Identification of SSR Markers to Study Genetic Diversity of *Colletotrichum navitas*, the Causal Organism of Switchgrass Anthracnose

Christopher Mann1, Vincenzo Averello2, Jennifer Vaicunas3, Christine Kubik3, Lisa Beirn3, JoAnne Crouch1, Josh Honig2, Stacy Bonos2

1State University of New York. College of Environmental Science and Forestry. 2Rutgers University, Department of Plant Biology and Pathology, USDA-ARS, Beltsville, MD.

NEWBio
Northeast Woody/Warm season Biomass Consortium

Introduction

Switchgrass (*Panicum virgatum L.*) is a warm season perennial prairie grass that has recently garnered attention as a potential renewable energy resource. As a North American native plant, switchgrass could be more resistant to diseases that could potentially affect biomass.1 Anthracnose, a fungal disease affecting a wide variety of plants, has been identified on switchgrass cultivars and isolated as the recently discovered species *Colletotrichum navitas*. To avoid potential biomass losses and expensive continual fungicide treatments, C. navitas resistance could be bred into switchgrass. To begin an effort in breeding anthracnose resistance into switchgrass the genetic diversity of the wild C. navitas pathogen must first be understood to determine the diversity within the fungus which help to understand inheritance of resistance.

Objective

**Questions:**
How diverse is the *Colletotrichum navitas* fungus in switchgrass? How genetically similar are individuals to each other and to other *Colletotrichum* species?

**Experiment:**
Identify effective SSR primers from genome sequences to amplify SSR markers in the *Colletotrichum navitas* genome in order to capture the best image of *Colletotrichum* diversity. This information will assist with more intensive diversity studies and future breeding efforts by providing information on type of inheritance involved in resistance in switchgrass.

Materials and Methods cont.

- *Colletotrichum* isolates included multiple species: *C. caudatum, C. cereale, C. eremochloae, C. navitas, C. sublineola,* and *C. zosiae*
- Approximately 90 *C. navitas* isolates were also included in the study. These were collected by Shannon Hennessey, the NEWBio scholar in 2013 from Adelphia, Somerset and New Brunswick, NJ, and State College and Phillipsburg, PA from standard cultivars and experimental selections of switchgrass.
- DNA was extracted from *Colletotrichum* isolates using the ArchivePure Cell/Tissue Kit.
- Samples were delivered by Dr. Jo Anne Crouch from the USDA.
- Bioinformatics programs Sciroko, ActivePerl, and Primer3 were used to find, quantify, and design primers respectively around Simple Sequence Repeat (SSRs) located in the *C. navitas* genome.

Results

- A total of 96 samples of *Colletotrichum* spec. DNA were screened with 50 primer sets to find reliably quantifiable SSR markers to use for future *Colletotrichum* genotype characterization.
- Microsatellite DNA samples tagged with fluorescent markers and quantified with the Applied Biosystems 3500xl Genetic Analyzer.
- Results were scored for allelic composition with Applied Biosystems Genemapper Software.
- Genetic similarity was calculated using PowerMarker 3.25.

Table 1.

<table>
<thead>
<tr>
<th>C. navitas Genetic Diversity Summary Statistics</th>
<th>Allele Frequency</th>
<th>Simple Sequence Flanking</th>
<th>Unique Flanking</th>
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<tbody>
<tr>
<td>Marker Allele Frequency</td>
<td>Sample Size</td>
<td># observations</td>
<td>Allele # Availibility</td>
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</table>

Table 1: Simple Sequence Repeat regions of DNA. SSR markers are highly variable in individuals. The non-repeat flanking regions determine the site of primer selection.

Looking Forward

- The high degree of genetic diversity observed within switchgrass anthracnose isolates indicate that the fungus has probably been present in switchgrass for some time and is probably a pervasive member of the Northeastern U.S. ecosystem.
- The finding of multiple isolates on a single switchgrass plant suggests that breeding for resistance may be somewhat difficult.
- Future studies will include increasing the number of *C. navitas* isolates from different regions throughout the country and comparing isolates from the same cultivar evaluated in multiple environments.

References


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