Grant Code: New

Title: Wheat - weed competition: Understanding the response of spring wheat to multi-generational weed competition

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Justification: Among the economically important agricultural pests, weeds cause the greatest potential and actual yield losses (Oerke 2006). Estimates from North America showed that yield loss from uncontrolled weeds would be up to 47% in spring wheat (Flessner et al. 2021), which translates into several hundred million dollars. Kochia and Italian ryegrass are among the most widespread and serious weed problems in small grain production systems (Appleby et al. 1976, Nandula 2014, Hashem et al. 1998). The general expectation is that when plants are subjected to repeated stress (e.g., weeds), their progeny perform better under the stress than the parents. Singh and Roberts (2015) posited that *in agriculture, transgenerational priming of plant defense has the potential to contribute to sustainable intensification*. It was argued that once induced, *primed plants should require fewer pesticide applications to reach the same level of protection* (Singh and Roberts 2015). Transgenerational priming has been demonstrated in plant defense against pathogens and herbivory (Agrawal 2001, Ramírez-Carrasco et al. 2017). However, we are not aware of any research on how transgenerational priming affects crop-weed interaction. *If memory response is indeed an important component of plant defense, it is expected that crops subjected to multiple generations of weed competition may produce progeny more competitive in the presence of weeds.* The basic knowledge discovered in this project could form the basis for future studies aimed at developing strategies to reduce the competitive effects of weeds on crop yields.

Objectives

1. Assess growth and phenotypic plasticity in wheat in response to multigenerational weed competition.
2. Assess epigenetic patterns and gene expression in spring wheat response to weed competition.
3. Generate preliminary data to apply for a federal grant

Methods/Plan of Work: Greenhouse studies were initiated in 2021 at the University of Idaho Kimberly Research and Extension Center to evaluate the response of spring wheat to repeated exposure to weed competition. There were four treatments (Figure 1) arranged in a completely randomized design with 15 replications. Treatments were as follows:

1. Wheat-only: one wheat per pot
2. Wheat-wheat: one wheat in the center surrounded by 8 wheat
3. Wheat-Italian ryegrass: one wheat in the center surrounded by 8 Italian ryegrass
4. Wheat-kochia: one wheat in the center surrounded by 8 kochia

One spring wheat ('UI Cookie') was planted in the center of a 3 L pot filled with potting soil and surrounded with either wheat, Italian ryegrass, kochia, or no plants. There were 8 surrounding plants to maximize competition and ensure plants were not too crowded to prevent seed production. The wheat in the center of the pot was grown to maturity. At heading, wheat in the center of each pot was covered with pollination bags to prevent cross-pollination or gene flow. Seeds from the first generation were used to plant the second generation and the process was repeated to obtain five generations. The original seed (G0), as well as seeds from generations 1 to 5 (G1 to G5), were saved for a common garden experiment.

Figure 1 Illustration of competition treatments used in greenhouse experiments

Common garden experiment #1

This experiment will consist of 84 treatments as depicted in Figure 2. The 84 treatments will consist of the five generations (G1 to G5) by four competition levels from each generation (as described above), in addition to the G0. These 21 treatment combinations will be subjected to the four competition levels in the common garden experiment to give a total of 84 treatments. Each of the 84 treatments will be replicated 14 times.

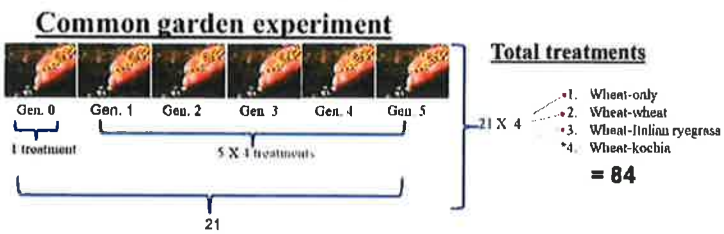

Figure 2. Treatment combinations for the common garden experiment

Common garden experiment #2

For specific obj #2, a second common garden experiment will be established where the 21 treatments (Figure 4) (G0 + G1 to G5 (by four competition levels) will be grown (one wheat per pot) without any competition. This will ensure that any differences observed were truly due to differences among the generations. There will be four replicates for this study and plants will be sampled 5 weeks after planting.

Specific objective #1. *Assess growth and phenotypic plasticity in wheat in response to multigeneration weed competition*

We will conduct three destructive samplings (3 replicates at each sampling) at 20, 40, and 60 days after planting to measure relative growth rate (RGR), number of leaves, number of tillers, leaf area, and stem biomass. Photosynthesis/gas exchange and leaf greenness (SPAD) will be measured before each destructive sampling. At maturity, 5 replicates of each treatment will be harvested to measure stem biomass, number of seed heads, number of seeds, and seed weight.

Results will be analyzed following standard statistical procedures.

Specific objective #2: *Evaluate epigenetic patterns and gene expression in spring wheat response to multigenerational weed competition.*

RNA-seq analysis will be performed on plants from common garden experiment #2 using TruSeq method to compare gene expression differences in the 5 generations and G0 (Chu et al. 2021). Leaf samples from the 5-week-old plants will be isolated and immediately put into liquid nitrogen. RNA will be extracted using a Qiagen-RNeasy Mini kit following the manufacturer's instructions. Sequencing libraries will be prepared using the TruSeq RNA Sample Preparation kit v2. mRNA will be purified using poly-T oligo-attached magnetic beads, cDNA will be synthesized after fragmentation, followed by A-tailing, adaptor ligation, and PCR amplification. Libraries will be checked for quality followed by deep sequencing on a single lane of Illumina HiSeq 2000 to generate 75 bp pair-end reads.
Results will be analyzed following standard statistical procedures.

Duration: This is a 1-year study.

Cooperation/Complementation: One PhD student (Albert Kwarteng) has been recruited to work on this project as part of his PhD dissertation. Albert's PhD committee Drs Kuhl, Xiao, and Murdoch will provide lab space for lab analysis.

Anticipated Benefits/Expected Outcomes: This is basic resaerch with no short-term benefit to Idaho wheat growers. The long-term benefit would be developing strategies to reduce the competitive effects of weeds on wheat yields.
Data generated from this study will also be used as preliminary data for a larger federal grant application. Also, this will be a part of Albert Kwarteng's PhD disseration.
Results will be presented at professional meetings. Final results will be published in Weed Science journal.

Literature review: Plants are constantly exposed to different types of stresses, and the exposure of a plant to a previous stress event could alter its physiological, biochemical, and transcriptional response to a subsequent stress of the same or similar nature (Alves de Freitas Guedes et al. 2019). Plants have evolved mechanisms by which they can store a memory of previous stress events and prime their responses in order to have a stronger and rapid response in case the stress event recurs in the near future (Fleta-Soriano and Munné-Bosch 2016, Hilker and Schmülling 2019). The ability of plant stress memory to be passed on to the next generation has been reported in *Arabidopsis thaliana* (Molinier et al. 2006), shoots and roots of barley (*Hordeum vulgare*) (Nosalewicz et al. 2016), maize (*Zea mays*) (Ding et al. 2014), and a host of other plants.

From an evolutionary perspective, stress memory could be seen as an effective strategy for preparing the plant for subsequent stress, thereby providing it with an adaptive or beneficial response to subsequent stress (Godwin and Farrona 2020). For instance, when plants are exposed to drought stress over two years, Rubisco and several anti-oxidative enzymes are upregulated in the subsequent generation and act in concert to produce a long-term stress memory, which persists over several weeks (Lukić et al. 2020). Stress memory may sometimes be associated with negative effects including delayed growth and development and drastic reductions in yield resulting in maladaptive memory (Skirycz and Inzé 2010, Crisp et al. 2016).

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FY2025
COMMODITY COMMISSION BUDGET
Principal Investigator: *Albert Adjesiwor*

Allocated by ____________ *(Commission/Organization)*	during FY2023	$	-
Allocated by ____________ *(Commission/Organization)*	during FY2024	$	-

REQUESTED SUPPORT Budget Categories	*Awarded for FY2024*	*Requested for FY2025*
(10) Salary *(staff, post-docs, et NOTE: Faculty salary/fringe NOT allowed*	$ -	$ 11,000
(12) Temporary Help/III	$ -	$ -
(11) Fringe Benefits	$ -	$ 275
(20) Travel	$ -	$ -
(30) Other Expenses	$ -	$ 18,984
(40) Capital Outlay >$5k	$ -	$ -
(45) Capital Outlay <$5k	$ -	$ -
(70) Graduate Student Tuition/Fees	$ -	$ 6,354
TOTALS	$ -	$ 36,613

TOTAL BUDGET REQUESTED FOR FY2025:	$ 36,613

BREAKDOWN FOR MULTIPLE INDEXES:

Budget Categories	*(Insert Co-PI Name)*	*(Insert Co-PI Name)*	*(Insert Co-PI Name)*	*(Insert Co-PI Name)*
(10) Salary *(staff, post-docs, et*	$ -	$ -	$ -	$ -
(12) Temporary Help	$ -	$ -	$ -	$ -
(11) Fringe Benefits	$ -	$ -	$ -	$ -
(20) Travel	$ -	$ -	$ -	$ -
(30) Other Expenses	$ -	$ -	$ -	$ -
(40) Capital Outlay >$5k	$ -	$ -	$ -	$ -
(45) Capital Outlay <$5k	$ -	$ -	$ -	$ -
(70) Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ -	$ -	$ -	$ -
			Total Sub-budgets	$ -