AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2021– 2023

TITLE: Tools for genomic breeding in western white pine: Improving resistance to white pine blister rust

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SUMMARY/ABSTRACT:
Western white pine (WWP), an economically and ecologically important conifer, has been severely impacted by blister rust, a disease caused by an introduced fungal pathogen. Resistance breeding is important for restoring WWP on the landscape, and these breeding programs can be accelerated by adopting a new marker-assisted breeding approach called "genomic selection." Currently, there is no information on the feasibility of using genomic selection to improve resistance to white pine blister rust. Our project is based on single-nucleotide polymorphism (SNP) markers that were recently developed for WWP. SNP markers are single-letter differences in the DNA code. In previous work, we laid the foundation for genomic selection by (1) developing ~1.9 million potential SNP markers for WWP using transcriptome sequencing, (2) developing simulation software (Tree Genome Simulator) that we will use to test genomic selection strategies, and (3) conducting quantitative genetic analyses of WWP progeny tests. Our objectives are to use these preliminary results to (1) design a high-density Axiom screening array with Thermo Fisher (420K SNPs), (2) design a low-density Flex-Seq SNP assay with Rapid Genomics, LLC (5K SNPs), and (3) prepare preliminary results to include in proposals for external grant funds (see below).

OBJECTIVES:
Broad objectives for the overall project. Our project will help by giving tree breeders new tools to genetically improve resistance to white pine blister rust. Using Agricultural Research Foundation (ARF) funds and subsequent funds from national competitive grant programs, we propose to design and manufacture a (1) high-density (50K SNP) Axiom genotyping array and (2) low-density Flex-Seq SNP assay for western white pine. These tools will allow breeders to use genomic selection to improve disease resistance and growth for this iconic and important species.

Our ARF objectives are to use existing genomic data for western white pine to:

1. Design a high-density Axiom screening array with Thermo Fisher (420K SNPs)
2. Design a low-density Flex-Seq SNP assay with Rapid Genomics, LLC (5K SNPs)
3. Prepare preliminary results to include in proposals for external grant funds (see below)

Our objectives for future grant proposals are to:

1. Include the ARF preliminary results in new grant proposals

2. Manufacture the Axiom screening array and test it on ~100 western white pine trees
3. Develop the final Axiom genotyping array (50K SNPs)
4. Test the Rapid Genomics SNP assays on ~100 western white pine trees
5. Develop the final Rapid Genomics genotyping assay (3.5K SNPs)
6. Genotype western white pine trees in breeding programs, test genomic selection, and develop breeding recommendations

PROCEDURES:

1. Design a high-density Axiom screening array with Thermo Fisher (420K SNPs)

For technical and genetic reasons, only some of the SNPs detected using bioinformatics will be successfully genotyped on genotyping arrays. In Douglas-fir, for example, only 28K SNPs were successfully genotyped on our 50K SNP array (Howe et al. 2020). So, rather than genotype thousands of trees and only obtain data for ~28K SNPs, we will use a (1) SNP screening phase and (2) final genotyping phase. By completing the design of the screening array, we will increase our chances for successfully competing for funds for our research on marker-assisted breeding.

As part of earlier work, we completed the bioinformatics needed to design the WWP screening array (i.e., selection of genes, regions, sequences, and SNP types). Because the foundational bioinformatic analyses are complete, our methods are straightforward. We will design a 420K SNP Axiom array with Thermo Fisher, using the methods described in Howe et al. (2020). Thus, although the remaining steps are not trivial, we can design the screening array using 1.5 months of a post-doc's time over the course of the project. Details on the bioinformatic approaches are described in Howe et al. (2020). Technical details on the Axiom array and the design process are also available online (Axiom myDesign Genotyping Arrays; https://www.thermofisher.com/us/en/home/life-science/microarray-analysis/agrigenomics-solutions-microarrays-gbs/axiom-genotyping-solution-agrigenomics/axiom-mydesign-genotyping-arrays.html).

2. Design a low-density Flex-Seq SNP assay with Rapid Genomics, LLC (5K SNPs)

We recently completed the design of a low-density Flex-Seq SNP assay for Douglas-fir in collaboration with Rapid Genomics, LLC. Now, we propose to design similar assays for WWP. The low-density Flex-Seq SNP assays will be ideal for fast, routine genotyping of WWP. This will allow us to test genomic selection and routine genotyping operationally.

3. Prepare preliminary results to include in proposals for external grant funds

The ARF project is designed to provide preliminary results needed to be competitive for external grant funds. By completing the key technological steps needed to practice marker-assisted selection, we will demonstrate we are ready to directly test marker-assisted selection for improving blister rust resistance in western white pine. We will design the Axiom array and Flex-Seq assays, and then prepare tables, figures, and other summary results that will demonstrate the expected performance of these new tools.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

1. We completed the Phase 1 bioinformatics needed to develop the Axiom screening array.

The development of an Axiom screening array (420K SNPs) involves four phases of bioinformatics to be conducted by OSU and our collaborator, Thermo Fisher Scientific. These

phases are (1) SNP discovery and the preparation of input files for SNP assay design (OSU), (2) use of the design files to predict SNP genotyping success (e.g., pConvert statistic) using proprietary software (Thermo Fisher), (3) ranking of SNP assays using pConvert plus other SNP statistics (OSU), and (4) final design of the screening array (Thermo Fisher) (Howe et al. 2020). In 2021, we completed Phase 1, and are now working with Thermo Fisher to begin Phase 2 (see below).

2. We worked with Thermo-Fisher to develop a new Memorandum of Understanding (MOU) for the development and manufacture of Axiom SNP arrays for western white pine. As a Group Leader in the Conifer SNP Consortium (https://faculty.cnr.ncsu.edu/fikretisik/research/, I helped develop an MOU with Thermo-Fisher for the development and manufacture of Axiom genotyping arrays for multiple conifer species. This MOU provided priority pricing for a 420K SNP screening array, a 50K SNP final genotyping array, and sample processing. Because the original MOE (completed in 2018) has expired, we have been working with Thermo Fisher and other members of the Conifer SNP Consortium to develop a new MOU. The costs associated with this new MOU are still being negotiated, but we know the per-sample genotyping costs will increase from $20/sample to $23/sample. We hope the cost of the screening array will remain the same (i.e., $45K), and there will be no required minimum number of samples, but these are still being negotiated. Although the bioinformatics has progressed, ARF project funds will be spent only after we have finalized the MOU with Thermo Fisher. The completion of this MOU will be critical for obtaining future grant support.

3. We analyzed SNP data from Rapid Genomics. We designed and analyzed Flex-Seq SNP assays based on a targeted genotype-by-sequencing approach. This was done in association with Rapid Genomics, another collaborator on this project. Although these data are for Douglas-fir, these analyses will help us optimize our approach for western white pine, and will contribute to tables, figures, and other summary results we will include in future grant proposals.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: None

FUTURE FUNDING POSSIBILITIES:
In February 2021, we will submit a proposal to the Gene Conservation, Resistance, and Restoration Program administered by USDA-Forest Service Forest Health Protection. This proposal will seek funds for our longer-term objectives of (1) manufacturing and testing the Axiom screening array (420K SNPs), (2) manufacturing and testing the final Axiom genotyping array (50K SNPs), and (3) using the final genotyping array in resistance breeding programs. We will seek additional funding for long-term objectives via national competitive grants programs such as those administered by the USDA National Institute of Food and Agriculture.

CITATIONS:
Howe, G., Jayawickrama, K., Kolpak, S.E., Kling, J.G., Trappe, M., Hipkins, V.D., Ye, T., Guida, S., Cronn, R., Cushman, S.A., and McEvoy, S. 2020. An Axiom SNP genotyping array for Douglas-fir. BMC Genomics. 21(9).