Grant Code: New

Title: Discovering wheat mutations for herbicide resistance

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Justification/Rationale: Common wheat (*Triticum aestivum* L.) is a stable food crop in the world. Wheat is also the top cereal crop in Idaho, USA. Dr. Daolin Fu's group recently treated the Idaho wheat variety 'Brundage' using ethyl methanesulfonate (EMS), fast neutron, and gamma array and has generated more than 10,000 mutant lines, which can be used to identify novel trait for breeding.

Herbicide resistance is one value-added trait in US farming. Gain of herbicide resistance is common in weedy plants due to mutations in genes that are targeted by specific herbicides (Devine and Shukla 2000). In wheat, an A2004V mutation (Ostlie et al. 2015) in the Acetyl co-enzyme A carboxylase (*ACC1*) gene has been successfully used to assemble the CoAXium Wheat Production System (www.coaxiumwps.com). It will be important to screen the Brundage mutant population in order to identify herbicide-resisting wheat, which will then be used to innovate cropping systems for effective weed control. To accomplish this, it is necessary to conceive three layers of research: 1) to identity wheat mutants with gained resistance to one or several herbicide groups, 2) to stack effective mutations for assembling novel and broad herbicide resistance, and 3) to establish practical cropping systems that will take advantage of novel herbicide resistance in wheat.

Roundup Ready (RR) crops' successes indicate that modern agriculture highly depends on the use of herbicide resistance trait. However, RR crops are genetically modified organisms (GMO), which causes global controversy. It is desirable to generate herbicide resistant wheat through traditional approaches (e.g. mutagenesis) or other acceptable biotechnology (e.g. gene editing). In wheat, the Clearfield Production Systems are widely applied to control grasses and broadleaf weeds. In addition to the Clearfield and CoAXium Production Systems, it is urgent to develop novel herbicide resistance trait in wheat. Successful use of herbicide resistant wheat will help growers to get the most out of every acre.

Hypothesis & Objectives: Herbicides kill weeds by inhibiting essential biological processes, specifically by repressing essential enzyme proteins. Herbicide-susceptible weed species gain herbicide resistance by acquiring an insensitive enzyme gene by natural or induced mutagenesis. Wheat is an amenable species to gain herbicide resistance. Our long term goal is to develop herbicide-resistant wheat in Idaho. Towards this end, we propose five objectives: 1) screen insensitive mutants to herbicides with different modes of action (Table 1), 2) identify wheat nuclear genes that are potentially targeted by herbicides, 3) screen mutations in "target-site genes" in wheat mutant population, 4) test "target-site mutations" for their responses to specific herbicides, and 5) stack effective mutations for controlling grassy weeds in wheat fields.

Procedures/Plan of Work: Main approaches are to identify wheat lines with gain of resistance to selected herbicides, identify "target-site mutations" using high throughput genotyping, and to stack effective "target-site mutations" using traditional breeding. Objectives and procedures are discussed as below.

Objective 1: Screen insensitive mutants to herbicides with different modes of action. To date, we have generated > 10,000 mutant lines in the wheat variety 'Brundage'. As in Table 1, we have started to test herbicides with different modes of action. Large sets of composite M_3 seeds were planted in the Parker Farm, University of Idaho. They have been treated with six different herbicides (Table 1) and will be treated with other types of herbicides that are applicable in the Spring growing season. Small sets of composite M_3 seeds have been tested in the 6^{th} street greenhouse, University of Idaho. Tolerant or insensitive wheat mutant to selected herbicides will be identified in greenhouse and field tests.

Objective 2. Identify wheat nuclear genes that are potentially targeted by herbicides. Target site mechanisms is a common phenomenon in herbicide resistance (Devine and Shukla 2000). In many cases, a modified site in the herbicide target protein reduces its affinity to specific herbicide (Devine and Shukla 2000). In contrast, enormous amplification/overexpression of a target protein also counteracts specific herbicides (Gaines et al. 2010). Known herbicide target proteins including acetyl-CoA carboxylase, acetolactate synthase, tubulin polymerization, and many others (Devine and Shukla 2000) will be fully reviewed. Using them as entry, we will study their wheat orthologues, such as their copy numbers and expression profiles in common wheat (e.g. 'Chinese Spring"). The single or low copy functional genes will be selected as preferable candidate for mutation screening.

Objective 3. Screen mutations in "target-site genes" in wheat mutant population. We will identify mutations of selected target-site genes in the 'Brundage' mutant population. Mutation screening in genes of interest can be retrieved using traditional method (Uauy et al. 2009) or high throughput amplicon sequencing (Tsuda et al. 2015). The 'Brundage' mutant population has over 10,000 M_3 lines. Leaf tissues of 100 lines in a row will be mixed to make a DNA row pool, and leaf tissues of 100 lines in a column were mixed to make a DNA column pool. Two hundred DNA pools will represent 10,000 mutant lines in a 100×100 design. In the leading PI's lab, amplicon sequencing technology has been successfully used to identify mutations of the alpha amylase (*Amy3*) gene from nearly 700 transgenic plants. Similar approach will be used to identify mutation in genes of interest using multiple amplicon sequencing from 200 DNA pools.

Objective 4. Test "target-site mutations" for their responses to specific herbicides. Any target-site mutation may impact its affinity to specific herbicide. Among all identified target-site mutations, we will test their response to selected herbicides as done in Objective 1.

Objective 5. Stack effective mutations for controlling grassy weeds in wheat fields. Mutants with high levels of resistance or tolerance to herbicides (Objectives 1 and 4) will be stacked to assemble more sustainable herbicide resistance in wheat, which will be designed to reduce dose rates of herbicides while maintaining its efficacy in weed control.

Duration: Four years, FY 2020 is the first year.

Year 1 (FY 2020): a) Screen insensitive mutants to six selected herbicides (Table 1) and b) identify wheat nuclear genes that are potentially targeted by herbicides.

Year 2 (FY 2021): a) Identify mutations in "target-site genes" in wheat and b) test their responses to herbicides with selected mode of action.

Year 3 (FY 2022): a) Stack effective mutations in homologous or non-homologous "target-site" genes and b) test how the stacked mutations response to herbicides with single or combined modes of action.
Year 4 (FY 2023): Release elite mutation lines for herbicide resistance breeding.

Cooperation/Collaboration: Dr. Daolin Fu and Joan Campbell will lead the project and work with visiting scholars, graduate students and/or temporary help on mutant identification and herbicide tests. Fu and Campbell will present research findings at professional conferences, and communicate research progress and results to the Idaho Wheat Commission. Visiting scholars, graduate students and other personnel working on mutation screening and herbicide resistance will report progress to Fu and Campbell. The research team will regularly communicate via e-mail, phone, and face-to-face meetings. Herbicide resistance will be tested in facilities accessible to the University of Idaho.

Anticipated Benefits, Expected Outcomes and Impacts and Transfer of Information:
Thousands of growers and stakeholders will benefit from these efforts. Beneficiaries of this project will be all growers affected or potentially affected by grassy weeds, as well as the Idaho wheat industry as a whole by providing new genetic material in wheat for resistance to herbicides.
The generation of herbicide-resistant wheat by mutagenesis offers a fast toolbox for understanding gene function, which in combination with gene editing can deliver more desirable wheat variety with value-added trait. In comparison to Round-up crops (GMO), both mutagenesis and gene editing are more plausible for growers and wheat industry.
The success of the project will be measured by presentation of research results at the annual ASA and CSSA Meeting (American Society of Agronomy & Crop Science Society of America, over 1,500 scientist attendants) and publication of 1-2 research articles in the peer-referred journals.

Literature Review: Common wheat (*Triticum aestivum* L.) is a staple food crop that provides about 20% of global calories for human consumption (Shewry and Hey 2015). The increasing human population worldwide (United-Nations 2015) will place an even greater demand for wheat production globally. However, wheat production can be compromised by arable weeds. Overall, average yield loss with no weed control can be up to 40% in wheat[1]. With no weed control, potential loss in value for wheat will be approximately $3.5 billion based on the 2017 data in US.
Herbicides play an important role in ensuring wheat output as they can reduce weed populations that compete for resources in nature. However, resistant weeds can survive herbicide application via target-site resistance (TSR) and non-target-site resistance (NTSR) (Délye et al. 2013), which negates a wide choice of effective herbicides. Herbicides exhibit different modes of action (Délye et al. 2013; Duke 1990; Duke 2012; Wallace et al. 2018; Yan et al. 2018). Breeding wheat with resistance to herbicides of different modes of action will facilitate Integrated Pest Management (IPM) for weed control in wheat production. Herbicide resistant crops can be developed through genetic transformation, mutagenesis, and other methods (Rizwan et al. 2015). In wheat, an A2004V mutation of the Acetyl co-enzyme A carboxylase (ACC1) gene (Ostlie et al. 2015) is successfully used to assemble the CoAXium Wheat Production System[2]. Therefore,

[1] www.bigyield.us/weeds-may-cause-40-percent-yield-loss-in-wheat/
[2] www.coaxiumwps.com/

discovery and application of wheat with resistance to herbicides is important for wheat production in US.

Table 1: Herbicides used to screen wheat mutants in 2018-2019

Herbicides	Application timing	Dose rate	Active ingredients	Location
Axiom	Pre-emergence	24 oz/acre	Metribuzin Flufenacet	Field
Anthem Flex	Pre-emergence	15 oz/acre	Carfentrazone Pyroxasulfone	Field
Valor	Pre-emergence	10 oz/acre	Flumioxazin	Field
Dual Magnum	Pre-emergence	5 pints/acre	s-metolachlor	Field
Outlook	Pre-emergence	64 fl oz/acre	Dimethenamid-P	Field
Metribuzin	Pre-emergence	30 oz/acre	Metribuzin	Field
Dual Magnum	Pre-emergence	Various doses	s-metolachlor	GH
Zidua	Pre-emergence	Various doses	Pyroxasulfone	GH
Liberty	2 to 3 leaf	Various doses	Glufosinate-ammonium	GH

Note: Herbicides have been used to screen the mutant population of 'Brundage'.

FY2020

IDAHO WHEAT COMMISSION - BUDGET FORM

Principal Investigator: Daolin Fu

Allocated by	Idaho Wheat Commission	during FY 2018	$ -
Allocated by	Idaho Wheat Commission	during FY 2019	$ -

REQUESTED FY2020 SUPPORT:

Budget Categories	(10) Salaries (*staff, post-docs, etc.*)	(12) Temp Help	(11) Fringe	(20) Travel	(30) OE	(70) Graduate Tuition/ Fees	TOTALS
Idaho Wheat Commission	$ -	$ 31,000	$ 2,697	$ -	$ 14,000	$ -	$ 47,697

TOTAL BUDGET REQUEST FOR FY 2020: $ 47,697

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

Budget Categories	*Daolin Fu*	*Joan Campbell*	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*
(10) Salaries	$ -	$ -	$ -	$ -
(12) Temp Help	$ 25,000	$ 6,000	$ -	$ -
(11) Fringe Benefits	$ 2,175	$ 522	$ -	$ -
(20) Travel	$ -	$ -	$ -	$ -
(30) Other Expenses	$ 12,000	$ 2,000	$ -	$ -
(70) Graduate Student Tuition/F	$ -	$ -	$ -	$ -
TOTALS	$ 39,175	$ 8,522	$ -	$ -

Total Sub-budgets $ 47,697

Explanatory Comments:

Fall 2018 Version