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North American Pulse Improvement Association (NAPIA) Biannual Meeting

2-3 November 2017

The Kellogg Center East Lansing, Michigan, USA

Wednesday, November 1, 2017

8:00-17:00 NAPIA tour to Frankenmuth

17:30 NAPIA Board meeting

18:00-20:00 NAPIA Registration/Reception

Thursday, November 2, 2017

	Tiitle	Speaker
7:00-8:15	Registration and Continential Breakfast (Provided)	
8:15-8:30	Opening Session and introduction for the keynote speaker by NAPIA President	Weidong Chen
8:30-9:15	Keynote presentation: Pulses in wheat-based cropping systems modify soil nitrogen level, root-associated communities and soil function, when sufficient moisture is present	Chantal Hamel
Session 1	Chair: Julie Pasche	
9:15:9:30	Soil residual water and nutrients explain about 30% of the rotational effect	Yantai Gan
9:30:9:45	Does intercropping affect biodiversity and adaptive dynamics in plant pathogen complexes?	Christophe Le May
9:45-10:00	Potential of ABA Analogs as Plant Growth Regulators for Pulse Crops	Sue Abrams
10:00-10:30	Refreshment Break	

Session 2	Chair: Syama Chatterton	5 · 0 · 1
10:30:10:45	Temporal and Spatial Variation in a large collection of Chickpea (<i>Cicer arietinum</i>) landraces.	Eric Bishop von Wettberg
10:45-11:00	Utilizing Phenomics and GWAS in Cultivated Lentil	Derek Wright
11:00-11:15	The picture underground: wild lentils as genetic resources	Linda Gorim
11:15-11:30	LegumeInfo and Legume Federation resources for pulses and cool-season legumes	Jacqueline Campbell
11:30-11:45	Pulses as a promising whole foods for prevention of obesity	Dil Thavarajah
11:45-12:00	Enhancing nutritional value: biofortification of pea and lentil	Tom Warkentin
12:00-13:00	Lunch (Provided)	
Session 3	Chair: Tom Warkentin	
13:00-13:15	Faba Bean Research and Development in Saskatchewan	Bert Vandenberg
13:15-13:30	CRISPR/Cas9-mediated gene editing in chickpea	Yurdagul Ferhatoglu
13:30-13:45	Chickpea <i>Ca-elf3</i> a key gene for photoperiod responsiveness and adaptation to short growing regions	Bunyamin Tar'an
13:45-14:00	Phenological study of diverse lentil (<i>Lens culinaris</i> Medik.) germplasm in temperate and sub-tropical savannah growing environments*)	Sandesh Neupane
14:00-14:15	Days to flowering in Lentil (<i>Lens culinaris</i> Medik.) under long-day greenhouse and Temperate field conditions*).	Taryn Heidecker
14:15-14:30	Development of SNP markers for rust (<i>Uromyces viciae-fabae</i>) resistance gene controlling hypersensitivity in faba bean cultivar selection Doza#12034 (<i>Vicia faba</i> L.)*)	Usman IJAZ

14:30-14:45	Yield, nutrient uptake by four faba bean cultivars at four sites in Saskatchewan*)	Serena Klippenstein
14-45-15:00	Evaluation of Field Pea Susceptibility to Pea Seed-Borne Mosaic Virus (PSbMV) and Seed Transmission*)	Amanda Beck
15:00-15:30	Refreshment Break	
Session 4	Chair: Dil Thavarajah	
15:30-15:45	Characterization of Heat Responsive Genes in Pea Pollen and Leaves via RNA-Seq*)	Shaoming Huang
15:45-16:00	A high throughput root based phenotyping protocol for drought tolerance in Faba bean (<i>Vicia faba</i> L.)*)	Md Abdul Muktadir
16:00-16:15	Screening on salt-tolerance genes and functional analysis of two genes of myo-inositol-O-methyltransferase and aquaporins in <i>Sophora alopecuroides</i> L*)	Yin Zhichao
16:15-16:30	Evaluation of genome wide association study panel for Fe, Zn, and Se concentration in field pea*)	Dilanganie Dissanayaka
16:30-16:45	Evaluation of X-ray fluorescence spectroscopy for phenotyping pea seeds for mineral concentration*)	Ramandeep Bamrah
16:45-17:00	Using overhead images to determine volume and ground cover of lentil (<i>Lens culinaris</i> Medik.)*)	Karsten Nielsen
	*) Student Presentations	
18:00-20:30	NAPIA mixer & Poster viewing (Author presents)	

Friday, November 3, 2017

	Title	Speaker
7:30-8:15	Continential Breakfast (Provided)	
Session 5	Chair: Kevin McPhee	

8:15-8:45	Key presentation: Comparative genetics of Aphanomyces root rot resistance and root architecture in pea	Marie-Laure Pilet-Nayel
8:45-9:00	Introgression Breeding of Major Aphanomyces Root Rot Disease Resistance QTLs into Elite Pea Varieties	Shivappa Hukkeri
9:00-9:15	Quantitative Trait Loci Associated with Yield, Seed Yield Per Plant, and Seed Size in Dry Field Peas	Jamin A. Smitchger
9:15:9:30	Increasing Field Pea Yield with Leaf Type Varietal Blends	Lena D Syrovy
9:30:9:45	Exploring resistance in <i>Lens ervoides</i> to foliar fungal pathogens	Sabine Banniza
9:45-10:00	Updates to the Cool Season Food Legume Genome Database: Resources for pea, lentil, faba bean and chickpea genetics, genomics and breeding	Sook Jung
10:00-10:30	Refreshment Break	
Session 6	Chair: Sabine Banniza	
10:30:10:45	Rapid generation cycling of RILs from <i>Lens culinaris</i> x <i>Lens ervoides</i> after aphanomyces-root rot screening	Monika Lulsdorf
10:45-11:00	Identifying Sources of Resistance in Chickpea to Seed Rot and Pre-emergence Damping-off Caused by	George Vandemark
	Mefenoxam-Resistant Isolates of Pythium ultimum	
11:00-11:15	Mefenoxam-Resistant Isolates of <i>Pythium ultimum</i> Assessment of the efficacy of four seed treatment fungicides on <i>Didymella lentis</i> of lentil.	Cecilia Peluola
11:00-11:15 11:15-11:30	Assessment of the efficacy of four seed treatment	Cecilia Peluola Lyndon Porter

11:45-12:00	Insect pests and fungal pathogens associated with faba bean production in Alberta, Canada.	Syama Chatterton
12:00-14:00	Lunch (Provided) & Announcements	
14:00-16:00	Business Meeting Pisum CGC Meeting Cool Season Legume CGC Meeting	

North American Pulse Improvement Association (NAPIA) Biannual Meeting

POSTERS

1	Identification of White Mold Resistance Genes in Pea using time series RNA-Seq and Genome-wide Association Mapping	Hao-Xun Chang
2	Can endophyte seed treatments increase yield, protein and/or nitrogen fixation in peas and lentils?	Christopher Graham
3	Differential Gene Expression Regulating Protein Synthesis in Field Peas	John Lu
4	Variability within Pseudomonas syringae pathovars infecting pea in Montana	Josephine Mgbechi-Ezeri
5	Exploring Sclerotinia sclerotiorum effectors during infection of pea	Hyunkyu Sang
6	Refining seeding rate recommendations for different lentil classes	Lena D Syrovy
7	SEQUENCE CHARACTERIZATION OF PGIP1 OF CHICKPEA*)	Vishnutej Ellur
8	Identification of quantitative trait loci associated with cold tolerance in an interspecific chickpea recombinant inbred line population.*)	Deus Mugabe
9	Sampling strategies and identification of <i>Aphanomyces euteiches</i> in Montana soils*)	Murphy, Carmen
10	Physiological Response to PEG induced drought stress At Flowering Period in Adzuki Bean*)	Guo Wenyun

Promotive effects of ABA antagonist on seed germination of Brassica napus under low temperature*)
 Milling efficiency of the four major seed coat types in red lentil (Lens culinaris Medikus)*)
 LegumeInfo and Legume Federation resources for pulses and cool-season legumes

Junsheng Zhou
Maya Subedi
(LegumeInfo and Legume Federation resources for pulses and cool-season legumes
Campbell

*) Student Posters

Keynote speaker:



Chantal Hamel Ph.D.

Principal Research Scientist, Agriculture and Agri-Food Canada Adjunct Professor, Department of Soil Science, University of Saskatchewan

Chercheuse associée, Institut de recherche en biologie végétale

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A native of Québec City, Dr. Hamel obtained her PhD from the Department of Plant Science of McGill University in 1990 and Postdoctoral Fellow at Université de Montréal in 1991-92. She was a research scientist at the Service de phytotechnie de St-Hyacinthe of the Ministère de l'Agriculture des pêcheries et de l'alimentation du Québec in 1993-94, taught soil fertility and soil microbiology and biochemistry in the Department of Natural Resource Science of McGill University in 1995-2002, worked as a soil microbiologist with Agriculture and Agri-Food Canada in the semiarid prairie of Saskatchewan in 2003-2015, and now in the St. Lawrence River valley. Chantal's research concerns the efficiency of nutrient cycling in the continuum between the plants and the soil, and has generated over 130 co-authored publications. In the last decade she has lead several national studies concerning the influence of crop management on soil biology, mainly on the fungi and in particularly on the arbuscular mycorrhizal fungi. Chantal was member of the Executive Committee of the Conseil des productions végétales du Québec in 1997-1999, and is Secretary of the International Mycorrhiza Society from 2009 to 2015.

Keyspeaker:



Dr. Marie-Laure Pilet-Nayel

INRA, Institut de Génétique Environnement et Protection des Plantes, Le Rheu, France

INRA-Terres Inovia TMU PISOM, Ideotypes, Systems, Surveys of pea and faba bean Main diseases, Le Rheu, France

(Marie-Laure.Pilet-Nayel@inra.fr)

Dr. Marie-Laure Pilet-Nayel is a Research Scientist at INRA IGEPP, Rennes, France, graduated with a PhD degree in genetics and plant breeding since 1999. She has developed expertise in quantitative genetics, genomics, marker-assisted-selection and disease resistance in plants. After her post-doc position at USDA-ARS, WA, USA (1999-2000), she has managed research programs in genetics and genomics of Aphanomyces root rot resistance in legumes for the past 17 years, in tight collaborations with pathologists, physiologists and breeders especially in France and the USA. Her recent accomplishments are the development of NILs and GWA studies to validate QTL involved in partial resistance to Aphanomyces root rot in pea and to study the QTL-underlying mechanisms. Dr Pilet-Nayel has coordinated large local, national and international collaborative projects or workpackages and has experience of working at the interface between public research and breeding companies. She has authored or co-authored >35 publications in peer-reviewed international journals, > 90 communications in congress and >40 transfer documents or communications to breeders and technical institutes.

ABSTRACTS

Keynote presentation:

Pulses in wheat-based cropping systems modify soil nitrogen level, root-associated communities and soil function, when sufficient moisture is present

Chantal Hamel^{a,b}, Yantai Gan^b, Chao Yang^b, Navid Bazghaleh^b, Adriana Navarro-Borell^b, Walid Ellouze^b, and Luke D. Bainard^b

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^bSwift Current Research and Development Centre, Agriculture and Agri-Food Canada, 1 Airport Road, Swift Current, SK, S9H 3X2, Canada

Diversifying cropping systems by including pulses in wheat-based systems in semiarid regions is seen as a sustainable strategy for intensive grain crop production. As soil microorganisms are major contributors to nutrient cycling, understanding pulses' influence on soil biology is expected to help optimizing the efficiency of cropping systems. Plants influence the broad soil microbial community through the provision of organic matter, but can also influence differentially different microbial taxa through the production of bioactive molecules, including fixed nitrogen. Ten years of research on the influence of pulses on the microbial community of the roots and rhizosphere soil in the Canadian prairie has shown N₂-fixation as a key component of the pulse effect in wheat-based system. Pulse crops could modify the structure and diversity of roots and rhizosphere soil communities, leading to changes in soil function at the level of eleven groups of KEGG pathways. The effect of pulses on bacteria was related to increased soil N level more than to pulse identity, and wheat yield was related with soil nitrate level and Rhizobium abundance in soil. Cropping practices such as pesticide use and harvest time are components of the pulse effect on the microbial communities associated with roots. The roots of different pulse genotypes may host different microbial communities, a selective effect that may be attributed to a differential production of phytochemicals. Overall, it appears that pulse crops influence especially the rhizosphere soil community. The legacy effects of pulse on the diversity associated with the roots of a following crop of wheat is relatively modest, presumably due to the strong influence of wheat on its environment. The effects of pulse in wheat-based cropping systems are mitigated by drought in the Canadian prairie.

Session 1

Soil residual water and nutrients explain about 30% of the rotational effect

Yining Niu^{1,4}, Luke D. Bainard¹, Manjula Bandara², Chantal Hamel³, Yantai Gan^{1*}

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⁴Gansu Provincial Key Laboratory for Aridland Crop Science, Gansu Agricultural University, Lanzhou, 730070, China

Diverse crop rotations enable the best use of residual soil water and nutrients, thus, decreasing production inputs. Here, we determined the effect of cropping sequences on soil residual water and nutrients, and the performance of subsequent wheat. Nine rotation systems were evaluated from 2010 to 2014. Pea (P) and lentil (L) as preceding crops before wheat (W) or the rotation systems with pea (PPPW) or lentil (LLLW) included more than once in the 4-yr rotations had the highest residual soil water and N in the 30-90 cm depths. Preceding pea and lentil increased the subsequent wheat yield by 26% and 18%, respectively, as compared with the continuous wheat. Redundancy analysis revealed that soil residual water and N explained 12.4 to 42.7% (average 30%) of the yield variation in the subsequent wheat, with the rest of the rotational benefits unexplainable. Investigation of the factors other than soil water and nutrients that contribute to the succeeding wheat yield may further enhance the rotational effect.

Does intercropping affect biodiversity and adaptive dynamics in plant pathogen complexes?

Dutt A. ^{1, 2}, Andrivon D. ¹, Le Roy G. ¹, Jumel S. ^{1, 2}, Baranger A. ^{1, 2}, Le May C. ^{1, 2, 3}

¹ INRA, UMR 1349, Institut de Génétique Environnement et Protection des Plantes, Le Rheu, France

The sustainability of conventional agricultural systems is currently more and more questioned. New systems maximising ecosystemic services thus need to be developed. Within-field diversification through intercropping is one, favouring, among a large set of potential services, the control of plant diseases (Boudreau, 2013). However, not all

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intercrops are beneficial, as competition between or co-occurrence of hosts, largely depending on species, varieties, and cultural practises, can sometimes lead to unexpected interactions, especially regarding plant diseases succession and/or complexes evolution during the season. Complementary to quantitative assessments, a to date unexplored theoretical approach, based on mechanistic understanding of processes, would help us to better define the impact of intercrops on plant pathogen and pathogen complexes adaptative and evolving processes. Our work will thus attempt to describe, quantify and predict the development of a plant pathogen complex in intercrops using both experimental and modelling approaches.

The basic assumption underlying this project is that intercrops, by modifying plant nutritional status and canopy microclimate, will contribute to develop new ecological niches within the canopy, which in turn will select specific components of the disease complex according to life history traits (LHT) of the complex member species. By focusing on a winter pea/wheat intercropping system, and on the pea ascochyta blight disease complex, we are adressing two questions: *i)* which trade-offs between LHT within each member of the pathogen complex will determine the colonization and exploitation of the different niches? *ii)* do different niches developed from intercropping select fungal species with particular LHT, and consequently modify the composition and dynamics of the parasitic complex?

Boudreau MA., 2013. Disease in intercropping systems. Annual Review of Phytopathology 51: 499–519.

Potential of ABA Analogs as Plant Growth Regulators for Pulse Crops

Abrams, SR

University of Saskatchewan, Department of Chemistry (sue.abrams@usask.ca)

Abscisic acid (ABA) is present in all plants and is a signaling molecule that regulates a host of physiological processes in plant growth and development as well as responses to abiotic stresses. Natural ABA produced by fermentation, is used commercially to promote ripening of table grapes and also as a seed treatment for hybrid seed production of wheat and rice. The applications of ABA itself are limited by its short half life in plants.

No derivatives or analogs of ABA are yet on the market, although there are many applications for agonist analogs that have greater persistence or activity than natural hormone. There are also applications for ABA antagonist compounds that directly counter act ABA. Antagonists could have application for pulse crops, in promoting seed germination in cold, wet soils, in breaking dormancy in recalcitrant species, and in reducing green seed at harvest.

Examples of applications of agonists and the development and potential application of antagonists will discussed in the presentation.

Session 2

Temporal and Spatial Variation in a large collection of Chickpea (*Cicer arietinum*) landraces.

Kassaye Negesh, Syed Gul Abbas, Elena Plekhanova, Bahattin Tamolac, Peter Chang, Noelia Carrasquila-Garcia, RV Penmetsa, Asnake Fikre, Kassahun Tesfaye, Nina Noujdina, Maria Samsonova, Sergey Nuzhdin, Doug Cook, Eric von Wettberg

Landraces of crops are important genetic resources, reflecting traditional cultivation practices and potential adaptation to diverse climates and agroecologies. We examine landrace diversity in chickpea, a unique crop with a wild ancestor with an extremely narrow geographic distribution in Southeastern Anatolia but landrace cultivation spanning six continents and conditions from sub-tropical South Asia and East Africa, to Mediterranean climates worldwide, and to temperate prairie conditions. By assembling a diverse collection of landraces from multiple genebanks spanning nearly century of germplasm collections and the three primary centers of diversification for chickpea, we examine changing patterns of diversity. We find little evidence of declining landrace diversity from the oldest collections in the Vavilov Institute for Research to contemporary collections. We document shifts in diversity in the center of domestication in Turkey to secondary diversification centers in South Asia and East Africa. Comparison to diversity in a recently assembled large collection of the wild relatives allows us to build a demographic model of multiple bottlenecks and diversifications impacting the diversity of chickpea.

Utilizing Phenomics and GWAS in Cultivated Lentil

Wright D., Neupane S., Heidecker T., Bett K. & Vandenberg A. University of Saskatchewan, Department of Plant Science

The recent development of genomic resources for *Lens culinaris*, including the release of its sequenced genome (knowpulse.usask.ca), has brought this orphan crop into the genomics era. Through the Application of Genomics to Innovation in the Lentil Economy (AGILE) project, we have developed, and genotyped, a population of 324 *L. culinaris* accessions, representative of the global genetic diversity of cultivated lentil. Field trials are being carried out, over two years, in some of the major lentil growing regions: Canada, USA, Morocco, Spain, Italy, India, Nepal and Bangladesh. Utilizing the power of genome-wide association studies (GWAS), we hope to identify genes of economic importance and elucidate upon mechanisms of adaptation to the different growing environments. This GWAS approach has already shown success with simple traits such as cotyledon color, in which the causal gene location is already known, and shows promise for more complex traits such as days to flower.

THE PICTURE UNDERGROUND: WILD LENTILS AS GENETIC RESOURCES

Gorim, L.Y. 1*, and Vandenberg, A. 2

^{1, 2} Department of Plant sciences, University of Saskatchewan, 51 Campus Drive Saskatoon SK S7N 5A8 (Corresponding author: linda.gorim@usask.ca)

With increase in the world population, there is greater need to maximize available resources such as water and amount of fertilizer used for crop production. There is a need to breed lentil cultivars that are both water efficient and capable of fixing more of their own nitrogen. Wild lentil species have their centers of origin located in marginal and drought prone areas, making them good candidates for the evaluation of root traits and genetics that can subsequently be transferred to cultivated lentil. The root systems of seven wild lentil genotypes were evaluated under two levels of drought and compared to fully watered conditions. Plants were sown indoors in tubes, 60-cm long containing soil from different soil horizons, collected from the field. Wild lentils used different strategies to evade or tolerate drought and develop variation in distribution patterns in different soil horizons. Variable nodule shapes were also observed in wild lentils, and what this variation implies with respect to nitrogen fixation ability require further investigation.

LegumeInfo and Legume Federation resources for pulses and cool-season legumes

Campbell JD^{1*}, Berendzen J², Cannon EKS¹, Chan A³, Cleary A², Dash S², Hokin S², Huang W¹, Krishnakumar V³, Weeks NT⁴, Wilkey AP¹, Lyons E⁵, Town C³, Fernández-Baca D¹, Farmer AD², Cannon SB^{1,4}

An impressive collection of online tools is available for legume research, with many available for pulse species and other cool-season legumes (chickpea, Medicago, lupin, red clover; soon lentil, pea, and others). We will describe several tools from the "Legume Information System" and "Legume Federation" projects (https://legumeinfo.org and https://legumeinfo.org and https://legumeinfo.org and https://legumeinfo.org and

- InterMine instances for chickpea and several other legumes.
- Geographic information system (GIS) viewer for visualizing legume germplasm collections globally against high-resolution maps.
- Legume transcript annotation tool.
- Sequence search tools for genomes and gene sets.

¹ Iowa State University, Ames, IA

² National Center for Genome Resources, Santa Fe, NM

³ J. Craig Venter Institute, Rockville, MD

⁴ USDA-ARS-CICGRU, Ames, IA

⁵ University of Arizona, Tucson, AZ

- Tools for visualizing sequence matches with respect to a genome or a gene.
- Gene family viewers for legume species.
- Synteny viewers for exploring genome-wide and gene-focused synteny across the legumes.
- New interactive genetic map viewer.

We will demonstrate how your data can be analyzed using these tools and visually displayed into these tools or related resources.

Pulses as a promising whole foods for prevention of obesity

Dil Thavarajah

Associate Professor, Pulse Quality and Nutrition, Plant and Environmental Sciences, Clemson University, SC, USA.

Obesity has been a severally neglected global public health concern for decades, today obesity results in 36 million deaths globally each year. Lentil, a moderate-energy high-protein pulse crop, provides significant amounts of essential micronutrients (e.g., minerals, carotenoids, folates) with very low concentration of phytic acid. Our recent research indicates that lentil also contains a wide array of low-molecular weight carbohydrates (LMWC) or prebiotic carbohydrates, such as mono- and di-saccharides, raffinose-family oligosaccharides (RFO), fructooligosaccharides (FOS), and sugar alcohols, and high molecular weight resistant starches. For example, lentil provides more than 13-15 g of prebiotic carbohydrates per 100 g serving, and this level increases almost two-fold upon cooking, cooling, and re-heating. Further, our recent intestinal microbiome studies suggest a prebiotic-rich, low-calorie diet can reduce the prevelence of obesity and related non-communicable diseases. Lentil thus represents a whole food source of prebiotics that can play a role in efforts to reduce obesity and non-communicable diseases.

Enhancing nutritional value: biofortification of pea and lentil

Thomas D. Warkentin¹, Albert Vandenberg¹ and Raymond Glahn²

Email: tom.warkentin@usask.ca

Field pea and lentil seeds, like those of other pulse crops, are rich in protein, slowly digestible carbohydrates, and fiber. To further enhance their nutritional value, research is in progress to increase the concentration and bioavailability of Fe, Zn, Se, carotenoids and folates. A 100 g dry weight serving of field pea or lentil supplied a substantial portion of

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the recommended daily allowance for adults of Fe, Zn, and Se. Association analysis in pea identified 9 single nucleotide polymorphisms (SNPs) associated with Fe concentration, and 2 SNPs associate with Zn concentration. Evaluation of bi-parental pea recombinant inbred line populations revealed QTLs associated with Fe, Zn, and Se concentration. A panel of 138 diverse cultivated lentil accessions phenotyped in four environments and genotyped using 1150 SNP markers exhibited a wide range of variation for seed Fe and Zn concentration, and marker-trait association analysis detected two SNPs tightly linked to seed Fe and one linked to seed Zn concentration. A number of putative candidate genes underlying detected loci encode Fe- and Zn-related functions. Selected pea RILs which segregated for Fe concentration showed positive correlation (r=0.38) between their Fe concentration and iron bioavailability. Field pea lines were identified with a 60% reduction in phytate-phosphorus concentration in seeds, while the inorganic (available) phosphorus concentration increased by a similar amount. A single recessive gene controls the low phytate trait and was mapped on pea chromosome 3. Phytate concentration was negatively correlated with iron bioavailability (r = -0.34 to -0.37) in pea lines segregating for phytate concentration. Dehulling lentil improved iron bioavailability, as did reducing the polyphenol concentration by introducing the zero tannin gene. Wild lentil species represent a potential resource for biofortification since 5 of 6 wild species are crossable with cultivated lentil and these accessions show diversity in Fe and Zn concentration. Total carotenoid concentration of 20 lentil cultivars ranged from 6-28 µg g⁻¹. Total carotenoid concentration of 12 pea cultivars ranged from 7-22 µg g⁻¹. Lutein was the most abundant carotenoid in lentil and pea seeds. Lutein concentration was positively correlated (r = 0.41) with iron bioavailability in a pea population. Low phytate-high carotenoid pea lines had up to 3-fold greater iron bioavailability than normal phytate checks. Total folate concentration of a small set of cultivars of chickpea was greater than that of lentil and common bean, while pea cultivars tested had substantially lower concentration. Current research is exploring more diverse germplasm for folate profile. This research shows potential positive associations between low phytate, high Fe, and high carotenoid concentration with improved iron bioavailability in pea and lentil seeds to improve Fe nutrition of foods.

Session 3

Faba Bean Research and Development in Saskatchewan

A. Vandenberg, H. Khazaei, R. Purves, H. Zhang, S. Zanotto, J. Hughes, A. Feyissa, and B. Tar'an

Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, S7N 5A8, Canada

Faba bean, the forgotten pulse crop, is now becoming an object of desire in Canada and elsewhere. Agronomy, quality, genetic and pathology research is now moving rapidly in concert and collaboration with international partners in research and development. We

will present what is going on, why it is happening, and what it all means to the future of faba bean in our farm and food systems.

CRISPR/Cas9-mediated gene editing in chickpea

Ferhatoglu Y^{1*}, Bowmik P², and Tar'an B¹

^{1,} University of Saskatchewan, Department of Plant Sciences (yuf183@mail.usask.ca)

Genome editing in plant species specifically the CRISPR/Cas9 system allows for the production of plants with altered genomes by producing double strand breaks leading to genome modifications during the repair process. This system has been used in multiple important crops for editing specific genes, a gain of function in the development of profitable traits such as herbicide tolerance. Currently, in literature, there are no studies involving gene editing in pulse crops with this technology and Cas9 delivery into plastids which are necessary for gene editing in chloroplasts. In this study, we aim to test CRISPR/Cas9 for precise editing of one plastidic gene - psbA for metribuzin tolerance and one cytosolic gene- PDS using chickpea protoplasts. These results along with the challenges in validation of CRISPR/Cas9 cleavage efficiency in chickpea protoplasts will be presented.

Chickpea Ca-elf3 a key gene for photoperiod responsiveness and adaptation to short growing regions

Stephen Ridge, Amit Deokar and Bunyamin Tar'an
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Chickpea (*Cicer arietinum* L.) is currently grown in over 53 countries representing a wide range of environments and cropping systems. Phenology (time to flowering, podding and maturity) is an important component of crop adaptation in these environments. Crop maturity ranges from 80 to 180 days depending on genotype, soil moisture, time of sowing, latitude and altitude. However, in at least two-thirds of the chickpea growing area, the available crop-growing season is short (90-120 days) due to risk of drought or temperature extremes at the end of season during pod filling stage. Early phenology is important in Canada to escape from frost at the end of season. Hence, development of early maturing cultivars is one of the major objectives in chickpea breeding program in Canada. To this end, we have mapped, sequenced and characterized what is likely to be *Efl-1*, an orthologue of the Arabidopsis gene *ELF3* that confers early flowering and

² National Research Council of Canada, Saskatoon, Saskatchewan

photoperiod insensitivity. In a RIL population derived from a cross between CDC Frontier and ICCV 96029, this gene was mapped to chromosome 5 that explained approximately 60% of flowering time variation under short days. Sequencing of the *Efl-1* in chickpea cultivar ICCV 96029 revealed an 11-bp deletion in the first exon that was predicted to result in a premature stop codon. This polymorphism does not appear to be widely-distributed within global chickpea germplasm, indicating that this mutation arose relatively recently. The effect of this polymorphism on flowering time is confirmed through transgenic complementation of the *Arabidopsis elf3-1* mutant.

Phenological study of diverse lentil (*Lens culinaris* Medik.) germplasm in temperate and sub-tropical savannah growing environments

S. Neupane^{1*}, D. Wright¹, T. Heidecker¹, A. Vandenberg¹ and K. E. Bett¹

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Three hundred twenty-four different accessions of lentil (*Lens culinaris* Medik.) consisting of 40 temperate, 235 Mediterranean and 39 South Asian types, were evaluated at two locations in Saskatchewan, Canada in summer 2016 and 2017, and one in Bardiya, Nepal in winter 2017 to understand the adaptation response of germplasms to specific environments. Field experiments were set in the randomized complete block design in all locations. Data on days to emergence, to flower, to swollen pod, and to maturity were collected along with the temperature and day length. Site specific comparison was made on the basis the origin of the accessions. Results showed that the accessions from South Asia were the first to flower, have swollen pods and to mature in all locations in both years and temperate accessions were late. Less variation was found in terms of days to emerge in Saskatchewan irrespective of the origin, however, a broader range was observed in Nepal.

Days to flowering in Lentil (Lens culinaris Medik.) under long-day greenhouse and Temperate field conditions.

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Lentils (*Lens culinaris* Medik.) are an important pulse crop in Western Canada, and are consumed worldwide for their nutritional benefits. Adaptations for flowering time have contributed to genetic isolation of lentil germplasm between the three major production groupings, which are: Temperate, Mediterranean and South Asian. The identification of

markers for selecting for flowering habit in lentil would make breeding with diverse material more feasible, and undesirable material could be easily selected away from. A diverse panel of 324 diverse lines from the major climatic regions was replicated three times in a greenhouse on the campus of the University of Saskatchewan, with artificial light supplemented to ensure long (17hr) days. The same panel was grown during the 2016 and 2017 growing seasons at two field sites near Saskatoon, Saskatchewan. The differences in flowering time responses between the greenhouse and field experiments was investigated. A previously identified marker which had a significant effect on days to flowering time on a population grown under short-days was also run on the diverse lentil panel. The marker control was less conclusive in these two long-day experiments as it was in the prior, short-day, study. Future work will involve developing markers which account for the differences in lentil flowering time when grown in long-day, temperate conditions.

Development of SNP markers for rust (*Uromyces viciae-fabae*) resistance gene controlling hypersensitivity in faba bean cultivar selection Doza#12034 (*Vicia faba* L.)

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Faba bean rust is a major fungal disease worldwide that can potentially damage 70-80% crop produce. To develop SNP (Single nucleotide polymorphism) markers, the F₆ recombinant inbred line (RIL) population was developed by crossing a selection (#12034) from resistance cultivar 'Doza' with susceptible cultivar 'Fiord'. The genetic linkage map was produced by the analysis of transcriptome data (RNA-Seq) generated by Illumina Infinium® 1536-SNP sequencing platform. A total, 618 SNPs were distributed to 12 linkage groups (LGs) covering 1025.63 cM with an average marker density of 1 locus per 1.66 cM. The phenotyping was performed under glasshouse conditions where two weeks old seedlings were inoculated with urediniospores of rust isolate Uvf-8. The SNP markers *C241205*, *C250539*, *C246203* and *C229467* were found co-segregated at 1.7 cM distal to rust resistance gene on chromosome-III. Moreover, PCR based KASP (Kompetitive allelespecific primers) assay of these SNP markers on 40 diverse faba bean genotypes demonstrated its robustness for merker-assisted selection for this rust resistance gene.

Yield, nutrient uptake by four faba bean cultivars at four sites in Saskatchewan

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More legume crops like faba bean are being included in rotations to maintain soil N fertility through biological N fixation. The study purpose is to determine fababean yield and nutrient uptake to assist in crop nutrition planning. Four recent zero tannin faba bean cultivars were used in a two-year field study at four sites in Saskatchewan. In 2016, faba bean grain yields at the sites ranged from $^{\sim}$ 3 to 7 tonnes ha- 1 with limited response to fertilization, and large amounts of nitrogen ($^{\sim}$ 250 kg N ha- 1 averaged across sites and varieties) found in above ground biomass and derived from fixation, with a large proportion ($^{\sim}$ 91 %) contained in the grain removed at harvest. The faba beans were also high P users with $^{\sim}$ 30 kg P ha- 1 ($^{\sim}$ 70 kg P₂O₅ ha- 1) in the grain and straw, with the majority in the grain.

Evaluation of Field Pea Susceptibility to *Pea Seed-Borne Mosaic Virus* (PSbMV) and Seed Transmission

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Pea seed-borne mosaic virus (PSbMV), a non-persistently aphid-transmitted potyvirus, has been reported in field pea (Pisum sativum L.) growing regions worldwide. Reductions in yield and seed quality due to PSbMV are influenced by virus pathotype, host genotype, and infection frequency. PSbMV can exist asymptomatically, or elicit a variety of symptoms including stunting, shortening of the internodes, deformed, crescent-shaped pods, aborted seed, and delayed plant maturity. Isolate ND14-1, recovered from North Dakota infected seed and preliminarily identified as pathotype 4 (P4), was mechanically inoculated onto twenty field pea cultivars under greenhouse conditions. PSbMV infection frequency was evaluated using enzyme-linked immunosorbent assay (ELISA). Two cultivars were resistant to ND14-1, one cultivar exhibited partial resistance (20 to 40% infection), and all others were susceptible (>40%) to ND14-1. The rate of virus transmission from seed grown in infected plants, to daughter plants was less than 35%. Yield in virus positive plants of some cultivars increased slightly due to delayed plant maturity; however, reductions were observed in most cultivars. In PSbMV positive plants, the number of pods per plant increased up to 137%. Symptoms of seed cracking and water-soaked lesions were present in 14 to 50% of seeds harvested from infected plants depending on cultivar; however, the presence of seed symptoms was not an indicator of PSbMV transmission. A risk index was developed based on cultivar susceptibility, yield reduction, and PSbMV seed transmission. An index value (0 to 9) was assigned to each field pea cultivar to represent the inherent risk assumed when the cultivar is grown in an area where PSbMV is present.

Session 4

Characterization of Heat Responsive Genes in Pea Pollen and Leaves via RNA-Seq

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Heat stress is one of the major abiotic stresses limiting the world's agricultural production including pea. With the development of next generation sequencing, RNA-Seq has been increasingly utilized for transcriptome profiling in abiotic stress research. This technology will be applied to the discovery of heat responsive genes in field pea. Prior to that, a RT-PCR of *PsHsp 18* and *70* genes, which were identified to be high heat inducible in previous pea heat shock protein studies, is underway at four heat stress time intervals (3h, 6h, 12h, 24h) for the validation of the most heat responsive timing for sample collection as well as the selection of a pair of pea varieties with the most contrasting gene expressions of *PsHsp 18* and *70* genes.

A high throughput root based phenotyping protocol for drought tolerance in Faba bean (*Vicia faba* L.)

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Among different drought adaptation strategies, root traits have a major influence on plant health. We developed a new screening technique based on seedling survivability, drought score and root morphological traits of faba bean plants exposed to drought under hydroponic conditions and sand. The hydroponic technique involved removing 3 week old seedlings of 96 genotypes from the nutrient solution and exposing them to air for 5 h daily for 7 days. In a sand culture technique, three week old plants were subjected to drought treatment by withholding water for a week. A small seeded genotype AC805#4912 from Yemen showed maximum seedling survivability and minimum reduction in the growth parameters with a drought score of 0.0–0.4 indicating higher tolerance to drought both in hydroponics and sand. Results recommended that hydroponics is a simple, high-throughput and reliable phenotyping tool for screening a large number of faba bean genotypes for drought tolerance.

Screening on salt-tolerance genes and functional analysis of two genes of myo-inositol-O-methyltransferase and aquaporins in Sophora alopecuroides L

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Sophora alopecuroides is the leguminous plants of the genus robinia, alias manufacture, and plants of perennial herbs, roots underground bud, xerophytic and salt-tolerant. For significant drought and salt resistance, it is an abundant genetic resources of resistant gene. In this study, salt-tolerance relevant genes was screened from cDNA yeast expression library of sophora alopecuroides, and two salt-tolerance genes was screened, named SalMT and SaAQP, preliminary analysis was conducted for its structure and function at the same time, in order to further validate the salt-tolerance gene, in which lay a theoretical and technical basis for resistant breeding.

Evaluation of genome wide association study panel for Fe, Zn, and Se concentration in field pea

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Iron (Fe), zinc (Zn) and selenium (Se) are essential nutrients for human health. Our previous studies indicated substantial variation in micronutrient concentration in field pea (Pisum sativum L.). In this research, 177 diverse pea accessions were evaluated from three location-years (2013 Saskatoon, 2016 Saskatoon and 2016 Rosthern) using atomic absorption spectroscopy. Genotypes differed significantly (P < 0.001) for Fe and Zn in all location-years, but not for Se. Year, location and year*location*genotype effects were significant for all three micronutrients. Mean concentration for the combined analysis were 50.17, 31.56, and 0.88 and the concentration ranged from 29.22 to 74.76, 16.78 to 51.47 and 0.06 to 6.12 in ppm in the solid material, for Fe, Zn, and Se, respectively. Evaluation of two additional location-years, i.e., North Dakota 2014 and Saskatoon 2015 are in progress. These phenotypic data will be associated with genotypic data generated from genotyping-by-sequencing using genome wide association study to identify significant SNPs associated with Fe, Zn, and Se concentration.

Using overhead images to determine volume and ground cover of lentil (*Lens culinaris* Medik.)

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One of the most time-consuming components in plant breeding programs is the process of phenotyping, or identifying traits of interest in a field environment. This data collection process is typically carried out using human labor, and consumes considerable time and money. Analyzed overhead images may instead be used as an equivalent or improved source of information. A diversity panel of 324 lines of lentil (*Lens culinaris* Medik.), arranged in randomized complete block design (RCBD) and consisting of three replicates, were grown in microplots in two locations in Saskatchewan, Canada in 2016 and 2017. An additional subset of 6 varieties was grown in 3 locations in Saskatchewan in 2017 for the purpose of collecting whole-plot biomass once every two weeks from emergence until maturity. A further 16 varieties were grown in yield plots at two locations in Saskatchewan in 2016 and 2017 in 3 replicates and arranged in a RCBD. Biomass samples were taken from these plots once every two weeks. All trials were imaged using ground and/or aerial-based overhead imaging techniques which produce high resolution images.

This research is ongoing. For final analysis, two-dimensional merged images (orthomosaics) will first be produced. Then, utilizing Structure from Motion (SfM) techniques, 3-dimensional point clouds will be constructed and utilized as an estimate of volume. Volume estimates will be compared with biomass measurements. In partnership with the University of Saskatchewan's Department of Computer Science, deep learning and machine learning techniques may be utilized to correlate UAV imagery with plot biomass. This has potential to allow rapid, non-destructive biomass estimations and predictions with high temporal resolution.

Session 5

Comparative genetics of Aphanomyces root rot resistance and root architecture in pea

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In a sustainable agriculture context, breeding for varieties combining genetic resistance and plant architecture can be of major interest to limit disease impacts in crops. The aim of this study was to compare genetic determinants of root architecture and resistance to *Aphanomyces euteiches*, a major soilborne pathogen of peas. A genome wide association analysis among a 266-pea-line collection enabled us to pinpoint a total of 150 loci associated with resistance and/or plant architecture, and to compare them with QTL

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detected in previous studies. A common major locus was associated both with resistance and root architecture traits. At this locus, the resistance-enhancing allele contributed to an increased total root projected area and reduced root losses in response to infection. Several other loci were detected for both resistance and disease-induced architecture. The combination of resistance and some architecture traits in some selected pea lines was associated with reduced yield losses in infested fields. Results provide original knowledge about comparative genetics of disease resistance and plant architecture for root rot diseases. They provide tools and information for the choice of resistance and architecture QTL to combine in breeding strategies to improve resistance to *A. euteiches* in future pea varieties.

Desgroux A, Baudais V, Aubert V, Le Roy G, de Larambergue H, Miteul H, Aubert G, Boutet G, Duc G, Baranger A, Burstin J, Manzanares-Dauleux M, Pilet-Nayel M-L*, Bourion V* (2017). Comparative Genome-Wide-Association Mapping identifies common loci controlling root system architecture and resistance to *Aphanomyces euteiches* in pea. Frontiers in Plant Sci., submitted

Introgression Breeding of Major Aphanomyces Root Rot Disease Resistance QTLs into Elite Pea Varieties

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Pea productivity is diminishing due to Aphanomyces root rot (ARR) occurrence in high rainfall seasons. ARR disease is caused by *Aphanomyces euteiches* of the Saprolegniaceae. Several quantitative trait loci (QTLs) associated with ARR resistance, particularly *Ae-Ps7.6* and *Ae-Ps4.5* with major effects, were identified by Pilet-Nayel and colleagues (INRA, France). Research is in progress to introgress these QTLs based on single nucleotide polymorphism markers assisted backcross selections. Eight pea crosses were made between pea lines (PI 660736, PI 660733, PI 660729 and 90-2079) carrying ARR QTL *Ae-Ps7.6* and *Ae-Ps4.5* and six agronomically elite, ARR susceptible CDC pea varieties (CDC 2950-19, CDC 2936-7, CDC 2847-21, CDC 3007-6, CDC 4053-4 and CDC 3422-8). BC₂F₂ seeds were recently harvested for genotyping and phenotyping for ARR resistance. Best performing pea lines will be tested under field conditions.

Quantitative Trait Loci Associated with Yield, Seed Yield Per Plant, and Seed Size in Dry Field Peas

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In pea, increasing yield is the most important focus of plant breeding. In order to discover the quantitative trait loci (QTLs) influencing yield, a recombinant inbred line (RIL) population was created from a relatively wide cross between the commercial variety Delta and an unnamed pea variety. The RIL population was grown for 3 site-years in Bozeman, MT, USA, and phenotypic data was collected for yield, yield per plant, seed size, and 18 quantitative traits, in addition to seven categorical traits which were thought to be associated with crop yield. Genotypic data was derived from genotype by sequencing, microsattelite markers, and cleaved amplified sequence tagged sites. A layered QTL analysis was performed to determine whether QTL for yield, yield per plant, and seed size co-located in the same positions. Yield was found to be controlled by eight QTLs. All QTLs for yield had pleiotropic effects on lodging resistance and yield per plant. Seed size was not correlated with yield, and a model was created which explained why no association between yield and seed size was found. The results of this study further refine the ideotype for pea, and can be used for marker assisted selection in this crop.

Increasing Field Pea Yield with Leaf Type Varietal Blends

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In recent years field pea breeding has moved towards development of semi-leafless cultivars, which have strong tendrils, rigid stems, and lack leaflets on tendrils. These cultivars have superior lodging resistance over leafed (wild type) cultivars, however leafed cultivars can have higher yield potential if lodging can be prevented. Recent research suggests that seeding blends of leafed and semi-leafless types may overcome the poor lodging resistance of leafed pea, increasing overall yield. Leafed pea breeding lines near-isogenic to high-yielding modern semi-leafless pea cultivars were developed at the Crop Development Centre in Saskatchewan, Canada, to test this hypothesis. An experiment was conducted near Saskatoon, Canada in 2017, which compared ten sibling and non-sibling combinations of four pairs of near-isogenic leafed and semi-leafless lines, sown in blends of 50, 67, and 83% semi-leafless pea, with leafed and semi-leafless monocultures. A significant overall yield increase of 4% was recorded in leafed and semi-leafless blends compared with single leaf type monocultures. There was a significant quadratic response of yield to percentage of semi-leafless pea in the mixture, with different varietal blends

having different optimum percentages of semi-leafless pea. The optimum mixing percentage ranged from 30-70% semi-leafless pea. The yield increase of near-isogenic blends above monocultures at their respective optima ranged from 2-10%. Data analyses are still in progress, and this experiment will be replicated at multiple locations in 2018 and 2019 to confirm these preliminary results.

Exploring resistance in Lens ervoides to foliar fungal pathogens

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Lens ervoides, a species in the tertiary gene pool of Lens, has been recognized as a valuable source of resistance to several fungal pathogens commonly found in cultivated lentil. Accessions have been identified with high levels of resistance to Ascochyta lentis causing ascochyta blight of lentil, Stemphylium spp. causing stemphylium blight, and both races of Colletotrichum lentis, the causal agent of anthracnose. Interspecific hybrids have been created with the help of embryo rescue, but segregation distortion in the resulting populations has prevented genetic studies and development of markers. An intraspecific L. ervoides population (LR-66) was developed from L01-827A \times IG 72815 to study the genetic control of resistance to these pathogens and develop closely linked markers to be used in interspecific populations. A 94 F₉ RIL population was phenotyped for resistance to all three diseases and genotyped on the Illumina HiSeq 2500 platform. SNP-based mapping resulted in 7 linkage groups. Parents and RILs segregated for responses to stemphylium blight (S. botryosum) and anthracnose (C.lentis), so three QTLs for S. botryosum resistance, five for C. lentis race 0, and six QTLs for C. lentis race 1 resistance could be identified. Genes that may be involved in resistance were identified through QTL dissection combined with proteome mapping. Further characterization and validation of these putative resistance genes will be performed by gene expression and effector - receptor interaction studies.

Updates to the Cool Season Food Legume Genome Database: Resources for pea, lentil, faba bean and chickpea genetics, genomics and breeding

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The Cool Season Food Legume Genome database (CSFL, www.coolseasonfoodlegume.org) is an online resource for genomics, genetics, and breeding research for chickpea, lentil,



pea, and faba bean. The user-friendly and curated website allows for all publicly available map, marker, trait, gene, transcript, germplasm, and genome data to be easily searched and retrieved as downloadable tables and files. CSFL now utilizes the Tripal BLAST module which

allows for hyperlinks from the results to JBrowse, NCBI, and data detail pages in CSFL. The available BLAST databases include chickpea, lotus, Medicago, and soybean genome assemblies; annotated reference transcriptomes (RefTrans) and unigenes assemblies for pea, chickpea, lentil and faba bean; and ESTs from NCBI for each of the four crops. CSFL also has several tools for data exploration such as JBrowse for viewing chickpea and related model organism genomes and the corresponding annotations or alignments and PathwayTools for viewing predicted metabolic pathways in the Cicer genomes. CSFL features a new integrated genetic MapViewer module that replaces CMap and allows for easier comparison, display and retrieval of all map and associated data. MapViewer pulls data from the CSFL Chado database and integrates the maps into the CSFL genetic map details pages and allows users to zoom and select regions dynamically through clicking and dragging. MapViewer also allows users to compare linkage groups from the same map or different maps and display the corresponding markers in both maps. In the near future CSFL will also utilize the Tripal Breeding Information Management System (BIMS) module and allow for breeders to manage their private data and use tools that will assist with marker-assisted breeding. This project is supported by USDA NRSP10, the USA Dry Pea and Lentil Council, Northern Pulse Growers Association, USDA-ARS and Washington State University.

Session 6

Rapid generation cycling of RILs from *Lens culinaris* x *L. ervoides* after aphanomyces-root rot screening

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Aphanomyces root rot (ARR) is a destructive lentil disease that has appeared in recent years in Saskatchewan fields under wet conditions. Wild lentil species may represent a valuable source

of resistance to ARR. A F_2 RIL population derived from a cross between *Lens culinaris* 'Eston' and *L. ervoides* LO1-827A was screened for root rot resistance. The average population score was 3.9 (scale from 0 = resistant to 5 = dead), and the best RIL achieved a score of 2.4.

After disease assessments were completed, a simple rapid generation cycling technique (RGT) was applied to all RILs that scored less than 4. Each RIL was advanced for five generations in less than one year using a single seed descent method. The generation length was 74 days for the ARR-screened population and 56 days for subsequent generations resulting in the production of five generations in less than one year (Fig. 1). Mature, dry seeds were harvested from the 6th generation and the 7th generation was again screened for ARR. The nearly homozygous F₇ population scored 2.7 on average with one RIL achieving 1.4 indicating that aphanomyces root rot resistance can be improved in lentil through crossing with wild species.

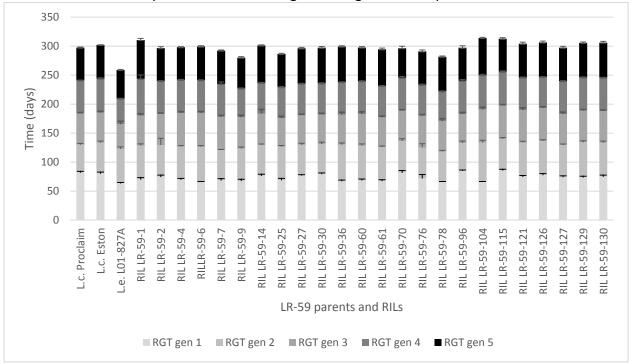


Fig. 1. Life cycle length (days) for generations 1 (F_2) through 5 (F_6) for RIL populations derived from a cross between *L. culinaris* var. Eston (L.c.) and *L. ervoides* L01-827A (L.e.).

Identifying Sources of Resistance in Chickpea to Seed Rot and Pre-emergence Dampingoff Caused by Mefenoxam-Resistant Isolates of *Pythium ultimum*

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Seed rot and pre-emergence damping off of chickpea caused by *Pythium ultimum* has been historically controlled by seed treatments with R-metalaxyl (mefenoxam). Since 2014, failure of stand establishment in chickpea production fields caused by mefenoxam-resistant (MR) strains of *P. ultimum* has become an emerging problem in the U.S. Pacific Northwest. Currently the disease has only been reported in the cultivar 'Sierra', which is the most widely grown large seeded kabuli chickpea cultivar in the U.S. We are interested in identifying sources of resistance in chickpea to diseases caused by mefenoxam-resistant isolates of *P. ultimum*, and this report describes efforts to develop a reliable screening method.

Several different methods were evaluated to screen the ability of chickpea seeds to germinate and emerge in the presence of mefenoxam-resistant *P. ultimum* including 1. Petri dish assays (PDA and H₂O agar), 2. Greenhouse assays using mycelial plugs as inoculum, and 3. Growth chamber assays using soil infested with sporangia and oospores. The most repeatable results were observed using a growth chamber assay in which seeds were sown in soil that was infested with known quantities (colony forming units /gm) of sporangia and oospores of *P. ultimum*. Results suggest that the cultivars Sierra and 'Nash', and the breeding line FLIP 84-92C, which are all large seeded café kabuli chickpeas, are susceptible while the desi chickpea cultivar 'Myles' is resistant (Table 1). Current efforts include introgressing resistance from Myles into kabuli type chickpeas and identifying markers associated with resistance to MR-isolates of *P. ultimum* using a RIL population derived from the cross FLIP 84-92C (*C. arietinum*)/PI599072 (*C. reticulatum*).

Table 1. Mean number of seeds¹ germinated per pot after two weeks when sown in soil infested with different amounts of inoculum (*Pythium ultimum* oospores).

()					
	Treatment				
Entry	Healthy	5,000 CFU per	10,000 CFU per	20,000 CFU per	40,000 CFU per
	Control	pot	pot	pot	pot
Myles	4.75	4.13	2.5	2.5	0.63
Nash	4.38	0.13	0.25	0	0
Sierra	4.38	0.5	0	0	0
FLIP84-92C	4.38	0.88	0.13	0	0

¹Five seeds were planted in each pot. Pots were maintained in a growth chamber (18°Cday/14°C night, 12 hr daylength). Means represent the average of two experiments. Each experiment included four replicate pots for each entry-treatment combination.

Assessment of the efficacy of four seed treatment fungicides on *Didymella lentis* of lentil.

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Ascochyta blight of lentil caused by *Didymella lentis* (DL) can be a major constraint to lentil production. Seed treatment fungicides are excellent management options, and efficacy data is lacking in the literature. Efficacy of four seed treatment fungicide mixtures were

studied in vitro on DL-infected lentil that were naturally infested. Infected seeds (variety Richlea) were treated with four different fungicides coded AA (Racona 3.8 FS + Belmont 2.7 FS + Florite 1127 at 2.335floz/wt), AB (Racona V100 FS + Belmont 2.7 FS + Thiram 480 DP at 4.5 floz/wt), AC (Racona V100 FS + Belmont 2.7 FS at 3.0floz/wt) and AD (Racona 3.8 FS + Belmont 2.7 FS + Thiram at 3.085 floz/wt). Two trials were conducted. For each trial, 400 seeds were treated with each of the four fungicides. A set of 400 untreated seeds was used as a control. Treated and untreated seeds were plated on potato dextrose agar. Incidence of Ascochyta sp. (Ascochyta primordial) were assessed from 5-14 days after plating (DAP) for trial 1 and trial 2. Data on incidence of Ascochyta pathogen and seed germination were analyzed using generalized linear model of SAS version 9.4; means were separated using Tukey's at P = 0.05. The incidence of DL in treated lentil seeds were significantly reduced (P = 0.001) relative to control in both trials. Compared to the other three fungicides, the incidence of DL on AB was significantly lower (P = 0.001) at 0.0-0.8% (41 -56.0% control) and 0.5-2% (45-47% control) in trials 1 and 2, respectively at 7 - 8, and 10 DAP. Effect of seed treatment on seed germination of treated seeds were significant (P = 0.05) and similar at 7-8 and 10 DAP. Lentil seeds treated with AA significantly differed in germination rate (76, 78.0%) from control (87, 87.0%) (P = 0.001 and P = 0.002) at 7 and 10 days, respectively in trial 1 and was similar in trial 2. All other treatments gave comparable results with the control.

Characteristics and management of metalaxyl-resistant *Pythium ultimum* found in irrigated pea cropping systems in the Columbia Basin of Washington

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Pythium ultimum is a major seed rotting pathogen in irrigated organic and conventional pea cropping systems in the Columbia Basin of Washington. This pathogen is managed by using the compound metalaxyl as a seed treatment, however, the pathogen has developed resistance. The geographic distribution, EC-50 values, management and growth habits of metalaxyl-resistant verse metalaxyl-sensitive isolates will be presented.

Regional Pulse Crop Diagnostic Lab: Progress and challenges

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The Regional Pulse Crop Diagnostic Laboratory was established in 2014 with the I supporting grant from USDA-APHIS Farm Bill. The laboratory provides pulse crop disease diagnostic services to the growers in the United States. Early disease detection is vital for effective management of plant diseases. As the pulse crop production in the United States

continues to increase, so are the crop disease risks. Therefore, growers and producers need information on the health status of their seedlots before making planting and exporting decisions. The Pulse lab has monitored seedlots from 2015 and 2016 growing seasons, almost exclusively from Montana, and will be presenting data on the pathogens detected. Also, we shall be highlighting the research efforts of the laboratory. Economic thresholds of seedborne pathogens and seed-to-seedling transmission of pulse crop pathogens are subjects we will be soliciting inputs. Conference participants are encouraged to suggest unmet pulse pathogen diagnostic needs, as Pulse lab improves its services and extend them beyond Montana's pulse growers and producers. Our goal is to mitigate pulse crop losses resulting from diseases.

Insect pests and fungal pathogens associated with faba bean production in Alberta, Canada.

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Faba bean (Vicia faba L.) is an emerging pulse crop in the Canadian Prairies, with approximately 40,000 hectares seeded in Alberta and Saskatchewan in 2017. As production of this crop increases, it is anticipated that disease and insect threats will also increase. A survey was conducted for diseases, fungi and insect pests in Alberta in 2015 and 2016. Crops were rated for disease during flowering, and insect sweeps were conducted at the same time and also at bud and pod stage. Leaves with lesions were returned to the lab for isolations followed by pathogenicity tests. Seeds were collected from the same fields at maturity, rated for necrotic spots, and plated to recover fungi. A total of 201 fungi were isolated from leaf lesions over the 2 years. Alternaria spp. were the most frequently isolated from foliar lesions, followed by Botrytis, Fusarium and Stemphylium spp. Fusarium spp. were most frequently isolated from seeds, followed by Botrytis and then Alternaria, although rankings did change between the 2 years. Botrytis spp. were highly pathogenic, Fusarium and Stemphylium spp. moderately pathogenic, and Alternia spp. were weak pathogens. Lygus spp. were the most abundant insects recovered, but other species, such as pea leaf weevil (Sitona lineatus L.), pea aphids (Acyrthosiphon pisum), leaf hoppers (Macrosteles quadrilineatus F.) and grasshoppers were also frequently collected. Results indicate that Lygus species such as L. lineolaris and L. keltoni were the most significant contributing factor to poor seed quality, and while disease pressure was fairly low, there are several pathogens that may impact production and seed quality as acreage increases.

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Poster Presentation

P1

Identification of White Mold Resistance Genes in Pea using time series RNA-Seq and Genome-wide Association Mapping

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Sclerotinia sclerotiorum is a fungal pathogen, which infects a broad range of crops including pea (Pisum sativum L.) and causes white mold (WM). WM is a destructive disease in major pea production areas of the United States such as Michigan and Washington State, but the genetics of WM resistance and mechanism in pea has not been intensively studied. Prior knowledge on WM resistance from other crops point out the disease resistance is quantitative, making resistance breeding difficult. This study aims to discover WM resistance in pea using two approaches. Firstly, genome-wide association (GWA) is applied to discover single nucleotide polymorphisms (SNPs) in the USDA pea germplasms associated with WM resistance. Secondly, a time series RNA-Seq is designed to discover genes with differential expression between a WM partially resistant line 'PI 240515' and a WM susceptible line 'Lifter'. Results from GWA and RNA-Seq will be integrated and SNPs that locate within differentially expressed genes will receive priority of interest. Putative resistance genes including two leucine-rich repeat genes were discovered using GWA. For these putative resistance genes, time-series RNA-Seq expression analyses will offer additional characterization and novel insights of WM resistance. The broad impacts of this study will not only provide understanding of the WM genetic resistance source in the USDA pea germplasm collection, but also the molecular mechanism of WM resistance in pea.

P2

Can endophyte seed treatments increase yield in peas and lentils?

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Endophytes are certain types of fungal and bacterial species that live within the plant. Certain endophytes have the ability to form symbiotic relationships with plants and are thought to confer certain benefits to the plant host such as drought, temperature or

salinity tolerance. However, very little is known about what degree endophytes can protect their host plants from any given abiotic stressor.

This study examined the use of several endophytic seed treatments as a mechanism to promote growth and yield under drought stress in field peas (Pisum sativum) and lentils (Lens culinaris). Field peas and lentils were grown at two field locations in central South Dakota over two growing seasons. Both years received below average rainfall, which provided an opportunity to assess drought stress. Preliminary results will be presented on yield, protein and nitrogen fixation (2017 only) potential in this stressed environment.

P3 Differential Gene Expression Regulating Protein Synthesis in Field Peas

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Protein content is an important quality trait in pulse crops. Field peas normally contain approximately 23% protein. Novel germplasm with approximately 30% protein was identified in AAFC field pea breeding program. This study characterizes gene transcripts differentially expressed in high protein pea. Two pea genotypes, a green pea cultivar Cooper and a high protein germplasm line MI3391, were used and total RNA from developing seeds at 8 and 20 days post anthesis was extracted. Four corresponding RNA-seq libraries were prepared and sequenced using Illumina paired-end technology. Reads from the RNA-seq datasets were aligned and 37,753 transcripts were mapped to pea reference transcriptomes. Differential transcript expression between pea genotypes was analyzed and 62 transcripts were significantly up-regulated in the high protein pea line. The identification of SNPs in these up-regulated transcripts is under way.

P4 Variability within *Pseudomonas syringae pathovars* infecting pea in Montana

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Bacterial blight caused by *Pseudomonas syringae* pv. *pisi* (Ppi) and P.s. pv. *syringae* (Pss) is an important disease of pea worldwide. This study was conducted to identify *Pseudomonas syringae* pathovars causing bacterial blight of pea in Montana and determine their pathogenic and phylogenetic diversity. From a survey of pea fields in 8 counties (Cascade, Daniels, Gallatin, Glacier, Hill, Valley, Roosevelt and Sheridan) during the 2016 growing

season, fluorescent and non-fluorescent Pseudomonads were isolated from infected pea plants on Kings B medium. For preliminary identification of *Pseudomonas syringae*, 200 bacterial colonies from all the counties were subjected to biochemical tests and hypersensitivity on tobacco plant. Of the 200 colonies analyzed, 134 (67%) were gram negative, positive for levan production, negative for the presence of oxidase, arginine dihydrolase, potato soft rot and induced hypersensitive response on tobacco leaves suggesting Pseudomonas syringae (LOPAT group 1a). To differentiate pathovar pisi from syringae and determine their pathogenicity, the 134 Pseudomonas syringae were amplified with syrB, ANS 3 &7 primers specific to Pss and Ppi and then inoculated into lemon fruits and bean pods. In total, 108 (81%) of the strains were amplified with the specific markers ANS 3 (24) and ANS 7 (84) for Ppi, 14 (10%) possess the syrB gene for Pss, and 12 (9 %) lack the syrB gene and were designated as unclassified Pseudomonas spp. (Ps). Symptoms of infection such as water soaking (37%), necrosis (54%) and reddish-brown discoloration (4 %) observed on bean pods compared with the water control indicated that both Pss and Ppi strains were pathogenic. There was variability in the response of the lemon fruits to inoculation with the Pss, only 7 (27%) of the Pss strains induced necrosis where none of the Ppi caused infection. In addition, fifty selected strains of Ppi and Pss which reacted differentially to the test above were assessed for pathogenicity on two of the most cultivated pea cultivars: Aragorn (green pea) and Montech (yellow pea). The Pss and Ppi strains in this study were categorized into four pathogenic groups: non-pathogenic (3 Pss), weakly pathogenic (5 Pss and 4 Ppi), moderately pathogenic (1 Pss, 17 Ppi and 5 Unclassified Ps) and highly pathogenic (3 unclassified Ps). Fifteen of the strains showed strain x cultivar interaction. Forty *Pseudomonas* strains representatives of the total population were further characterized by multilocus sequence typing (MLST) using four housekeeping genes qapA, cts, qyrB and rpoD. Phylogenetic analysis is ongoing. This study presents information needed to facilitate development of specific detection protocols for diagnostics and disease resistance screening.

P5 Exploring Sclerotinia sclerotiorum effectors during infection of pea

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Sclerotinia sclerotiorum is a plant pathogenic fungus with over 400 host species including pea (*Pisum sativum* L.). The fungus causes white mold, which is a significant problem in many areas where pea is produced. To improve white mold control in pea, understanding the mechanisms of both pea resistance and *Sclerotinia* pathogenicity is essential. Generally, fungal effectors are used as a probe to search for resistance components in

plants, but *Sclerotinia* effectors to pea have been poorly studied. RNA-seq analysis of a partially white mold resistant cultivar "Pl240515" and susceptible cultivar "Lifter" in response to *S. sclerotiorum* infection (12, 24, and 48 hours post inoculation; hpi) was conducted. 149 putative *S. sclerotiorum* effectors were identified and subjected to gene expression pattern analysis during infection of two pea cultivars, and on agar plugs incubated in Petri dishes containing potato dextrose agar medium. A total 28 of putative effectors were significantly induced in both cultivars compared to the expression on the medium. Most of the effectors were highly expressed in the late infection time point (48 hpi), but one effector showed induced expression at 12 hpi only and four effectors showed increased expression at 24 hpi only. Interestingly, three putative effectors were significantly less expressed in the resistant cultivar than the susceptible cultivar at 12, 24, and 48 hpi, respectively. The interaction of these three uncharacterized effectors with two pea cultivars are currently being investigated using molecular and biochemical techniques. This study will shed new light on white mold resistance mechanisms in pea and *Sclerotinia* pathogenicity to other economically important crops as well.

P6 Refining seeding rate recommendations for different lentil classes

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Optimum crop seeding rates are influenced by many factors including seed size, the yield density response of the crop, plant foliar diseases, and the growing environment. A target plant population of 130 plants m⁻² is currently recommended for all lentil classes in Western Canada, however the initial agronomic research was conducted on a single seed size class, large green lentil. Recent research suggests that this recommendation may be below that necessary for maximum yield and economic return for some lentil classes. Research was undertaken to determine the effect of seeding rate on yield and profitability of six different seed size classes of lentil. Six lentil varieties representing different seed classes (extra small red (CDC Imperial, TSW 34), small red (CDC Maxim, TSW 40), large red (CDC KR-2, TSW 56), small green (CDC Invincible, TSW 34), medium green (CDC Imigreen, TSW 57) and large green IBC-768, TSW 56) were grown at five target seeding rates (60, 120, 180, 240, 320 plants m⁻²) over 7 site-years in central Saskatchewan, Canada between 2013 and 2016. Four of the six lentil classes reached maximum yield at target plant populations very close to the currently recommended seeding rate of 130 plants m⁻². Target plant populations of 144, 133, 124, and 128 plants m⁻², or seeding rates of 48.3, 82.5, 47.2, and 82.3 kg seed ha⁻¹, were the most profitable for extra small red, large red, small green, and medium green lentil classes, respectively. Small red and large green lentil were exceptions to the trend observed in the majority of lentil classes. Yield and profit of small red lentil was maximized at a target plant population of 250 plants m⁻², which amounted to approximately 111.1 kg seed ha⁻¹, or double the current recommended

seeding rate. Large green lentil was most profitable when targeting a plant population of 170 plants m⁻², which amounted to approximately 107.7 kg seed ha⁻¹, or 1.3x the current seeding rate recommendation. Seeding rate recommendations for small red and large green lentil classes should be revised upward to enable producers to increase yield and profit.

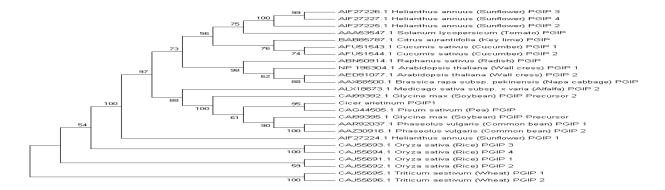
P7 SEQUENCE CHARACTERIZATION OF *PGIP1* OF CHICKPEA

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Chickpea (*Cicer arietinum* L.) is the second most important pulse crop in the world. Chickpea is important because of its high protein content, lack of major anti-nutritional factors, and drought tolerance. Chickpea production is constrained by many stresses which include biotic factors like diseases and insect-pests and abiotic factors like drought, heat and cold. In general, soil borne diseases (Fusarium wilt, dry root rot, stem rot) and foliar diseases (Ascochyta blight) are important. All the diseases involve degrading plant cell wall at some point of the disease process.

Host resistance plays very important roles in disease control. PGIPs (polygalacturonase-inhibiting proteins) are plant cell wall-bound proteins capable of inhibiting actions of polygalacturonase (PG) enzymes produced by pathogens. PGIPs can be utilized to confer disease resistance in the crop plants. Up to now more than 170 complete or partial *PGIP* genes from dicot and monocot plants have been deposited in nucleotide databases (e.g., http://www.ncbi.nlm.nih.gov/). Most of these genes have been identified as *PGIP* genes based on sequence identity but only a few of them have been shown to encode proteins with PG-inhibitory activity (R. M. Kalunke *et al.*, 2015).

In order to explore the usefulness and demonstrate the biological functions of chickpea PGIPs in diseases resistance, we have PCR-amplified *CaPGIP1* from chickpea cultivar "Dwelley" and cloned it to *E. coli* using TA cloning method. The inserts were completely sequenced in both directions. The sequence was 100% match with the predicted chickpea *PGIP1* gene sequence from the GenBank. The deduced PGIP amino acid sequence was aligned with available *PGIP* sequences in the GenBank and a phylogenetic tree was constructed using the neighbor-joining method and bootstrap tests with replicated 1000 times to show the confidence level. Phylogenetic analysis demonstrated that the deduced chickpea CaPGIP1 amino acid sequence shared identity with pea, soybean, common bean and alfalfa. The phylogenetic trees exhibited a close relationship with previously reported PGIP genes in legumes.



The successful cloning of the chickpea *PGIP1* gene will allow us to express the gene and study its functions in inhibiting fungal polygalacturonases, and in transforming and expressing in model plants and to examine its effect on disease development. Completion of the project will empirically demonstrate the functions of chickpea *PGIP1*.

P8 Identification of quantitative trait loci associated with cold tolerance in an interspecific chickpea recombinant inbred line population

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Chickpea, Cicer arietinum L., is one of the oldest grain legumes in modern agriculture and is grown in more than 50 countries. The seeds are rich in carbohydrates, minerals, and protein. Increases in production are slowed by several abiotic and biotic stresses. In regions with Mediterranean climates that have mild winters, yields can almost double when chickpea is planted in the fall rather than the spring. However, there are no cultivars that can successfully overwinter in regions with harsher Continental climates. This project focuses on understanding the genetic basis of cold tolerance in chickpea. The objectives are to identify QTL and genomic regions associated with cold tolerance. An interspecific recombinant inbred line (RIL) population was evaluated for cold tolerance in field and controlled conditions and was genotyped using genotyping-by-sequencing (GBS). The RIL population, developed from the cross ICC 4958 / PI 489777, is F6-derived and consists of 129 lines. ICC 4958 is a cold sensitive desi type (C. arietinum); PI 489777 is a cold tolerant wild relative (C. reticulatum). The field evaluations were conducted at two locations with different winter conditions; Central Ferry, WA (46.63 N; 117.49 W; 195 m asl) and the Washington State University Spillman Research Farm, Pullman, WA (46.73 N; 117.17 W; 720 m asl). Assessment of cold tolerance was based on stand counts and leaf damage, both assessed in late autumn and again in early-spring. A freezing experiment in controlled conditions will provide additional cold tolerance data for specific growth stages. Preliminary data indicate that post-winter plant leaf damage is highly correlated to mean spring stand count.

P9

Sampling strategies and identification of Aphanomyces euteiches in Montana soils

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In the pulse-producing regions of Montana, wet conditions can give rise to an increase in root rots of pulse crops that limit plant yield. *Aphanomyces euteiches* was discovered in three Montana pea fields in a 2016 disease survey in northeastern Montana. There is a need for efficient, sensitive and accurate sampling strategies for field detection of Aphanomyces to implement management strategies. Three fields with known infestation of *A. euteiches* were sampled by taking soil cores 6 inches deep to test differing spatial sampling strategies in the fall of 2017. Data analysis, identification of species and pathogenicity tests are ongoing. The results of this study will be important for understanding the distribution, identity and disease risk for Aphanomyces root rot in Montana.

P10 Physiological Response to PEG induced drought stress at Flowering Period in Adzuki Bean

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At the preliminary stage, four kinds of adzuki bean varieties with different drought-resistance were chosen as materials for drought-resistant physiological responses; In this study, polyethylene glycol 6000 (PEG6000) was used to simulate the water stress, with different treatment concentration to measure the adzuki bean leaf, and analyzed the influence of different degree of drought on the drought resistance physiological indexes, and finally determine the indexes of drought- resistance identification on flowering stage; The results showed that 10% PEG treatment of 3 days' superoxide anion (O²⁻ •) producing rate, 5% PEG treatment of 6 days' relative electric conductivity, 10% PEG treatment of 6 days' abscisic acid (ABA) levels, 5% PEG treatment of 9 days' soluble sugar concentration could be used as physiological indexes of flowering drought resistance identification.

P11

Promotive effects of ABA antagonist on seed germination of brassica napus under low temperature

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Short growing seasons in Canada hinder canola to express their full growth potentials. Low temperature in early spring could induce seed dormancy, therefore, early seeding will delay germination. A series of ABA antagonist to act against ABA, which could be used in releasing dormancy and promoting seed germination under low temperature were tested. Among all, ABA 1009 has a significant promoting effect and relatively simple for mass production. The hormone analysis on ABA 1009 treated seeds suggested that even though the application of 1009 induced ABA biosynthesis, it also increased ABA degradation through hydroxylation of 8' carbon of ABA. The RNA expression analysis will be done on genes that involved in ABA biosynthesis or degradation pathway to examine the effects of ABA 1009 at the gene level.

P12 Milling Efficiency of the Four Major Seed Coat Types in Red Lentil (*Lens culinaris* Medikus)

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Milling efficiency characteristics are key export quality traits for the red lentil industry. Various factor including seed coat color and chemistry may influence these qualities. The objectives of the study were to determine effect of seed coat color on milling traits. This study compares four basic seed coat background colors (green, gray, tan, and brown) of red cotyledon genotypes grown in two locations at Saskatchewan and two years. The percentages of dehulling efficiency, milling and football recovery varied with the different seed coat colours conferred by specific genotypes. Seed coats of lentil genotypes with homozygous recessive tgc allele (green and gray seeds) had significantly higher percentages of dehulling efficiency (DE%) and milling recovery (MR%) compared to brown or tan seed coat types while latter had significantly higher football recovery (FR%) percentages in two-site years. This result suggests lentil with green or gray seed coat color with red cotyledons and uniform seed dimension would be beneficial for the millers to optimize dehulling and milling recovery percentages. To optimize football portion, it would be beneficial to select brown seed coat color genotypes.

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Kimberly	Zitnick-	North Dakota State University, Fargo, ND
	Anderson	

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Meeting Rooms Floor Plan & Dimensions

