Abstracts of Forever Green Projects Funded with Clean Water Legacy Funds Minnesota Department of Agriculture, 2019

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Walid Sadok and Michelle Dobbratz, Department of Agronomy and Plant Genetics, Unlocking the Physiological and Environmental Mechanisms of Kernza Interannual Yield Decline

Abstract: Intermediate wheatgrass (IWG) is a promising perennial grain crop for Minnesota which offers unique new economic opportunities for farmers, since its grain is of interest to the baking and brewing value chains, two industries that are critical to Minnesota's economy. This crop is also highly valuable environmentally as it reduces erosion and nitrate leaching risks, sequesters atmospheric CO_2 and provides numerous ecosystem services. A challenge, however, is that despite progress in breeding and domestication, IWG still exhibits large yield decreases of up to 80% following the first year, resulting in substantial economic burden to the farmer. Thus, the ability to close this interannual yield gap will be critical to the economic viability of this promising crop in Minnesota. This proposal specifically addresses this key objective. This objective is not unrealistic since IWG is still in the process of being domesticated, that is, it still harbors physiological attributes that are unsuitable for modern crops. Therefore, building on previous research carried out by the IWG team and new promising developments in the physiology of IWG, our goal is to uncover the physiological basis of the interannual yield decline and its environmental controls. To this end, our objectives are to identify: i) promising physiological traits to be incorporated in advanced breeding lines to mitigate the yield decline and ii) environments in which to deploy such genotypes in order to maximize yield potential across the state. To this end, we have assembled an inter-disciplinary IWG team with expertise in physiology, agronomy and breeding.

Robert Stupar, Department of Agronomy and Plant Genetics, Developing Spring and Winter Pea as Profitable and Environmentally Friendly Crops for Minnesota

Abstract: Pea is a potentially transformative forever green crop. It is an annual species that has the potential to be grown at different seasonal time points within crop rotations, including winter and early spring seasons, thereby providing a ground cover that benefits soil and water quality. It is a legume that fixes atmospheric nitrogen, thereby enhancing soil fertility. Furthermore, it is a key source of plant-based protein for the food industry. The pea protein ingredients market is the fastest growing segment of the global plant protein market, providing economic opportunities for farmers. However, there are knowledge and resource gaps that currently limit the use of pea in Minnesota, including susceptibility to disease and freezing temperatures, and limitations to using pea seed protein in the food industry. This project proposes a multi-faceted approach to address these gaps. First, a nascent breeding program will be initiated to improve specific traits of interest for the Minnesota pea industry, namely protein

functionality for food uses and cold hardiness for winter survivability. To enable this program, existing pea germplasm will be screened to assess genetic variation (DNA), seed protein functionality, and winter survival traits. The physiological basis of pea winter survival will be dissected using cellular and biochemical assays, enabling the development of high-throughput phenotyping tools for this trait. High-performing varieties will be introduced into a breeding pipeline to develop improved spring and winter pea varieties for Minnesota, specifically focusing on the traits desired by the food industry and adaptations suited to our environment.

Neil Anderson, Department of Horticulture Science, Genetic Structure of Perennial Flax to Enable Identification of Genes Influencing Agronomic and Horticultural Traits

Abstract: Flax remains a high-value oilseed crop because of its unusually high content of omega-3 fatty acid (linolenic acid) with health benefits. Flax is an important ornamental garden plant and has the potential to be a new, blue-flowered cut flower crop (filler material in floral designs). Our long-term goal is to provide MN producers with high-yielding perennial flax varieties with as many as two harvests/season, enhanced quality and field performance, while providing forage for pollinating insects. Domestication of perennial flax (*L. perenne, L. austriacum,* and *L. lewisii*) commenced with the current FGI grant wherein crop ideotypes were created for agronomic and horticultural phenotypes; numerous genotypes with superior winter hardiness (USDA Z3, Z4) were selected and trialed. Likewise, several ornamental perennial genotypes with superior plant habit, flowering throughout the daytime, and with lengthy vase life potential were selected; 1-2 are slated for release to the market. Our proposed flax research is a continuation of our current work funded by FGI and has five objectives designed to produce phenotyping and molecular data, SNPs of the flax germplasm, conduct genetic structure analyses to determine species-specific SNPs and relatedness of accessions/hybrids/inbreds. This research refines the flax breeding program and brings it to a higher level of integrating phenotypic and genotypic markers for more rapid advancement of breeding objectives and serves as a gateway for a genome sequencing and genome-wide association study (GWAS) of domesticated traits to make a viable perennial flax for oilseed and ornamental production.

Kevin Smith, Department of Agronomy and Plant Genetics, Advancing breeding of *Silphium integrifolium* as a perennial oilseed crop for Minnesota

Abstract: *Silphium integrifolium* is a native perennial related to sunflower that has potential to serve as a perennial oilseed crop in Minnesota. The eventual deployment of this perennial crop on the landscape could help to protect natural resources, improve water quality, and provide sustainable value to the agricultural community. The University of Minnesota Silphium research team is part of a multi-disciplinary international effort organized through The Land Institute to domesticate silphium as a perennial oilseed crop to contribute to the array of new crops developed under the umbrella of the Forever Green Initiative. Over the past four years, we have conducted agronomic trials evaluating seeding density, nitrogen fertilizer rates, and planting date on yield and related traits and established a Silphium Domestication Panel to investigate important breeding traits. We have initiated development

of silphium populations with improved agronomic characteristics. Because of the perennial nature of silphium it takes several years to complete a breeding cycle. Thus, the overall goal of this project is to develop tools that will increase the speed and efficiency of breeding and to simultaneously develop improved germplasm. Specifically, we will develop a genotyping platform, extend our field studies from single plant to plot trials, characterize genetic variation for yield decline with age, conduct genetic mapping studies, and develop and implement a genomic-selection breeding program. This work will be carried out in close collaboration with scientists at The Land Institute and complement our ongoing efforts in silphium research.

Kathryn Bushley and Senyu Chen, Departments of Plant and Microbial Sciences and Plant Pathology, Pennycress and Soybean Cyst Nematode: A Solution Oriented Approach

Abstract: Soybean cyst nematode (SCN, *Heterodera glycines*) is the most prevalent pest affecting soybean yield in the Midwest. Pennycress is being evaluated as a cash cover crop in the corn-soybean production system to provide both economic opportunity and ecosystem benefits. Pennycress has been found to be a moderate host of SCN in greenhouse studies. Pennycress as an alternate host could be problematic in the corn-soybean rotation as SCN population density may increase and thereby cause yield loss in the soybean crop. Growers will not adopt a cover crop system which increases pest pressure for their commodity crop. Previous research has shown variation in SCN development on pennycress in the field, especially with changing environmental conditions. We have also identified variation in SCN susceptibility in genetically unique pennycress lines. The research proposed in this grant is intended to build on this previous research through three complementary objectives. First, we will determine the environmental conditions and management strategies in the field that cause SCN development on pennycress. Second, we will use high-throughput screening to detect changes in SCN susceptibility in a variety of wild- germplasm and EMS derived mutant lines. Lastly, we will use transcriptomics to compare susceptible versus resistant phenotypes and identify candidate genes involved in SCN resistance. We will validate these candidate genes using mutant lines to identify sources of resistance for breeding efforts. The information gained from this research mitigate of any risks pennycress poses for SCN and enable incorporation of pennycress into soybean-corn production systems for economic and environmental benefits.

James Anderson and Katherine Frels, Department of Agronomy and Plant Genetics, Expanding the Field Pennycress Breeding Program for Variety Development and Rapid Response to New Challenges

Abstract: Pennycress (*Thlaspi arvense* L.) is an extremely winter hardy Brassica plant that can be used as a cash cover crop in Minnesota. When planted as a cover crop in the fall, pennycress provides both ecosystem services such as reduced soil erosion and nutrient loss and pollinator forage and economic benefits with an oilseed harvest that allows farmers to harvest two cash crops in one year. The UMN pennycress breeding program has identified genes that modify key domestication traits and developed breeding lines with rapid, uniform emergence and increased yields. However, these traits and lines need additional performance evaluation prior to variety release. Increased testing will also be used to identify and rapidly respond to unforeseen challenges in pennycress production and produce seed needed for

acquiring Generally Recognized as Safe (GRAS) status for pennycress oil. We will also work to develop genetic solutions for ongoing challenges such as seed dormancy and pest pressure in conjunction with researchers in Plant and Microbial Biology, Entomology, and Plant Pathology. With data from these trials, we expect to release the first pennycress variety for use as a harvestable cover crop by Minnesota farmers in the next four years.

James Anderson, Department of Agronomy and Plant Genetics, Applied Genomics Assisted Breeding to Improve Long-Term Yield Potential of Intermediate Wheatgrass

Abstract: Perennial crops require less labor and inputs and cover the landscape year-round and could solve many of the challenges of annual cropping systems. One such perennial grain crop is intermediate wheatgrass (IWG, *Thinopyrum intermedium*), selected for domestication because of its potential food applications and ecosystem services. The University Of Minnesota (UMN) has been breeding IWG since 2011 with considerable progress made in increasing grain yield and improving domestication traits. In our breeding program, we combine targeted phenotyping and high-throughput DNA sequencing with robust statistical models to predict the performance of large IWG breeding populations and select the best parents for inter-crossing. As a result, seed weight has increased by 60%, percent free threshing grain has increased by 34%, and resistance to seed shatter has improved by more than 15%. A signature achievement of the UMN IWG breeding program was the release of 'MN-Clearwater' in August 2019, the first commercial Kernza® variety in the world. Despite the progress made, IWG needs higher grain yield, minimal seed shattering, and higher free threshing to become more widely adopted by growers and improve its future economic viability. In particular, long-term sustained grain yield needs to be addressed as grain yield tends to decline by the second year of production. This project seeks to improve these traits through next-generation genomics technologies and advanced plant breeding methods resulting in improved cultivars. In addition, we will quantify the rate of breeding progress and genetic gain made in the program during 2011-2019.

Jake Jungers, Department of Agronomy and Plant Genetics, Low-risk, High-reward Agronomic Trials to Enhance Kernza Development and Deployment

Abstract: Commercialization of Kernza intermediate wheatgrass is occurring rapidly and farmers across Minnesota will soon have access to the world's first Kernza variety – 'MN-Clearwater' – developed here at UMN. For Kernza acreage to expand and provide ecosystem services, it is essential that the early adopter farmers of this new crop have a positive experience, which is only possible with solid agronomic recommendations for profitable Kernza production. Based on research results and targeted grower feedback, we have identified four high-priority agronomic issues to address in this proposal. This project will 1) identify effective and ecologically sustainable methods to terminate Kernza for successful transition to a subsequent crop, 2) identify optimum combinations of nitrogen and phosphorus fertilizer to enhance yield and promote soil health, 3) develop a breeding selection system based on genotypic performance to nitrogen fertilization to select the parents of variety candidates that perform well under optimal agronomic management, and 4) collect weed suppression and crop safety data for three herbicides applied to Kernza for potential regulatory approval and use. This project will provide

agronomic information and deliverables based on mechanisms learned from detailed research on plant-soil-microbe interactions. Experiments will focus on enhancing and optimizing economic success and environmental sustainability. This project will also develop and test a new strategy to improve breeding methods that will lead to future varieties better suited to positively respond to agronomic management; a goal that will substantially increase the rate of progress in Kernza development and commercialization.

George Annor, Department of Food Science and Nutrition, Improving the Commercial Viability of intermediate wheatgrass (IWG) Through the Development of Value-added Ingredients and New Products

Abstract: Our **long-term goal** is to increase the market demand for IWG by diversifying its food uses and thereby increasing landscape scale use, groundwater quality conservation and farm income. Our **short-term goal** is to develop value-added ingredients and new products from IWG for commercialization. Our **objectives** are: 1) Evaluate breeding germplasm for nutritional quality and storage stability as affected by breeding and agronomic practices. 2) Use sprouted and puffed IWG grains and flour in novel recipes, products, and brewery applications, and evaluate their nutritional quality and acceptability with potential consumers 3) Develop novel recipes and new products, especially ready-to-eat products from sprouted and puffed IWG grains and flour and assess their quality. Our **approach** will be to assess the nutritional quality and storage stability of IWG grains from field trials as affected by breeding and agronomic practices. We will also produce value-added ingredients and products from IWG using sprouting and puffing techniques for new food applications. We will collaborate with Beth Dooley's kitchen to develop recipes from sprouted and puffed IWG grains and flours. Undergraduate Capstone students will be engaged to develop new products from sprouted and puffed IWG grains and flours. Student teams with industry mentors will develop new products and assess their nutritional quality, market feasibility and consumer acceptance. Sprouted grains will be used to brew beers, which will be evaluated for sensory attributes and consumer acceptability. This research will generate important nutrition product development, and consumer acceptability data to support the commercialization team and to guide variety selections.

David Mulla, Department of Soil, Water and Climate, Impacts of Kernza on Water Uptake and Nitrogen Leaching in SW Minnesota Wellhead Protection Areas

Abstract: Intermediate wheatgrass (IWG) is a cool-season perennial grass being domesticated to serve as the world's first commercially-viable perennial grain crop. This new grain crop, "Kernza", has potential to reduce nitrate leaching to groundwater and protect rural drinking water supply from nitrate contamination. Previous work by this team has prompted policy proposals to support the planting of Kernza in wellhead protection areas for groundwater protection. However, little is known about water uptake by Kernza or how uptake varies with growth. Water uptake dynamics affect percolation of water below the rooting zone and the associated nitrate leaching, and these dynamics must be understood to optimize grain yield and water quality benefits. This project will utilize new and existing production-scale Kernza plantings positioned in wellhead protection areas in SW Minnesota, where drinking water is severely compromised as a result of nitrate leaching. We will measure water use dynamics (e.g.

transpiration, percolation), carbon cycling and nitrogen leaching in IWG fields varying in age and managed with and without nitrogen fertilizer. A suite of hydrology parameters, soil physical and chemical parameters, and soil solution nitrate data will be used to calibrate and validate the DSSAT crop simulation model to predict production and water quality impacts of Kernza across various soil types in SW Minnesota Drinking Water Source Management Areas. We will use experimental and satellite remote sensing data to calibrate and validate the model for Kernza productivity. Model results will be used to estimate regional productivity and benefits of Kernza for drinking water quality.

M. David Marks, Department of Plant and Microbial Sciences, Utilizing Genetic Tools to Identify Winter Camelina Lines with Early Maturity, High Protein and Reduced Glucosinolates

Abstract: There are three urgent problems that improved agricultural systems can address: hunger, climate change, and water pollution. The United Nations Food and Agriculture Organization and the Minnesota Pollution Control Agency have both called for innovations in plant-based strategies to address multiple pressing crises simultaneously. As a winter annual, camelina has the potential to protect Minnesota's soil and water without sacrificing the production of food, feed, and fuel. The climate of the Midwest appears to be well suited to the cultivation of camelina, and with advances in next generation DNA sequencing and other similar technologies, we can rapidly improve the oilseed camelina to promote its adoption. Our strategy for improving traits in camelina is multi-faceted. We are evaluating wild-germplasm and their progenies for variation in key traits, and developing new variation using mutation breeding and gene-editing techniques. At the end of this project, we will generate elite germplasm for winter camelina with improved traits. In addition, as this project progresses we hope to train the next-generation of scientists who are passionate about solving ecosystem challenges and food security.

Pam Ismail, Department of Food Science and Nutrition, Evaluating Camelina and Pennycress as Novel Sources of Plant Protein

Abstract: Demonstration of camelina and pennycress proteins as equivalent or superior sources compared to existing protein alternatives is essential to their market success. In order to secure a competitive place in the market, the functional and nutritional value of these crops, specifically their protein component, need to be evaluated. The proposed work is multifaceted involving a concerted effort from breeders as well as food and nutrition scientists will lead to the production of pennycress and camelina lines that are viable sources of plant protein for various food applications. Specific objectives are: 1) Develop flavor-guided protein extraction methodology for optimal quality and yield following innovative approaches; 2) Screen lines for protein nutritional quality and functionality to develop optimal lines for food use. One pennycress line and one camelina line will be selected for optimization of protein isolation conditions. Based on recent work with pennycress (work funded by FGI) and camelina (work funded by GMI) wild type seeds, defatted meals will be subjected to two protein extraction procedures, alkaline and salt extraction, coupled with membrane filtration. Extraction conditions will be optimized for yield, purity, flavor, and functionality. Seeds from 5 camelina and 5 pennycress lines with potential variation in protein composition and profile, provided by the breeders,

will be used to produce protein isolates following the flavor guided optimized extraction. Protein structural, functional, and nutritional properties will be determined. Generated data will feed into the breeding program that aim at successfully adapting pennycress and camelina as edible protein crops for food applications.

Josh Gamble, USDA-ARS Department of Soil, Water and Climate, Production Scale Deployment of Forever Green Cropping Systems: Agronomic, Economic, and Environmental Aspects

Abstract*:* The Forever Green Initiative (FGI) has worked to develop a broad portfolio of exciting perennial and winter annual cover crops to enhance rural economies, improve agricultural sustainability, and bolster agricultural resilience. However, **long-term agroecological strategies for integrating new FGI crops into field-scale agricultural systems have yet to be explored.** This project aims to understand the benefits and challenges of integrating emerging FGI crops into field-scale production systems that include common annual and perennial crops, and also to quantify the potential environmental benefits at large spatial and temporal scales. We propose evaluation of two field-scale cropping systems: a winter camelina (*Camelina sativa*) relay system in a corn-soybean rotation (annual grain and oilseed relay system), and a Kernza® intermediate wheatgrass (*Thinopyrum intermedium*) - alfalfa (*Medicago sativa* L.) rotation (perennial forage and grain system). Our objectives are 1) to quantify field-scale yield and potential economic returns for novel Forever Green crops integrated into rotational systems; 2) to quantify the seasonal, interannual, and spatial variability of CO_2, water, and energy exchange in these systems; and 3) Improve our understanding of how Forever Green crops influence soil health at the field-scale. The FGI cropping systems will be contrasted with a data from a native grassland ecosystem and a conventional corn-soybean rotation as benchmarks. Through this work we will improve best management guidelines, refine crop enterprise budgets based on field-scale data, and provide valuable new insights about the water and energy use, carbon storage potential, and soil health benefits of key Forever Green crops.