

# Minnesota Wheat Research and Promotion Council

## RESEARCH PROPOSAL GRANT APPLICATION

<b>1. NAME AND ADDRESS OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE</b>  <b>Name:</b> Regents of the University of Minnesota <b>Address:</b> Sponsored Projects Administration 454 McNamara Alumni Center, 200 Oak Street SE Minneapolis, MN 55455-2070		
<b>2. TITLE OF PROPOSAL</b> <b>Coordinated Effort to Isolate a Fusarium Head Blight Resistance Gene</b>		
<b>3. PRINCIPAL INVESTIGATOR(S)</b>  Gary J. Muehlbauer	<b>4. PI #1 BUSINESS ADDRESS</b>  Department of Agronomy and Plant Genetics 1991 Buford Circle 411 Borlaug Hall University of Minnesota St. Paul, MN 55108	
PI# 2 Name:		
PI# 3 Name:		
<b>5. PROPOSED PROJECT DATES (calendar years)</b>  1/01/11 – 12/31/12 Note: Research Reports are Due November 15th of Each Year	<b>6. TOTAL PROJECT COST</b>  \$ 67,000	<b>7. PI #1 PHONE NO.</b>  612-625-6228
<b>8. RESEARCH OBJECTIVES:</b> (List objectives to be accomplished by research grant)  Objective 1. Identify candidate genes for <i>Fhb1</i> .  Objective 2. Validate the candidate <i>Fhb1</i> gene.  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).		
<b>Signature Of Principal Investigator</b>	<b>Date</b>  11/22/10	<b>Phone Number</b>  612-625-6228
<b>Signature Of Authorized Representative</b>	<b>Title</b>	<b>Date</b>
<b>Address Of Authorized Representative</b>  Kevin McKoskey, Branch Mgr., McNamara Bldg. Suite 450, 200 Oak St Minneapolis MN 55455-2070		<b>Phone Number</b>

# Minnesota Wheat Research and Promotion Council

## RESEARCH PROJECT PROPOSAL

### (2-pages maximum)

**Project Title:** Coordinated effort to isolate a Fusarium head blight resistance gene

**Importance:** Fusarium head blight (FHB) is a major disease problem for Minnesota wheat growers. In the United States in 1993 alone, one billion dollars of losses was attributed to FHB (McMullen et al., 1997).

**Background:** Fusarium head blight (FHB) is a major disease problem for Minnesota wheat growers. There are few genes that have been identified that exhibit resistance to FHB. A major resistance gene located on chromosome 3BS referred to as *Fhb1*, exhibits partial resistance to FHB. *Fhb1* has been incorporated into breeding programs and resulted in new varieties with improved resistance. However, the new varieties are still susceptible during a severe FHB epidemic. Unfortunately, the *Fhb1* gene that underlies resistance has not been isolated.

A large collaboration of scientists from the University of Minnesota (Jim Anderson), Kansas State University (Bikram Gill, Eduard Akhunov) and Washington State University (Mike Pumphrey) has formed to isolate and characterize the *Fhb1* gene. We plan to use a combination of genetic and physical mapping, gene expression analysis and mutant characterization to isolate the gene. Having the gene will result in the perfect marker for marker-assisted selection for FHB resistance in breeding programs and be an ideal candidate for genetic engineering. This will ultimately benefit the growers through improved FHB resistant varieties. **There are two objectives: (1) identify candidate genes for *Fhb1*; and (2) validate the candidate *Fhb1* gene.**

**Relationship To Past Projects:** We have studied gene expression in wheat and barley during *F. graminearum* infection (e.g., Boddu et al., 2007; Jia et al., 2009; 2010; Gardiner et al., 2010). We have also developed transgenic wheat with enhanced resistance to FHB (Mackintosh et al., 2006; Shin et al., 2008). We have identified one gene (UDP-glucosyltransferase) that exhibits deoxynivalenol (DON) resistance (Schweiger et al., 2010) and have three other genes that we have preliminary evidence that they also exhibit DON resistance. We are developing transgenic wheat carrying the UDP-glucosyltransferase gene.

**Procedures: Objective 1. Identify candidate genes for *Fhb1*.** Dr. Jim Anderson has developed genetic stocks carrying either the resistant or the susceptible allele for the *Fhb1* gene. We inoculated these lines with *Fusarium graminearum* and sequenced the expressed genes. To date, we have over 40 million sequence reads from each of these genetic stocks. Our hypothesis is that the resistant genotype will carry the gene and the susceptible genotype will not carry the gene. Currently, we are becoming familiar with handling this size of dataset and are in the initial stages of examining it for genes that are specifically expressed in the resistant compared to the susceptible genotype. We plan to conduct a similar sequencing experiment with the same genotypes inoculated with DON. The *Fhb1* gene is thought to detoxify or regulate the detoxification of DON. Taken together, this work will reveal candidate genes for *Fhb1* based the expression of the gene in the resistant genotype and lack of expression of the gene in the susceptible genotype.

The Kansas State University and Washington State University groups will clone and sequence the region containing *Fhb1* from the FHB resistance source Sumai3. This approach is highly complementary to the approach that my laboratory will conduct. The five groups working on this project will combine the results from the two approaches and identify candidate genes for the *Fhb1* gene.

**Objective 2. Validate the candidate *Fhb1* gene.** Various approaches will be used by the group to test the *Fhb1* candidate genes including: developing and testing transgenic plants, and examining mutations in the candidate genes. My group will take the mutation approach as follows. Dr. Mike Pumphrey at Washington State University has developed a mutagenized population in the Sumai3 background. Sumai3 carries the *Fhb1* resistance gene. His group will identify mutations in the *Fhb1* candidate genes. My group in collaboration with Dr. Anderson will screen the mutants in the greenhouse following standard disease assays for FHB. Our expectation is that if we identify a mutation in the *Fhb1* gene the resulting plants will be susceptible to FHB.

#### **Research group:**

University of Minnesota – Gary J. Muehlbauer, Jim Anderson, Anna Sawyer (graduate student)

Kansas State University – Eduard Akhunov, Bikram Gill

Washington State University – Mike Pumphrey

**Regional Linkages To Other Research Activities:** Dr. Anderson has developed genetic stocks that carry either the resistant allele or susceptible allele for the *Fhb1* gene that will be used in the proposed study. Drs. Anderson, Gill, Ahkunov and Pumphrey have been working to isolate the *Fhb1* gene through standard map-based cloning approaches. The line of research proposed here is complementary to these ongoing research efforts to isolate the *Fhb1* gene.

**Additional Sources of Funding:**

Molecular genetic approaches to develop scab resistance, USWBSI, USDA-ARS, \$109,320, 5/10 – 5/11  
Enhancement of scab resistance in small grains by molecular genetics, Minnesota Small Grains Initiative, \$94,656, 7/09 – 7/11  
Barley Coordinated Agricultural Project: Leveraging genomics, genetics, and breeding for gene discovery and barley improvement, USDA-CSREES-NRI, \$5,000,000, 4/06 – 8/11  
Genetics of malting quality, USBGP, USDA-CSREES, \$59,062, 8/09 – 8/11  
Exploiting wild barley for crop improvement, USDA-NIFA, USBGP, \$37,600, 8/10 – 7/11  
Analysis of soybean fast neutron gene deletion mutants for root architecture, MSRPC, \$66,265, 5/10 – 5/11  
Advancing the barley genome, USDA-NIFA, \$1,000,000 (\$92,838 to Muehlbauer), 10/09 – 9/11  
Genomic analysis of shoot meristem function in maize, NSF, \$5,219,153 (\$848,304 to Muehlbauer), 9/08 – 10/12

**References:**

Boddu, J., S. Cho and G.J. Muehlbauer. 2007. Transcriptome analysis of trichothecene-induced gene expression in barley. *Mol. Plant-Microbe Interact.* 20:1364-1375.  
Gardiner, S.A., J. Boddu, F. Berthiller, C. Hametner, R. Stupar, G. Adam, and G.J. Muehlbauer. 2010. Transcriptome analysis of the barley-deoxynivalenol interaction: evidence for a role of glutathione in deoxynivalenol detoxification. *Mol. Plant-Microbe Interact.* 23:962-976.  
Jia, H., S. Cho and G.J. Muehlbauer. Transcriptome analysis of a wheat near-isogenic line pair carrying fusarium head blight resistant and susceptible alleles. *Mol. Plant-Microbe Interact.* 22:1366-1378.  
Jia, H., B. Millett, S. Cho, H. Bilgic, K.P. Smith, and G.J. Muehlbauer. Transcriptome analysis of three barley near-isogenic line pairs carrying contrasting alleles for different Fusarium head blight resistance QTLs. *Functional & Integrative Genomics* DOI 10.1007/s10142-010-0192-1.  
Mackintosh, C.A., J. Lewis, L.E. Radmer, S. Shin, S.J. Heinen, L.A. Smith, M.N. Wyckoff, R. Dill-Macky, C.K. Evans, S. Kravchenko, G.D. Baldrige, R.J. Zeyen and G.J. Muehlbauer. 2006. Overexpression of defense response genes enhances the resistance of wheat to Fusarium Head Blight. *Plant Cell Rep.* 26:479-488.  
McMullen, M., R. Jones and D. Gellenberg. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Dis* 81:1340-1348.  
Schweiger, W., J. Boddu, S. Shin, B. Poppenberger, F. Berthiller, M. Lemmens, G.J. Muehlbauer, and G. Adam. 2010. Validation of a candidate deoxynivalenol-inactivating UDP-glucosyltransferase from barley by heterologous expression in yeast. *Mol. Plant-Microbe Interact.* 23:977-986.  
Shin, S., C.A. Mackintosh, J. Lewis, S.J. Heinen, L. Radmer, R. Dill-Macky, G.D. Baldrige, R.J. Zeyen and G.J. Muehlbauer. 2008. Transgenic wheat expressing a barley class II chitinase gene has enhanced resistance to Fusarium Head Blight. *J. Exp. Bot.* 59:2371-2378.

**Budget justification:** Funding is requested for the salary of a graduate student in year 1 (\$10,359) and year 2 (\$20,717). Fringe benefits (76.68%) are also requested for the graduate student in year 1 (\$7,943) and year 2 (\$15,886). Funding for materials and supplies are also requested for molecular biology reagents in year 1 (\$3,000) and year 2 (\$3,095). Funding is requested for sequencing costs in year 1 (\$5,000) and growth chamber and greenhouse rental in year 2 (\$1,000). Total request is \$67,000.

# Minnesota Wheat Research and Promotion Council

## RESEARCH PROJECT PROPOSAL BUDGET

<b>ORGANIZATION AND ADDRESS</b>			
<b>Name:</b> Regents of the University of Minnesota			
<b>Address:</b> Sponsored Projects Administration 450 McNamara Alumni Center, 200 Oak Street SE Minneapolis, MN 55455-2070			
<b>Principal Investigator(s) / Project Directors(s)</b>  Gary J. Muehlbauer	<u>Funds Requested For</u>		
	Year 1 (2011)	Year 2 (2012)	Year 3 (2013)
A. Salaries and Wages	\$	\$	\$
1. Co-principal Investigator(s)			
2. Senior Associates			
3. Research Associates - Post Doctorate			
4. Other Professionals			
5. Graduate Students	10,359	20,717	
6. Prebaccalaureate Students			
7. Secretarial - Clerical			
8. Technical, Shop and Other			
B. Fringe Benefits	7,943	15,886	
C. Nonexpendable Equipment (Planting and harvesting equipment use)			
D. Materials and Supplies	3,000	3,095	
E. Travel			
F. Publication Costs			
G. Computer Costs			
H. All Other Direct Costs (Attach supporting data) See Attached Yr 1 - \$5,000 for sequencing costs, yr 2 – \$1,000 for greenhouse and growth chamber rental	5,000	1,000	
<b>I. TOTAL AMOUNT OF THIS REQUEST (per year)</b>	<b>\$ 26,302</b>	<b>\$ 40,698</b>	<b>\$</b>