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: North Dakota State University
Address: Sponsored Programs Administration
NDSU Dept. 4000, PO Box 6050
Fargo, ND 58108-6050

2. TITLE OF PROPOSAL
Organismal Responses During Drought and Recovery in Globally and Regionally Selected Wheat Varieties

3. PRINCIPAL INVESTIGATOR(S)

Dr. Zhikai Liang

PI# 2 Name:

PI# 3 Name:

4. PI #1 BUSINESS ADDRESS
North Dakota State University
Department of Plant Sciences
Dept. 7670, PO Box 6050
Fargo, ND 58108-6050

5. PROPOSED PROJECT DATES (calendar years)
01/01/2024 – 12/31/2025

Note: Research Reports are Due November 15th of Each Year

6. TOTAL PROJECT COST

$21,770

7. PI #1 PHONE NO.
701-231-8892

8. RESEARCH OBJECTIVES: (List objectives to be accomplished by research grant)
Year 1 – Seed propagations, data recording, and PlantExplorer Pro+ Operation Training and Sample Testing.

Year 2 – Fluorescence image collections and numeric value extractions and data analysis

Attach a 2-page detailed discussion of importance of the proposal to wheat profitability; how study complements previous research in area; procedures to be used; and competency of the research group in achieving research objectives. (Please keep the proposal concise, only 2 pages will be provided reviewers).

Signature Of Principal Investigator

Date 12/26/2023

Phone Number
701-231-8892

Signature Of Authorized Representative
Valrey V. Kettner

Title Assoc. VP
Sponsored Programs
Administration

Date
12/28/2023

Address Of Authorized Representative

NDSU Dept. 4000

PO Box 6050

Fargo, ND 58108-6050

Phone Number
701-231-8045

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

Project Title: Organismal responses during drought and recovery in globally and regionally selected wheat varieties

Importance of this project to the profitability of wheat producers: Drought is an on-set environmental stress event in the Northern Great Plains that impacts agricultural productions. Nearly 95% Hard Red Spring Wheats (HRSW) in the United States are planted in this region. Producers in North Dakota alone generate an average of 265 million bushels of HRSW annually. However, the uncertain prevalence of drought causes significant losses in wheat production across the Northern Great Plains. For instance, the 2021 drought event resulted in a nearly 32% yield drop for spring wheat in North Dakota (source: https://asmith.ucdavis.edu/news/drought-upper-great-plains). Even as of July 2023, drought monitor data indicated that 44% of the state's spring wheat was in drought-affected regions Based on hard red spring (HRS) futures at $7.14/bu reported by the March 2024 MGEX (Minneapolis Grain Exchange), this suggests a market worth at least $1.8 billion for HRSW in North Dakota, with even larger market values across the Northern Great Plains. Enhancing the drought stress tolerance of local wheat germplasms will reduce potential economic losses for wheat producers in the Northern Great Plains caused by future drought events. In our study, we will develop precise fluorescence image-based methods for early-stage drought stress monitoring and understanding drought tolerance variations across a wide range of representative wheat germplasms. These methods will guide field management strategies and enhance genetic diversity in drought stress tolerance among local wheat germplasms in the Northern Great Plains.

Procedures: Overall, this is a two-year project. Core materials include 19 wheat germplasms including 15 germplasms used for wheat pan-genome assemblies (Walkowiak et al. 2020), 3 hard red spring wheats (Sumai3, Glenn, Rollag) with genome assemblies that are under planning (https://www.ars.usda.gov/research/project/?accnNo=438467) and 1 Chinese Spring that was used as the germplasm for assembling the first wheat reference genome (IWGSC et al. 2018). PlantExplorer Pro+ will be the major equipment for capturing high-resolution images to quantify wheat drought tolerance and recovery abilities.

- ***In Year 1***, we will focus on three goals, ***1) Seed Propagations***. To ensure we will have enough seeds for drought stress quantifications in this project, we need to propagate seeds for germplasms we requested from the John Innes Centre (JIC) in the United Kingdom. Currently we have 20 seeds generated from Single Seed Descent for each ordered germplasm ***2) Data recording***. We will record growth data and other conventional phenotypes of 19 germplasms in this study including germination rate, tillering time, flowering time and entire growth period; ***3) PlantExplorer Pro+ operation training and sample testing***. Our ordered PlantExplorer Pro+ will arrive at NDSU campus in the beginning of 2024 from the Netherlands. Experts from the PhenoViation in the Netherlands will come in person at NDSU to train people in our group on operating the machine and we will use a couple of wheat samples for understanding images generated from this machine and optimization.
- ***In Year 2***, we will focus on another three goals, ***1) Fluorescence image collections***. For each of 19 germplasms in this experiment, we will perform drought stress measurement experiments in two independent stages – tillering stage and grain filling stage. For each stage per germplasm, we will run a ~1-week drought stress and return to the regular watering at the end of the drought stress period. About 7 individual time points will be selected based on the experimental design and one image will be collected for each plant (3 replicates for each germplasm) per time point; ***2) Numeric value extractions and data analysis***. We will use commercial software provided by PlantExplorer Pro+ for photosynthetic value extractions. For segmenting individual organs, we will develop our own Python-based program to extract photosynthetic values for individual organs and pixels. All collected image-based photosynthetic parameters will be used as input data and analyzed by statistical methods (e.g. Linear Discriminant Analysis, Principal Component Analysis, Linear Mixed Model, Hierarchical Clustering) using Python/R programming.; and ***3) Result deliveries***. Personnel from this project will attend a national/international conference to communicate with other experts and deliver our discoveries to the community. We will also wrap up results for publishing a paper in a scientific journal.

Regional linkages to other research activities Multiple PIs at the Department of Plant Sciences at North Dakota State University are also working on the drought tolerance in wheat. As a Co-PI, I have worked with other faculty at NDSU including PI Dr. Xuehui Li (Associate Professor), Co-PI Dr. James Alan Staricka (Soil Scientist) and Co-PI Dr. Elias Elias (University Distinguished Professor) on applying a USDA-NIFA grant entitled "Develop Durum Wheat Pre-Breeding Germplasm with Improved Grain Yield under Drought Environment of Northern Great Plains". This is a 4-year research program that requests total funds at $610,025 and focuses on selecting durum wheats with strong drought tolerance in a durum wheat breeding program at North Dakota. Other personnels in this project had years of experience on studying drought stress in wheat. This opportunity to work with them will ensure the success of this proposed project. Drought stress evaluation system developed from this durum wheat drought tolerance breeding program can provide valuable information for this image-based drought stress quantification project.

List any other secured, pending, or planned submissions to outside funding sources for this work: Currently I do not have directly secured/pending grants from outside funding sources for this work. However, research outcomes from this work will be used for preparing two submissions soon. The first submission is about using a multi-omics and statistical modeling approach to understand genetic architecture underlying spring wheat drought tolerance. Our group has already requested ~400 spring wheat germplasm from USDA. Outcomes from this project sponsored by MN wheat research council will establish a system for us to quantify drought tolerance in wheat using image-based approaches and help us prepare this submission. Another submission is about developing an artificial intelligence method to ameliorate barriers between fluorescent camera and portable multi-spectral camera on crop stress quantifications. Our group will plan to purchase a portable multispectral camera which will enable us to measure crop stress easily in both controlled environments or field settings. However, the multispectral camera is cheaper and less accurate on stress qualifications than the PlantExplorer Pro+ in this project. We will develop models to bridge the cap and make portable multispectral cameras easier applied to field-based stress evaluation works. USDA-NIFA will be the potential funding agency for consideration of these two submissions.

Research Group: Dr. Zhikai Liang at the department of Plant Sciences will be the only group that leads this project. However, Dr. Liang's group will collaborate with Dr. Xuehui Li (Associate Professor) at the department of Plant Sciences for drought stress measurements/quantifications in wheat and Dr. Jason Fiedler (Research Plant Molecular Geneticist) at the Cereal Crops Research of U.S. Department of Agriculture for some spring wheat germplasms.

Relationship to past projects: Since I am new to the department, there are no past projects from my current lab at NDSU related to this project. However, there are two projects in my past that are related to this project. The first one was the project that I was involved in during my PhD study at University of Nebraska-Lincoln. I was involved in a nationwide collaborative project in maize entitled "The Genomes To Fields (G2F) Initiative" with >50 PIs from >15 states in North America (https://www.genomes2fields.org/home/). My role in this project is to develop a python-based high-throughput image data processing pipeline to extract numeric values from images captured by multiple cameras including RGB, fluorescence and hyperspectral in the Nebraska Automated Greenhouse. One paper that I was the lead author on was published under the support of this project (Liang et al. 2018). Another project was from my Postdoctoral training at University of Minnesota. In this project, I used a PAM camera (fluorescence camera) for quantifying heat stress tolerance in leaves of ~100 maize inbreds. I demonstrated that duration of heat stress exposure can be linked with photosynthetic parameters including Y(NPQ), Y(II) and Y(NO) (Figure 1). Broad-sense heritabilities were calculated for all three measured photosynthetic parameters and they ranged from 0.538 to 0.692, indicating 53.8% to 69.2% variations of photosynthetic parameters are controlled by genetic factors (Liang et al. 2022).

Estimate the budget requirements: Total estimated budget is $21,770. Budget Justification is listed as below,

- **Salaries and Wages ($9,473):** Two-month salary will be paid for a graduate student each year. Annual base salary starts at $28,000/yr with 3% annual increase. Year 1: $2,333.33/month x 2 months = $4,667; Year 2: $2,403.33/month x 2 months = $4,806. This graduate student will work on plant growing, collecting image data using PlantExplorer Pro+ and data analysis.
- **Fringe benefits ($284):** Fringe benefits are calculated at 3% for the graduate student. Year 1: $4,667 x 3% = $140; Year 2: $4,806 x 3% = $144.
- **Travel ($3,000):** Travel is requested in Year 2 to support one personnel attending a national conference (e.g. North American Plant Phenotyping Network Annual Conference, ASA, CSSA, SSSA International Annual Meeting). This travel fund includes registration: $1,000, Airfare: $600, Lodging: $1,000, Meals: $400
- **Supplies and Services ($2,000):** $1,000 x 2 years = $2,000 requested for supplies: items like soil moisture monitors/sensors, seed envelopes/bags, disposable gloves, collection tools, and chemical reagents.
- **Other expenses ($7,013):** $3,000 - publication fee will be requested in Year 2. One publication is anticipated to be published in Year 2. $4,013 – Greenhouse fees are requested at $0.80/square feet x 418 square feet x 6 months/year x 2 years (Year 1 and Year 2) = $4,013.

References:

- International Wheat Genome Sequencing Consortium (IWGSC). 2018. "Shifting the Limits in Wheat Research and Breeding Using a Fully Annotated Reference Genome." *Science* 361 (6403). https://doi.org/10.1126/science.aar7191.
- Liang, Zhikai, Zachary A. Myers, Dominic Petrella, Julia Engelhorn, Thomas Hartwig, and Nathan M. Springer. 2022. "Mapping Responsive Genomic Elements to Heat Stress in a Maize Diversity Panel." *Genome Biology* 23 (1): 234.
- Liang, Zhikai, Piyush Pandey, Vincent Stoerger, Yuhang Xu, Yumou Qiu, Yufeng Ge, and James C. Schnable. 2018. "Conventional and Hyperspectral Time-Series Imaging of Maize Lines Widely Used in Field Trials." *GigaScience* 7 (2): 1–11.
- Walkowiak, Sean, Liangliang Gao, Cecile Monat, Georg Haberer, Mulualem T. Kassa, Jemima Brinton, Ricardo H. Ramirez-Gonzalez, et al. 2020. "Multiple Wheat Genomes Reveal Global Variation in Modern Breeding." *Nature* 588 (7837): 277–83.

Minnesota Wheat Research and Promotion Council

RESEARCH PROJECT PROPOSAL BUDGET

Project Title:			
Principal Investigator(s) / Project Director(s)	Funds Requested For		
	Year 1 (2024)	Year 2 (2025)	Year 3 (2026)
A. Salaries and Wages	$ 4,667	$ 4,806	$
1. Co-principal Investigator(s)	0	0	
2. Senior Associates	0	0	
3. Research Associates – Post Doctorate	0	0	
4. Other Professionals	0	0	
5. Graduate Students	4,667	4,806	
6. Prebaccalaureate Students	0	0	
7. Secretarial - Clerical	0	0	
8. Technical, Shop and Other	0	0	
B. Fringe Benefits	140	144	
C. Consulting and Professional Services	0	0	
D. Supplies and Services	1,000	1,000	
E. Travel	0	3,000	
F. Sub-Contracts	0	0	
G. Repairs & Maintenance	0	0	
H. Rentals & Lease	0	0	
I. Other Expenses	2,007	5,006	
TOTAL AMOUNT OF THIS REQUEST (per year)	**$ 7,814**	**$ 13,956**	**$**

Approved Brian Sorenson Int'l: _____Date:_____