

Final Technical Report

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Title: Genetics and Genomics of Pathogen Resistance in Switchgrass

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This is a report of the accomplishments of Dr. Gary Yuen, University of Nebraska – Lincoln in the project “Genetics and Genomics of Pathogen Resistance in Switchgrass” conducted in collaboration with Serge Edme et al., USDA-ARS Wheat, Sorghum, and Forage Research Unit. This project investigated the genetic and genomic bases of resistance/tolerance to disease in switchgrass (*Panicum virgatum*) populations developed as bioenergy feedstock. The focus was placed on populations adapted to the Central Great Plains derived from the hybridization of upland and lowland ecotypes, represented by cultivars Summer and Kanlow, respectively. Resistance was investigated against the rust disease caused by the fungus *Puccinia novo-panici* (syn. *P. emaculata*) and mosaic disease caused by the virus complex of *Panicum mosaic virus* (PMV) and Satellite *Panicum Mosaic virus* (SPMV).

The major goals of the project

- 1) understand the genetic and genomic bases of resistance/tolerance in locally adapted upland and lowland switchgrass breeding populations with contrasting disease symptoms under field conditions;
- 2) determine the molecular underpinnings of why cv Kanlow has broad resistance/tolerance to pathogens;
- 3) discover the molecular differences that permit systemic viral infections in some switchgrass plants but not in other genetically-related plants.

Accomplishments in goal 1

The Yuen lab played a critical role in phenotyping three generations of a switchgrass breeding population (Summer [female] x Kanlow [male]) for resistance/susceptibility to rust disease and the viral mosaic complex. The phenotyping included evaluation of more than 3,000 individual field-grown plants, in 2016, 2017, and 2018 growing seasons, for viral mosaic severity in June and twice (August and September) for rust severity. The rust and viral mosaic severity data was analyzed in conjunction with biomass yield and quality (cell-wall) traits to estimate their phenotypic and genetic correlations and to predict the breeding values of parents and progeny. Dry matter yield was found to be genetically but negatively correlated with mosaic ratings, indicating that higher yielding genotypes were more resistant/tolerant to virus

infection. 'Liberty' a cultivar released from the Summer x Kanlow population was intermediate in resistance to rust and viral pathogens, which imply introgression of resistance factors from the paternal parent (Kanlow) into the maternal genome.

Accomplishments in goal 2

The Yuen lab established methodology critical to this objective. Different schemes were tested to determine the best method for viral and fungal inoculations of switchgrass under greenhouse conditions. Yuen lab personnel conducted rust inoculations of greenhouse-grown Kanlow and Summer plants used in RNA-seq analysis of their responses to infection. Changes in basal and temporal expression of genes related to leaf functions were evaluated for greenhouse grown 'Kanlow', and 'Summer' plants. Three biological replicates of the 4th leaf pooled from 15 plants per replicate were harvested at regular intervals beginning from leaf emergence through senescence. Increases and decreases in leaf chlorophyll and N content were similar for both cultivars. Likewise, multidimensional scaling (MDS) analysis indicated both cultivar-independent and cultivar-specific gene expression. Cultivar-independent genes and gene-networks included those associated with leaf function, such as growth/senescence, carbon/nitrogen assimilation, photosynthesis, chlorophyll biosynthesis, and chlorophyll degradation. However, many genes encoding nucleotide-binding leucine rich repeat (NB-LRRs) proteins and wall-bound kinases associated with detecting and responding to environmental signals were differentially expressed. Several of these belonged to unique cultivar-specific gene co-expression networks. Analysis of genomic resequencing data provided several examples of NB-LRRs genes that were not expressed and/or apparently absent in the genomes of Summer plants. It is plausible that cultivar (ecotype)-specific genes and gene-networks could be one of the drivers for the documented differences in responses to leaf-borne pathogens between these two cultivars.

Accomplishments in goal 3

The Yuen lab established methodology critical to this objective. PMV and SPMV synergistically interact in cointfected proso and pearl millet plants resulting in a severe symptom phenotype. In this study, we examined synergistic interactions between the isolates of PMV and SPMV by using PMV-NE, PMV85, SPMV-KS, and SPMV-Type as interacting partner viruses in different combinations. Cointfection of proso millet plants by PMV-NE and SPMV-KS elicited severe mosaic, chlorosis, stunting, and eventual plant death compared with moderate mosaic, chlorotic streaks, and stunting by PMV85 and SPMV-Type. In reciprocal combinations, cointfection of proso millet by either isolate of PMV with SPMV-KS but not with SPMV-Type elicited severe disease synergism, suggesting that SPMV-KS was the main contributor for efficient synergistic interaction with PMV isolates. Cointfection of proso millet plants by either isolate of PMV and SPMV-KS or SPMV-Type caused increased accumulation of coat protein (CP) and genomic RNA copies of PMV, compared with infections by individual PMV isolates. Additionally, CP and genomic RNA copies of SPMV-KS accumulated at substantially higher levels, compared with SPMV-Type in cointfected proso millet plants with either isolate of PMV. Hybrid viruses between SPMV-KS and SPMV-Type revealed that SPMV isolates harboring a CP

fragment with four differing amino acids at positions 18, 35, 59, and 98 were responsible for differential synergistic interactions with PMV in proso millet plants. Mutation of amino acid residues at these positions in different combinations in SPMV-KS, similar to those as in SPMV-Type or vice-versa, revealed that A35 and R98 in SPMV-KS CP play critical roles in enhanced synergistic interactions with PMV isolates. Taken together, these data suggest that the two distinct amino acids at positions 35 and 98 in the CP of SPMV-KS and SPMV-Type are involved in the differential synergistic interactions with the helper viruses.

Publications in peer-reviewed journals

Chowda-Reddy, R.V., Palmer, N., Edme, S., Sarath, G., Kovacs, F., Yuen, G.Y., Mitchell, R. and Tatineni, S. 2019. A Two-Amino Acid Difference in the Coat Protein of Satellite panicum mosaic virus Isolates is Responsible for Differential Synergistic Interactions with Panicum mosaic virus. MPMI <https://doi.org/10.1094/MPMI-09-18-0247-R>

Palmer, N.A., Chowda-Reddy, R.V., Muhle, A.A., Tatineni, S., Yuen, G., Edme, S.J., Mitchell R.B., and Sarath, G. 2019. Transcriptome divergence during leaf development in two contrasting switchgrass (*Panicum virgatum* L.) cultivars. PLoS ONE 14(9): e0222080. <https://doi.org/10.1371/journal.pone.0222080>

Edm , S.J., Sarath, G., Palmer, N., Yuen, G.Y., Muhle, A.A., Mitchell, R., Tatineni, S., and Tobias, C. 2020. Genetic (co)variation and accuracy of selection for resistance to viral mosaic disease and production traits in an inter-ecotypic switchgrass breeding population. Crop Science (published 14 April, 2021). <https://doi.org/10.1002/csc2.20392>

