PROJECT NO: AP3846

TITLE: Discovering wheat mutations for herbicide resistance

PERSONNEL: Daolin Fu, Saket Chandra, Joan Campbell, Department of Plant Sciences, University of Idaho.
ADDRESS: 875 Perimeter Drive, Moscow, ID 83844 (208-885-1542, dlfu@uidaho.edu)

JUSTIFICATION:

Wheat is an important cereal crop worldwide; it ranks third in the U.S.A[1] in terms of total yield and acreage. According to FAO the global wheat production is decreased by 1 million tons in 2019. Weeds are important biotic factors that suppress crop production by competing resources that are essential for crops(Rojano-Delgado *et al.*, 2015). Noxious weeds can cause yield losses up to 50% in wheat(Jabran *et al.*, 2017).

Herbicide resistance is one value-added trait in crop management. Cereal crops that contain a herbicide resistant trait are considered to be cost effective and could assist growers to better manage weed problems(ISAAA, 2016). Traditional breeding has long been implemented in crop improvement. However, it takes long time and tedious work to advance from selecting desirable parents, making crosses, to releasing an elite cultivar. In recently years, introduction of foreign genes into cultivars has been used to harness desirable traits(Prado *et al.*, 2014). However, many people worry that genetically modified crops possess environmental and health threat, which demands much time and huge expenditure before releasing a genetically modified cultivar(Cui & Shoemaker, 2018, Napier *et al.*, 2019). For instance, Roundup Ready (RR) crops are featured by its resistance to a broad-spectrum herbicide glyphosate, but as genetically modified organisms, RR crops and the wide use of glyphosate have caused global controversy. Hence, a more efficient and sustainable breeding is required. In wheat, the Clearfield and CoAxium Production Systems(Tan *et al.*, 2005, Nakka *et al.*, 2019) were produced by Ethyl methanosulphate mutagenesis. Both of these are successful cases with an herbicide resistance trait. In addition to the Clearfield and CoAXium wheat, it is urgent to develop novel herbicide resistance in wheat. Today, genome editing(Chen *et al.*, 2019) and TILLING(Uauy *et al.*, 2009) can be used to accelerate plant breeding for herbicide resistance.

Wheat is the top cereal crop in Idaho, USA. We have generated more than 10,000 mutant lines from the Idaho wheat 'Brundage' and established genome editing approaches in polyploidy wheat. Using genome editing and/or TILLING, we aim to study herbicide-target genes and their mode of inheritance in conferring herbicide resistance and release herbicide-resistant wheat. Successful use of herbicide resistant wheat will help growers to get the most out of every acre.

HYPOTHESIS & OBJECTIVES:

Weeds cause considerable amount of loss in wheat production in the U.S.A and Canada. Wheat growers prefer to cost-effective methods to manage weeds and their removal from the field.

[1] https://www.ers.usda.gov/topics/crops/wheat/

Herbicides as one group of pesticides are used to kill or suppress weeds. The use of herbicide resistant crops is important for increasing wheat production. Herbicide-susceptible weeds gain herbicide resistance by acquiring an insensitive enzyme 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 six 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; 5) edit genes of interest to install insensitive target sites that confer herbicide resistance; and 6) stack effective mutations for controlling grassy weeds in wheat fields.

PROCEDURES:

Main approaches are to identify wheat lines with gain of resistance to selected herbicides, identify "target-site mutations" using high throughput genotyping, install insensitive target sites using gene editing, 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 6th 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. Several studies related to herbicide resistance is linked with modification, overexpression and duplication of genes in weeds that is responsible for imparting resistance against herbicides(Powles & Yu, 2010, Délye *et al.*, 2013). In most cases, an altered site in the herbicide target protein reduces its affinity to specific herbicide and results in its resistance against that particular herbicide (Devine & Shukla, 2000). Known herbicide target proteins are: acetyl-CoA carboxylase, acetolactate synthase, 5-enolpyruvylshikimate-3-phosphate synthase, Glutamine synthetase, 4-hydroxy phenylpyruvate dioxygenase, phytoene desaturase, protoporphyrinogen oxidase and many others (Devine & Shukla, 2000). All of these genes will be fully reviewed. The orthologs of these genes will be retrieved from the latest release of wheat genome. The extracted genes sequence will be further characterized *in silico*, in common wheat (e.g. 'Chinese Spring"). The single or low copy functional genes will be selected as preferable candidate for mutagenesis.

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. Precise genome editing for herbicide resistance. Plant genome editing in the specific region is now possible due to the availability of engineered nucleases like clustered regularly interspaced short palindromic repeats (CRISPR). This technology is helpful in fast-tracked molecular breeding. The specific single guide RNA (sgRNA) will be designed for accurate gene editing. For implementing this technology, the detailed review of the gene will be performed to identify the sites that makes the gene insensitive to herbicides in weeds(Han & Kim, 2019). The site that has been reported to bring herbicide resistance naturally in weed species will be selected for genome editing in wheat. The prime editing approach(Anzalone *et al.*, 2019) will be used to introduce mutations in wheat.

Objective 5. 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 6. Stack effective mutations for controlling grassy weeds in wheat fields. Mutants with high levels of resistance or tolerance to herbicides (Objectives 1, 3, 4 and 5) 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. Once, the causal gene is identified then genome editing approach will be initiated.

Year 2 (FY 2021): a) Identify mutations in "target-site genes" in wheat, b) install insensitive target sites using genome editing and c) test plant 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: Dr. Daolin Fu and Dr. Joan Campbell will lead the project and work with Dr. Saket Chandra, graduate students and/or temporary help on mutant identification and herbicide tests. Fu, Saket and Campbell will present research findings at professional conferences, and communicate research progress and results to the Idaho Wheat Commission. Saket Chandra, graduate students and other personnel working on mutation screening, gene editing 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/INFORMATION TRANSFER:

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:

Wheat (*Triticum aestivum* L.) is an important part of the global agricultural economy and accounts for 218.5 Mha of cultivated land per year(FAOSTAT, 2017). Sustainable production of wheat is important for securing food for an increasing world population. Weed intrusion during different stages of wheat growth considerably reduces global wheat production. Without weed management, wheat production can be decreased by 50%(Oerke, 2005, Oad *et al.*, 2007, Gharde *et al.*, 2018). Managing weeds with herbicides has been proven very efficient in developed countries(Gianessi, 2013). Due to wide and lasting usage of herbicides, numerous cases of weeds, together with crops, develop herbicide resistance. Until now, herbicide resistance has been reported in about 255 weed species(Heap, 2019). Worldwide the wheat field ranks first with recorded herbicide resistant weeds, including 140 incidences from 77 species(Heap, 2019, Nakka et al., 2019). There are basically two modes of herbicide resistance: a) detoxification of herbicides before reaching the target site; or b) modification in the target gene. The target site herbicide resistance is generally achieved by alteration or overexpression of genes responsible for herbicide resistance. There are several reports of achieving herbicide resistance trait by altering the target genes(Nakka et al., 2019). For example, acetolactate synthase, 5-enol pyruvylshikimate 3-phosphate synthase, and glutamine synthetase are all involved in amino acid biosynthesis. They are targeted by the herbicide groups 2, 9, and 10, respectively(Peterson *et al.*, 2015). In wheat, the Clearfield and CoAxium systems (Tan et al., 2005, Nakka et al., 2019) are resistant against Imidazolinone and Aryloxyphenoxypropionate, respectively. The Clearfield wheat is based on a single amino acid change (Ser653Asp) in the Acetolactate synthase(Rojano-Delgado et al., 2015).; the CoAXium wheat is based on a single amino acid substitution (Ala2004Val) in the Acetyl CoA Carboxylase(Ostlie *et al.*, 2015). The success of these two-production system motivated us to screen our mutant lines for the value-added trait of herbicide resistance. In addition, precise

genome editing is practical to engineer insensitive target sites for herbicide resistance(Han & Kim, 2019).

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'.

References:

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., *et al.* (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature,* **576,** 149-157.

Chen, K., Wang, Y., Zhang, R., Zhang, H. and Gao, C. (2019) CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual Review of Plant Biology,* **70,** 667-697.

Cui, K. and Shoemaker, S. P. (2018) Public perception of genetically-modified (GM) food: A nationwide Chinese consumer study. *npj Science of Food,* **2,** 10.

Délye, C., Jasieniuk, M. and Le Corre, V. (2013) Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics,* **29,** 649-658.

Devine, M. D. and Shukla, A. (2000) Altered target sites as a mechanism of herbicide resistance. *Crop Protection,* **19,** 881-889.

FAOSTAT (2017) *Crop statistics*. Rome: Food and Agriculture Organization of the United Nations.

Gharde, Y., Singh, P. K., Dubey, R. P. and Gupta, P. K. (2018) Assessment of yield and economic losses in agriculture due to weeds in India. *Crop Protection,* **107,** 12-18.

Gianessi, L. P. (2013) The increasing importance of herbicides in worldwide crop production. *Pest Management Science,* **69,** 1099-1105.

Han, Y.-J. and Kim, J.-I. (2019) Application of CRISPR/Cas9-mediated gene editing for the development of herbicide-resistant plants. *Plant Biotechnology Reports,* **13,** 447-457.

Heap, I. (2019) The international survey of herbicide resistant weeds. Online.

ISAAA (2016) *Global status of commercialized biotech/GM crops: 2016*. Ithaca, NY: The International Service for the Acquisition of Agri-biotech Applications (ISAAA).

Jabran, K., Mahmood, K., Melander, B., Bajwa, A. A. and Kudsk, P. (2017) Chapter Three - Weed dynamics and management in wheat. In: *Advances in Agronomy.* (Sparks, D. L., ed.). Academic Press, pp. 97-166.

Nakka, S., Jugulam, M., Peterson, D. and Asif, M. (2019) Herbicide resistance: Development of wheat production systems and current status of resistant weeds in wheat cropping systems. *The Crop Journal,* **7,** 750-760.

Napier, J. A., Haslam, R. P., Tsalavouta, M. and Sayanova, O. (2019) The challenges of delivering genetically modified crops with nutritional enhancement traits. *Nature Plants,* **5,** 563-567.

Oad, F. C., Siddiqui, M. H. and Buriro, U. A. (2007) Growth and yield losses in wheat due to different weed densities . . *Asian Journal of Plant Sciences,* **6,** 173-176.

Oerke, E. C. (2005) Crop losses to pests. *The Journal of Agricultural Science,* **144,** 31-43.

Ostlie, M., Haley, S., Anderson, V., Shaner, D., Manmathan, H., Beil, C., *et al.* (2015) Development and characterization of mutant winter wheat (*Triticum aestivum* L.) accessions resistant to the herbicide quizalofop. *Theoretical and Applied Genetics,* **128,** 343-351.

Peterson, D. E., Thompson, C. R., Shoup, D. E. and Jugulam, M. (2015) Herbicide mode of action. Manhattan, KS: Kansas State University Agricultural Experiment Station and Cooperative Extension.

Powles, S. B. and Yu, Q. (2010) Evolution in action: Plants resistant to herbicides. *Annual Review of Plant Biology,* **61,** 317-347.

Prado, J. R., Segers, G., Voelker, T., Carson, D., Dobert, R., Phillips, J., *et al.* (2014) Genetically engineered crops: From idea to product. *Annual Review of Plant Biology,* **65,** 769-790.

Rojano-Delgado, A. M., Priego-Capote, F., Luque de Castro, M. D. and De Prado, R. (2015) Mechanism of imazamox resistance of the Clearfield® wheat cultivar for better weed control. *Agronomy for Sustainable Development,* **35,** 639-648.

Tan, S., Evans, R. R., Dahmer, M. L., Singh, B. K. and Shaner, D. L. (2005) Imidazolinone-tolerant crops: history, current status and future. *Pest Management Science,* **61,** 246-257.

Tsuda, M., Kaga, A., Anai, T., Shimizu, T., Sayama, T., Takagi, K., *et al.* (2015) Construction of a high-density mutant library in soybean and development of a mutant retrieval method using amplicon sequencing. *BMC Genomics,* **16,** 1014.

Uauy, C., Paraiso, F., Colasuonno, P., Tran, R., Tsai, H., Berardi, S., *et al.* (2009) A modified TILLING approach to detect induced mutations in tetraploid and hexaploid wheat. *BMC Plant Biology,* **9,** 115.

FY2021

IDAHO WHEAT COMMISSION - BUDGET FORM

Principal Investigator: Daolin Fu, et al.

If applicable,	**Allocated by**	**Idaho Wheat Commission**	**during FY 2019**	**$ -**
If applicable,	**Allocated by**	**Idaho Wheat Commission**	**during FY 2020**	**$ 21,200**

REQUESTED FY2021 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	$ -	$ 34,000	$ 3,026	$ 2,000	$ 19,000	$ -	$ 58,026

TOTAL BUDGET REQUEST FOR FY 2021: **$ 58,026**

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

Budget Categories	*Daolin Fu*	*Joan Campbell*	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*
(10) Salaries	$ -	$ -	$ -	$ -
(12) Temp Help	$ 30,000	$ 4,000	$ -	$ -
(11) Fringe Benefits	$ 2,670	$ 356	$ -	$ -
(20) Travel	$ 2,000	$ -	$ -	$ -
(30) Other Expenses	$ 18,000	$ 1,000	$ -	$ -
(70) Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ 52,670	$ 5,356	$ -	$ -

Total Sub-budgets **$ 58,026**

Brief Explanatory Comments: (see FY2021 RFP for guidance)

Fall 2019 Version

ANNUAL REPORT

PROJECT NO: AP3846

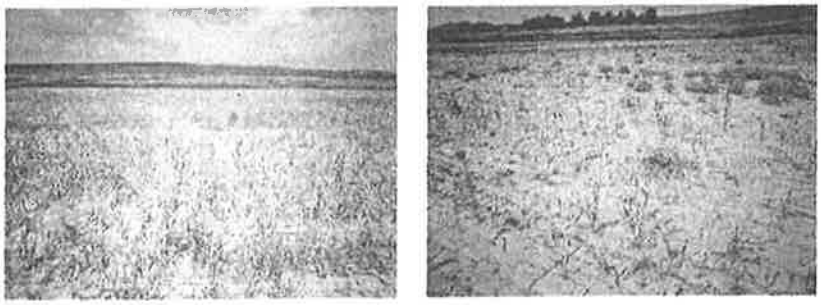
TITLE: Discovering wheat mutations for herbicide resistance

PERSONNEL: Saket Chandra, Huifei Zhang, Mengmeng Lin, Joan Campbell, Daolin Fu

ADDRESS: Daolin Fu, 875 Perimeter Dr., Moscow, ID 83844; 208-885-1542; dlfu@uidaho.edu

ACCOMPLISHMENTS:

***Wheat mutant screening with selected herbicide groups*:** The current focus is to identify wheat mutations that may confer resistance to herbicides with specific mode of action. In field, the wheat Brundage population were treated with six herbicides, including Axiom, Anthem Flex, Dual Magnum, Metribuzin, Outlook and Valor (Figure 1). Unfortunately, a large number of wheat plants survived the applied dose, which prevented the selection of promising herbicide resistant mutants. In greenhouse tests, we identified three mutant plants that are potentially resistant to Dual Magnum (metolachlor). Progeny test of the selected lines will be conducted in 2020.



Anthem Flex at 15 oz per acre Valor at 10 oz per acre

Figure 1. Herbicide test in the Parker Farm, Moscow, ID

***Wheat mutation identification for herbicide target genes*:** To facilitate DNA-based screening, we planted 10,404 M_3 wheat mutants in greenhouse, which were arranged in a 102×102 matrix. Every 102 plants in a row or a column were collected as one DNA pool. We have extracted 102 row DNA pools and 102 column DNA pools. Using PCR, a desirable mutation can be located by cross matching the positive row and column DNA pools.

The acetyl co-enzyme A carboxylase (*ACC1*) is the target of the group 1 herbicides(Nikolskaya *et al.*, 1999). In wheat, the A1992V (or called A2004V)(Ostlie *et al.*, 2015) mutation is resistant to quizalofop-P-ethyl (Aggressor), the key herbicide of the CoAXium Wheat Production System. We decided to target three residues, including Ala_{1992}, Asp_{2066}, and Gly_{2084} (Table 1). Using a customized PCR approach, we have identified three potential *ACC1* mutation. We will test whether the identified mutations confer herbicide resistance in 2020.

We also started to look for other herbicide target genes. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme that is found only in microorganisms and plants. The non-availability of this enzyme in animals makes it an important target for herbicides, like glyphosate. This gene has been reported to be present in 8,833 species(El-Gebali *et al.*, 2018). In nature, most of the glyphosate resistance is due to mutation at position Thr_{102} and Pro_{106}, which are located to the most conserved region in EPSPS. Therefore, we plan to initiate mutant screening for these two sites for further investigation.

Table 1. Target residues in the grass ACC1 protein

Species	Genotype	Gene Name	Gene ID	Residue 1992	Residue 2066	Residue 2084
A. myosuroides	V1	*AmACC1*	AJ310767	Ala	Asp	Gly
Triticum aestivum	CS	*TaACC-A1*	TraesCS2A01G069400	Ala	Asp	Gly
T. dicoccoides	Zavitan	*TdACC-A1*	na	Ala	Asp	Gly
T. aestivum	CS	*TaACC-B1*	TraesCS2B01G082500	Ala	Asp	Gly
T. dicoccoides	Zavitan	*TdACC-B1*	na	Ala	Asp	Gly
T. aestivum	CS	*TaACC-D1*	TraesCS2D01G068100	Ala	Asp	Gly
T. aestivum	Tam 107	*TaACC-D1*	AF029895	Ala	Asp	Gly
Aegilops tauschii	AL8/78	*AeACC1*	na	Ala	Asp	Gly
Hordeum vulgare	Morex	*HvACC1*	HORVU2Hr1G010440	Ala	Asp	Gly
H. vulgare	Morex	*HvACC1*	MK481065	Ala	Asp	Gly
H. vulgare	GP	*HvACC1*	MK492375	Ala	Asp	Gly
H. vulgare	GP	*HvACC1*	MK492376	Ala	Asp	Gly
H. vulgare	Tamalpais	*HvACC1*	MK481066, MK481069	Ala	Asp	Gly
Herbicide resistant residues				Val	Gly	Ala
Resistance to APPs				H	L-H	H
Resistance to CHDs				nd	L-H	S-L

Abbreviations: *Alopecurus* (*A.*), aryloxyphenoxypropionates (APPs), cyclohexanediones (CHDs), Chinese Spring (CS, Golden Promise (GP), not available (na), not determined (nd). Herbicide resistance indexes are not resistant (S), low resistance (L), and high resistance (H).

Acetolactate synthase (ALS) enzyme is the most important enzyme for the biosynthesis of branched chain amino acid like isoleucine, leucine and valine. This enzyme is the primary target for five classes of herbicides namely, imidazolinones, pyrimidinylthiobenzoates, sulfonylureas, triazolopyrimidin -es and sulfonylaminocarbonyltriazolinone. Herbicide resistant *ALS* genes occurred in wheat cultivar recently (Pozniak *et al.*, 2004, Li *et al.*, 2008, Zhang *et al.*, 2019a, Zhang *et al.*, 2019b). We plan to target four sites (Ala_{122}, Pro_{197}, Ala_{205} and Ser_{653}) for mutant screening in 2020.

***Prime editing with herbicide target genes*:** Prime editing is a powerful tool for gene manipulation(Anzalone *et al.*, 2019). We plan to edit *ACC1*, *ALS* and/or *EPSPS* in wheat to generate herbicide resistant alleles. Currently, we have requested the prime editing vectors and are preparing the plant-adapted systems.

PROJECTIONS:

In the past half a year, we performed both field and greenhouse-based screening for herbicide resistant wheat. At the same time, we have identified three herbicide target genes, *ACC1*, *ALS* and *EPSPS* in

wheat. Our goals are to identify the induced mutations of these target genes, or introduce desirable mutations in them. Identified mutants will be tested for herbicide resistance.

Actionable knowledge for Idaho Wheat Growers: It is feasible to introduce and identify desirable mutations in herbicide target genes. Herbicide resistance may be identified from the induced wheat mutant populations or may be acquired by gene editing.

Next Steps-Strategy: The study is ongoing. In 2020, we will continue to generate desirable mutations in the herbicide target genes *ACC1*, *ALS*, and/or *EPSPS*. Research results will be presented at the IWC research review.

Intentions for FY2021: Daolin Fu will lead to seek funding to continue the on-going research for herbicide resistance in wheat. We have submitted a renewal application to IWC for FY21.

PUBLICATIONS: Mengmeng Lin has submitted a manuscript, entitled as "CRISPR/Cas9-based editing of the acetyl CoA carboxylase (*ACC1*) gene in barley", to the Journal of Northeast Agricultural University (English Edition).

REFERENCES:

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., *et al.* (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature,* **576,** 149-157.

El-Gebali, S., Mistry, J., Bateman, A., Eddy, S. R., Luciani, A., Potter, S. C., *et al.* (2018) The Pfam protein families database in 2019. *Nucleic Acids Research,* **47,** D427-D432.

Li, D., Barclay, I., Jose, K., Stefanova, K. and Appels, R. (2008) A mutation at the Ala122 position of acetohydroxyacid synthase (AHAS) located on chromosome 6D of wheat: improved resistance to imidazolinone and a faster assay for marker assisted selection. *Molecular Breeding,* **22,** 217-225.

Miki, D. and Shimamoto, K. (2004) Simple RNAi vectors for stable and transient suppression of gene function in rice. *Plant and Cell Physiology,* **45,** 490-495.

Nikolskaya, T., Zagnitko, O., Tevzadze, G., Haselkorn, R. and Gornicki, P. (1999) Herbicide sensitivity determinant of wheat plastid acetyl-CoA carboxylase is located in a 400-amino acid fragment of the carboxyltransferase domain. *Proceedings of the National Academy of Sciences,* **96,** 14647-14651.

Ostlie, M., Haley, S., Anderson, V., Shaner, D., Manmathan, H., Beil, C., *et al.* (2015) Development and characterization of mutant winter wheat (*Triticum aestivum* L.) accessions resistant to the herbicide quizalofop. *Theoretical and Applied Genetics,* **128,** 343-351.

Pozniak, C. J., Birk, I. T., O'Donoughue, L. S., Ménard, C., Hucl, P. J. and Singh, B. K. (2004) Physiological and molecular characterization of mutation-derived imidazolinone resistance in spring wheat. *Crop Science,* **44,** 1434-1443.

Ulrich, J., Dao, V. A., Majumdar, U., Schmitt-Engel, C., Schwirz, J., Schultheis, D., *et al.* (2015) Large scale RNAi screen in Tribolium reveals novel target genes for pest control and the proteasome as prime target. *BMC Genomics,* **16,** 674.

Zhang, J., Khan, S. A., Hasse, C., Ruf, S., Heckel, D. G. and Bock, R. (2015) Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. *Science,* **347,** 991-994.

Zhang, R., Liu, J., Chai, Z., Chen, S., Bai, Y., Zong, Y., *et al.* (2019a) Generation of herbicide tolerance traits and a new selectable marker in wheat using base editing. *Nature Plants,* **5,** 480-485.

Zhang, Y., Malzahn, A. A., Sretenovic, S. and Qi, Y. (2019b) The emerging and uncultivated potential of CRISPR technology in plant science. *Nature Plants,* **5,** 778-794.