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 #4050 PO Box 6050, Fargo, ND 58108-6050

2. TITLE OF PROPOSAL **Transfer Of Leaf And Stem Rust Resistance Genes To Hard Red Winter Wheat Genetic Backgrounds**

3. PRINCIPAL INVESTIGATOR(S)

G. Francois Marais

PI# 2 Name: Maricelis Acevedo (NDSU)

PI# 3 Name:

4. PI #1 BUSINESS ADDRESS
NDSU Plant Sciences Department
NDSU Department 7670
474G Loftsgard Hall
PO Box 6050
Fargo ND 58108-6050

5. PROPOSED PROJECT DATES (calendar years)
2011, 2012, 2013

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

6. TOTAL PROJECT COST

$ 60,915

7. PI #1 PHONE NO.

(701) 231-8155

8. RESEARCH OBJECTIVES: (List objectives to be accomplished by research grant)

- Transfer 21 leaf rust resistance (*Lr*) and three stem rust resistance (*Sr*) genes into well-adapted winter wheat backgrounds. The genes/translocations in question are:
  - Of recent origin and generally not present in winter wheat germplasm
  - Effective against North American rust pathotypes
  - The *Sr* genes are also effective against UG99 variants
  - Five of the genes are closely linked to stripe rust resistance genes
- Provide valuable breeding stock that can be used to establish a productive winter wheat breeding gene pool that:
  - Supplements existing North American winter wheat breeding programs
  - Combines the new resistance traits with adequate levels of winter hardiness and processing quality
  - Can be used to initiate and sustain resistance gene pyramiding through recurrent mass selection pre-breeding.

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).

Signature Of Principal Investigator	**Date**	**Phone Number**
	December 1, 2010	701-231-8155
Signature Of Authorized Representative	**Title**	**Date**

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

Project Title: Transfer of leaf and stem rust resistance genes to hard red winter wheat genetic backgrounds

Importance: A new winter wheat breeding program is being launched at NDSU. A first priority is to develop a unique and versatile pool of winter wheat germplasm from which breeding parents can be drawn and segregating generations can be derived. In view of the long generation interval of winter wheat, this has to be done with urgency. The envisaged breeding stock should be winter-hardy and should contain ample exploitable variability for disease resistance (primarily rust and fusarium), processing quality, adaptation and yield. This project will be one of three integrated attempts to broaden the very narrow base of variability that is currently available.

The wheat rust fungi cause the potentially most devastating diseases of wheat. Genetic control is a highly effective and cost-saving alternative to chemicals which are only justified under intensive production systems where one chemical controls several diseases. Wheat cultivars are bred to be genetically uniform and are grown over large areas, often for many years, a practice which encourages the evolution of pathogen virulence. The situation is aggravated when resistance genes are employed singly in new varieties as this significantly reduces the commercial life span of a gene and increases crop vulnerability. As a result, the number of commercially employed rust resistance genes that are still widely effective has steadily dwindled over time. However, a wealth of knowledge regarding resistance mechanisms, available genes, closely linked markers and breeding strategies for pyramiding resistance genes has accumulated in recent years. Numerous new resistance genes have been identified in wheat or were transferred from wild grass relatives of wheat; yet, the majority of these genes remain poorly utilized, particularly in winter wheat breeding. This project will draw on more recently developed resistance genes to broaden the winter wheat gene pool.

Background: Depending on their mode of action, strength and durability of suppression of the pathogen attack, rust resistance genes can be broadly described as major hypersensitive response (HR) type genes or minor non-hypersensitive response (non-HR) type genes. Major HR genes are more abundant, have stronger phenotypic effects and are easier to manipulate in breeding. However, HR genes may be more easily overcome by pathogen mutation than minor non-HR genes. Durability of especially HR genes can be improved through gene pyramiding which also prolongs the commercial life of resistance genes. Ideally, multiple minor, non-HR and major HR genes should be combined in new varieties to optimize both the level of resistance and its durability. Non-HR genes such as *Lr34*, *Lr46, Lr67* and *Sr2* occur at very low frequency in winter wheat germplasm. In the case of the non-HR leaf rust resistance genes *Lr34*, *Lr46, Lr67* this may partially be the result of indirect selection against their pleiotrophic leaf tip necrosis effects. Leaf necrosis might be accentuated under low temperature growing conditions putting carrier plants at a disadvantage. Thus, if this presumption is correct, employment of at least these three non-HR genes in winter wheat breeding might be compromised. Gene pyramiding in the very cold growing regions may then need to rely more strongly on the assembly of diverse, major HR genes. In the case of the non-HR stem rust resistance gene *Sr2*, there is no current evidence to suggest that it could not be employed in pyramids with HR genes to protect winter wheat against this pathogen.

A comparatively small group of major HR genes are still commercially effective and presently resistance breeding relies strongly on genes such as *Lr16, Lr21, Lr22a, Lr24/Sr24* and *Sr23*. However, highly effective major HR genes for resistance to leaf and stem rust of wheat are continuously being discovered or transferred from related grasses. Furthermore, the utility of certain alien-derived HR genes that previously occurred on translocations with deleterious agronomic effects has been improved through chromosome engineering. Such genes, especially those of more recent origin, are generally poorly utilized and in addition occur almost exclusively in spring wheat backgrounds. Systematic evaluation and transfer of such genes will strongly aid the breeding of winter wheat varieties with stable, complex resistance.

Relationship To Past Projects: This is a first-time project.

Procedures: The 24 sources of resistance are listed in Table 1 which also provides an indication of those genes for which reliable molecular markers have been developed. Each source will first be crossed with Norstar which is noted for its high level of winter hardiness and good processing quality. Each F_1 (with minimal vernalization requirement) will then be crossed with Jerry (well adapted, winter hardy, average quality). Following the second cross to winter wheat, resistant F_1 plants will be identified (seedling screening or markers) for pollination with Flourish, a winter-hardy, good quality winter wheat. Resistant F_1 will once again be selected for seed multiplication and F_2 plants will be artificially hardened and selected (cold chamber) for winter-hardiness (plus presence of the resistance). Towards this end an appropriate winter-hardiness test procedure will be worked out based on existing literature. Selected winter-hardy F_3 families will be identified which can:

1. be used as parents in crosses with the better selections from the main pedigree breeding program (2013 crossing block),
2. be field planted in September 2013 and subjected to continued selection and inbreeding within the segregating populations of the main program, and,
3. be used for the initiation of an ongoing pre-breeding program (recurrent mass selection).

Table 1. List of stem rust (*Sr*), leaf rust (*Lr*) and stripe rust (*Yr*) resistance genes targeted for transfer. The gene origin and availability of diagnostic markers[1] are also shown.

Lr19-149-299.478 (Thinopyrum ponticum)[1]	*Lr53/Yr35 (T dicoccoides)*	*Lr62/Yr42 (Ae neglecta)*
Lr42 (Aegilops tauschii)	*Lr54/Yr37 (Ae kotschyi)*[1]	*Lr63 (T monococcum)*
Lr47 (Ae speltoides)[1]	*Lr55 (Elymus trachycaulus)*	*Lr64 (T dicoccoides)*
Lr48 Bread wheat APR	*Lr56/Yr38 (Ae sharonensis*	*Lr66 (Ae speltoides)*
Lr49 Bread wheat APR	*Lr57/Yr40 (Ae geniculata)*[1]	*Lrbi (Ae Biuncialis)*
Lr50 (Triticum timopheevii)[1]	*Lr58 (Ae triuncialis)*	*Sr26 (Th ponticum)*[1]
Lr51 (Ae speltoides)[1]	*Lr59 (Ae peregrina)*	*SrR* (Imperial rye)[1]
Lr52 (Bread wheat)	*Lr61 (T turgidum var durum)*	*Sr2 (T turgidum* var *dicoccum)*[1]

At the onset of the project it will be necessary to characterize (seedling rust screening; molecular markers) the donor material and the winter wheat recipients and to identify specific leaf rust pathotypes/marker polymorphisms that can be used to track the resistance genes. The material that will be developed here will eventually form one of three independent attempts to broaden the available variability. A second and a third set of supplementary crosses (not budgeted for in this application) will respectively focus on (a) the acquisition of diverse Fusarium resistance genes and (b) the incorporation of diverse genes for rust resistance (*Lr16, Lr21, Lr22a, Sr23*), sprouting tolerance and processing quality.

Research Group: The greenhouse and field work will be executed by Francois Marais (wheat geneticist), Travis Sanderson (research associate) and a student assistant. Rust seedling resistance screening will be done in close cooperation with Dr Maricelis Acevedo (wheat pathologist) who will also supply the rust cultures. A student assistant will be employed to do the DNA marker analyses.

Regional Linkages To Other Research Activities: The material will be made available to other winter wheat breeders who may wish to utilize it. The NDSU has already benefited significantly from germplasm that was shared by the Montana (Dr Phil Bruckner) and South Dakota (Dr Bill Berzonsky) programs and we would like to reciprocate.

Additional Sources of Funding: The NDSU winter wheat breeding program is being initiated with a grant from Ducks Unlimited/Bayer CropScience (2010-2014).

References:

Jones JDG and Dangl JL 2006. The plant immune system. Nature 444, 323-329
Marsalis MA and Goldberg NP 2006. Leaf, Stem and Stripe Rust Diseases of Wheat. New Mexico State University, College of Agriculture and Home Economics , Guide A-415 (www.cahe.nmsu.edu).
Mcintosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, D.J. Somers, R. Appels and K.M. Devos. 2008. Catalogue of Gene Symbols for Wheat. (http://www.shigen.nig.ac.jp/wheat/komugi/genes/download.jsp)

McIntosh, R.A., J. Dubcovsky, W.J. Rogers, C. Morris, R. Appels and X.C. Xia. 2010. Catalogue of gene symbols for wheat: 2010 supplement. (http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp)
Dubcovsky, J 2010. MASWheat – Marker assisted selection in wheat. Dept of Plant Sciences, University of California website (http://maswheat.ucdavis.edu/)

Minnesota Wheat Research and Promotion Council
RESEARCH PROPOSAL BUDGET

ORGANIZATION AND ADDRESS

Name: North Dakota State University
Address: Office of Sponsored Programs Administration
Dept #4050 PO Box 6050
Fargo, ND 58108-6050

Principal Investigator(s) / Project Directors(s) Francois Marais	Funds Requested For 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			
6. Prebaccalaureate Students	10,641	10,641	10,641
7. Secretarial - Clerical			
8. Technical, Shop and Other			
B. Fringe Benefits @ 10%	1,064	1,064	1,064
C. Nonexpendable Equipment (Planting and harvesting equipment use)			
D. Materials and Supplies	8,600	8,600	8,600
E. Travel			
F. Publication Costs			
G. Computer Costs			
H. All Other Direct Costs (Attach supporting data) - Purchase of Service -			
I. **II. TOTAL AMOUNT OF THIS REQUEST (per year)**	**$ 20,305**	**$ 20,305**	**$ 20,305**

Budget justification

Prebaccalaureate Students: The students will help with emasculations and crosses, DNA preparation for PCR marker analyses, seedling rust resistance screening and the development of an artificial screen for cold tolerance.

Materials and Supplies: The amount budgeted will be used to acquire chemicals and consumables related to greenhouse rust screening, chromosome counts, DNA extraction, PCR and visualization of amplification products.