Genetics and Genomics of Pathogen Resistance in Switchgrass

Serge Edmé[1*] (serge.edme@ars.usda.gov), Gautam Sarath[1], **Nathan Palmer**[1], Rob Mitchell[1], Satyanarayana Tatineni[1], Gary Yuen[2], Anthony Muhle[2], and R.V. Chowda Reddy[1]
[1]USDA-ARS Wheat, Sorghum and Forage Research Unit, Lincoln, NE; [2]Plant Pathology Department, University of Nebraska, Lincoln, NE

Project Goals: This project aims at applying genomic selection (GS) in switchgrass, a key bioenergy crop in the US, on the genic regions causally linked to high biomass, ethanol yield, and durable resistance to fungal and viral diseases. The specific objectives are:
(1) Understand the genetic and genomic bases of pathogen response in regionally-adapted upland and lowland switchgrass breeding populations with contrasting disease symptoms under field conditions,
(2) Dissect the molecular underpinnings of the broad resistance or tolerance to pathogens in 'Kanlow' vs 'Summer', and
(3) Discover the molecular differences that permit systemic viral infections in some switchgrass plants, but not in other genetically-related plants.

Diseases, such as viral mosaic (caused by *Panicum mosaic virus*, PMV) and rust (caused by *Puccinia emaculata* and *Uromyces graminicola*), can cause significant losses in yield and quality traits in switchgrass (*Panicum virgatum*) bred for forage and bioenergy. Breeding by phenotypic recurrent selection can be effective at increasing genetic resistance in switchgrass populations. However, GS approaches are expected to increase the accuracy of selection, to concurrently improve yield and disease traits in switchgrass, and to ultimately generate greater genetic gains. Dense markers, such as single nucleotide polymorphisms (SNP) that span the whole genome, are required by genomic selection in order to predict breeding values of selection candidates by combining genotypes, pedigree, and SNP effects with phenotypic data.

Two interconnected reciprocal switchgrass populations derived by crossing 'Kanlow' (lowland) with 'Summer' (upland) are being followed across three generations. A reference germplasm set of switchgrass cultivars (from several US geographic regions) and selections was assembled to constitute a good allelic representation of the genome. Parental and progeny populations were phenotyped for biomass yield, Klason lignin content, ethanol yield, and disease ratings and genotyped with DArTseq to develop SNP markers. Two RNA-seq experiments (3 replicates of 10 pooled individual plants/timepoint/treatment) were sequenced and are being analyzed. In the first experiment, the 4th leaf from greenhouse-grown Kanlow and Summer plants were collected at seven timepoints over the course of two months in order to examine basal gene expression differences between the two populations. In the second experiment, the response of Kanlow and Summer plants to rust infection was examined by collecting the 4th leaf of infected and uninfected plants at 2, 7, 11, and 18 days after inoculation.

Genetic variation was assessed for viral mosaic and yield traits (dry matter yield, Klason lignin, and predicted ethanol yield) in an inter-ecotypic Summer x Kanlow population using linear and generalized linear mixed model approaches with restricted maximum likelihood. The two models were compared in their assessment of the genetic parameters, estimation of breeding values, and prediction of genetic gain. The analyses were also performed in the context of a multivariate animal model, which traces the pedigree back through three generations. A pedigree (Fig. 1) was

built to follow the flow of genes from parents to progeny across generations and to increase the efficiency of prediction models by including the pedigree and molecular relationship matrices. The resulting genetic parameters were used to update the selection index with disease resistance and determine the relative importance and contributions of each trait to maximizing genetic gains. Heritability estimates were 0.53 for dry matter yield, 0.36 for KL, 0.39 for ETOH, and 0.49 for mosaic ratings. Dry matter yield was genetically but negatively correlated with mosaic ratings (-0.44) and KL (-0.12ns), indicating that higher yielding genotypes were more resistant/tolerant to the virus (Edmé et al., 2017). '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 (Muhle et al., 2017).

The synergistic interaction between PMV and sPMV (*satellite PMV*) was investigated, using infectious cDNA clones of NE and TX isolates of PMV and clones of KS and TX isolates of sPMV (Chowda-Reddy et al. 2018). Both PMV-NE and TX elicited mild mosaic symptoms on proso millet (*Panicum miliaceum*) whereas co-infection by PMV-NE+sPMV-KS elicited severe mosaic, yellowing, and stunting symptoms, compared with moderate symptoms by PMV-TX+sPMV-TX. The severe symptoms caused by PMV-NE or PMV-TX with sPMV-KS indicated that sPMV-KS was the main contributor to an efficient synergistic interaction. The genome sequences of sPMV-KS and sPMV-TX differ by 11 nucleotides with four non-synonymous and three synonymous changes in the coat protein ORF. These genomic differences between sPMV isolates provide the basis for the differential synergistic interaction with PMV.

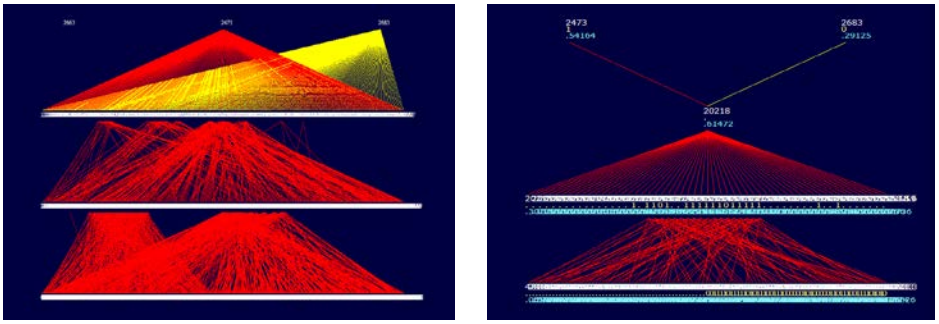

Fig. 1. Three cycles in the Pedigree of the Summer x Kanlow population. Left panel: female parents in red and male parents in yellow. Right panel: Original fullsib population with disease ratings and breeding values and successive generations bred by open-pollination.

References

Chowda-Reddy et al. 2018. Mapping synergistic interaction determinants between *Panicum mosaic virus* and *Satellite Panicum mosaic virus*. Intl Congress Plant Pathology. July 29-Aug 3, Boston, Mass.

Edmé et al. 2017. Realized and Expected Genetic Gains after Three Cycles of Breeding Switchgrass (*Panicum virgatum*) for Bioenergy. Manuscript under review.

Edmé et al. 2017. Genetic variation for resistance to viral mosaic in a Summer x Kanlow switchgrass breeding population based on the generalized linear mixed animal model. Switchgrass IV. Aug. 7-10, Lincoln, NE.

Muhle A. et al. 2017. Resistance of 'Liberty' switchgrass to rust and viral diseases. Switchgrass IV. Aug. 7-10, Lincoln, NE.

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