FOR ADMINISTRATIVE USE
Program Area Code   Proposal Code

Minnesota Wheat Research and Promotion Council

RESEARCH PROPOSAL GRANT APPLICATION

1. NAME AND ADDRESS OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE

Name: Regents of the University of Minnesota
Address: Sponsored Projects Administration
450 McNamara Alumni Center, 200 Oak Street SE
Minneapolis, MN 55455-2070

2. TITLE OF PROPOSAL
A Novel High-Throughput Phenotyping Pipeline to Deliver More Productive and Stress Resilient Minnesota Wheat Varieties

3. PRINCIPAL INVESTIGATOR(S)	**4. PI #1 BUSINESS ADDRESS**
PI# 1 Name: Walid Sadok	Department of Agronomy and Plant Genetics 411 Borlaug Hall
PI# 2 Name: José R López	1991 Upper Buford Circle St. Paul, MN 55108
PI# 3 Name: Brian J Steffenson PI# 4 Name: James A Anderson	

5. PROPOSED PROJECT DATES (calendar years)	**6. TOTAL PROJECT COST**	**7. PI #1 PHONE NO.**
January 1st, 2021 – December 31, 2021 Note: Research Reports are Due November 15th of Each Year		612-625-8291

8. RESEARCH OBJECTIVES:
Wheat canopies are key sources of carbohydrates, water and nitrogen compounds that will be remobilized into the grain during seed-fill. Therefore, healthy, highly active canopies that are resilient to stresses are key to enhancing yields. The challenge for the breeder, however, is that 'lazy' and well-performing canopies look identical to the naked eye. If breeders had access to a technology that enables to 'see' these differences and identify them rapidly in hundreds of genotypes, it would significantly accelerate the breeding effort. To address this challenge, we developed a promising new technology based on remote sensing using a drone-mounted thermal camera that can be deployed in the field to rapidly measure canopy health and performance on wheat. Data gathered last summer on 44 wheat genotypes revealed that this technology is not only able to identify differences between lines but also to capture differences in alleles of genes controlling canopy conductance.

Given these promising results, our goals for this year are to: (i) further fine-tune and scale-up this new phenotyping technique and (ii) quantify the extent of genotypic variability in these thermal signatures within a population of 360 breeding lines. To achieve this, we will combine the use of (1) a drone-mounted high-resolution thermal camera, (2) an additional hand- held thermal camera (on days where flying is not possible), (3) thermocouples (temperature sensors that are in direct physical contact with canopies) and (4) an in-house energy balance model to reliably extract 'clean' physiological signatures from 'noisy' field conditions. At the end of the year, we expect to validate this new technology and its effectiveness in screening a large number of breeding lines to support the U of M wheat breeding program and fast-track the delivery of superior wheat varieties to Minnesota growers.

Signature Of Principal Investigator	**Date**	**Phone Number**
[signature]	**1/12/2021**	**612-625-8291**
Signature Of Authorized Representative	**Title**	**Date**
[signature]	**Principal Grant Administrator**	**1/12/2021**

Address Of Authorized Representative	**Phone Number**
Office of Sponsored Projects Administration 450 McNamara Alumni Center, 200 Oak Street SE, Minneapolis, MN 55455-2070	612-624-5599

Minnesota Wheat Research and Promotion Council
RESEARCH PROJECT PROPOSAL
(2-pages maximum)

Project Title: A Novel High-Throughput Phenotyping Pipeline to Deliver More Productive and Stress Resilient Minnesota Wheat Varieties

Importance of this project to the profitability of wheat producers:

Enhancing wheat canopy health and productivity under adverse environmental stresses is critical to increasing or at least stabilizing wheat yields to achieve economically viable yields for the farmer (Monnens and Sadok, 2020). This is because a healthy canopy will maximize capture of light and carbon dioxide through canopy photosynthesis and uptake of nitrogen and water through canopy transpiration (Schoppach et al., 2017; Tamang et al., 2019). Since these resources (carbohydrates from photosynthesis, nitrogen compounds and water) will be invested in grain during seed fill, a fully active, healthy canopy will tend to maximize grain yields and grain protein content (Sadok et al., 2019).

The challenge, however, is to determine whether a given genotype is equipped with a highly active or under-performing canopy since these canopies look identical to the naked eye, especially among breeding lines or commercial cultivars. Based on experiments conducted during the summer of 2020, we have developed a novel remote-sensing, drone- based thermal imaging technology that could enable us to rapidly screen, under field conditions, hundreds of wheat varieties for enhanced canopy photosynthesis and transpiration. This method is also effective as a diagnostics tool, enabling detection of early signs of stress (biotic or abiotic) which typically depress photosynthesis and transpiration in wheat.

Last summer, this high-throughput technique was successfully tested by our team, enabling us to confirm these drone-based differences in canopy performance using low-throughput, ground-based equipment used to measure actual canopy photosynthesis and transpiration. Furthermore, these field drone-based thermal images enabled us to consistently detect allelic differences for previously identified gene marker controlling canopy conductance on 44 lines that segregated for this marker, which we have discovered based on previous support from the MWR&PC. In addition, this method was successfully applied to predict canopy tolerance to heat stress and grain yield among locally-grown oats, further highlighting its promising potential for wheat.

All of these findings make this approach extremely attractive to the U of M wheat breeding program as it will substantially accelerate screening of breeding material for enhanced yield potential. We seek to further develop this promising technology as a new breeding infrastructure enabling high- throughput phenotyping to identify the genetic basis of traits enhancing yield potential under optimal and stressful conditions. The associated genetic markers will then be used to fast-track the delivery of next-generation wheat cultivars to Minnesota wheat growers. Therefore, the immediate gain to wheat producers will be to benefit from more productive and stress-resilient wheat varieties.

Procedures:

The Sadok lab recently developed a method for a rapid screening of wheat varieties for canopy health and productivity (photosynthesis and transpiration) using a drone-based method, pioneered by postdoctoral associate José López, a collaborator on this project. While there are existing drone-based approaches to monitor crops, this method is unique as it relies on advanced thermal imaging technology coupled with energy balance modeling, and informed by physiology-based ground- truthing techniques. This combination of cutting-edge methods ensures that differences in thermal images among genotypes actually captures differences in plant physiology rather than differences due to the plant's environment. This is critical to any breeding program, because traits that are 'masked' by the environment will tend to have low heritability and are more difficult to genetically improve.

During the first year, we propose to test and validate our new approach by phenotyping a group of 360 breeding lines developed by wheat breeder James Anderson in collaboration with plant pathologist Brian Steffenson. The 360 lines will be our 2021 Preliminary Yield trial lines that include 8 check varieties, each planted at least 5 times in the field. Genotypes will be machine-planted in 5-by-8 ft field plots on the St. Paul campus of the University of Minnesota, managed per the local recommendations and machine-harvested to determine yield and seed protein content. At multiple times during the season, measurements will be made to (i) fine-tune the phenotyping method to maximize capture of the physiological status (health) of the canopy and minimize environmental "noise" and (ii) quantify the extent of genotypic variability in these physiological signatures within the available germplasm. To this end, we will combine the use of:

(1) a drone-mounted high-resolution thermal camera
(2) an additional hand- held thermal camera (on days where flying is not possible)
(3) thermocouples (sensors that are in direct physical contact with canopies to measure temperatures as

experienced by the plant) for ground-truthing
(4) an in-house energy balance model to extract physiological signatures from canopy images
(5) seasonal workers who will help with ground equipment installation and record plant phenology and various observations related to plant health.

A first round of data analysis will be based on advanced image feature extraction algorithms combined with energy balance modelling to extract key physiological features from thermal images and reduce environmental noise. Based on the previous step, analyses will be conducted to compute heritability and quantify differences among genotypes and their relationship with grain yield and grain protein content. If successful, this approach will enable, for the first time, to conduct large-scale phenotypic screens to identify the genetic basis of canopy health and use the associated genetic markers in the breeding program to develop more productive and stress-resilient wheat varieties.

Regional linkage to other research activities:

This project is part of a joint effort by the Sadok lab and breeders to enhance small grain (oats, barley and wheat) yields in the region (ND, SD and MN) by combining physiology and genetics. For instance, the drone-based technique that will be used in this project was first developed in a regional project aiming at enhancing oat yields in ND, SD and MN. In addition, this technique has been successfully used to detect differences in heat/drought stress tolerance in oats based on regional trials. Because such stresses are increasingly common in the region, we expect that our cost-effective technology would be of interest to various other university and industry small grain breeders throughout the region.

List current or potential other funding sources for this project: The PI is currently supported by an MDA-funded project which is being used to fine-tune the drone-based technology that will be deployed in this project. He has also submitted additional proposals to MDA and FFAR (on oat and wheat, respectively) based on this technology, which -if funded- will complement this project. Furthermore, this research is currently supported in part by project funded by USDA-NIFA through the Minnesota Agricultural Experiment Station. Finally, data from this project will be also used to support another USDA-NIFA project in preparation.

Research Group:

Walid Sadok and the postdoctoral research associate (Dr. José R López) will lead the drone technology development work, the phenotyping effort and data analyses. Jim Anderson will provide the population and genetic map, help with planting, harvesting, yield and protein measurement. Brian Steffenson will provide expertise on canopy health in response to biotic stress and remote sensing.

Relationship to past projects:

This project builds on a successfully completed project aiming at enhancing canopy conductance in wheat that was previously supported by the MWR&PC. The effect of a major quantitative trait loci (QTL) controlling canopy conductance that was identified under controlled conditions in that project was confirmed using the drone-based approach in the field. Therefore, without the previous project, we would not have validated this new, rapid and cost-effective thermal imaging technology.

Estimate the budget requirements:

Funding is requested to support a 50% postdoctoral research associate (salary: $26,585 and fringe: $6,753 at 25.4%). Funds are requested to support salary for undergraduate summer field workers: $2,860 (220h at $13/hr). Total: $36,197.

References (* indicate peer-reviewed scientific papers acknowledging support from MWR&PC):

- ***Monnens, D., & Sadok, W. (2020). Whole-plant hydraulics, water saving, and drought tolerance: a triptych for crop resilience in a drier world. Annual Plant Reviews, 3(4), 661-698.
- Sadok, W., Schoppach, R., Ghanem, M. E., Zucca, C., & Sinclair, T. R. (2019). Wheat drought-tolerance to enhance food security in Tunisia, birthplace of the Arab Spring. European Journal of Agronomy, 107, 1-9.
- Schoppach, R., Fleury, D., Sinclair, T. R., & Sadok, W. (2017). Transpiration sensitivity to evaporative demand across 120 years of breeding of australian wheat cultivars. Journal of Agronomy and Crop Science, 203(3), 219-226.
- ***Tamang, B. G., Schoppach, R., Monnens, D., Steffenson, B. J., Anderson, J. A., & Sadok, W. (2019). Variability in temperature-independent transpiration responses to evaporative demand correlate with nighttime water use and its circadian control across diverse wheat populations. Planta, 250, 115-127.