Genetics and Genomics of Pathogen Resistance in Switchgrass

Final Report 2016-2021

S.J. Edmé (PI), G. Sarath, N. Palmer, S. Tatineni, S, R. Mitchell, and G. Yuen
USDA-ARS Wheat, Sorghum, and Forage Research Unit
251 Filley Hall (Univ. of Lincoln, NE - East Campus)
Lincoln, NE 68583

1. Executive Summary

This project was funded by DOE under Grant no. *DE-SC0016108*. Originally approved for the 2016-2019 period, two no-cost extensions were solicited and approved, which prolonged the lifespan through July 2021. This final report informs on the results obtained so far from the research implemented. The research hinged on integrating genomics (genomic selection, RNAseq, virus-plant interactions) with classical genetics (conventional breeding) to incorporate durable resistance to fungal (rust) and viral (mosaic) diseases in switchgrass (*Panicum virgatum*) populations being bred for bioenergy. Higher biomass yield, higher quality (low lignin content), and durable disease resistance are key features to make lignocellulosic switchgrass feedstocks economically competitive and sustainable. Genomic selection is being applied on three generations of a switchgrass population derived from crossing two ecotypes (Kanlow as lowland female and Summer as upland male) with differential performance in terms of biomass yield and quality, disease resistance, and winter survivability. Target populations were screened for rust and mosaic in field and/or lab and phenotyped for biomass yield and quality traits. Genetic analyses were applied across generations to capture the joint inheritance of the targeted traits and predict breeding values for parents and progeny with greater accuracy. Parental and a panel of different switchgrass populations were genotyped with the DArTseq technology to develop SNP (0, 1, 2) and in-silico (presence/absence) DArT markers. Rust inoculations techniques were developed and applied successfully on switchgrass. The original populations (Kanlow and Summer) were sequenced with RNAseq to capture the gene expression profiles across sequential time-points and appraise the basis of greater resistance in the Kanlow vs the Summer ecotype. Constructs of PMV and sPMV mosaic virus were assembled and tested first on proso millet to find the best protocol to use later on switchgrass. Results from the preliminary analyses indicate that 1) ample additive genetic variation is available for selection and improving this inter-ecotypic population for yield, quality, and disease traits, 2) significant gains are to be expected with the genetic correlations being favorable between yield and lignin content and between yield and disease ratings, 3) substantial differences exist in the genetic regions controlling rust resistance in the two ecotypes, 4) co-infection with PMV isolates from Nebraska and its satellite from Kansas elicit severe mosaic symptoms, and 5) two different genetic systems are responsible for imparting resistance to rust and virus in switchgrass.

Project Objectives

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.

2. Project Overview and Results

1. Genetics and Genomics

Appraising the amount of variation available for selection in breeding switchgrass for bioenergy. Several populations were created to appraise the genetics of yield, quality, and disease traits in switchgrass. A panel of six populations was used to develop SNP (single nucleotide polymorphisms) against the version IV of the switchgrass reference genome and then against version V when it was updated at the Joint Genome Institute (JGI) website. The panel included the parental tetraploid populations (Kanlow and Summer), their progeny cultivar (Liberty), and three octoploid cultivars (Cave-in-Rock, Pathfinder, and King's County). Two crossing blocks and their derived progeny tests, representing two consecutive generations, were planted and used in genetic analyses. A mapping population was build using two genotypes from the last generation with differential expression in a rust screening test in a greenhouse.

There is an increasing interest in the world to convert plant feedstocks and residues into solid or liquid biofuels. Switchgrass, with its potential to produce high yield under low inputs, is one of the crops dedicated to fulfilling these needs. Understanding the genetic underpinning of traits (low lignin content, high biomass yield, low disease incidence) amenable to low-cost production of ethanol is a crucial step in making gains over time in biofuel switchgrass. Edmé et al (2017) investigated the genetic bases of biomass and ethanol yield in two generations of a switchgrass population. Two progeny tests were planted in 2007 and 2011 near Mead, NE and respectively analyzed for 2 and 4 yr. Each test was a randomized complete block design, with four replicates of 34 halfsib families in single-row plots of 10 seedlings in C1 and with three replicates of 111 halfsib families in single-row plots of five seedlings in C2. Multivariate mixed linear models revealed ample additive genetic variation for dry matter yield (DMY), Klason lignin (KL), and predicted ethanol yield (ETOH) and determined that ample genetic variation was present: heritability ranging from 0.40±0.18 to 0.5±0.14 at the family level, from 0.22±0.17 to 0.36±0.22 at the individual level, and from 0.25 to 0.31 within family in C1. Matching values in C2 were: from 0.42±0.09 to 0.63±0.07, from 0.10±0.07 to 0.34±0.13, and from 0.12 to 0.48. More opportunity exists to improve DMY, with a coefficient of additive genetic variation of 11 to 32%, than KL (3–5%) or ETOH (3–6%). The traits were genetically aligned favorably for optimizing gains for bioenergy. The strengths of the correlations were reduced in the second generation even though the signs remained unchanged. In the second generation, selecting for high biomass yield would meet the goal of simultaneously decreasing lignin content and increasing the ethanol yield of the plants. In the C2 generation, the same relationships between biomass yield and lignin or between lignin and ethanol yield existed, but the strengths were reduced by about half. The relationship between biomass yield and ethanol yield decreased from significant to zero. As the relationships weigh considerably in the selection of potential parents and progeny, Edmé et al (2017) determined that the best strategy is to recurrently assess their genetic merit at transmitting genes that confer high biomass and ethanol yields and low lignin content in every generation. The traits were properly aligned for joint improvement for high DMY and reduced KL in C1, owing to favorable genetic correlations (r_A=-0.33±0.11) and each having respective r_A of 0.60±0.05 and -0.62±0.07 with ETOH. In C2, the r_A between DMY and KL (-0.19±0.10) or ETOH (0.04±0.04) decreased towards zero, and that between KL and ETOH was moderately less negative (-0.35±0.15). These results suggest a strong genetic basis for improvement of the traits and monitoring of their patterns every cycle to find the proper weights that maximize the breeding goal of designing the ideal bioenergy switchgrass.

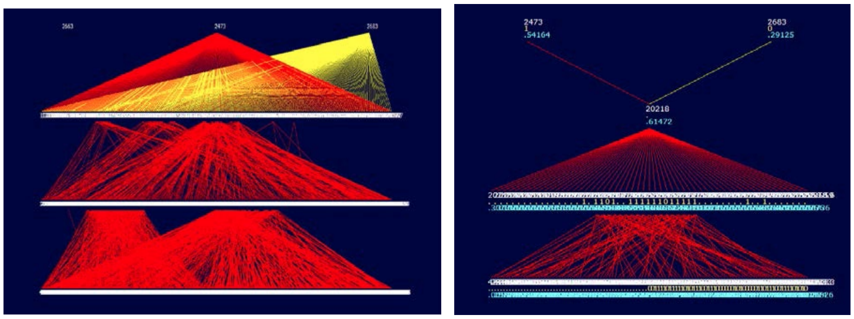


Figure 1. Pedigree of the Summer x Kanlow population used in genetic analyses. **Left panel:** Maternal population in red and paternal population in yellow. **Right panel:** Original fullsib population with subsequent generation bred by open-pollination along with disease ratings and breeding values.

Estimating the accuracy of selection for resistance to mosaic virus and for production traits in breeding switchgrass. Assuring good accuracy of selection prediction is key to making gains from breeding a population. The animal breeders have taught us that integrating the pedigree into genetic analyses will improve estimates of the genetic parameters and of breeding values, particularly when the analyses are based on the multivariate mixed modeling approach. Genetic variation was assessed for mosaic (caused by Panicum mosaic virus and treated as categorical or Virc and binary or Virb), yield (dry matter or DMY and predicted ethanol or Etoh), and quality traits (lignin content as Klason or KL and acid detergent or ADL) in a Summer x Kanlow switchgrass (Panicum virgatum L.) population using restricted maximum likelihood under a phenotypic (PBLUP) and multivariate animal (ABLUP) models. The ABLUP traces the pedigree back through three generations comprising 1622 halfsibs to integrate the relationship matrix in the evaluation. The PBLUP model analyzed the complete dataset without the pedigree. Edmé et al. (2021) compared the two models in their precision in assessing the genetic parameters (standard errors) and in estimating the breeding values (accuracy and reliability). The two models were similar in most aspects, allocating the highest h^2_i values to DMY (0.38±0.035 vs 0.41±0.035), Etoh (0.46±0.031 vs 0.42±0.033), and Virc (0.43±0.046 vs 0.37±0.047) and the lowest values (0.17±0.032 to 0.30±0.044) to KL, ADL, and Virb. The genetic correlations were always larger than the non-significant residual and phenotypic correlations, though of the same sign. An instance of resource-allocation competition was depicted in the case of the DMY-KL covariation based on r_G and r_E being of opposing signs. All six traits were found to be under intermediate or strong additive genetic control, whereby selecting for high-biomass genotypes will slightly increase lignin content and simultaneously impart mosaic tolerance. Mitigating the increase in lignin content will require including Etoh in a selection index based on its much stronger negative correlation (r_G=-0.63) with lignin. In this population, the accuracy values ranged from 0.06 to 0.94 (PBLUP) and from 0.26 to 0.92 (ABLUP) and corresponding reliability values from 0.004 to 0.89 (PBLUP) and from 0.07 to 0.87 (ABLUP). However, looking at each trait separately, the ABLUP model improved the average accuracy and reliability of DMY (h^2_i =0.41) and Etoh (h^2_i=0.42) by 11% and those of the remaining traits (h^2_i=0.17-0.37) by 4-5% over the PBLUP model. The ABLUP was evidenced as being a better model over PBLUP, which itself is a valid analysis in the absence of a pedigree. Decisions were made to carry inter-generational genetic analyses in predicting breeding values of parents and progeny and to integrate the pedigree for better precision.

Estimating genetic variation and gains for rust resistance in an inter-ecotypic population of switchgrass bred for bioenergy. Switchgrass (*Panicum virgatum* L.) is being developed as a forage and bioenergy crop for the U.S. However, rust, caused by a fungus named *Puccinia novopanici*, threatens the ability of future producers to obtain high biomass yield and quality of switchgrass. Genetic resistance to rust is a critical mechanism to control the disease. Maintaining low levels of rust incidence in switchgrass breeding populations is a priority for the USDA-ARS program engaged in improving cultivars for high biomass yield and quality. Essential to this goal is the unbiased and accurate estimation of genetic parameters to predict the merits of parents and progeny. Spores of the fungus were inoculated in greenhouse-grown seedling progeny of 31 halfsib families in generation 2 (Gen 2) of a composite Summer x Kanlow population for evaluation of rust incidence on the leaves with a 0-9 rating scale. Two parents were later chosen to cross and develop a linkage mapping population as Gen 3. The Gen 2, 3, and Kanlow seedlings were transplanted into the field in early June 2020 located near Mead, NE and laid out as a replicated row-column design with six blocks of single-row plots of 5 plants each. The field trial was rated in September 2021 and 2022 with a 0-4 scale. Lab and field data were subjected to univariate linear mixed models via the restricted maximum likelihood to extract the variance components needed to predict the breeding values (Edmé et al., 2022). The additive genetic variation was substantial ($P<0.01$), enough to result in high heritability estimates ranging from 0.42±14 to 0.73±0.09 at the individual and family-mean levels. This result implies that rust resistance is under strong genetic control to use mass selection for obtaining satisfactory gains. A possible rust-incidence x year interaction was detected with a Spearman correlation of breeding values of -0.38, caused by significant rank changes of the Gen 3 genotypes in 2022 (a high heat and drought year). Genetic gains were predicted to reduce rust incidence scores by at least 2 points on the rating scale when selecting backwards and by 1 point when selecting individual candidates as parents of the next generation. Faster gains (31 and 59%) were realized relative to the second generation by respectively selecting the top 10% of the families in Gen 3 or the top 10% of genotypes within this group.

Genomic selection. Parental populations of switchgrass in two crossing blocks and two progeny tests composed each of 111 and 121 halfsib families were genotyped with the DArTseq sequencing technique to call for SNP against version V of the switchgrass genome. The SNP were previously identified from sequencing a panel of six switchgrass cultivars, including the original parental Kanlow and Summer populations. A large proportion of the SNP markers fall within or near gene coding regions in the switchgrass genome, based on overlapping the sequences with protein coding regions (CDS), non-coding gene regions (UTRs/introns), ±5kb up/downstream of a gene coding region, or 5-10kb up/downstream of a gene coding region. A multidimensional scaling plot indicates that Kanlow and Summer are genetically different and also from their hybrids located midway between the two parental populations. The G1 and G2 parents represent a panmictic (mixed) population, still with a large variation along the 2nd dimension.

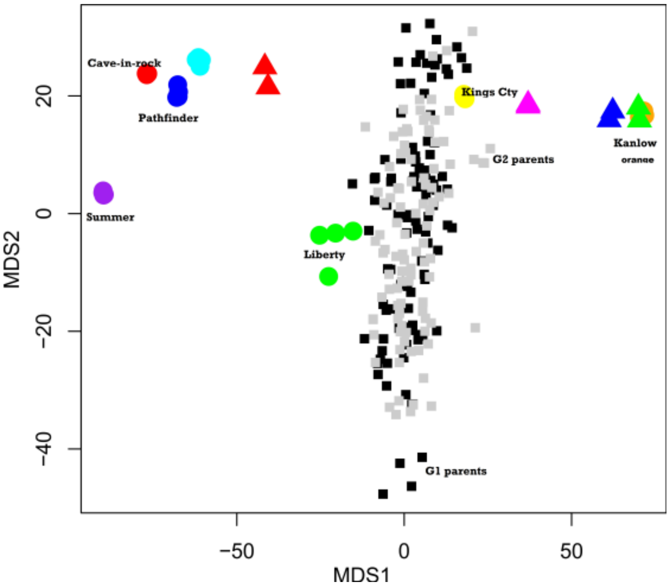


Figure2 . Multi-dimensional scaling plot applied to the SNP data to separate the different genetic populations included in the SNP discovery panel. Kanlow and Summer are on opposite sides of the first dimension. The clouds of black and grey squares are the respective G1 and G2 parents (derived from Summer♀ x Kanlow♂), with Liberty (a G1 cultivar) in close proximity. G1 and G2 parents show large variation along the 2nd dimension.

The genomic selection as anticipated was realized for several reasons: labor for extraction of high-quality DNA, ordering for the markers (internal bureaucracy), the COVID situation, special hardware to handle this large volume of data for ultimate analyses. Edmé is analyzing the data and a manuscript is anticipated this year on predicting genomic breeding values for yield, ethanol, and disease traits in switchgrass using a single-step genomic selection procedure. Future manuscripts in the genomic selection category include (to cite a few): ascertaining the paternal contribution in the pedigree of the progeny, genomic selection using the fullsib family structure across generations, QTL mapping across generations, determining the contribution of dominance in the inheritance of the traits, evaluating the potential of genomic selection using linear and threshold models in predicting rust and mosaic ratings, etc. Even though we are behind in executing this part of the project, the genomic selection planned for this project will, nevertheless, serve as a steppingstone for breeding switchgrass in the 2024-29 CRIS plan for this USDA-ARS Research Unit.

2. <u>Gene Expression of the broad resistance or tolerance of Kanlow to pathogens vs Summer</u>

Expression of defense-related genes differentiate lowland and upland switchgrass cultivars. Upland and lowland cultivars of switchgrass can differ in their ability to withstand attack by plant pathogens. Changes in basal and temporal expression of genes related to leaf function and defense could underlie the differences in the response of Kanlow or lowland and Summer or upland ecotypes to several biotic stressors. Possible molecular dynamics that drive these differences in non-infected plants were assessed via RNA sequences of greenhouse-grown Kanlow (lowland) and Summer (upland) switchgrass cultivars from leaf samples collected from emergence through leaf senescence. Overall development of leaf functions and transition to senescence were studied (Palmer et al., 2019). The 4th emerging leaf from greenhouse-grown Kanlow and Summer were collected from emergence through leaf senescence. RNA extracted from these leaves were subjected to high-throughput next generation sequencing. Harvested samples were analyzed for chlorophyll content to monitor leaf aging and by RNA-Seq using 3’-libraries to study gene-expression profiles. Increases and decreases in leaf chlorophyll content were similar for both ecotypes indicating no apparent differences in leaf developmental ontogeny. Likewise, non-metric multidimensional scaling (NMDS) analysis showed similar temporal changes in the leaf transcriptomes for both ecotypes indicating ecotype-independent gene expression, along with a clear population division, indicating ecotype-specific gene expression. Genes and gene-networks that were apparently ecotype-independent included those associated with leaf function, such as growth/senescence, carbon/nitrogen assimilation, photosynthesis, chlorophyll biosynthesis, and chlorophyll degradation. In contrast, many genes belonging to nucleotide-binding leucine rich repeat (NB-LRRs), wall-bound kinases, and gene classes such as WRKY transcription factors and others associated with detecting and responding to environmental signals were differentially expressed, and several belonged to co-expression networks unique to one or the other ecotype. 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 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. Incorporating broad resistance to plant pathogens in elite switchgrass germplasm could improve sustainability of biomass production under low input conditions. Data had been deposited at the Publisher website.

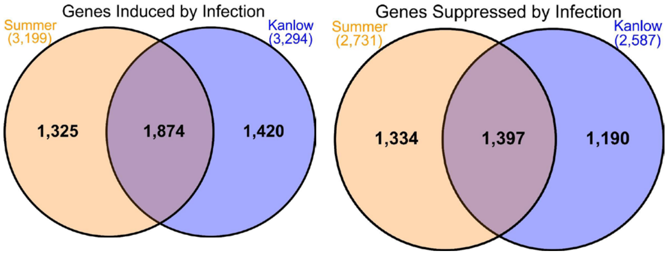


Fig. 3. Rust Infection Responsive Genes. Venn diagrams showing shared and population-specific genes induced (left) or suppressed (right) by rust infection in Summer (orange) and Kanlow (blue) plants.

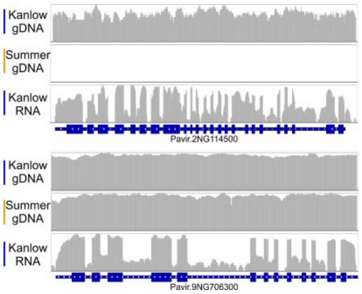


Fig. 4. Genes potentially found in Kanlow and not in the Summer genome.

Comparing the coverage of ~40x genomic resequencing data from Kanlow and Summer in gene-coding regions. Top panel reveals that a subset of Kanlow genes are not present in Summer. Bottom panel shows that the majority of the gene-coding regions had relatively similar genomic resequencing coverage in both populations.

Proteomic analyses provide gene targets that could improve rust resistance in switchgrass. It was not possible to successfully replicate, in switchgrass, Dr. Tatineni's (Co-PI) research in wheat related to engineering the virus genome to accept insertion of GFP-ORF to examine the mechanism of resistance in Kanlow and Summer. The size of the genome of the PMV virus prevented the integration of the foreign particle into its cells and that resulted in instability of the constructs. We reverted to carry a gene expression using proteomics and to follow the changes in protein profiles before and after inoculating Kanlow and Summer plants with the rust fungus. Switchgrass (Panicum virgatum) can be infected by the rust pathogen (*Puccinia novopanici*) resulting in lowering biomass yields and quality. Two cultivars with divergent resistance to rust infection were used to evaluate the dynamic changes in proteomes following rust inoculation. Label-free shotgun proteomics was conducted on leaf extracts harvested from a susceptible cultivar Summer at 7, 11, and 18 DAI, and leaves collected at 18 DAI from controls and infected plants of the more resistant cultivar Kanlow. A total of 3,908 proteins were identified with high confidence of which 3,710 were mapped to the switchgrass proteome and 198 proteins were mapped to different Puccinia proteomes. Across all comparisons, 1,835 DAPs were identified. EDAPs and DDAPs were subjected to a STRING analysis using Arabidopsis orthologs to deduce switchgrass cellular pathways impacted by rust infection. Proteins associated with plastid functions and primary metabolism were part of the DDAP modules in Summer plants at all harvest dates. In Kanlow at 18 DAI, DDAPs were also associated with plastid pigment biosynthesis, but evidence of linages to primary metabolism was less obvious. In Summer plants ribosomal, immunity signaling, and defense-related proteins were part of the EDAPs at 7 DAI, and by 11 DAI proteins with immunity, chaperone functions, and phenylpropanoid biosynthesis were significantly enriched. Among EDAPs at 18 DAI, 587 and 296 proteins were found to have Arabidopsis orthologs in Summer and Kanlow plants respectively. Of these proteins, 212 were found in common between the two cultivars. Many of 212 EDAPs were part of STRING modules linked to mitigation of cellular stress and defense. However, the large difference in the number of EDAPs in Summer plants relative to Kanlow plants at 18 DAI, indicated a divergence in the defense responses that could underpin susceptibility and resistance to rust in this switchgrass germplasm. Data will be deposited at the Publisher website.

3. Virology: Molecular differences explaining systemic viral infections in switchgrass

Synergistic interaction between panicum mosaic virus and satellite panicum mosaic virus in switchgrass. A productive and successful switchgrass cultivar needs to have good levels of resistance or tolerance to known diseases affecting its performance in forage and bioenergy production systems. To understand the interplay between virus and plants, a greenhouse study was carried out with two switchgrass cultivars, Kanlow as a lowland ecotype and Summer as an upland ecotype (Muhle et al., 2022). Both cultivars were challenged with the Panicum mosaic virus (PMV alone) and in combination with its parasite satellite SPMV (i.e. PMV + SPMV). In field studies, Kanlow is known to be resistant and Summer susceptible to these viruses. The plants were observed for visual symptoms and for the number of RNA copies of the virus in their cells at different times after inoculation under two different temperatures (24 C and 32 C). Panicum mosaic virus (PMV), the type member of the genus Panicovirus in the family Tombusviridae, naturally infects switchgrass (Panicum virgatum L.). PMV and its molecular parasite, satellite panicum mosaic virus (SPMV), interact synergistically in co-infected proso millet (Panicum miliaceum L.) and pearl millet [Cenchrus americanus (L.) Morrone] plants with exacerbated disease phenotype and increased accumulation of PMV, compared to plants infected only by PMV. In this study, we examined the reaction of switchgrass cvs. Summer and Kanlow to PMV and PMV+SPMV infections at 24°C and 32°C. Switchgrass cv. Summer was susceptible to PMV and SPMV at both temperatures. In contrast, cv. Kanlow was resistant to PMV and SPMV at 24°C but not at 32°C, suggesting that Kanlow harbors temperature-sensitive resistance against PMV and SPMV. At 24°C, both viruses were readily detected in inoculated leaves but not in upper non-inoculated leaves of Kanlow plants, suggesting that resistance to PMV and SPMV was likely mediated by abrogation of long-distance virus transport but did not impact cell-to-cell movement. Co-infection of cv. Summer with PMV and SPMV caused a mild disease synergism with a slightly increased accumulation of PMV genomic RNA compared to those infected only by PMV. In contrast, no detectable disease synergism was observed in cv. Kanlow plants co-infected with PMV and SPMV. However, co-inoculation of cv. Kanlow by PMV+SPMV caused an enhanced symptomless infection, suggesting a role for SPMV in enhancing symptomless infection in a resistant cultivar. These data suggest that interaction between PMV and SPMV enhanced symptomless infections in virus-resistant switchgrass cultivar that could serve as a source for virus spread and play an important role in panicum mosaic disease epidemiology under field conditions.

***Differential synergistic interaction with panicum mosaic virus*.** Viral infection can significantly affect plant growth, biomass yield and quality, and particularly bioenergy switchgrass. Switchgrass and millets can be co-infected by *Panicum mosaic virus* (PMV) and its parasite virus, *satellite panicum mosaic virus* (SPMV). Proso millet is a convenient surrogate host for evaluating viral diseases of switchgrass. Panicum mosaic virus (PMV) (genus Panicovirus, family Tombusviridae) and its molecular parasite, Satellite panicum mosaic virus (SPMV), synergistically interact in coinfected proso and pearl millet (Panicum miliaceum L.) plants resulting in a severe symptom phenotype. In this study, Chowda-Reddy et al. (2019) 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. Coinfection 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, coinfection 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. Coinfection 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 SMPV-Type in coinfected 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.

3. Summary and Impact:

Classical plant breeding: This research will offer a new paradigm in breeding perennial crops, like switchgrass (and other perennial grasses), that captures and optimizes/maximizes genetic gains in traits of economic importance (yield, quality, and disease). This will lead to a new philosophy of breeding perennial grasses with integration of the information on family with that on the selection candidates. The two sources of information will be combined in an index to predict the ideal bioenergy/forage switchgrass cultivar, while taking into consideration the long-term effect of selection on trait architecture. Even though the research emphasizes bioenergy switchgrass, the results can be easily ported to forage switchgrass bred for livestock as the traits are similar, but the importance differs in the selection index. This project serves in large as a starting point to apply genomic selection in the breeding program carried by USDA-ARS in Lincoln NE.

Genomic selection: this research is capitalizing on the appreciable knowledge gained in genomic selection over the years to predict successive generations from previous ones while exploring the multivariate approach of predicting several traits simultaneously and integrating index selection methodology, and that in a polyploid background. We have developed a pedigree for the population under study, and we intend to follow the example from animal breeding that favors integrating the additive relationship matrix with the genomic matrix into a combined matrix to better predict the performance of experimental genotypes. Other populations, not under genomic selection, will benefit also from the multivariate mixed animal (pedigree-derived) model approach that jointly predicts breeding values for parents and progeny across generations. The ultimate goal is the rapid deployment of cultivars (forage and bioenergy) that sustain the productive and economic life of the agricultural sector, by using perennial crops that are indigenous to the USA, crops that do not compete with food crops as they are adapted to marginal land conditions, and by increasing their economic importance with regards to bioenergy and forage.

4. Invited Talks and Posters

- **Talks**
  - ***Serge Edmé,*** Optimizing Selective Breeding in Switchgrass (*Panicum virgatum*) combining Genetics and Genomics, PAG Jan 12-16, 2019, Switchgrass Workshop. San Diego, CA.
  - ***Serge Edmé,*** Genetic Variation for Resistance to Mosaic and Rust in a Switchgrass Breeding Population Based on the Linear Mixed and Threshold Models. ASA, CSSA 2018. Baltimore.
  - ***Serge Edmé et al.,*** Genetics and Genomics of Pathogen Resistance in Switchgrass**.** 2017 DOE Genomic Sciences Annual PI Meeting. Crystal City, VA (Febr. 5-8)
  - ***Serge Edmé et al.,*** Genetics and Genomics of Pathogen Resistance in Switchgrass**.** 2018 DOE Genomic Sciences Annual PI Meeting. Crystal City, VA (Febr. 25-28)
  - **Anthony Muhle et al**. Resistance of Liberty Switchgrass to rust and viral diseases. Switchgrass IV Annual Meeting. Aug 2017. Lincoln NE. Switchgrass IV was organized by the PI and Co-PI of this project.
  - **Anthony Muhle**, Resistance in Switchgrass to Rust and Viral Disease. Seminar, March 2018. Pathology Dept. Univ of Lincoln, NE.
  - ***Serge Edmé et al.***, Genetic variation for disease resistance in a Summer x Kanlow switchgrass breeding population. Switchgrass IV Annual Meeting. Aug 2017. Lincoln NE. Switchgrass IV was organized by the PI and Co-PI of this project.
  - ***Serge Edmé et al.,*** Switchgrass V Annual Meeting. July 22-25, 2019. Champaign Illinois.
- **Poster**

  Nathan A. Palmer*, Serge Edmé, Rob Mitchell and Gautam Sarath,* Switchgrass Transcriptional Responses to a Range of Biotic Stresses. PAG Jan 13-19, 2019. San Diego, CA.
  Nathan A. Palmer*, Serge Edmé, Rob Mitchell and Gautam Sarath,* Switchgrass Transcriptional Responses to a Range of Biotic Stresses. PAG Jan 13-19, 2019. San Diego, CA.

- **Workshop:**
  Nathan A. Palmer. **JGI 24th Microbial and Metagenomics Workshop.** Walnut Creek, CA. Feb. 27 – Mar. 4, 2017.

5. PostDoc and Students supported by the DOE funding

1) **Dr. R. V. Chowda-Reddy** was hired as a Postdoc in July 2017 and worked for 1 year in the virology component of the project. He worked under the supervision of Dr. Tatineni (Co-PI, USDA)
2) **Anthony Muhle** obtained his MS degree at the University of Lincoln NE under the direction of Dr. Gary Yuen, Pathologist Co-PI. **Thesis Title**: “Rust and Viral Mosaic Diseases in Biofuel Switchgrass” (Aug. 2019). Muhle, after completing his MSc study, replaced the Postdoc to help finish the rust and mosaic virus research under the direction of Drs Sarath and Palmer (Co-PI).

3) **Five other students** from the Univ. of Lincoln NE worked as part-time employees in the breeding and genomics research during the lifetime of the project under the directions of Drs Edmé (PI) and Sarath/Palmer (Co-PI).

6. Publications

Edmé, S.J., Mitchell, R., Sarath, G. 2017. Genetic parameters and prediction of breeding values in switchgrass bred for bioenergy. Crop Science. 57:1-11. doi:10.2135/cropsci2016.09.0770.

Edmé, S.J.; Palmer, N.A.; Sarath, G.; Muhle, A.A.; Mitchell, R.; Yuen, G. Genetic Resistance of Switchgrass to Rust Evaluated in a Composite Upland × Lowland Population in Lab and Field Settings. *Agronomy* 2022, *12*, 3137. https://doi.org/10.3390/agronomy12123137

Edmé, S.J., Sarath, G., Palmer, N.A., Yuen, G., Muhle, A.A., Mitchell, R., Tatineni, S., Tobias, C.M. 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. 61(3):1652-1665. https://doi.org/10.1002/csc2.20392. DOI**:** https://doi.org/10.1002/csc2.20392

Palmer Nathan A., Sophie Alvarez, Michael J Naldrett, Anthony Muhle, Gautam Sarath, Serge J Edmé, Satyanarayana Tatineni, Robert B Mitchell, and Gary Yuen. Dynamic Reconfiguration of Switchgrass Proteomes in Response to Rust (*Puccinia novopanici*) Infection. To be Submitted.

Palmer, N.A., Rekalakunta Venka, C., Muhle, A., Tatineni, S., Yuen, G., Edmé, S.J., Mitchell, R., 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. DOI: https://doi.org/10.1371/journal.pone.0222080

Muhle, Anthony A., "Rust and viral mosaic diseases in biofuel switchgrass" (2019). *Thesis, Dissertations, and Student Research in Agronomy and Horticulture*. Under the Supervision of Professor Gary Y. Yuen. Lincoln, Nebraska: August 2019.

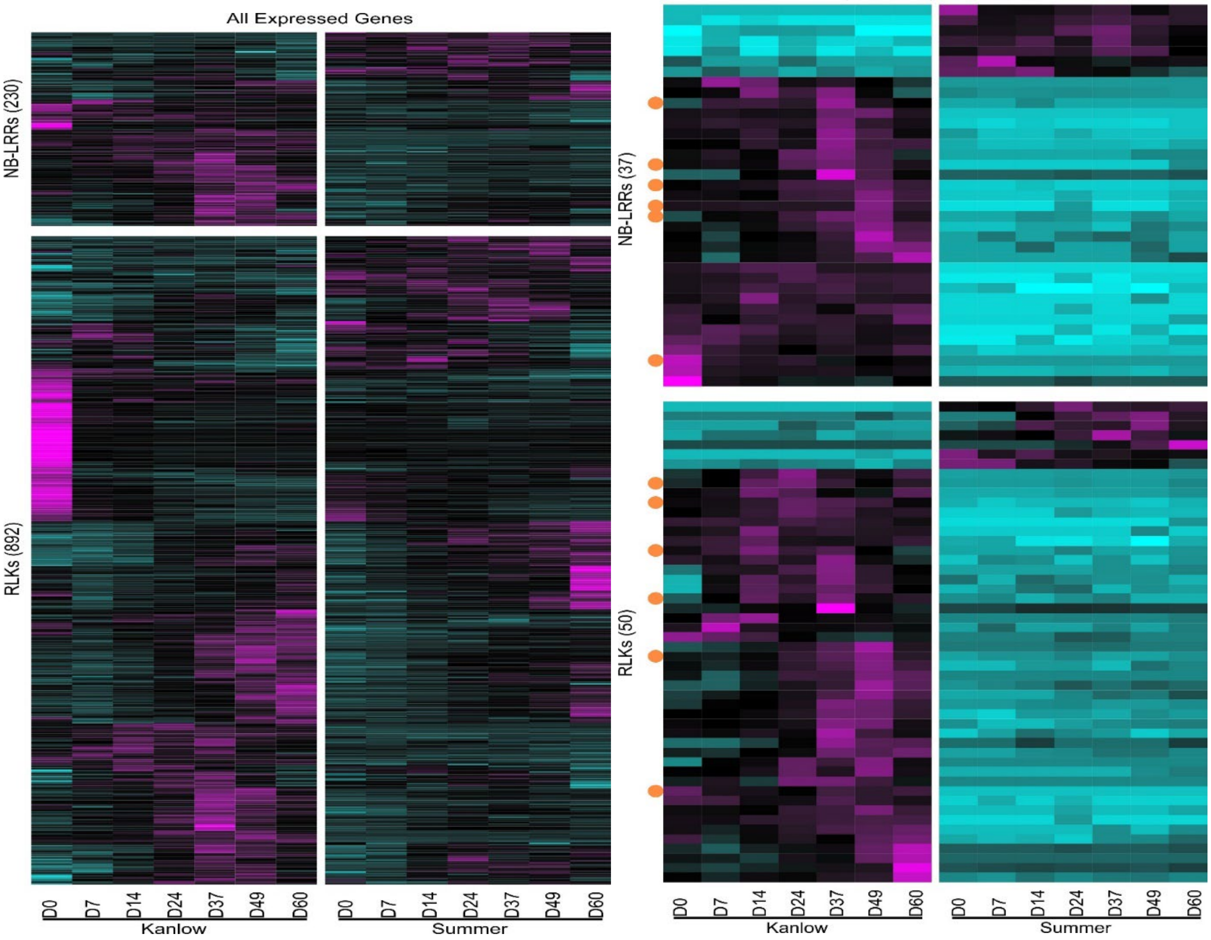
Muhle, A.A., Palmer, N.A., Edmé, S.J. Sarath, G., Yuen, G., Mitchell, R., Tatineni, S. Effect of cultivar and temperature on the synergistic interaction between panicum mosaic virus and satellite panicum mosaic virus in switchgrass. *Arch Virol* **167**, 1247–1256 (2022). https://doi.org/10.1007/s00705-022-05412-y.

R. V. Chowda-Reddy, Nathan Palmer, Serge Edme, Gautam Sarath, Frank Kovacs, Gary Yuen, Robert Mitchell, and Satyanarayana Tatineni. 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*. Molecular Plant-Microbe Interactions 32:4, 479-490.

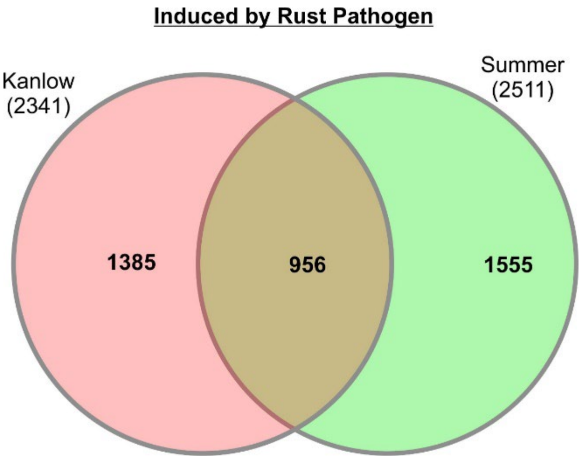
7. Appendices

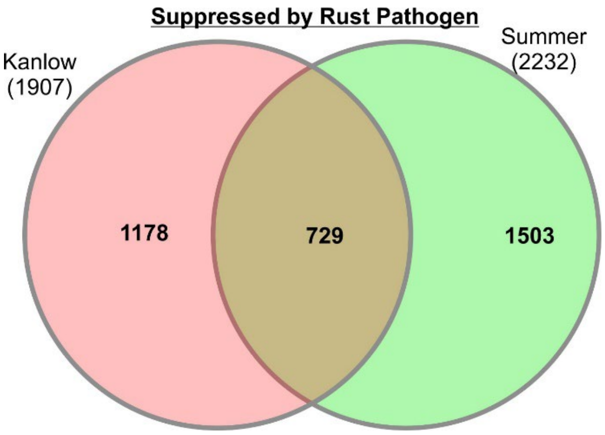
TRANSCRIPTOMICS: Rust and Mosaic

Leaf Development: Reference expression profiles in Kanlow and Summer

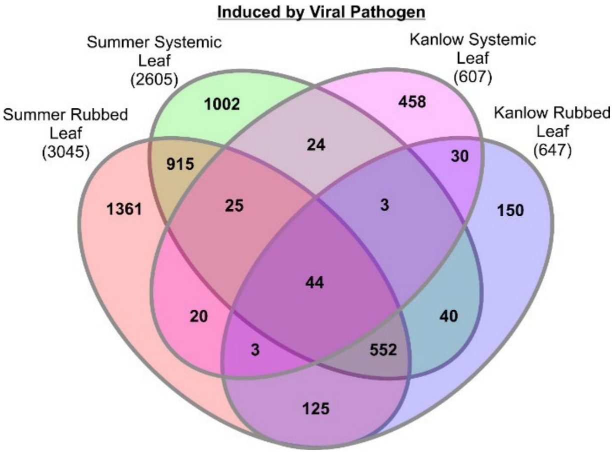


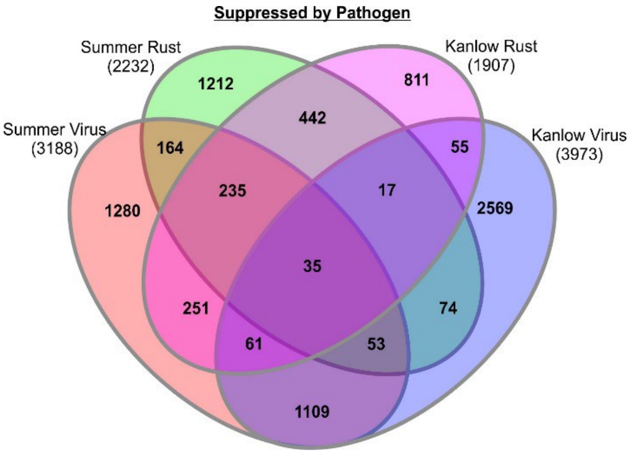
Response to Rust (*P. emaculata*) and Mosaic (PMV + sPMV) in Kanlow and Summer


Induced by Rust Pathogen

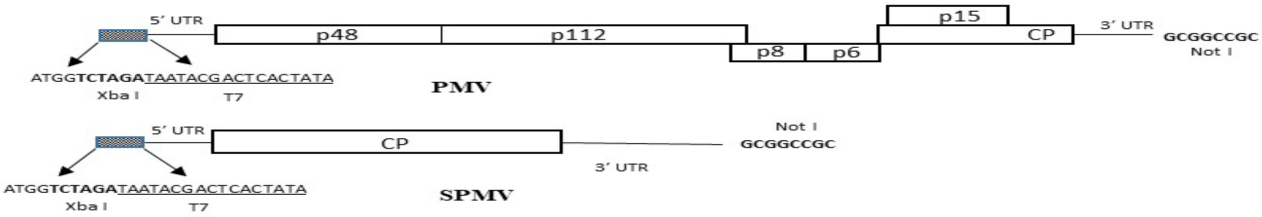

Suppressed by Rust Pathogen

Differential expression profiles across 7 timepoints in Kanlow and Summer with some crosstalk between genes involved in mosaic and rust resistance.

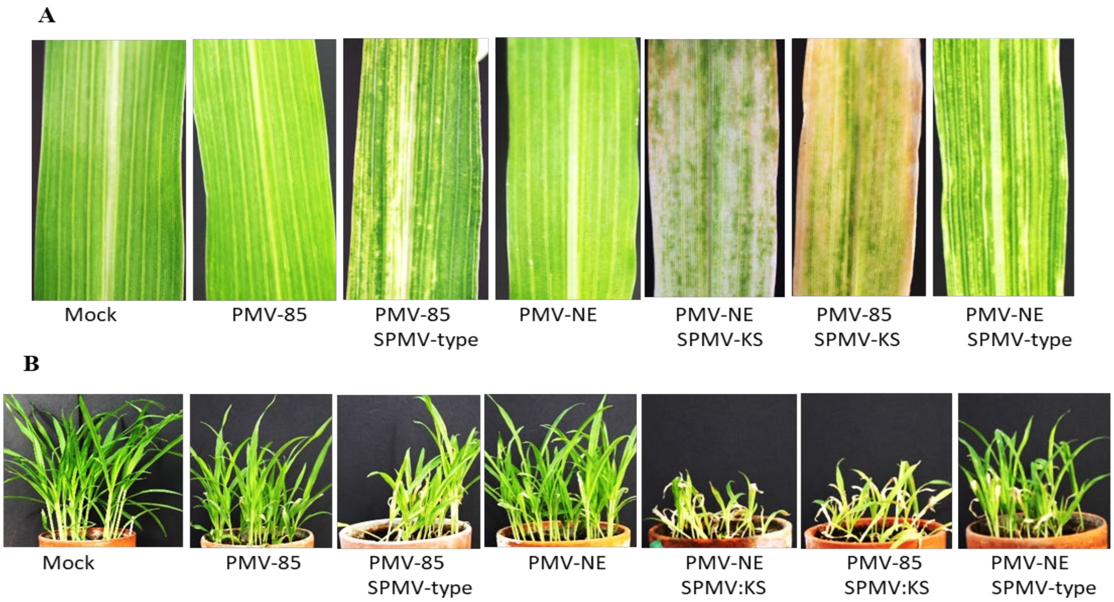

Induced by Viral Pathogen


Suppressed by Pathogen

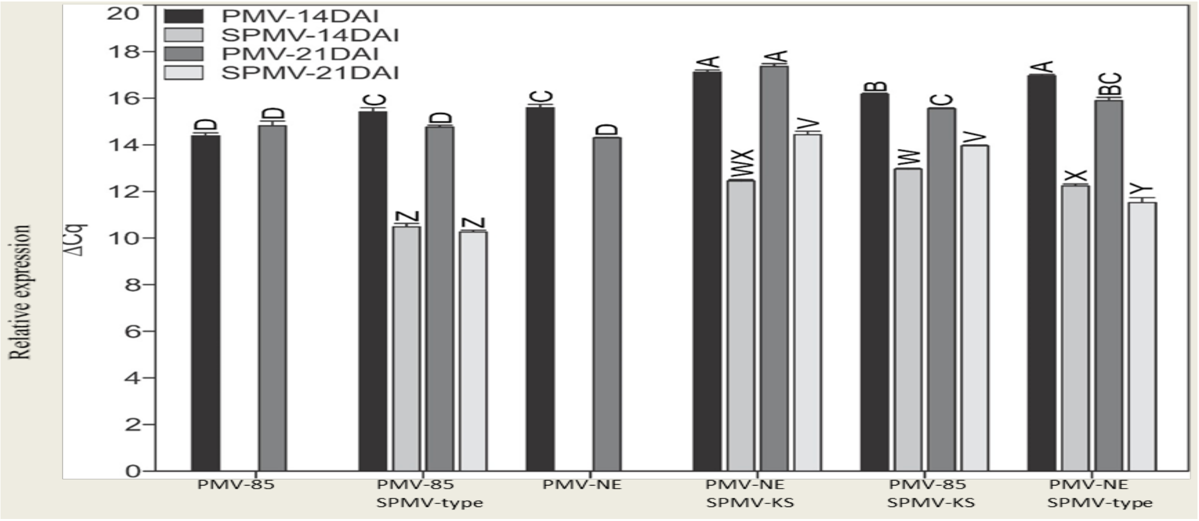
PLANT-VIRUS INTERACTIONS/SYNERGYSM: PMV and SPMV



Schematic representations of PMV and SPMV genomes



Disease symptoms in proso millet or switchgrass infected with PMV or PMV+SPMV at 14 dpi. PMV-NE or PMV-KS with SPMV-KS isolate elicited more severe symptoms than those elicited by SPMV-Type isolate.



RT-PCR analyses of accumulation of PMV and SPMV genomic RNA. Different alphabets (A, B, C, D for PMV and W, X, Y, Z for SPMV) indicate significant levels of viral genomic RNA in plants. Same alphabet: non significant.

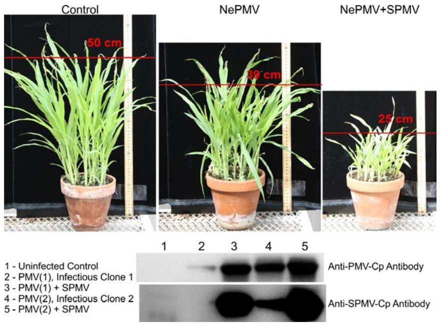
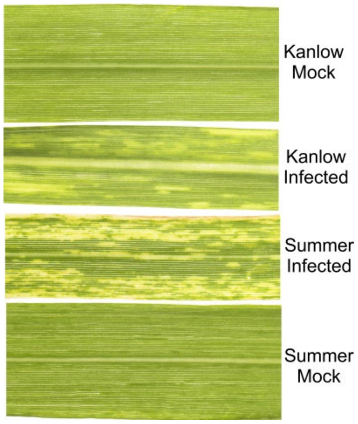


Figure 1: Co-infection of proso millet plants with the Nebraska isolate of PMV and its sattelite virus (SPMV) leads to significant stunting. Lower panel are results from an immunoblot, using antibodies to PMV and SPMV coat proteins. Lane numbers are as described.



Evaluation of different isolates of the Panicum mosaic virus on proso millet (left panel) determined that PMV-NE + SPMV-K was more virulent than either virus alone. This combination was repeated on switchgrass plants of the parental populations (Kanlow and Summer). In general, Summer shows more severe symptoms with 50% more plants than Kanlow after inoculations (right panel).