

WASHINGTON TURFGRASS SEED COMMISSION

PROGRESS REPORT FORMAT FOR 2019 PROJECTS

Project No.: WSU ACCOUNT #13C-3019-6780 and #17A-3019-9823

Title: Characterization of vernalization genes and flowering in Kentucky Bluegrass

Personnel: Michael M. Neff Ph.D. (PI) and Xin Xin (Graduate Student)

Reporting Period: 7/16/17 – present

Accomplishments: In the first year of this project, John Hadish, a rotating Molecular Plant Sciences (MPS) graduate student, cloned fragments of two flowering time genes, *VRN1* and *VRN3*, from the KBG genome. A new postdoc, Dr. Gaganjot Sidhu, and a Molecular Plant Sciences graduate student, Xin Xin, joined the Neff lab in July and August 2018, respectively, and began working on this project. Xin is supported by a scholarship from the China Scholarship Council, requiring approximately \$2500/semester plus summer salary coming from this project. Gaganjot identified nine publicly available KBG transcriptomic/genomic studies, the best of which was published by USDA/ARS grass geneticist, Dr. Shaun Bushman, in Logan, Utah. With this resource, Gaganjot has performed *in-silico* studies to identify *VRN* genes from KBG using wheat *VRN1*, 2 and 3 coding sequence as a reference. The results from Gaganjot's, Xin's, and Evan Stowe's (an undergraduate student working in the Neff lab) suggested that there are eight copies of *VRN1*, three copies of *VRN2*, and two copies of *FT/VRN3* genes in KBG. They also started both growth chamber and vernalization chamber/greenhouse experiments to examine the expression of these flowering genes as outlined in this proposal. During this funding period, Dr. Neff worked with WSU to build a new Perennial Grass Breeding and Ecology Farm. That farm is now in the finishing stages of construction and should be completely functional when this new proposal, if funded, becomes active. The farm is located adjacent to the USDA ARS Plant Introduction Group and the USDA NRCS Plant Materials Center. The former group maintains the US Cool-Season Grass Collection, which is the basis for our breeding program, currently funded by the Brubbaken and Reinbold Monocot Breeding Fund. The latter group focuses on breeding native grasses for forage and cover crops. Since they cannot apply for Plant Variety Protection (PVP) status for their material, they will collaborate with us to finish the breeding process and apply for PVP. Licensing these and other varieties will help supply long-term funds for the breeding program at the WSU Perennial Grass Breeding and Ecology Farm. As a result of press coverage for the new farm, Dr. Neff was contacted by Dr. Shaun Bushman. Dr. Neff and Dr. Bushman have established a collaboration between their two groups and Dr. Bushman is now on Xin Xin's Ph.D. committee. Dr. Bushman recently sequenced the KBG genome using material that is a poly-haploid of the variety "Hampton". Even though the genome sequence has not been published, Dr. Bushman has agreed to share the VRN-related gene sequences once a Data Transfer Agreement (DTA) between

our two entities has been finalized. This DTA has been approved by WSU and should be finalized shortly. A summary of the VRN-related sequences, based on discussions with Dr. Bushman, is described below.

Results: Dr. Bushman used the long-read PAC-BIO sequencing platform to sequence the KBG genome. He has established a 100X coverage, which means that each nucleotide in the genome has been sequenced an average of 100 times. This deep coverage eliminates the potential errors that can occur with PAC-BIO sequencing. The average read lengths are 35,000 nucleotides long. He currently has 95% of the genome assembled into 4,000 contiguous segments. This has allowed him to not only identify VRN-related genes but also their proximity to other genes. He has discovered that there are ten copies of *VRN1* (compared to our prediction of eight), three copies of *VRN2* (as predicted) and also two or three copies of *FT/VRN3* (compared to our prediction of two). For the ten copies of *VRN1*, six of the ten are in two groups of three in tandem. These six *VRN1* genes each occupy about 13,000 nucleotides. The two tandem arrays of triplicate VRN1 genes each occupy about 290,000 nucleotides. The other four *VRN1* genes occupy approximately 17,000 nucleotides. The similarity between these different genes is high and explains why we have had difficulty cloning individual genes based on our previous approach. With this information and collaboration, we will have completed the first aim of our previous proposal by the time the funding period is finished.

We have begun spray experiments with the PGR RyzUp SmartGrass[®] using Dr. Ian Burke's spray chamber at WSU. During this process, we realized that our vernalization treatments were not strong enough for varieties such as "Midnight" though they were sufficient for other varieties. This vernalization treatment was based on what is done for winter wheat varieties at WSU. We have now changed our vernalization treatment to mimic growing conditions in the region of central Washington where most irrigated KBG fields are located. This change required having a dedicated chamber that closely mimics environmental conditions in early August when KBG is planted to allow the plants to grow large enough to be primed for cold temperature vernalization under SD conditions.

In addition, we now have a new WSU Perennial Grass Breeding and Ecology Farm. This farm occupies five acres and has 18 80ft x 80ft plots and one 50ft x 200 ft plot, all under irrigation. This farm, which is operated by Dr. Neff's research program, will be able to mimic both irrigated and dryland cropping systems. Support from the Washington Turfgrass Seed Commission and the Brubbaken and Reinbold Monocot Breeding Fund has played an integral role in demonstrating stakeholder support and the role of this farm in the Land Grant Mission of WSU. This farm will allow on-site field studies related to this research project as well as our grass breeding program.

Popular articles about the new farm: <https://news.wsu.edu/2019/09/05/wsu-cultivating-new-perennial-grass-breeding-ecology-farm-research-site/>

<https://app.flashissue.com/newsletters/9a1994ff74f38b145bf383408e41b56a600b4f00#ytp-48-2614>