Growing knowledge for the future

25th Annual Plant Science Graduate Students' Symposium

North Dakota State University | University of Manitoba / University of Saskatchewan

25
Silver Jubilee Year

University of Manitoba, Winnipeg, Canada
March 13-14 (Fri - Sat), 2009

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GREETINS FROM THE DEPARTMENT HEAD

March 13, 2009

To Participants of the 25th annual Plant Science Symposium

On behalf of the Department of Plant Science and the University of Manitoba, I extend to you a warm welcome to Winnipeg. Muthukumar Bagavathiannan and his committee have been working diligently to make this 25th Anniversary Plant Science Symposium a resounding success, continuing the tradition of previous symposia successes. The continuation of this symposia is a tribute to the founding organizers, to the annual participation of individuals such as yourselves, and to respective organizing committees from the three participating Universities - North Dakota State University, the University of Saskatchewan and the University of Manitoba. The first four years of these meetings involved only students from the University of Manitoba. In 1985 both NDSU and the U of S were invited and came to actively participate. In 1986 NDSU hosted the meeting and from that point on this international meeting has rotated among the three Universities. My congratulations to you on being part of this long tradition!

The world faces a number of challenges currently including economic meltdown, climate change and global warming. A dedicated switch from non-renewable to renewable sources of chemicals, chemical feedstocks, energy and fuels is underway to resolve some of the major issues facing the world today. Plants will provide answers to many of the pressing problems facing us today, providing a bright and promising future for plant science research and development. Supplying tomorrow's food, feed, fibers and fuels will need to be done more sustainably for the farmer, for the community, and for the environment. Your meeting will have friendly competition and there will be institutional pride displayed. However, I encourage you to get to know each other, for you are all on the same team for the significant challenges that lie ahead!

Have fun and enjoy Winnipeg. I look forward to meeting you during the breaks.

Peter B. E. McVetty
Professor, Head and NSERC Senior Industrial Research Chair
Department of Plant Science

GREETINS FROM THE ORGANIZING COMMITTEE CHAIR

On behalf of the symposium organizing committee, I would like to extend you a warm welcome to the 25th Annual Plant Science Graduate Students' Symposium. I am glad that you are able to take part in the 'Silver Jubilee' celebration of this very important event.

I believe that this a great opportunity for every one of us to not only present our research and perfect our presentation skills but also meet new friends and establish networks with people who are also passionate about plant science research. I hope that the contacts will continue and result in future research collaborations.

The funding received by the Plant Science Graduate Students' Symposium is the key for making this event happen. We thank all our sponsors for their generous donations and we look forward to their continued support in the future events.

The organizing committee has been diligently working to ensure that you enjoy your participation in this important event. As the chair of the organizing committee, I would like to thank all the committee members and volunteers for their countless efforts and commitments that made this event possible.

Thanks again for your participation. I hope that you will take home many good memories.

Sincerely,

Muthukumar Bagavathiannan
President
Plant Science Graduate Students' Association

Faculty of Agricultural and Food Sciences
"Gateway to the Future of Agri-Food Knowledge"

The faculty had its beginnings in Winnipeg in 1906 with the formation of the Manitoba Agricultural College, located on the South bank of the Assiniboine River (today's Tuxedo area of Winnipeg). The first agricultural diplomas were conferred in 1908 and the first agricultural degrees in 1911.

Home Economics students began enrolling in the college in 1910, but several years elapsed before degrees were conferred. It wasn't until eight years later, in 1918, that the first graduates of the degree program were recognized.

In 1913, the Manitoba Agricultural College moved to the site of the Fort Garry Campus which later became the University of Manitoba. In 1924, the administration of the Manitoba Agricultural College, now the Faculties of Agriculture and Food Sciences and Home Economics, were transferred to the University of Manitoba.

Agriculture and Home Economics became separate faculties in 1970 and, in July 1991, the name was changed from Faculty of Agriculture to the Faculty of Agricultural and Food Sciences.

Program at a Glance

Friday, March 13, 2009

12:00 to 1:00pm
Registration and presentation upload
Venue: Plant science atrium

1:00 to 1:45pm
Lab tours in plant science building
Venue: Meet at the atrium

2:00 to 5:15pm
Pre-symposium tours

ROYAL CANADIAN MINT
MONNAIE ROYALE CANADIENNE

The Royal Canadian Mint's facility in Winnipeg, designed by the local architect Etienne Gaboury, was established in 1976. The plant occupies a 14,864 m^2 state-of-the-art facility and produces billions of coins each year. The Winnipeg Mint is the high-tech, high-volume manufacturing facility in the country. This is where all Canadian circulation coins are made, as well as those for more than 60 governments all around the world. A fascinating guided tour includes the viewing of a 5-minute video in the theatre area followed by a 40-minute walking tour overlooking the manufacturing facility where the precise art, craft, and science of coin-making is revealed!

UNIVERSITY OF MANITOBA
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Fort Garry Brewing Company Ltd. is Manitoba's largest brewer and distributor of premium beers. Having successfully completed an Initial Public Offering in January 1999, the company has recently undergone an expansion of operations. The new 25,000 square foot facility houses the most modern and efficient brewing and packaging equipment available to today's regional brewery. Fort Garry's beers are presently available throughout Winnipeg, rural Manitoba and Alberta.

5:30 to 7:30 p.m
Opening reception and welcoming dinner
Triple B's

8:00 to 10:00 p.m
Indoor night event
Academy bowling lanes

CropLife CANADA

SYMPOSIUM PROGRAM
Saturday, March 14, 2009

Breakfast:7:30 to 8:15 a.m
Opening Remarks: 8:15 to 8:30 a.m
Venue: Plant Science Atrium

Session I: Agronomy and Physiology
Chair: Kevin Baron
Venue: Carolyn Sifton Lecture Theatre

Time	Presenter	Title
8:30	Fernando Eckert, North Dakota Sate University	The effects of row spacing and nitrogen fertilization on yield response of pinto bean varieties under conditions of direct harvest
8:45	Nicole Seerey, University of Saskatchewan	Effect of secondary dormancy trait segregation in volunteer *Brassica napus* populations
9:00	Harun Cicek, University of Manitoba	Ecophysiology and agronomic benefits of selected cool and warm season legume cover crops grown for organic winter cereal in southern Manitoba
9:15	Dilshan Benaragama, University of Saskatchewan	Use of genotypic variation of oat (*Avena sativa,* L.) cultivars to suppress wild oat (*Avena fatua,* L.) competition
9:30	Benilda Sable, University of Manitoba	Development of flowering synchrony indices for volunteer and crop canola (*Brassica napus* L.) to measure density and planting date interactions
9:45	Anne Kirk, University of Manitoba	Wheat Breeding for Organic Production – Is it beneficial?

Health break: 10:00 to 10:30am
Venue: Plant Science Atrium

Concurrent Session I: Plant Pathology
Chair: Richard Cuthbert
Venue: 138, Agriculture Building

Time	Presenter	Title
8:30	Tyler Guerrieri, University of Manitoba	The effect of three different spray inoculation protocols on FHB infection of cultivars of common wheat and duram wheat
8:45	Maryam Rezaey, University of Manitoba	Cytological studies on necrotic and chlorotic lesions induced by *Pyrenophora tritici-repentis* on wheat
9:00	Yuanjie Su, North Dakota State University	Double Haploids production of canola with improved resistance to *Sclerotinia sclerotiorum*
9:15	Holly Taylor, University of Manitoba	The role of abscisic acid (ABA) and other phytohormones in defense signaling in the *Solanum tuberosum-Verticillium dahliae* interaction
9:30	Yueqiang Leng, North Dakota State University	Development of transformation and RNA-mediated gene silencing systems for functional genomics of *Cochliobolus sativus*
9:45	Silvia Barcellos-Rosa, University of Manitoba	Toropi as a source of leaf rust resistance genes

Session II: Agronomy and Physiology
Chair: Kevin Baron
Venue: Carolyn Sifton Lecture Theatre

Time	Presenter	Title
10:30	Nityananda Khandal, University of Saskatchewan	Chlorophyll fluorescence imaging: an alternative tool for assessing the survival of plants after exposure to freezing temperature
10:45	Jin Li, University of Saskatchewan	Determining germination thresholds in the mixed-grass prairie using modeling approach and their implications to global change: a FACE study
11:00	Muthukumar Bagavathiannan, University of Manitoba	Feral alfalfa and its implications for novel trait confinement
11:15	Rohit Dhanda, University of Saskatchewan	Fatty acid composition in diverse oat germplasm
11:30	Iris Vaisman, University of Manitoba	Reducing tillage in organic agriculture on the Canadian prairies
11:45	Teresa Sutanto, University of Manitoba	Propagation studies of sugar maple (*Acer saccharum*)

Symposium Luncheon: 12:00 to 1:00 pm
Venue: Plant Science Atrium

Concurrent Session II: Plant Pathology
Chair: Richard Cuthbert
Venue: 138, Agriculture Building

Time	Presenter	Title
10:30	Victoria Gauthier, University of Manitoba	Effects of 3-ADON and 15-ADON chemotypes of *Fusarium graminearum* on spring wheat
10:45	Jennifer Menat, University of Saskatchewan	Mating system of *Colletotrichum truncatum*, the causal agent of lentil anthracnose
11:00	Maryam Rezaey, University of Manitoba	Simulating pathogen population shifts of *Pyrenophora tritici-repentis* on different Canadian wheat cultivars
11:15	Nitin Mittal, North Dakota State University	Characterization of the secreted proteome of *Ascochyta rabiei*
11:30	Li Ru, University of Manitoba	Assessment of the biocontrol resources of *Pseudomonas* species antagonism towards *Sclerotinia sclerotiorum* (Lib.) de Bary under different cropping systems
11:45	Ravneet Behla, University of Manitoba	Identification of quantitative trait loci for resistance against *Sclerotinia* stem rot in *Brassica napus* L.

Session III: Plant Breeding
Chair: Kevin Baron
Venue: JRI Auditorium

Time	Presenter	Title
1:00	Paul Werner, North Dakota State University	Genetics of pre-harvest sprouting in barley
1:15	Adithya Ramachandran, University of Saskatchewan	Carotenoid accumulation during grain fill in durum wheat
1:30	Junyun Yang, North Dakota State University	Identification of early maturing inbred lines and hybrids with fast rate of dry down
1:45	Meghan Rose, University of Manitoba	Characterization of the Agp-L locus in winter and spring wheat varieties
2:00	Eder Mantovani, North Dakota State University	Genetics studies on kernel and spike morphology in spring wheat (*Triticum aestivum* L.)
2:15	Hongzia Wang, North Dakota State University	Identification of molecular markers linked to x-disease resistance in chokecherry

Health break: 2:30 to 3:00 pm
Venue: Plant Science Atrium

Session IV: Genomics and Molecular Biology
Chair: Santosh Kumar
Venue: JRI Auditorium

Time	Presenter	Title
3:00	Arvind Hirani, University of Manitoba	Regulation of aliphatic glucosinolates biosynthesis in *Brassica* by gene re-placement
3:15	Guojia Ma, North Dakota State University	Cloning and characterization of the meiotic genes *Rec8* and *Cdc5* in wheat
3:30	Alain Ngantcha, University of Saskatchewan	DNA fingerprinting and assessment of genetic diversity among 35 biomass willow clones
3:45	Mohammad Tahir, University of Saskatchewan	Galactinol synthase activity in lentil seeds with contrasting raffinose family of oligosaccharides profiles
4:00	Andrej Noyszewski, North Dakota State University	Mitochondrial Sequence Similarity and Gene Expression Analysis in Alloplasmic Durum Wheat
4:15	Mohamed Elhiti, University of Manitoba	Molecular regulation during shoot apical meristem formation in canola microspore-derived embryos

Banquet and Awards Reception: 6:00 to 9:00pm
Venue: University Club, Pembina Hall

Banquet Key Note Speaker

Laura Rance is an award-winning journalist who has reported on farm and rural issues in daily and weekly publications for more than 25 years. She became editor of the Manitoba Co-operator in February 2007 after it merged with Farmers' Independent Weekly, a publication she and six partners started in 2002. Laura is also a weekly free-lance business columnist for the Winnipeg Free Press. She works from her home on a small acreage near the community of Carman about an hour outside of Winnipeg.

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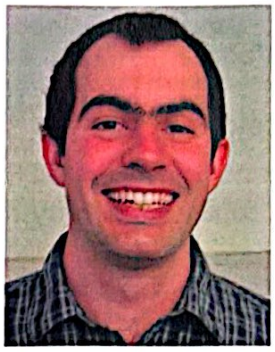

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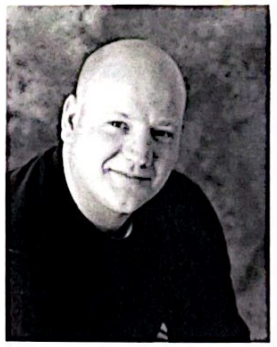

Kevin Baron
Symposium Chairing

Ravneet Behla
Judging and Awards

Anne Kirk
Local Arrangements

Holly Taylor
Local Arrangements

Special Thanks

Judging Panel

Agronomy and Physiology	Dr. Robert Gulden Gary Martens Santosh Kumar
Plant Pathology	Dr. Dilantha Fernando Dr. Fouad Daayf Dr. Kaveh Ghanbarnia
Plant Breeding, Genomics & Molecular Biology	Dr. Anita Brûlé-Babel Dr. Murray Balance Dr. Xiuqiang Huang
Session Chairs	Richard Cuthbert Santosh Kumar
Photography and symposium logo design	Bert Luit
Technical Assistance	David Treble

A very special thanks to all the volunteers who made the Plant Science Graduate Students' Symposium 2009 a great success.

Abstracts

Agronomy and Physiology

The effects of row spacing and nitrogen fertilization on yield response of pinto bean varieties under conditions of direct harvest

Fernando Eckert, Hans Kandel, Burton Johnson, Gonzalo Rojas-Cifuentes, Albert VanderWal, Chad Deplazes and Juan Osorno*

Department of Plant Sciences, North Dakota State University, Fargo, ND, USA. *Corresponding author, E-mail: juan.osorno@ndsu.edu

In 2007, the NDSU breeding program released two pinto bean varieties Lariat and Stampede. These new varieties with erect growth should help reduce seed loss during direct harvest. However, it is important to know how these varieties will respond under different row spacings and nitrogen availability. The objective of this study was to evaluate yield performance and yield loss due to direct harvesting of Lariat and Stampede, compared with the well known variety Maverick, which tends to be more prostrate or vining. This study was conducted at Carrington and Prosper, ND, in 2008. The experimental design was a RCB in a split-plot with three replicates. The study had three row spacings: solid seeded, narrow rows, and wide rows (30, 46, and 76 cm row spacings, respectively). Two nitrogen availability levels: 56 kg ha^{-1} N (soil N) and 112 kg ha^{-1} N (soil N + fertilizer N) were used with all row spacings and varieties. Characteristics evaluated included plant stand, flowering date, plant height, lowest pod height, seed yield, harvest loss, and seed weight. The varieties were planted in plots 7.62 m long at recommended seeding rates. A Hege 125B plot combine was used to direct harvest. Harvest losses, in each plot, were estimated by counting the seeds on the ground within an area bounded by a square metal hoop. Seed number was converted to seed weight for calculating yield loss. Preliminary conclusions are that Lariat was the highest yielding when direct combined and had the lowest seed loss. Yield potential of Lariat and Stampede were similar. There was no significant difference in yield between N levels. Yield increased with narrower rows in Prosper, whereas intermediate row spacing appears to be the best in Carrington. The research will continue in 2009 to obtain more accurate information under different growing season conditions.

Effect of secondary dormancy trait segregation in volunteer *Brassica napus* populations

Nicole Seerey* and Steve Shirtliffe

Department of Plant Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Canada - S7N 5A8. *Corresponding Author, E-mail: NJS741@mail.usask.ca

Due to the large acreage of canola (*Brassica napus* L.) grown in western Canada, inherent harvest losses, and the genetic potential to maintain secondary dormancy volunteer canola has become a common, reoccurring weed in producer fields. Commonly grown canola varieties are developed by hybrid genetics which segregate in subsequent generations. The segregation of traits creates volunteers with unknown dormancy capabilities. The objective of this study was to evaluate the effect of volunteer canola genotype and generation on secondary dormancy in volunteer canola. Two hybrid and one open pollinated canola variety with three consecutive generations were used in a dormancy assay. It remains unknown whether generational differences or interaction between generation and genotype has the ability to affect secondary dormancy potential. Initial analysis shows some F2 populations with increased secondary dormancy potential. Seed dormancy remains the complex mechanism governing volunteer canola persistence.

Ecophysiology and agronomic benefits of selected cool and warm season legume cover crops grown for organic winter cereal in southern Manitoba

Harun Cicek* and Martin Entz

Department of Plant Science, University of Manitoba, Winnipeg, Canada - R3T 2N2. *Corresponding author, E-mail: umcicek@cc.umanitoba.ca

Increasing environmental awareness, along with the rising energy prices, has been contributing to the adoption of cover cropping techniques by a growing number of farmers. Cover cropping involves planting a second crop before or after the main crop, thus, potentially, providing services to the system, such as, nitrogen supply, weed and pest suppression, and food for soil biota. Prairie organic grain farms often do not include livestock, reducing the opportunity for nutrient recycling. These farms depend on legumes for their nitrogen; nitrogen is fixed biologically from various green manure legume species. In order to assess the nitrogen benefits of common (i.e. red and sweet clover, lentil, and forage pea) and novel (i.e. hairy vetch, soybean, cowpea) legume cover crops in organic agriculture, field experiments will be established at two Manitoba and one Saskatchewan location. A randomized complete block design with 4 replicates will be used. Both cool season (most legumes) and water season (soybean and cowpea) plant species will be tested for plant establishment and growth under conventional tillage and reduced tillage organic management under dry (Saskatchewan plots) and wet conditions (Manitoba plots). N contribution from the legume cover crops will be determined using a number of techniques: 1) measure N uptake in a wheat bioassay crops planted on plot areas in year 2 of the study; 2) bioassay conducted in the growth chamber on surface soils (0 to 15 cm) harvested from experimental plots after cover crops reach maturity; and 3) resin ion exchange strips used to measure N supply rate (conducted in growth chamber and in field). In supplemental controlled environmental studies, physiology of warm and cool season legume cover crop species will also be studied. Specifically, the response to temperature and moisture regime will be evaluated in order to assess the feasibility of growth of these legumes in organic farms across the diverse climate on the Canadian prairies.

Use of genotypic variation of oat *(Aven sativa* L.) cultivars to suppress wild oat (*Avena fatua* L.) *competition*

Dilshan Benaragama*[1], Steven Shirtliffe[1] and Brian Rossnagel[2]

[1]Dep. of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada - S7N 5A8; [2]Crop Development Center, Univ. of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada - S7N 5A8. *Corresponding author, E-mail: dib777@mail.usask.ca

Wild oat (*Avena fatua* L*)* is one of the most troublesome weeds in oat cultivation due to its difficulty to control using herbicides. Genotypic variation in oat cultivars can be used as a potential strategy to suppress the wild oat competition. Seven oat lines generated from a cross of the forage oat CDC Baler and the semi-dwarf oat, Ronald were evaluated for the competitive ability with wild oat. The lines were grown with and without wild oat at 250plants m^{-2} at two locations in 2008. Plant height, Light interception, shoot biomass, and grain yield data were recorded. According to the preliminary data analysis the selected cop genotypes shows a significant ($P =<0.05$) difference in plant height among the genotypes. Also there was a significant variation among grain yields among all the treatments. Therefore from these preliminary studies there may be variation in competitive ability between selected oat genotypes.

Development of flowering synchrony indices for volunteer and crop canola (*Brassica napus* L.) to measure density and planting date interactions

Benilda Zamora-Sable*[1], and Rene Van Acker[2]

[1]Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada; [2]Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada. *Corresponding author, E-mail: umsable@cc.umanitoba.ca

Flowering synchrony is frequently identified as a precursor for pollen-mediated gene flow but there is limited information on the factors that could potentially increase or decrease synchronization, and its relation to consequent amount of genetic exchange. In addition, previous studies measure synchrony solely based on

number of overlap flowering days between populations. This approach is inadequate when analyzing flowering synchrony in the context of intraspecific gene flow in canola because it does not take into account directionality, flower abundance of gene source and pollen receptors, and timing of three main flowering phases. Canola follows a mixed mating system, a mass-flowering pattern and develops flowers indeterminately, and therefore pollen load can be highly variable during its three main flowering phases. There is a need for a robust method to estimate flowering synchrony that includes factors other than overlap to generate more realistic estimates of gene flow. This study developed indices to measure flowering synchrony between simulated volunteers and crop canola stands to investigate the effects of density and emergence on synchronization under field conditions, and compare the robustness of this approach to previously generated indices solely based on overlapping days relative to gene flow estimation. Results showed significant interactions among density and emergence in terms of flowering synchrony. Detection and estimation of gene flow detection is currently on-going, but preliminary results indicate that the new approach can facilitate a more-in-depth analysis of synchrony-gene flow.

Wheat breeding for organic production – is it beneficial?

Anne Kirk*[1,2], Stephen Fox[2], and Martin Entz[1]

[1]Department of Plant Science, University of Manitoba, Winnipeg, MB - R3T 2N2, Canada; [2]Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB - R3T 2M9, Canada.
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Organic agricultural systems differ from conventional systems in terms of soil fertility, soil microflora distribution and weed management. Organic agriculture may benefit from wheat cultivars specifically tailored to this environment, as wheat cultivars are currently selected under conventional management. In 2004 a collaboration was initiated between Agriculture and Agri-Food Canada and the University of Manitoba to breed wheat targeted for organic production. Populations of wheat from the same crosses were grown and selected under both organic and conventional breeding programs. After five generations populations from both breeding programs were bulked and compared under both organic and conventional growing conditions at nine locations over two years. While differences existed between populations, overall the populations bred under organic conditions yielded higher in organic conditions than the conventionally bred populations. This study indicates that there is value in an organic breeding program.

Chlorophyll Fluorescence Imaging: an alternative tool for assessing the survival of plants after exposure to freezing temperature

Nityananda Khanal*[1], Swati Gupta[2] and Gordon Gray[1,2]

[1]Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada; [2]Department of Biochemistry, University of Saskatchewan, Saskatoon, Canada. *Corresponding author, E-mail: nik370@mail.usask.ca

Photosynthesis is one of the most rapidly perturbed processes during plant stresses, such as freezing. Because of this property, the degree of disturbance in photosynthetic efficiency can be a potential indicator of the severity of freezing stress. The extent of freezing tolerance is popularly assessed through the electrical conductivity test of the tissues or regrowth scoring of plants. Both of these processes are cumbersome and time consuming, limiting their utility for screening of large number of genotypes. In this study, a potential use of chlorophyll *a* fluorescence imaging as an alternative to the electrical conductivity or regrowth method is ascertained. Two model plant species, *Arabidopsis* and *Thellungiella,* were exposed to various cold temperatures between +5°C and -25°C with the cooling rate of 2.5°C per hour. A nearly perfect correlation ($r > 0.88$) was found between the maximum quantum efficiency (F_v/F_m) of stressed plants after 8 hours thawing and their regrowth scores after two weeks of recovery. It is evident that the plants displaying F_v/F_m values greater than 0.60 upon cold exposure tended to survive the stress while those displaying the F_v/F_m values of less than 0.40 indicated lethal state of plants. The temperature, at which the F_v/F_m values of about 0.50 occur, can be the approximation of lethal temperature for 50% plants (LT_{50}). These evidences clearly demonstrate that chlorophyll fluorescence imaging can be used as an alternative tool for freezing tolerance assessment. Because chlorophyll fluorescence imaging is efficient, it can be used in screening large numbers of breeding lines.

Determining germination thresholds in the Mixed-grass Prairie using modeling approach and their implications to global change: a FACE study

Jin Li* and Yuguang Bai

Department of Plant Science, University of Saskatchewan, Saskatoon, Canada - S7N 5A8. *Corresponding author, E-mail: jil095@mail.usask.ca

Global climate change is an escalating concern because of its numerous economic and ecological consequences. Increases in the concentration of greenhouse gases in the atmosphere are thought to be the causes of global climate change, which can affect all aspects and processes of ecosystems. Plant reproduction in response to rising CO_2 has important implications in both natural and agro-ecosystems. The effects of CO_2 enrichment, increasing temperature and other parental conditions during plant growth and seed production, and their influences on seed germination, have been reported in many species. However, there are no consistent trends both within and among plant functional groups regarding seed quality and germinability as affected by climate change conditions. We propose to use thermal and hydrothermal time models to identify the shifts in temperature and water thresholds during germination in selected species from the Mixed-grass Prairie brought about by elevated CO_2 and warming conditions. Seeds are collected from the PHACE (Prairie Heating and CO_2 Enrichment) experiment. Germination tests will be conducted under six alternating temperatures with temperature amplitude of 10°C and increments of 5°C for the thermal time model; and under six water potentials at three alternating temperatures for the hydrothermal time model. As the significantly increased germination rate under elevated CO_2 has been reported in many species, it is predicted that either a decrease of thermal time requirement or a lower base temperature for germination under elevated CO_2 will be found.

Feral alfalfa and its implications for novel trait confinement

Muthukumar Bagavathiannan*[1], Robert Gulden[1] and Rene Van Acker[2]

[1]Department of Plant Science, University of Manitoba, Winnipeg, Canada - R3T 2N2; [2]Department of plant agriculture, The University of Guelph, Ontario, Canada - N1G2W1. *Corresponding author, E-mail: umbagava@cc.umanitoba.ca

Medicago sativa (alfalfa) is an important forage crop in North America. Apart from cultivated fields, alfalfa is also found along road sides and other natural and semi-natural habitats. However, limited information is available on the establishment capabilities of alfalfa in non-cultivated areas and the potential of these founding populations to become feral. This information will help us to understand whether feral alfalfa populations will become a barrier for the co-existence of cultivated alfalfa containing novel traits with conventional alfalfa. The objective of this study was to investigate the demography and dynamics of alfalfa populations occurring in natural and semi-natural environments. Research sites were identified in three rural municipalities in Southern Manitoba (Western Canada) in 2006. The dynamics of different life stages including soil seedbank, seedling recruitment, vegetative growth and reproductive output were investigated. Mowing was usually done by the rural municipality two times per season (late June, late August). In general, almost 93% of the non-mowed alfalfa plants and about 29% of the mowed plants were reproductively successful and this may be sufficient for replenishing a seed bank. We found a viable and active seed bank at the roadside sites. Seedling recruitment varied greatly among the sites and on average, 1.2 seedlings successfully recruited around each mother plant studied. Herbicide spray in the road verges (often 2,4-D) killed the above ground life stages but its effect on below ground stages is not clear. This data is used to develop a matrix population model for feral alfalfa population establishment and growth.

Fatty acid composition in diverse oat germplasm

Rohit Dhanda*[1], Brian Rossnagel[1] and Axel Diederichsen[2]

[1]Crop Development Centre, University of Saskatchewan; [2]PGRC, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada. *Corresponding author, E-mail: rkd318@mail.usask.ca

Oat is a very important crop for livestock feed and human nutrition. Increased interest in health benefits from seeds, such as oat, has lead to a need to explore current germplasm of oat for nutritional qualities. The objective of this research is to study the fatty acid profiles of a subset of some 1000 diverse accessions from the

oat collection preserved in the Canadian national seed genebank, Plant Gene Resources of Canada (PGRC), at the Agriculture and Agri-Food, Canada Research Centre, Saskatoon, Saskatchewan, Canada. The accessions include a wide range of *Avena sativa* L. and other selected species from the genus *Avena*. Gas Chromatography will be used to determine fatty acid profiles and selected accessions will be grown in replicated field trials to gain insights to the influence of the environment on fatty acid composition. The understanding gained from this research has direct applications on improving the fatty acid profiles of future oat cultivars for both food and feed.

Reducing tillage in organic agriculture on the Canadian prairies

Iris Vaisman* and Martin Entz

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In organic crop production, tillage is used to terminate and incorporate green manures, and also as a major tool for weed control. However, tillage has been shown to decrease organic matter and can lead to erodible soil. Zero tillage cropping systems, on the other hand, have been shown to have better soil health and decreased risk of erosion as compared to tillage systems. Organic farmers are therefore faced with the challenge of managing green manures in a way that can maintain soil health, while minimizing weed populations. In response to this challenge, the Rodale Institute developed the roller crimper. The roller crimps crop stems and lays the crop flat, eventually drying and killing the plant. The residue left on the soil surface creates a barrier that can suppress weeds, reduce water evaporation, and also contribute to soil organic matter. The roller crimper can therefore help reduce tillage in organic agriculture. This study compared the termination of green manures in tillage, low tillage, and zero tillage conditions, and the effect on soil nitrogen, soil water, and yield of a subsequent wheat crop. In the spring of Year 1, green manures were seeded. At full bloom, the green manures were subject to varying intensities of tillage. Disking was used in tillage treatments and the roller crimper was used in the low and zero till treatments. In Year 2, wheat was seeded. At the time of seeding, nitrogen and soil water were measured down to 120cm, and at harvest, yield was assessed. The study showed that using the roller crimper to terminate green manures provided the same amount of nitrogen and soil water to the subsequent wheat crop as tillage. Wheat yield was not affected by tillage intensity. The roller crimper therefore has the potential to replace tillage of green manures while still providing adequate nitrogen and wheat yield.

Propagation studies of sugar maple (*Acer saccharum*)

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Sugar maple (*Acer saccharum*) is a very important tree species known for its sap in the making of maple syrup, and also for its wood quality, commonly used for furniture and flooring. The project aims to study the effectiveness of procedures of propagating sugar maple by tissue culture and rooting of softwood cuttings under mist. The development of an effective propagation method for sugar maple will open many doors of opportunities for the nursery industries to improve their cultivar selection and increase the production capacity, or to the further expansion to an establishment of a biotechnology industry focusing on sugar maple lines for its ornamental and/or economic values. Currently, bud grafting is the only propagation method used to vegetatively produce sugar maple trees in Manitoba. Axillary buds from young sugar maple trees (< 15 years old) were plated on Murashige-Skoog (MS) media containing 0.01 mg/L thidiazuron (TDZ) and 2 mg/L 2- isopentenyl adenine (2iPA) to induce formation of multiple shoots, which were excised to be rooted on a half-stength MS with 1 mg/L α-naphthaleneacetic acid (NAA) and 0.03 mg/L pheenylacetic acid (PAA). Sugar maple softwood cuttings were collected on three dates; July 3, 10 and 15 of 2008. Two cultivars, Unity and Fall Fiesta, were used in the experiment. The cuttings were treated by dipping the bottom 1-inch stem in solution of either NAA or indole-3-butyric acid (IBA) at 5 g/L or 10 g/L or a combination of NAA and IBA for ten seconds, before planting them in sand bed with timed-interval misting. StimRoot #2, which is composed of IBA at 4000 ppm, served as a control. Among the three collection dates, the first collection on July 3, 2008 yielded the best rooting percentage. In addition, treatment with 5 g/L of IBA gave the highest rooting percentage in both cultivars.

Plant Pathology

The effect of three different spray inoculation protocols on FHB infection of cultivars of common wheat and durum wheat

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Fusarium head blight (FHB) caused by the fungal pathogen *Fusarium graminearum* is a devastating disease of wheat that leads to losses in yield and grain quality. Mycotoxins such a deoxynivalenol (DON) in FHB infected grain also limit the use of the grain for food and feed purposes. Reliable screening methods that can effectively identify differences in host resistance and susceptibility are essential to successful breeding for FHB resistance. Screening methods vary in the source of inoculum used, physiological stage the plant at the time of inoculation, and number of inoculations. The objective of this study was to compare the effect of three different inoculation protocols and an uninoculated check on Fusarium head blight infection in eight common wheat (*Triticum aestivum*) genotypes and two durum wheat (*Triticum turgidum* spp. *durum*) genotypes that differed in response to *F. graminearum*. Conidial suspensions of a mixture of four *F. graminearum* isolates were used for all three conidial suspension inoculation protocols. The spray inoculation protocols started at different physiological growth stages: first heading, first anthesis, and fifty percent anthesis, and differed in the number of inoculations. Disease incidence and severity were recorded from first development of visual symptoms and subsequently every three days until symptoms were indistinguishable from natural senescence. FHB index was calculated from the product of the incidence and severity measurements. Area under the disease progress curve was determined for each treatment. The percentage of Fusarium damaged kernels and the DON content was determined on harvested grain samples. Preliminary analysis of data showed that the relative ranking for resistant and susceptible wheat genotypes did not change with the different inoculation protocols. However, there were absolute differences in disease severity and disease incidence among the different inoculation protocols. Further data collection and analysis will be conducted to determine which protocol gives the best separation among the wheat genotypes.

Cytological studies on necrotic and chlorotic lesions induced by *Pyrenophora tritici-repentis* on wheat

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Pyrenophora tritici-repentis causes necrosis and chlorosis on susceptible wheat hosts. Ptr ToxA and Ptr ToxB are responsible for inducing necrosis and chlorosis, respectively in sensitive wheat lines/cultivars. Using a fluorescence microscopic technique, the pre-colonization and colonization events of necrosis- and/or chlorosis-inducing isolates of the pathogen were studied on four wheat lines/cultivars, three Ptr toxin-sensitive and one resistant. There was no significant difference between Ptr ToxA- and/or Ptr ToxB-producing isolates in terms of germination percentage, number of germ tubes and appressoria per spore, as well as the penetrated epidermal cells per conidia. There was also no significant difference between isolates in their incompatible interaction with wheat lines/cultivars in terms of percentage of mycellial area coverage (mycelium %) in the mesophyll layer. But, there was a significant difference between Ptr ToxA- and Ptr ToxB-producing isolates in their compatible interaction with wheat lines/cultivars after 24 hours and thereafter till 72 hours postinoculation, necrosis-producing isolates had a higher mycelium percentage than the chlorosis-inducing isolates.

Double haploids production of canola with improved resistance to *Sclerotinia sclerotiorum*

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Sclerotinia Stem Rot (SSR), caused by *Sclerotinia sclerotiorum*, is the most serious yield-reducing fungal disease of canola (*Brassica napus* L.) in ND. Up to 13% of the yield loss is caused by SSR. Currently no canola cultivars available are resistant to SSR. In this study, double haploid populations are being developed using microspore culture. The plant materials used for double haploid production are *Brassica napus* accessions collected by USDA National Plant Germplasm System (NPGS). These accessions have been screened for SSR resistance based on a three-year evaluation in the Department of Plant Pathology at NDSU. The research result showed that genotype and culture temperature significantly affect microspore embryogenesis. Treatment of isolated microspores with 32°C for 24 hours is essential to induce embryogenesis for four out of seven lines. A few haploid and double haploid (need to be confirmed) plants showed SSR resistance after inoculated with *Sclerotinia sclerotiorum* in the greenhouse. Resistant plants have nearly no symptom while the susceptible ones resulted in irreversible wilt of foliage in 5-7 days after inoculation. These plants are being cloned *in vitro* for further evaluation.

The role of abscisic acid (ABA) and other phytohormones in defense signaling in the *Solanum tuberosum-Verticillium dahliae* interaction

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Verticillium dahliae is a soil-borne pathogen causing Verticillium wilt in potato (*Solanum tuberosum*). It infects plants via their roots and proliferates through the vascular system of the plant. Interestingly, its growth *in planta* is very similar between plants that are either highly susceptible or moderately resistant. Plant resistance functions through a complex network of signaling pathways, including phytohormones such as salicylic acid, jasmonic acid, and ethylene. The concept of crosstalk between these pathways is an important issue because they may have an antagonistic interaction or may cooperate in different steps of the defense reaction. It is commonly believed that the jasmonate pathway is the main one involved in potato defense against *V. dahliae*, in analogy with this pathway's role in the interaction of *V. dahliae* with tomato. According to several recent studies, another hormone, abscisic acid (ABA), is involved in plant responses to both biotic and abiotic stress. This molecule may play a role in potato defense. To date, it remains unclear which of the major pathways are involved in the potato-*V. dahliae* signaling. The focus of this project is to elucidate the involvement of abscisic acid in the plant defense reaction of *S. tuberosum* after infection by *V. dahliae*. Of particular interest is at what stages of the interaction the phytohormone is involved and whether ABA interacts with other pathways. Using a model where both a susceptible and moderately resistant potato cultivars are inoculated with either a hypo-aggressive or a highly aggressive isolates of *V. dahliae*, I will investigate (i) the expression of genes representing the major signaling pathways, (ii) the expression of defense genes, and (iii) the production of SA, JA, and ABA. (i) and (ii) will be completed using real-time RT-PCR, and (iii) using chromatography/spectroscopy techniques.

Development of transformation and RNA-mediated gene silencing systems for functional genomics of *Cochliobolus sativus*

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Cochliobolus sativus (anamorph: *Bipolaris sorokiniana*) is an important fungal pathogen which causes many diseases such as spot blotch, common root rot and black point on wheat and barley. Good gene transformation and RNA-mediated silencing systems are very useful and necessary for studying gene functions. In this study, we used Tox A and GFP genes as the reporter genes and isolate of *C. sativus*, ND93-1 to develop such good systems. We have got transformants with Tox A and GFP gene by polyethylene glycol (PEG)-mediated

transformation. High level expression of GFP can be observed in both mycelia and conidia. Based on PCR amplification and infiltration on some differentials of wheat and barley we also find that Tox A gene can be expressed in this fungus. However, when a silencing vector modified from pSlient-1 to adapt to Gateway Cloning System, which can express the hairpin RNA construct was transformed into all transformants, we found that the expression of GFP and toxin were decreased. Based on the southern blot and real time PCR we confirmed that the decrease was due to the expression of the silencing vector. These systems would provide a powerful tool for large scale gene functional studying in this fungus and other filamentous fungi.

Toropi as a source of leaf rust resistance genes

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Leaf rust is one of the most prevalent diseases in wheat and is found almost everywhere wheat is grown. The most cost effective method to control rust is genetic resistance. Designation of 61 leaf rust resistance genes has been described. Most leaf rust resistance genes are race-specific, losing effectiveness by shifts in *P. triticina* population. Some adult plant resistance genes have provided more durable resistance than many genes expressed at the seedling stage. Toropi, a Brazilian cultivar released in 1965, and grown extensively for 15 years, has maintained its resistance for over 40 years. Two complementary recessive genes on 1AS and 4DS chromosomes were previously identified when *P. triticina* virulence phenotype LCG-RS was used. The objective of our study was to identify, characterize, and fine map the leaf rust resistance genes in Toropi. Resistant lines derived from crosses between Toropi and a susceptible parent IAC 13-Lorena, and both parents were inoculated at seedling and adult plant stages with 6 different isolates of *P. triticina* (BBBD, TDBG, TJBJ, MGBJ, MBDS, MBRJ) and with a mixture of different isolates. The results achieved to date demonstrated that Toropi has at least one race specific seedling resistance gene and three adult plant resistance genes, two of which were race non-specific. Crosses between Toropi and Thatcher are being conducted to develop new population to better characterize the source of resistance present in Toropi and to map these new genes.

Effects of 3-ADON and 15-ADON chemotypes of *Fusarium graminearum* on spring wheat

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Fusarium graminearum, the causal agent of Fusarium Head Blight (FHB) results in yield losses and decreased grain quality in wheat due to the presence of deoxynivalenol (DON). Recent research shows that the higher DON producer, 3-acetyl DON (3-ADON) is replacing the 15-acetyl DON (15-ADON) chemotype populations. The objectives of this study were to evaluate the effects of *F. graminearum* isolates on the incidence, severity, FHB index, Fusarium damaged kernels (FDK), DON accumulation, yield and area under the disease progress curve (AUDPC). Three wheat genotypes were used based on their resistance levels: FHB37 (resistant), AC Cora (intermediate) and CDC Teal (susceptible). A split plot design with three replicates was used. *F. graminearum* isolate was the main plot effect and wheat genotype was the sub plot effect. The plots were artificially inoculated with single spore cultured inoculum. There were 13 3-ADON and 12 15-ADON isolates tested. After two inoculations, the plots were mist irrigated every 10 minutes for 10 hours. Disease incidence and severity were measured at three day intervals until there was loss of green coloration of the control spikes. FHB index values were determined as percentages from the product of disease incidence and severity measurements. Plots were harvested to determine effects of the isolates on grain yield. Analysis of the results showed that there were significant differences among isolates, genotypes as well as the isolate-genotype interaction for yield and AUDPC. Partitioning out the isolate effects showed that there were no significant differences within the 3-ADON isolates, although there were significant differences within the 15-ADON isolates and between the 3-ADON and 15-ADON isolates. Analysis also showed that there were significant differences between isolates and among genotypes. Isolate-genotype interactions showed that the relative rankings stayed the same, although there were changes in magnitude. Further analyses will be done to determine if there is a difference between the 15-ADON and 3-ADON chemotypes.

Mating system of *Colletotrichum truncatum*, the causal agent of lentil anthracnose

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Fungal diseases are the major biological constraint to lentil seed productivity in Western Canada. Amongst them is lentil anthracnose, caused by *Colletotrichum truncatum* (Schwein.) Andrus & Moore, an ascomycete known to reproduce asexually in the field. Sexual reproduction, however, has been demonstrated under laboratory conditions. In order to describe the mating system of *C. truncatum*, crosses among 21 isolates from Saskatchewan and Manitoba were performed. Sterile lentil stems were soaked in spore suspensions of each isolate individually and of all possible pairs of isolates to test for cross- and self-fertility. Stems were incubated under optimum conditions for perithecium formation. All isolates were self-sterile, suggesting that *C. truncatum* is heterothallic. Isolates fell into two mating compatibility groups, which is consistent with a bipolar self-incompatibility mating system. In this type of system, mating types are usually determined by a single locus with two alleles called MAT1 and MAT2. Experiments to determine if those genes are present in *C. truncatum* are being conducted. This study will be completed by analyzing the distribution and frequencies of mating types in the field in order to detect potential outbreeding. Information from this project on the biology and population structure of *C. truncatum* will eventually provide direction for anthracnose-resistance breeding in lentil.

Simulating pathogen population shifts of *Pyrenophora tritici-repentis* on different Canadian wheat cultivars

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The fungal pathogen *Pyrenophora tritici-repentis* causes tan spot, an important leaf spotting disease of wheat worldwide. The epidemiological value of Ptr ToxA and Ptr ToxB, the host-selective toxins of *P. tritici-repentis*, was studied on seven Canadian wheat cultivars. Six susceptible and one resistant cultivar (five hexaploid and two tetraploid) were inoculated with a pathogen population consisting of a mixture of isolates from the eight known races of the pathogen (one isolate per race). Subsequent pathogen generations were constructed by mixing 100 single-spore isolates obtained from individual lesions from infected plants. These populations were re-inoculated to produce the next generation. In this study, four consecutive generations were constructed, and the population structure at the second and fourth generations was assessed by PCR. Ptr ToxA-producing isolates were predominant on all of the hexaploid and one tetraploid (4B160) wheat cultivars, followed by isolates that produce both Ptr ToxA and Ptr ToxB. Ptr ToxB-producing isolates, which are not part of the Canadian pathogen population, survived on all the major wheat cultivars grown in western Canada over the past century which were included in this study. This suggests that Ptr ToxB-producing isolates would have been a matter of concern for wheat production if they were part of the pathogen population. The production of Ptr ToxA and/or Ptr ToxB appears to confer a competitive advantage to the producing isolates, as determined by population shifts over four generations.

Characterization of the secreted proteome of *Ascochyta rabiei*

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Chickpea (*Cicer arietinum* L.) is the third most important food legume grown with 9 million ton produced globally each year. The necrotrophic fungus, *Ascochyta rabiei* (Pass.) Labr., the causal organism of ascochyta blight, is the most devastating pathogen of chickpea worldwide. Plant pathogens secrete a vast number of proteins to manipulate the host and to digest nutrients. Many of these proteins have been found to be important virulence factors. Therefore the study of these secreted proteins is especially important for understanding host–

pathogen interactions. We are characterizing proteins secreted by *A. rabiei* under different growth conditions including growth in Modified Fries Media, Czapek Dox media and *in planta*. Modified fries media has been used in many fungal systems for the production of proteinaceous toxins. In Czapek Dox media *Ascochyta rabiei* is known to produce the only well studied toxins known to be secreted by *Ascochyta rabiei*, the solanapyrone toxins. Characterization of proteins secreted *in planta* will be used to identify proteins secreted by pathogen during infection and will be compared to those observed from the other growth conditions. Proteins harvested from modified fries media and czapek dox media have shown similar patterns on SDS PAGE. Two-dimensional gel electrophoresis results have shown that the production of proteins with basic PI values is reduced in czapek dox media when compared to fries media while there is no significant difference in the production of acidic proteins in both media conditions. We are currently obtaining intracellular wash fluids from infected plants for comparison to these results.

Assessment of the biocontrol resources of *Pseudomonas* species antagonism towards *Sclerotinia sclerotiorum* (Lib.) de Bary under the different cropping systems

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Sclerotinia sclerotiorum (Lib.) de Bary is a devastating fungal pathogen that causes a disease on over 400 species of plants around the world, including many economically important vegetables and field crops. Sclerotinia, a pigmented, multi-hyphal structure produced by this fungal, can remain viable over long periods of time under unfavorable conditions and make it a very successful pathogen. Due to the lack of adequate levels of host resistance, crop rotation and fungicides have been major methods for sclerotinia diseases control. However, the fungicide could pose the high risk to pollute environment, and to develop fungicide resistance. Biocontrol of sclerotinia diseases is an alternative disease control strategy. The objective of this study was to investigate potential biocontrol resources of *Pesudomonas* species under different long term cropping systems in Manitoba Canada. The *Pseudomonas* strains were isolated from bulk soils and rhizosphere samples collected from different cropping systems (different rotations, monoculture and with and without pesticides) at the Carman and Glenlea Research Stations in Manitoba Canada from 2006 to 2008. An *in vitro* test screened the isolates for their antagonism against *Sclerotinia sclerotiorum* .They were further characterized using gene-specific PCR primers for their antibiotics, and antibiotics were then confirmed by HPLC. It was found that pyrrolnitrin-producing strains were predominant in several cropping systems, followed by Phenazine-producing strains, while 2, 4-DAPG and pyoluteorin –producing strains were much less. Differences between the treatments were also found based on the frequency of isolation of antibiotic producing Pseudomonas strains. Higher relative numbers of antibiotic-producing isolates were obtained from cropping systems without pesticide treatment than cropping systems with pesticide treatment. The results obtained in this study strongly indicate that different cropping systems influence the antibiotic-producing sub-population dynamic of *Pseudomonas spp* .

Identification of quantitative trait loci for resistance against sclerotinia stem rot in *Brassica napus* L.

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Sclerotinia stem rot causes significant losses on *Brassica napus* L. The first part of the project was to identify a suitable screening method. For this four methods; ascospore spray, detached leaf, cotyledon inoculation and petiole inoculation method were compared for their suitability and repeatability. Ascospore spray method requires ascospore spray at flowering stage under high humidity conditions. This method was discarded for its requirement of large humidity chambers and mature plants. Detached leaf and cotyledon methods were found to be non-reliable methods in this study. These methods could not clearly differentiate resistant and susceptible parents. Petiole inoculation method with certain modification was identified as most appropriate method. The main modification was to reduce the plant age at which plants were inoculated to three week as compared to four weeks as described in original method. This modification has not only reduced the total time taken for screening but also overcome the differential growth habit problem of winter and spring types of germplasm. This method is being used to identify QTL against sclerotinia stem rot. Six double haploid (DH) populations having

resistant parent and six susceptible parents are being used for this study. Each population is being screened three times with each line having twelve plants. The results so far show reliability of the method over the replications for most of the lines in each population. The overall objective here is to identify conserved QTL among these six DH populations and to develop molecular markers which could be used to identify tolerant plants in back cross populations and thus to fasten the breeding objectives.

Plant Breeding

Genetics of pre-harvest sprouting in barley

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Genetic variation in the dormancy level of barley (*Hordeum vulgare* L.) can have a drastic effect on the malting value of a cultivar. Excessive dormancy has been selected against by plant breeders to allow uniform germination during the malting process shortly following harvest. As a result of low levels of dormancy, sprouting can occur while still on the mother plant, known as pre-harvest sprouting (PHS), which renders the seed useless for malting. Field conditions prior to harvest that include cool temperatures and high levels of moisture greatly increase the losses due to PHS damage, as seen regularly in the upper Midwest since the mid 1990s. Dormancy genes have been identified in several mapping populations of barley using germination tests, and several QTL have consistently been identified. Identification of PHS differs from dormancy, in that there are whole plant and environmental interactions that are absent in germination tests. We have developed a test to identify PHS in the greenhouse using whole plant artificial misting tests. Currently, we are running experiments to optimize the length of misting treatments to identify differences between 14 genotypes with known PHS reaction. The misting test will be then be used to phenotype three mapping populations that have been previously evaluated for dormancy using germination tests to determine if dormancy and PHS are genetically related. Additionally, we are screening lines from eight breeding programs collaborating in the USDA-CSREES Barley Coordinated Project (CAP) for dormancy using a germination test. These data will be analyzed using association mapping methods to identify QTL. Preliminary evaluation of the 2006 and 2007 CAP lines has identified several genomic regions potentially involved with dormancy.

Carotenoid accumulation during grain fill in durum wheat

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Yellow pigment content, primarily caused by the carotenoid lutein, is an important, highly heritable quality trait in durum wheat (*Triticum turgidum* L. var *durum*). Carotenoids in durum are valued in the export market and have associated health benefits. The main objective was to measure the accumulation patterns of lutein, zeaxanthin, α-carotene, β-carotene and other carotenoids during grain fill in 13 durum lines. These lines were selected for their large variation in yellow pigment content. High performance liquid chromatography (HPLC) is currently being conducted to quantify these carotenoids. Lutein accumulation stopped early in grain fill in low pigment lines, and continued to the end of grain fill in medium and high pigment lines. A second objective was to obtain sequence information for the different alleles of *Psy1*, which is the gene coding for a phytoene synthase. This enzyme catalyzes the first committed step in the carotenoid biosynthetic pathway. Three alleles (*PsyA1a*, *PsyA1b* and *PsyA1c*) have been linked to significant differences in yellow pigment levels from previous studies. Sequencing confirmed the presence of *Psy-A1c* in one of the low-pigment lines from the accumulation study, which is in agreement with published data on this allele. Information from this study will help guide breeding for higher carotenoid content.

Identification of early maturing inbred lines and hybrids with fast rate of dry down

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Fast dry down is an economically important breeding target of corn (*Zea mays* L.) in the northern U.S. Corn Belt. However, phenotyping this trait precisely has been a challenge for corn breeders. The objectives of this research are to establish a simple, fast, and reliable method to phenotype field dry down, and to identify elite inbred lines and hybrids with fast rate of dry down. Electronic moisture meter BLD5604 was used to measure moisture content of ear samples. For establishing a regression model between meter reading and actual moisture, a total of 73 hybrid ears were randomly sampled to measure grain moisture starting at 30 days after pollination until harvest at 7-day intervals, using both electronic and oven-tried methods. Three North Carolina II (NCII) mating designs between elite NDSU and industry lines produced single-cross hybrids that were grown across ND and MN locations in experiments arranged in 12 x 12 partially balanced lattice designs in 2007 and 2008. Ear moisture was collected at four 7-day intervals starting 45 days after pollination (D1, D2, D3, and D4). The area under the dry down curve (AUDDC) was calculated based on D1 to D4. A model was established to estimate actual moisture ($R^2 = 0.82$). Experimental lines ND05-73, ND06-85, and ND06-211 would be promising sources of fast drying hybrids while ND06-144 and ND06-50 showed potential for grain yield. Results suggested AUDDC can be a good index for fast dry down selection and that using BLD5604 can reliably phenotype this trait, being much easier and faster than the traditional oven-dried method.

Characterization of the Agp-L locus in winter and spring wheat varieties

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Breeding for increased starch composition in winter wheat has become important due to the demand for a high starch, high yielding and disease free fermentable feedstock for ethanol production. The detection of natural sequence variation in the Agp-L locus associated with higher yields would be valuable to plant breeders and to the ethanol industry. The Agp-L locus encodes ADP glucose pyrophosphatase (AGPase) the rate limiting enzyme in starch biosynthesis. AGPase consists of four subunits, the large subunit, Agp-L, locus is found on group 1 chromosomes of common wheat and its manipulation has been shown to increase total seed weight. *Triticum aestivum* is an allotetraploid crop with three related genomes making isolation and sequencing of genomic DNA to detect polymorphisms difficult. Detecting single nucleotide polymorphisms, small insertions and deletions has involved the construction of genome specific primers, sequencing the Agp-L locus in 24 diverse spring and winter lines and assembly and alignment of these sequences. Preliminary results show several haplotypes based on 1B chromosome sequence. To determine if the sequence characteristics are linked to overall starch content these varieties are being grown under greenhouse conditions and upon harvest, grain yield, spikes per plant, seeds per spike and total percent starch will be measured. Using this data associations between the characterized haplotypes and phenotypic data will be analyzed.

Genetics studies on kernel and spike morphology in spring wheat (*Triticum aestivum* L.)

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Kernel morphology, texture, grain volume weight, shape of germ, crease, and brush are characteristics that determine the market value of wheat. For bread wheat, kernel shape and uniformity can significantly increase milling quality. Since productive spikes per unit area is one of the three major components of wheat grain yield, spike morphology has a significant influence on kernel traits, grain and flour yield. Therefore, using

recombinant inbreed lines (RIL) populations between diverse wheat germplasm, this study aims to: study the phenotypic variation of kernel and spike morphology; map and identify QTL associated with kernel and spike morphology; and determine the relationship between kernel and spike morphology and other agronomic and quality traits. To conduct this study two populations with 200 RIL will be used. The first population was developed from a cross between ND 705 and WCB 462. The second population was developed from a cross between WCB 414 and WCB 617. All of the parents are hard red spring wheat, except WCB 414 which is white wheat. The populations were developed using single seed descent method and the RIL will be evaluated during the F_9 generation. The experiments will be conducted in summers of 2009 and 2010 at two locations, Prosper and Carrington, ND. Each experiment will include the RIL, their parents, and checks and will be laid out in a randomized complete block design, with two replicates. The phenotypic data will be collected on agronomic traits, kernel morphology, and spike morphology. For the molecular studies, DNA will be extracted from the RIL and their parents and microsatellite markers associated ($P<0.05$) with the traits of interest will be used to genotype the entire populations for polymorphisms. Linkage maps will be constructed with a logarithmic of odds of 3 and Kosambi function.

Identification of molecular markers linked to x-disease resistance in chokecherry

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Chokecherry (*Prunus virginiana L.*) is an important native woody species in the northern Great Plains of North America. X-disease can cause severe damage in stone fruit production and can be hardly detected. Development of disease resistant plants would be the best management of X-disease. In this study, hybrid seedlings of populations derived from the cross between resistant and susceptible chokecherries were inoculated with X-disease phytoplasmas using a grafting system. The nested-PCR was used to early confirm the infection and proliferation of X-disease pathogen in grafted chokecherry plants. Phenotyping data were available in the coming growth season. Before searching the molecular markers linked to X-disease resistance, SSR, TARP, and AFLP markers were used for construction of molecular genetic map. BSA (Bulked Segregant Analysis) will be used for the identification of the X-disease linked markers in the next step.

Genomics and Molecular Biology

Regulation of aliphatic glucosinolates biosynthesis in Brassica by gene replacement

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Rapeseed is an important source of seed meal for animal after edible oil; however reduced glucosinolates content in the seeds is primary requirement for better meal quality. High concentration of glucosinolates is deleterious to animal health and cause severe nutritional problems in livestock industries. Among all the glucosinolates, aliphatic glucosinolates are major contributor and their reduced content resulting good quality seed meal. In Brassica species, 4C and 3C aliphatic glucosinolates are biosynthesized by ELONG and PRO genes respectively. However white cauliflower and broccoli (*Brassica oleracea*) possesses non-functional alleles $ELONG^-$ and PRO^- respectively. White cauliflower and broccoli are back crossed with *B. rapa* to replace functional alleles ($ELONG^+$ and PRO^+) in A genome by non-functional alleles ($ELONG^-$ and PRO^-) from C genome. Gene specific SCAR markers are used for marker assisted backcross selection of non-functional alleles in *B. rapa*. Leaves and seeds glucosinolates would be analyzed by HPLC to determine reduce level of 4C and 3C glucosinolate in backcross progenies. Gene replacement or introgression for $ELONG^-$ and PRO^- alleles would be confirmed by linkage map based functional gene specific markers analysis.

Cloning and characterization of the meiotic genes *Rec8* and *Cdc5* in wheat

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Meiosis is a crucial cell division involved in the sexual reproduction of eukaryotes. It governs gametegenesis and gene transmission over sexual generations. Genes conditioning various meiotic events have been identified and characterized in yeast, plants, animals, and humans. The objectives of this research are to clone and analyze cDNA and genomic DNA sequences of the homologs of the meiotic genes *Rec8* and *Cdc5* in tetraploid wheat, designated *wRec8* and *wCdc5*; to determine chromosomal location of these two genes and their expression profiles at different meiotic stages and tissues; and to localize wRec8 and wCdc5 proteins within meiocytes during meiosis using indirect immunofluorescence. *Rec8* and *Cdc5*, encode a cohesion protein and a Polo-like kinase, respectively, are required for co-orientation of sister kinetochores at meiosis I. We have obtained full length cDNA sequences and most of the full length genomic sequences of the putative *wRec8* and *wCdc5*, and will characterize their structure and organization in wheat genomes. We have purified the proteins encoded by these two genes and have raised antibodies against the proteins. This will allow us to localize the proteins at sub-cellular level and to characterize their function in meiosis. Results from this study will enhance knowledge of chromosome segregation during meiosis and manipulation of meiotic process for various genetic studies and improvement of plant species.

DNA fingerprinting and assessment of genetic diversity among 35 biomass willow clones

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Willow clones and species are being investigated for their use as short rotation crops for biomass production, to assess the impact of returning agricultural landscapes back to woody vegetation on the biogeochemical cycle of C and N in Saskatchewan. Morphological identification of the 35 willow clones and species used in that study is sometimes difficult to accomplish, due to intraspecific variation, superficial similarities and developmental variability. These difficulties can cause concerns about certifying that the correct species or clone is being used. Our work aims to use molecular techniques to identify the clones and assess the genetic relatedness among them. rRNA genes have been investigated through amplification and sequencing of internal transcribed spacer (ITS) and Non Transcribed Spacer (NTS). 35 ISSR primers have been tested, scored and data analysed through NTSYS. SCAR markers have been developed from informative SNPs of ITS sequences for individual identification of clones.

Galactinol synthase activity in lentil seeds with contrasting raffinose family of oligosaccharides profiles

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Raffinose family oligosaccharides (RFO) and sucrose are the major soluble carbohydrates of lentils (*Lens culinaris*. M). High concentrations of RFO affect lentil quality as food and affect its consumption. Galctinol synthase plays an important role in the biosynthesis of RFO by catalyzing the formation of galactinol from UDP-gal and myo-inositol. Galactinol acts as a galactocyle donor and is thought to be the first committed step in the biosynthesis of RFO and is thought to plays a key regulatory role in the carbon partitioning between sucrose and RFO in plants. The objective of our current study is to investigate GS activity at various stages in lentil seed development and to find any correlation between GS activity and total seed RFO concentrations. Initial work on galactinol synthase activity in lentil accessions with contrasting RFO concentrations showed that galactinol synthase has a pH optimum of 7.5; however, considerable activity was detected at pH range 5.0-8.0. Highest GS activity of 0.16 $\mu mole^{-1}min^{-1}mg$ protein was detected at 16 days after flowering (DAF) and reduced considerably as the seeds matured. No correlation, however, was found between GS activity and RFO concentration.

Mitochondrial sequence similarity and gene expression analysis in alloplasmic durum wheat

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Cellular organelle (chloroplasts, mitochondria) are known to exchange information with nuclei through "retrograde communication". Lack of this communication can be described as nuclear - cytoplasmic incompatibility. Hundreds of genetic diseases in animals and thousands of phenotypic variations in plants are known to result from such incompatibility. The species cytoplasmic specific (*scs*) gene plays a vital role in restoring compatibility between the nucleus and cytoplasm among various *Triticum* and *Aegilops* species. Information about genomic, proteomic and transcriptomic differences between particular wheat species and cytoplasm donors may help us understand the mechanisms of male sterility, plant vigor, seed size, and other characteristics resulting from nuclear-cytoplamic interaction. Four mitochondrial genomes will be sequenced, described and compared. The expression analysis of major mitochondrial genes will be performed. Mitochondrial sequences from thirteen different plant species were compared on the genomic and proteomic levels using BLAST and MultiAlign software. Protein and amino acid sequences for individual genes were used to create distance trees. Genes were compared and, based on consensus sequences, primers were designed. These primers are being utilized for analysis of mitochondrial expression patterns in alloplasmic plants and the early stages of seed development. The major mitochondrial genes involved in metabolism are highly conserved among species. Different plant species have mitochondrial proteins with similar composition on both the genomic and proteomic levels. These similarities can prove useful for understanding the regulation and expression of genes in species not yet sequenced. The objective of this research include: 1) determining the differences between four mitochondrial genomes of closely related species to durum wheat and 2) defining the role of the *scs* gene in relationship to expression of mitochondrial genes.

Molecular regulation during shoot apical meristem formation in canola microspore-derived embryos

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Our present study intends to identify the molecular mechanism that can guarantee the best performance of Shoot apical meristem (SAM) through embryogenesis. The relationship between the SAM structure and the expression of both *BnSHOOTMERISTIMLESS* (*BnSTM*) and *BnCLAVATA1* (*BnCLV1*), two SAM marker genes isolated from canola tissue, have been identified using RNA in situ and quantitative RT-PCR. We have used several different treatments to alter SAM formation. In the first treatment GSSG and buthionine sulfoximine (BSO), an inhibitor of reduced glutathione (GSH), were applied to the culture medium in order to enhance SAM formation and functionality (Belemonte et al, 2006). For other treatments we added triidiobenzoic acid (TIBA), an inhibitor of auxin transporter PIN1 protein, indole acetic acid (IAA), and reduced glutathione (GSH) to the medium. These compounds induce meristem deterioration resulting in poor pant regeneration. Our results indicate that the expression of *BnSTM* is directly associated by the SAM structure. Nevertheless, the expression of *BnCLV1* was not affected by BSO applications, although the expression of this gene was strongly suppressed by GSH. The expression of *BnCLV1* was repressed by TIBA and IAA, especially during the later stages of embryo development. This suggests that the expression pattern of *BnCLV1* is affected by the quality of the SAM during the late embryonic phases. RNA *in situ* experiments indicate that the expression domain for STM strongly increases in BSO treated embryos, while *CIV1* is not affected by this treatment. Both STM and CLV1 have been robustly repressed in deteriorated SAM. These results show that *STM* is the most significant gene that controls the stat-of-art for SAM. Ectopic expression of *BnSTM* in Arabidopsis significantly increases the number of somatic embryos per explant by 5 fold more than wild type. Also, the number of siliques is significantly increased in over expressed lines compared with wild type.

NOTES

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