthage" and "BN-309-69" that have been challenged with anthracnose in the field. We postulate that these primers may have been designed from sequences including intronic regions which are not present in cDNA. Thus, amplification did not occur.

FUTURE DIRECTIONS

Future work will include redesigning primers from the coding sequence only. Diverse switchgrass cultivars may yield different gene expression patterns. Genes amplified in cDNA will be used for Quantitative PCR. Anthracnose isolates from different regions can be used to inoculate the diverse cultivars.

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