FOR ADMINISTRATIVE USE
Program Area Code Proposal Code

Minnesota Wheat Research and Promotion Council

RESEARCH PROPOSAL GRANT APPLICATION

1. NAME AND ADDRESS OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE

Name: North Dakota State University
Address: Office of Sponsored Programs Administration
Dept #4000 PO Box 6050, Fargo, ND 58108-6050

2. TITLE OF PROPOSAL: Pre-breeding of HRWW to achieve multiple disease resistance

3. PRINCIPAL INVESTIGATOR(S)
PI# 1 Name: G. Francois Marais

PI# 2 Name:

PI# 3 Name:

4. PI #1 BUSINESS ADDRESS: NDSU, Dept. 7670, Loftsgard Hall, PO Box 6050, Fargo, ND 58108. **E-mail:** Gideon.marais@ndsu.edu

PI #2 BUSINESS ADDRESS:

5. PROPOSED PROJECT DATES (calendar years)
2016, 2017, 2018

Note: Research Reports are Due November 15th of Each Year

6. TOTAL PROJECT COST
$75,004

7. PI #1 PHONE NO.
701-231-8155

8. RESEARCH OBJECTIVES: (List objectives to be accomplished by research grant)
Current hard red winter wheat germplasm is generally lacking in resistance to major diseases such as Fusarium head blight, leaf rust, stem rust, bacterial leaf streak, tan spot and Septoria nodorum blotch. In the past five years, useful resistance has been transferred from many diverse sources of spring wheat and less winter-hardy winter wheat. The acquired genes occur mostly singly in diverse introgression lines that will now be utilized for the systematic development of inbred lines with broader resistance spectra and superior winter survival. The project therefore has the following objectives:

1. Annually, for three years, new crosses to combine increasingly complex subsets of genes will be produced.
2. Accelerated inbreeding and marker-assisted selection will be used to pyramid useful combinations of FHB, leaf rust and stem rust resistance genes in winter-hardy genetic backgrounds.
3. The genetic background of each cross will simultaneously be selected for increased levels of resistance to one or more of the diseases tan spot, Septoria nodorum blotch and bacterial leaf streak.
4. The best pyramided lines will be employed in routine crosses to quickly and effectively disperse the resistance genes throughout the pedigree breeding population.
5. The best pyramided lines will also be evaluated for commercial usefulness.

Signature Of Principal Investigator	**Date**	**Phone Number**
G. F. Marais	March 23, 2016	701-231-8155

Signature Of Authorized Representative	**Title**	**Date**
Amy B. Scott Amy Scott Assistant Director Office of Sponsored Programs Administration		3-31-16

Address Of Authorized Representative	**Phone Number**
NDSU Dept. 4000 **PO Box 6050, Fargo, ND 58108-6050**	**701-231-8045**

Minnesota Wheat Research and Promotion Council
RESEARCH PROPOSAL GRANT APPLICATION
(2-pages maximum)

Project Title: *Pre-breeding of HRWW to achieve multiple disease resistance*
Importance of this project to the profitability of wheat producers: The HRWW breeding program aims to develop improved varieties of winter wheat that will be better equipped to cope with the environmental and disease challenges of the Northern Prairies. Current varieties and breeding populations have limited disease resistance. In the past five years useful resistance genes have been acquired from spring wheat and less winter-hardy winter wheat that now have to be fully dispersed and utilized in the pedigree breeding population. Resistance genes with respect to the different diseases not only need to be combined with one another in the same genotypes, they also need to be combined with other advantageous traits such as cold-hardiness, adaptation to the northern Prairies and end-use quality.
Procedures: Funding from North Dakota Wheat Commission and Ducks Unlimited/Bayer CropScience allowed for the implementation of a breeding program at NDSU in 2011. In order to obtain disease resistance genes for this new program, Minnesota Wheat Research and Promotion Council provided funding (2011-15) for the transfer of rust resistance genes from HRSW and for the identification and selection of useful parental material with respect to tan spot (TS) and Septoria nodorum blotch (SNB). In addition, a project funded by the US Wheat and Barley Scab Initiative (USWBSI) provided for the transfer of Fusarium Head Blight (FHB) resistance genes from HRSW to winter wheat. Through these combined efforts **six pools** of very useful genetic variability have been established/ identified which are now being used to assemble transitional genotypes with pyramided resistance:

(1) The varieties Jerry and Decade as well as Canadian varieties such as Norstar, Peregrine, Accipiter, etc., have adequate to high levels of cold-hardiness and are adapted to growing conditions in the Northern Plains. **(2)** Leaf rust resistance genes not previously employed in HRWW in ND/MN have been transferred from spring wheat. Among the most useful are the minor (durable) resistance genes *Lr34*, *Lr46* and *Lr68* and the major (hypersensitive response) genes *Lr19*, *Lr53*, *Lr56*, *Lr59* and *Lr62*. **(3)** Stem rust resistance genes *Sr26* and *Sr39* (not previously employed in HRWW in ND/MN) were obtained from spring wheat. In addition, an improved version of the previously used *Sr50* translocation (lacks the detrimental rye *Sec1* locus) as well as the less frequently used minor gene *Sr2* were transferred. **(4)** FHB resistance genes not previously employed in winter wheat in the Northern Plains include *Fhb1*, *Qfhs.ifa-5A* (Sumai 3), *QTL*3A (Frontana), *QTL*5AS and 5AL (PI266012), and *Fhb6*; all of which have been acquired from spring wheat. **(5)** Few of the varieties currently grown in ND/MN have significant resistance to TS and SNB. Resistance in Decade, SY Wolf, Wesley and two breeding lines are being used for pre-breeding. Only Decade has good winter hardiness while all are susceptible to bacterial leaf streak (BLS). **(6)** Few varieties have significant resistance to BLS, however, intermediate resistance was detected in Jerry and strong resistance was found in the breeding lines Nord1401 and Crux. Unfortunately, these sources have virtually no TS or SNB resistance.

The newly assembled resistance genes occur mostly singly in different introgression lines and now need to be systematically combined in pyramided lines. This is a gradual process requiring hybridization of two donor parents at a time followed by selection of the segregating progenies for plants with the target gene combinations/pyramids. In this manner increasingly complex pyramids can be derived. The proposed project will involve successive sets of crosses of which the progeny will be subjected to (a) modified (with selection) single seed descent (SSD) and (b) random doubled haploid (DH) production to hasten the production of large numbers of inbred lines. Marker-aided selection will be used for detecting

major disease resistance loci, whereas phenotyping (field and greenhouse) will be used to assess cold-hardiness, agrotype, TS, SNB and BLS resistance. Gene sets that will be targeted in the first year are tabulated below. In parallel, a next generation of crosses to produce further subsets of pyramids will be initiated. The crosses of the second cycle will have more complex pyramids that will also involve *Fhb6*, *Lr19* and *Sr50*. The third set of crosses will be based upon the best and most successful pyramids from cycles one and two and will aim to derive pyramids of still higher complexity.

Targeted gene combination	**Background traits**	**Crosses**	**SSD**	**DH**
Fhb1, Lr34, Lr46, Lr68	Cold-hard	2		±130
Lr34, Lr46, Lr68, Lr56	Cold-hard	1		±50
Fhb1, Qfhs.ifa-5A, Lr56	Cold-hard	2		±100
Fhb1, Qfhs.ifa-5A	BLS, TS, SNB, cold-hard	12	±100	±150
Fhb1, Qfhs.ifa-5A, Lr53	BLS, Cold-hard; white grain	4	±50	±200
Fhb1, Qfhs.ifa-5A, Lr34, Lr56	BLS, TS, SNB, cold-hard	3	±350	±50
Fhb1, Qfhs.ifa-5A, Lr34, Lr53	BLS, TS, SNB, cold-hard	1	±50	
*Fhb1, Qfhs.ifa-5A, QTL*3A, *Lr56*	BLS, cold-hard	2		±50
*Fhb1, Qfhs.ifa-5A, QTL*3A, *Lr53*	BLS, cold-hard	2		±50
*Fhb1, Qfhs.ifa-5A, QTL*3A, *Sr26*	BLS, TS, SNB, cold-hard	3	±100	±50
*Fhb1, QTL*5AS&5AL		1	±100	

It will not be possible to combine all of the target genes in only three years. Rather, the aim will be to establish diverse pyramids of multiple disease resistance loci in winter-hardy backgrounds. Such gene pyramids will not only have direct value as potential cultivars, they also make very effective parents that will speed up the dispersal of the newly acquired resistance within the breeding population.

Marker-assisted selection for FHB resistance genes *Qfhs.ifa-5A*, the QTL on chromosomes 3A, 5AS and 5AL as well as *Fhb6* will be performed in the HRWW laboratory by Dr Seyed Pirseyedi (Postdoctoral Fellow) while *Fhb1* and *Lr34* will be tested for at the genotyping center in Fargo. Dr Pirseyedi will perform marker analyses for rust resistance genes including *Lr19, Lr46, Lr68, Lr56, Lr59, Lr62, Sr2, Sr22, Sr26, Sr35, Sr39* and *Sr50*. He will also do greenhouse FHB testing and SSD inbreeding.

Regional linkages to other research activities: Improved lines will be included in the HRWW FHB and Regional Germplasm Observation Nurseries as well as the Northern Regional Performance Nursery.

Research Group: Dr Marais will plan and coordinate crosses, selection and pyramiding schemes; Dr Pirseyedi will develop, phenotype and marker select parents and inbred lines. PhD student H. Tao will do specific aspects of the project. Certain automated marker analyses will be done by the USDA Genotyping Center (Dr S Chao), while Dr S Zhong will help with the FHB phenotyping.

Relationship to past projects: Single genes previously acquired from HRSW will be combined with the best available breeding material to establish inbred lines with multiple, effective disease resistance genes.

Current or potential other funding sources for this project: These are potential funding sources for this project. Ducks Unlimited (post-doc salary in 2016), the USWBSI (PhD stipend, running costs), NDWC (running costs for marker work, greenhouse screening, DH production).

Estimated budget requirements: $75,004 over 3 years.

Program's current and pending support: NDWC = $60,000 (7/1/2015 – 6/30/2016); USWBSI (ARS) = $19,000 (7/1/2015 – 6/30/2016); NDSU Appropriated = $70,000 (7/1/2015 – 6/30/2017); SBARE SCAB = $33,000 (7/1/2015 – 6/30/2017); Ducks Unlimited/Bayer = ±$52,000 (1/1/2016 – 12/31/2016).

References: Marker detail and protocols required for the project can be accessed through the MASWheat (http://maswheat.ucdavis.edu/) and Graingenes (http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker) websites. General marker procedures for wheat were described by: Liu S, Rudd JC, Bai G, Haley SD, Ibrahim AMH, Xue Q, Hays DB, Graybosch RA, Devkota RN, St Amand P 2014. Crop Sci 54:1.

Minnesota Wheat Research and Promotion Council
RESEARCH PROPOSAL BUDGET

PROJECT TITLE: Pre-breeding of HRWW to achieve multiple disease resistance

Principal Investigator(s) / Project Directors(s) G. Francois Marais	Funds Requested For Year 1 (2016)	Year 2 (2017)	Year 3 (2018)
A. Salaries and Wages	$	$	$
1. Co-principal Investigator(s)			
2. Senior Associates			
3. Research Associates - Post Doctorate	15,757	16,230	16,717
4. Other Professionals			
5. Graduate Students			
6. Prebaccalaureate Students			
7. Secretarial - Clerical			
8. Technical, Shop and Other			
B. Fringe Benefits @ (**54%**)	8,509	8,764	9,027
C. Nonexpendable Equipment (Planting and harvesting equipment use)			
D. Materials and Supplies			
E. Travel			
F. Publication Costs			
G. Computer Costs			
H. All Other Direct Costs (Attach supporting data)			
TOTAL AMOUNT OF THIS REQUEST (per year)	24,266	24,994	25,744

Budget justification:

(1) **Postdoctoral Fellow:** The success of the project will hinge on an ability to implement large scale molecular marker analyses for the routine selection of inbred lines. Dr Pirseyedi will develop large numbers of inbred lines from select crosses, and will then genotype and select these with molecular markers to derive the pyramided genotypes. He has extensive experience with analytical DNA work and the large scale application of wheat molecular marker procedures. This budget is with respect to his salary.