Grant Code: New
Title: Increasing Wheat Grain Size by Adjusting a Growth Regulating Factor
Personnel: Zonglie Hong, Ph.D., Associate Professor, Department of Plant Sciences
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Justification/Rationale:
Grain size is one of the determining factors for grain yield in cereals including rice and wheat. Although it could be affected by physiological and environmental factors, the grain size trait is largely controlled by genes. Over the past several decades, a great number of quantitative trait loci (QTLs) have been identified to affect grain size (Huang et al. 2013). However, it is until recently that genes underlying the grain size trait have been cloned and studied. Recent investigations by our group and several other independent teams worldwide have identified a key QTL that has a major effect on grain size in rice. The rice mutant isolated by our group produces drastically elongated and enlarged grains. Genetic mapping and molecular biology studies have revealed that a mutation in a Growth-Regulating Factor (GRF4) gene is responsible for the increase in grain size (Chen et al. 2019). Unlike rice that has only one GRF4 gene in its diploid genome, wheat has three copies of the *GRF4* gene in its hexaploid genome. This proposal will take advantage of the progress in the rice GRF4 studies and investigate potential roles of GRF4 in grain development in wheat. We reason that if a mutation in GRF4 could cause such a dramatic change in grain size in rice, expression of similar GRF4 mutations may also result in enlargement of grain size in wheat. Grain size has been a traditional prime target for wheat breeding programs. In addition to being a major component of grain yield, grain size also affects nutrition quality and market value of the wheat product. Successful completion of the proposed project would provide breeders with molecular markers for more efficient selection of grain traits.

Hypothesis & Objectives:
Recent studies in rice has firmly established that GRF4 plays a key role in grain size regulation, and mutations in the GRF4 gene could lead to formation of large grains. Although there are three GRF4 genes in the wheat genome, their potential functions in grain development remain unknown. The GRF4 gene is present on the long arm of the rice chromosome 2, and the three GRF4 genes are also found on the wheat chromosomes 2A, 2B and 2D, respectively. The high sequence homology (about 60% amino acid identity and 70% similarity) and similar chromosomal organization between rice and wheat implies that the wheat GRF4 genes may play a similar role in grain size regulation as in rice. We further hypothesize that expression of the dominant mutant allele of GRF4 that underlies the large-grain trait in rice would also allow development of similar large-grain phenotype in wheat in a dominant manner.

The long-term goal of this program is to help wheat breeders with molecular tools for efficient trait selection and cultivar development. The immediate objectives of this proposal are to investigate the roles of GRF4 genes in grain formation in wheat, and to create a GRF4 mutation that mimics the dominant allele of the rice GRF4 mutant that underlies the large-grain trait in rice. In the coming year of project, we will pursue the following three specific objectives:

1) Evaluation of the natural variation in the GRF4 DNA sequence on grain size in wheat. We will select representative wheat germplasm lines that develop either large grains and small grains, and investigate if the natural grain size variation is correlated with polymorphisms in GRF4. Primers corresponding to each of the three GRF4 cDNAs will be used to amplify GRF4 cDNAs

from the 10 wheat representative lines. The total 30 GRF4 cDNAs will be sequenced and analyzed in comparison with the control cultivar. It is expected that the 5 germplasms with large grains may contain some characteristic changes in DNA sequence in GRF4 genes.

2) Creation of a dominant mutation in one of the wheat GRF4 gene. The rice GRF4 mutant contains a change of two base-pairs (from TT to AA) in the third exon of the GRF gene. The mutated gene encodes a peptide that replace a highly conserved amino acid residue Serine with a Lysine, which is likely to serve as a constitutively active GRF4. In addition, the mutant mRNA of GRF4 becomes more stable because the mutation also eliminates a microRNA binding site. The dual effects of the mutation allow the mutated GRF4 behalves in a dominant manner. We will clone the wheat GRF4 cDNA and create an identical mutation using the PCR-based mutagenesis approach.
3) Expression of the dominant active allele of GRF4 in wheat and evaluation of grain size phenotype. The mutated GRF4 cDNA will be expressed under the GRF4 gene promoter. This expression construct will be made in a T-DNA vector and used for wheat transformation via *Agrobacterium*-mediated approach. Alternatively, gene-editing of the GRF4 genes via the CRISPR-Cas9 method could also be carried out. Because all three wheat GRF4 genes are conserved in the target region of gene-editing, this CRISPR-Cas9 approach could potentially change the two base-pairs from TT to AA in all three GRF4 genes simultaneously.

Procedures/Plan of work:

Seeds of wheat lines with large and small grains will be chosen from The National Small Grains Collection at USDA-ARS, Aberdeen ID. Wheat seedlings will be grown in a greenhouse. Total RNA will be isolated and cDNA will be prepared. Primers specific to each of the three wheat GRF4 genes will be synthesized and used for amplification of the GRF4 coding regions. DNA sequences of the three GRF4 cDNAs will be compared with the control cultivar, and potential correlations between the grain sizes and GRF4 DNA sequence variations will be analyzed. For Objective 2, we will use PCR-based mutagenesis approach to replace the two base-pairs from TC to AA, as in the rice GRF4 large-grain allele. This substitution would create a mutated peptide product in which a highly conserved Serine (S) residue (at amino acid position 163) would be replaced with a Lysine (K), generating a constitutively active dominant allele of GRF4. For Objective 3, the mutated and constitutively active allele of GRF4 will be expressed in transgenic wheat via *Agrobacterium*-mediated transformation. Transgenic wheat seedlings will be analyzed for expression of the GRF4 mutant allele, and grain size changes will be scored. The mRNA levels of the GRF4 mutant allele will also be assayed using real-time PCR. Transgenic wheat plants are expected to express and maintain an elevated level of the GRF4 mutant allele, and the grain size is expected to increase. If time permits, we may initiate direct genome editing via CRISPR-Cas9, to create transgenic wheat plants expressing the Serine-163-Lysine (S163K) mutation from all three GRF4 genes. We have the reagents, experience and facilities necessary to carry out the proposed work (Xie et al., 2011; Wang et al., 2012; 2013; Wang et al 2016).

Duration:

Obj. 1 will be initiated at the onset of the project and can be completed in 6 months. Obj. 2 will also be carried out as soon as wheat seedlings become available for RNA extraction. Mutagenesis of the GRF4 cDNAs and construction of DNA expression vectors will be completed in 6 months. Objective 3 will be started as soon as the DNA constructs are created. Wheat transformation is time consuming in general, but we have extensive experience with genetic transformation of cereals. We anticipate to complete all proposed work in the project year.

Cooperation/Collaboration:
Dr. Z. Hong will oversee the project and also carry out some proposed work. A graduate student will be recruited to work full-time on this project. With proper training, a graduate student should be able to carry out all the proposed tasks.

Anticipated Benefits, Expected Outcomes and Impacts, and Transfer of Information:
The direct outcome of this project is to provide evidence for the role of GRF4 genes in grain size regulation in wheat. If the hypothesis is proven correct, the mutant variant of GRF4 can be used to increase grain size and breeders can use this gene as a molecular marker to select and develop new cultivars with increased grain size. From the study in rice, we learnt that there are pleiotropic effects of the GRF4 mutation. In addition to producing larger grains, the GRF4 mutant is also more resistant to low temperature treatment. Therefore, it is highly likely that introduction of a similar mutation in wheat GRF4 may also lead to development of a new cultivar with increased grain size and enhanced cold tolerance.

Literature Review:
Grain size is genetically controlled by a large number of quantitative trait loci (QTLs). Recent genetic and molecular biology studies worldwide have led to the cloning of a group of genes that regulate grain size in rice and wheat (Huang et al., 2013). In addition, there are a great number of QTLs that have been mapped or associated with to the grain size trait. Recent studies by various independent groups worldwide on GRF4 have made a breakthrough in identification of genes that control grain size formation (Liu et al 2014; Che et al 2015; Duan et al 2015; Chen 2019). Growth-Regulating Factors are a family of highly conserved transcription factors that are only unique to plants. Members of this family have been implicated primarily in growth regulation of various tissues and processes in plants. GRF4 represents a key gene in regulating grain size, cold tolerance in rice and nitrogen assimilation (Li et al 2018). Recently, GRF4 has been implicated in grain weight regulation in the tetraploid wheat durum (Avni et al 2018). This proposal is focused on exploration of the possibility of increasing grain size in wheat by manipulating the function of the wheat *GRF4* genes. An improvement in grain size may eventually help breeders to develop new wheat cultivars with increased grain yield and improved grain quality.

Citations:
Avni R, Oren L, Shabtay G, et al (2018) Genes 9: 636
Che R, Tong H, Shi B, et al (2015) Nature Plants 2: 15195
Chen X, Jiang L, Zheng J, et al (2013) J Exp Bot 70: 3851-3866
Duan P, Ni S, Wang J, et al (2015) Nature Plants 2, 15203
Huang R, Jiang L, Zheng J, et al (2013) Trends Plant Sci 18: 218-226
Jiang L, Zheng J, Zhang Z, et al (2018) Bragantia 77: 452-465
Li S, Tian Y, Wu K, et al (2018) Nature 560: 595-600.
Liu H, Guo S, Xu Y, et al (2014) Plant Physiol 165: 160-174
Wang L, Wang L, Tan Q, et al (2016) Front Plant Sci 7: 1333.
Wang X, Gingrich DK, Deng Y, Hong Z (2012) Mol Bio Cell 23: 1446-1456
Xie B, Wang X, Zhu M, et al (2011) Plant J 65:1–14

FY2021

IDAHO WHEAT COMMISSION - BUDGET FORM

Principal Investigator: Zonglie Hong

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

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	$ 28,000	$ -	$ 952	$ 2,000	$ 5,000	$ -	$ 35,952

TOTAL BUDGET REQUEST FOR FY 2021: $ 35,952

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

Budget Categories	*(Insert PI Name)*	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*
(10) Salaries	$ -	$ -	$ -	$ -
(12) Temp Help	$ -	$ -	$ -	$ -
(11) Fringe Benefits	$ -	$ -	$ -	$ -
(20) Travel	$ -	$ -	$ -	$ -
(30) Other Expenses	$ -	$ -	$ -	$ -
(70) Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ -	$ -	$ -	$ -

Total Sub-budgets $ -

Brief Explanatory Comments: (see FY2021 RFP for guidance)

Fall 2019 Version