Abstracts and Short Papers from Oral Presentations at the North American Pulse Improvement Association (NAPIA) November 2-4, 2011 Meeting San Juan, Puerto Rico

Cool season food legume genome database: translating genomics for crop improvement

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Genomics-assisted breeding offers tools to optimize plant breeding efficiency by identify genes and/or markers related to traits of interest. The Cool Season Food Legume Genome Database (http://www.coolseasonfoodlegume.org) is being developed to assist in translating genomics into crop improvement. The main objective is to facilitate cool season food legume breeding and research by serving as a genomic, genetic and breeding data resource. Although databases exist for the model legumes, the Cool Season Food Legume Genome Database is specifically designed to collect and centralize data for pea, chickpea and lentil while using data from the model sequenced legumes for comparison and further curation. The database is built using Tripal which provides simplified site development by merging the power of Drupal, a popular web Content Management System (CMS), with that of Chado, a community derived database schema for storage of genomic, genetic and other biological data. Each page of the web site has a header bar composed of various categories for easy navigation: 'Home', 'About', 'Community', 'Crops', 'Maps', 'Tools', 'Search', 'Contact', 'Calendar', 'Publications', 'SCRI' and a link to join the 'Mailing List'. The 'Home', 'About', 'Community', 'Contact', 'Calendar', 'Publications' and 'SCRI' pages are self-explanatory and straight forward. The 'Crops', 'Maps', 'Tools' and 'Search' pages are described below. The 'Crops' page has a dropdown table of the currently available cool season food legume crops (pea, lentil and chickpea). For each crop, a unigene build for the publicly available ESTs along with the functional annotation data of the unigene set is available for users to browse. The functional annotation includes homologs in other model species, GO terms and KEGG pathway terms. The 'Crops' pages also have links to the genetic map data that can be viewed using the comparative map viewer, CMap, as well as links to NCBI and GRIN. The 'Tools' page provides SSR and BLAST servers, Medigaco GBrowse, Soybean GBrowse and Lotus GBrowse. The SSR server is an online tool that allows a user to upload sequences as a batch file, select the type of motifs they are interested in identifying and search the sequences for microsatellites. The results are returned by email for each SSR containing sequence. Sequences can be compared to the available datasets, such as the unigenes or the individual ESTs, using the BLAST server. The Genome Browsers are tools that allow the user to view the features of a genome such as predicted genes, markers and mapped transcripts. In the Cool Season Food Legume Genome Database, transcripts from pea, chickpea and lentil are mapped to regions/genes in the module legumes. This is useful for both function identification and fine mapping. The putative functions of transcribed genes can be assigned using various functional annotations. Functional annotation data of the EST unigenes can be browsed in web pages, downloaded in an excel file or queried using various categories in the EST/unigene 'Search' page. From the EST/unigene detail page, information on ESTs, gene ontology assignment, KEGG and InterProScan annotations along with homologs in other databases are available.

Sequences can also be downloaded in Fasta format. GO terms provide keywords of the type of processes/functions that the gene is involved in, KEGG annotation identifies potential pathways that the gene product belongs to, and the InterProScan analysis identifies functional domains in the gene product. These various annotations help build evidence for the function of a specific gene which can then be verified experimentally. Users can also query for sequences using a simple or advanced form. The advanced EST 'Search' page offers the option of EST or unigene search. Users can perform combinatorial search by name, assembly, sequence type, length or putative function. Registered users may acquire the collaborators status which allows them to view and analyze private research data. COS analysis, Gbrowse mapping, M. trunculata SSR analysis, and P.sativum SSR analysis are some of the private options stored under Research. Planned future development includes adding cool season food legume genome sequences as they become available, addition of more genetic maps and implementation of a publication search site, a breeder's survey form and an integrated breeder's toolbox such as the one we have developed for the genome database for Rosaceae (ww.rosaceae.org).

KnowPulse: A breeder-focused web portal that integrates genetics and genomics of pulse crops with model genomes

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Current sequencing technologies have the ability to deliver vast amounts of genotypic data to unsuspecting plant breeders. The past few years has seen a huge increase in the amount of genotypic information now available for pulse crops, including pea, chickpea, lentil and common bean. These data are of limited use to breeders until associated with phenotypic information and recently several projects have been initiated to address this both at the CDC and abroad.

The trouble with vast amounts of data is how to store it and how to access it in a manner that is useful to breeders and geneticists. Traditionally, literature searches or word-of-mouth techniques have been used to find markers for use in breeding programs. However, as the number of available markers increases, this becomes less and less feasible. A clear need exists for resources, designed with the breeder's objectives and viewpoint in mind, that provide centralized access to marker details. The web portal KnowPulse (http://pulse.usask.ca) is being developed to address these needs. It is designed for pulse crop breeders and currently includes genotypic information and molecular markers for common bean, chickpea, lentil and field pea.

Within KnowPulse, each genomic marker has an information page meant to facilitate the integration of the marker into a breeding program. This includes details like the type and location of the marker and any known protocols for marker detection. A summary of observed alleles and the genotypes of all germplasm surveyed to date is also included on the marker page, allowing a researcher to determine the likelihood of a marker being polymorphic for a given variety or population.

Genotypic data can also be accessed through dynamic tables which allow researchers to select germplasm entries of interest. Filters are available allowing researchers to select markers that are polymorphic in specific germplasm, or specific types of markers associated with a given project. Data are exportable as spreadsheets or marked-up FASTA files. The marked-up FASTA follows the submission

requirements for design of KBioscience KASP assays and allows bulk design of markers for this technology platform.

Genomic markers stored in KnowPulse are not required to be associated with phenotypes in the same species. If a phenotype-associated marker has not yet been developed for a trait of interest, KnowPulse can be used to find existing genomic markers in regions homologous to a gene thought to be involved in your trait based on studies in other species. A GMOD GBrowse (1) with a Medicago truncatula backbone makes it easy to visually inspect a region in Medicago for homologous sequences or genomic markers in multiple pulse species.

The cross-species GBrowse is also useful to geneticists due to the ease with which homology among pulse crop species can be visualized. This can help identify potential sequences for gene identification based on information from other species. KnowPulse hosts its own BLAST server (2, 3) with many pulse crop-specific and model plant datasets allowing researchers to find homologous sequences based on sequence similarity. Both of these tools are meant to make it easy to find either sequence or genotypic data based on information from other legume species.

Currently, KnowPulse contains all sequence data generated as part of 454 sequencing-based projects carried out at the CDC in conjunction with the NRC Plant Biotechnology Institute (PBI) in Saskatoon, the Lens culinaris EST collection generated previously at CDC/PBI (already deposited to NCBI), all legume DFCI Gene Indices (4) and the latest Medicago truncatula assembly (5). All genotypes and markers generated as part of the CDC-led Implementation of Markers for Pulses (iMAP) project are being loaded into KnowPulse. We intend to have most genotypic information generated in our lab publically available through this portal. Any sequence or genotypic information generated by other research groups is welcome and will be included upon request of the originators.

Future development plans for KnowPulse include the ability to store phenotypes including experimental details, environmental conditions and the phenotypes observed for any germplasm surveyed. Once this feature is available, phenotypes can be associated with genotypes, improving the searching capabilities for finding markers associated with a trait of interest. It is also our intention to promote inter-website communication and data-sharing between KnowPulse and other legume-specific web portals including the Legume Information System (6) and the Cool Season Food Legume Genome Database (7).

References

1. Stein L.D et al. The generic genome browser: a building block for model organism system database. 2002. Genome Research 12: 1599-1610.
2. Morgulis Aet al. Database indexing for production MegaBLAST searches. 2008. Bioinformatics 24(16): 1757-1764.
3. Papanicolaou A.,Heckel D.G. The GMOD Drupal Bioinformatic Server Framework. 2010. Bioinformatics 26(24):3119-3124.
4. Quackenbush J. et al. The TIGR Gene Indices: analysis of gene transcript sequences in highly sampled eukaryotic species. 2001. Nucleic Acids Research 29(1): 159-164.
5. Young N.D, et al., The Medicago genome provides insight into the evolution of rhizobial symbioses. 2011. Nature doi:10.1038/nature10625
6. Gonzales M.D. et al. The Legume Information System (LIS): an integrated information resource for comparative legume biology. 2005. Nucleic Acids Research 33(1): D660-D665.
7. Cool Season Food Legume Genome Database: http://www.gabcsfl.org/

Progress report on enhancing faba bean (Vicia faba L.) germplasm for improved winter-hardiness at Pullman, Washington

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Introduction

Numerous reports have documented that grain legumes have positive effects on the succeeding crop yield (1, 2). Winter-hardy peas and lentils are currently being used in rotation with wheat in the Palouse region of Washington and Idaho. Murray et al. (3) reported that faba bean (Vicia faba L.) has the same winter-hardiness as lentil and better than pea. Aiming at developing an alternative rotation crop for cool-temperate regions, we initiated a research project to enhance faba bean germplasm for improved winter-hardiness at Pullman, Washington in 2008. This report summarizes the progress made in the past three years.

Materials and methods

Available faba bean accessions maintained by the USDA-ARS Western Regional Plant Introduction Station in Pullman, WA were used for this project. For the first year experiment we used 43 accessions collected from various countries and 12 winter-hardy cultivars/breeding lines from W. Link of Georg-August University, Germany (4).

Two approaches were taken in screening for winter-hardiness. The first approach is the traditional replicated field trial of single row plots at two locations (Pullman and Central Ferry, WA). Thirty seeds from each entry were planted and observation notes were taken through the growing season. A weather logger was placed near the planting site to collect ambient and soil temperatures through the season. The second approach is a modified "mass selection". Seeds bulked from 466 accessions were planted at a high density in both locations in fall of 2010. Plants that survived through the winter at each location were kept to produce seeds for further testing.

Results and discussion

The first year results were reported earlier (5). We planted 43 accessions in both Pullman and Central Ferry and the 12 winter-hardy lines from Germany were planted in Central Ferry in October 2008. We observed a high level of variation in winter-hardiness among the accessions during the first year. The data loggers recorded minimum ambient temperatures that were at or below -14 °C with at least 5 consecutive nights of hard frost. The number of consecutive days that temperatures stayed below zero ranged from 7 to 14. The extreme was a period in Pullman that reached -22.2 °C and 11 consecutive nights of hard frost. All accessions survived in Central Ferry, while 30 of the 43 accessions were completely killed in Pullman. Accessions that had a higher survival rate in Central Ferry also had a few plants which survived in Pullman. We also observed that some accessions had the ability to send out shoots from the lower nodes of the stems from the plants that were damaged or killed by low temperatures. This ability to "regrow" could be used as one of the criteria to measure winter-hardiness of faba bean.

In October 2009, we planted 75 accessions in both Pullman and Central Ferry. These included 55 accessions that were tested in the first year plus 20 new accessions. Although the winter was not as cold as the previous year, there was little snow to cover the seedlings during the cold months. All the accessions in Pullman were completely dead. Approximately 20 accessions in Central Ferry survived and

the number of plants that survived of each accession varied from one to eleven plants. There was a significant location by year by genotype interaction. Based on the results of survival rate and seed yield potential obtained in the past two years, we selected 16 most winter-hardy accessions for further observation.

In October 2010, the selected accessions were planted in two locations. We also planted nine selected F3 families derived from a cross between a non-winter hardy vegetable-type variety 'Extra Precoce Vioetto' and a winter-hardy genotype 'Hiverna/2-5EP1' from Germany. All the selected accessions had plants that survived at both locations. Survival rates ranged from 16 - 100%. The plants that survived produced an average of 75g (range 33-150 g) of seeds per plant. All nine F3 families survived in Central Ferry while only two survived in Pullman. Of these two families, one had only one, and the other had two plants survived. These two families also had a higher survival rate in Central Ferry.

We tried a novel approach of "mass selection" for winter-hardiness. Seeds bulked from 466 accessions were planted at three locations in the Palouse region in October of 2010. The percentage of winter survival was estimated at <1% in Pullman, 5% in Central Ferry and 5-10% in Dayton. Seeds from these plants were harvested for further testing.

In summary, sufficient amount of variation has been captured in the USDA faba bean germplasm collection and satisfactory progress has been made towards establishing a winter-hardy faba bean population in the past three years. These genotypes that survived through the harsh winter in Pullman formed the foundation for developing an alternative fall-sown rotation crop for the Palouse region.

Acknowledgements

Technical assistance from Kristy Ann Ott, Landon Charlo, Wayne Olson, Kurt Tetrick, Sean Vail and Leslie Elberson is gratefully appreciated. Funding includes a Germplasm Evaluation Grant from the USDA Cool Season Food Legume Crop Germplasm Committee to WRPIS, Washington State University and USDA ARS CRIS Project 5348-21 000-026-00D.

References

1. Chalk P.M. 1998. Aust. J. Agric. Res. 4:303-316.
2. Plancquaert P., Desbureaux J. 1985. In: Hebblewaithe et al.(ed.) The Pea Crop. Butterworth, London. pp. 193-202.
3. Murray G.A., Eser D., Gusta L.V., Eteve G. 1988. In: Summerfield R.J. (ed.) World Crops: Cool Season Food Legumes. Kluwer, London. pp. 831-843.
4. Arbaoui M., Balko C., Link W. 2008. Field Crops Res. 106:60-67.
5. Hu J., Mwengi J.E., Coyne C.J. and Pan, W.L. 2009. Pisum Genetics. 41:57-58.

Revisiting strategies in lentil breeding: Wild species update

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Canada is the largest exporter and second largest producer of lentils *(L. culinaris* Medikus) in the world and 98% of it is produced in Saskatchewan. When first introduced to Saskatchewan, lentil was grown with relatively fewer problems, however as lentil acreage increased, the crop is now host for many

diseases, such as, anthracnose, ascochyta, stemphyllium, Gray mold, white mold, powdery mildew and root rot complex. A narrow genetic base led to lack of genetic diversity among lentil cultivars. Seven taxa in four species have been reported in lentil gene pool and only *Lens ervoides* species has the highest frequency of resistance followed by L. *nigricans* and L. *lamottei* to both anthracnose and ascochyta diseases. Little or no resistance has been found in the cultivated background for race-Ct0 of anthracnose.

In the breeding program, new lentil cultivars with greater productivity, enhanced quality traits and improved resistance to multiple diseases are becoming increasingly important as a way of maintaining genetic gain in yield. The first breeding approach is to establish interspecific genetic populations to study inheritance pattern and transfer resistance and other key traits to the cultivated background. Recombinant inbred lines (RILs) have been developed using *L. ervoides* and *L. culinaris,* sub sp. *culinaris* crosses. Also, a RIL development between *L. ervoides* accessions is underway. RILs for key traits were selected and backcrossed to adapted backgrounds to transfer favorable genes. Selections derived from the backcross entered into preliminary unreplicated yield trials and results so far are promising.

Another strategy to accelerate utilization favorable genes of wild species is the use of a broad array of genomic and genetic resources. It has been impossible to utilize wild species to improve yield because the superior traits of interest can not be identified phenotypically alone. Additional methods of identifying resistance and key quantitative traits (QTLs) are needed that are more rapid, reliable and useable in earlier generation breeding materials. Significant advances have been made in the development and use of embryo rescue technology, wide hybridizations, library of introgression lines, generating interspecific populations, BAC library, 454-SNPs, COS markers and resistance gene homologues (RGHs).

Developing technology platform for implementing marker assisted selection (MAS) is also another approach in breeding. The amount of introgressed segments can be assessed with SNP genotyping in backcross generations and the introduction of undesirable characteristics such as bushiness, small seededness, etc. during introgression will be minimized using a forground and background breeding. The goal is to broaden the working germplasm base and increase the genetic gain in yield. As a result, the number of useful new genes is likely to increase and the use of wild species is likely to continue to grow in importance for lentil breeders.

References

1. Vail, S., et al. (2012). Field Crops Research. 126: 145-151.
2. Tullu, A., et al. (2010). Plant Genetic Resources: Characterization and Utilization. 9(01):19-29.
3. Alo, F., et al. (2011). Journal of Heredity. 102 (3): 315-329.

Nitrogen fixation and amino acids in faba-developing a screening tool for improvement

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Pulses can supply most of their N requirements through N_2 fixation, a sustainable means of supplying N to high protein crops. Our goal was to assess 15 genotypes (cultivars and breeding lines of colored flower

types and white flower / low tannin types) of faba *(Viciafaba)* in the field. Faba was grown at two locations in SK in 2009 and 2010, and measured for their ability to take up N and to fix N2. The overall goal was to develop a screening method for detecting high N2 fixation via free amino acids found in leaf, stem and reproductive tissue.

Genotypes fixed N_2 (via a commercial inoculant *Rhizobium leguminosarum* which is marketed to include faba), and the fixed N2 accounted for 51 to 88% of its requirements depending on environment, the rest coming from root N uptake from the soil. Faba grew more with increasing moisture, but became more indeterminate, and despite higher N accumulation values, yield was not necessarily increased. A range of yield, biomass and N accumulation was seen for Rosthern 2009. Saskatoon 2009 was drier and yields were slightly lower, along with less biomass and slightly less N accumulated. Data for Rosthern for 2010 came from a record wet year, and so biomass was high and maturity was delayed. The 2010 Saskatoon site suffered from waterlogged soil, and despite limited sampling throughout the season, data were not analyzed due to missing plots and unrepresentative plants.

In 2009, Florent, Taboar, Melodie, FB2210 and Divine had the highest total plant N accumulation, coming from vegetative parts but especially from reproductive growth. The total shoot N content of the 15 genotypes ranged from 32 to 47 g N m^{-2}. The total shoot nitrogen content can be considered as 8 parts, with 1 part made up of leaf N, less than 1 part made up of stem N, about 1.5 parts being made up of pod shell, and the seed making up the remaining 5 parts. The nitrogen budget, as in the amount in stubble left for succeeding crops, was calculated and was far higher than reported data from Alberta (typical of western Canada), reflecting wet and excellent growing seasons. Additionally, our data are likely to be overestimates due to small plot edge effects. Assuming all N from above ground vegetative biomass at maturity became available to succeeding crops, faba genotypes supplied between 10 and 15 g N m^{-2}, or 100 to 150 kg N ha^{-1}. The yield portion removed an additional 20 to 35 g N m^{-2}, or 200 to 350 kg N ha^{-1}, due to faba being a high yielding crop with a high seed protein content (about 30%). For faba to be a good N-supplying crop to a succeeding crop in the rotation, breeding for less yield or lower seed protein concentration would enable more N to be retained in stover.

Despite being an indeterminate crop, faba was efficient at partitioning its above ground biomass to yield with harvest indices ranging from 0.34 to 0.45 in a cool, dry year. For Rosthern in 2009, cultivars had significantly different yields, ranging from 499 to 617 g m^{-2} (4,990 to 6,170 kg ha^{-1}). At Saskatoon, yield differences were less obvious among the 15 faba genotypes, ranging from 447 to 650 g m^{-2}. Snowbird had the highest yield at both locations in 2009. At Rosthern, Snowbird, NPZ5 7680, Divine and Imposa were in the high-yielding group (617-590 g m^{-2}). So far, faba's physiological characteristics show a reasonably efficient crop with lower variability when compared to chickpea and lentil (from previous research), but with high amounts of N accumulated in yield and stover. The above ground biomass partitioning of faba was <1 part leaf, >1 to 1.5 parts stem, 1 part pod shell, and the remaining 2 parts seed. The biomass range was 850 to 1670 g m^{-2} or 8,500 to 16,700 kg ha^{-1} in 2009, depending on genotype. Faba is a large biomass crop for an annual crop, likely at the upper end for the prairies.

Faba is classified as a cool season crop like pea and lentil, having amino acid metabolism where the main transport forms of N from N_2 fixation are typically the amides glutamine or asparagine, or amino acids aspartate and glutamate. But faba may have N2 fixation metabolism in the shoot that appears to be intermediate between cool season pulses and the warm season legumes like common bean *(Phaseolus)* and soybean. Warm season legumes use ureides, which are non-structural cyclic amino acids (allantoin and allantoate), as the major form of N arising from N2 fixation. Ureides are an efficient means of supplying amino N to the shoot, and may play a role in more stress-tolerant N2 fixation metabolism, making the crop better suited to dryland production. Ureides may also have a role in stress tolerant N metabolism, aside from N2 fixation as seen in warm season legumes. Our goal was to measure the ureide concentrations by a colorimetric assay (glyogylate production) for leaves, stem and pod material at

flowering, mid pod fill, and close to physiological maturity, and to relate these to plant nitrogen content and yield. Ureides were found in all plant partitions, and appeared to be associated with stress metabolism, as was the amino acid proline, due to consistent negative correlations of these amino acids with N accumulation, fixation and yield. Ureide concentration ranged from 2 to 16 umol g^{-1} dry tissue, depending on whether leaf, stem or pods were sampled, the environment, and growth stage. The range of ureides seen in faba leaf appear to be greater than in non-droughted chickpea and pea leaf , in stem they are about the same as non-droughted chickpea stem (2 to 5 umol g^{-1} stem) and less than non-droughted pea stem (3 to 15 umol g^{-1} stem). The ranges and concentrations of both leaf and stem ureides in faba are lower than soybean leaf (10 to 30 umol g^{-1} leaf) and petioles (15 to 50 umol g^{-1}).

Free amino acids were also measured by gas chromatography in various plant partitions throughout the lifecycle and correlated against yield, total plant N accumulated and the N2 fixed. Briefly, faba plants operate mainly with six amino acids, asparagine the major amide, and alanine, serine, tryptophan, cysteine and proline. The major N cycling amino acid is asparagine, followed by leaf metabolism using alanine. Glutamine also plays a role but less is present. Lesser to minimal amounts of aspartic acid and glutamic acid are used. Ureides and proline indicated more stress. We were expecting to find a single amino acid concentration or even a ratio using the amides asparagine or glutamine to aspartate and glutamate to be useful, based on limited studies where amino acid concentrations have been previously published. We found ratios of amino acids more indicative of plant N status than single amino acids, and we plan to use the sum of asparagine plus aspartic acid plus glutamic acid plus glutamine, compared to proline, and a second ratio of alanine to proline, in screening for higher N2 fixation and N accumulation in faba nurseries. We are currently verifying the amino acid ratios on 2011 plants to test whether this screening tool does find us elite yielding and high N2 fixing cultivars.

Acknowledgements

Funding from Saskatchewan Pulse Growers Association and the Agriculture Development Fund of Saskatchewan; ureide measurements were done by Ms. Denise Broersma.

Vicia faba - a potential rootstock for lentil breeding

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Wild lentil species are an increasingly important genetic resource for lentil breeding programs. An accession of each of the six wild *Lens* species was used as the scion in grafts to faba bean breeding line FB50-9 rootstock. Successful grafts were obtained for all species with survival of grafts to seed maturity between 70.7% and 87.7% except for *Lens orientalis* PI 72735 with 55.3% survival. Flowering time of grafted scions compared to ungrafted controls was not affected for four species but scions of *L. nigricans* PI 72560 and *L. orientalis* PI 72735 had flowering delays of 9 and 7 days respectively. For all six wild species, pod length, seed diameter and seed weight were not significantly different between non-grafted controls and scions grafted onto faba bean rootstocks. This simple approach opens the possibility of using intergeneric *in vivo* grafting techniques to rescue interspecific hybrids of lentil. The technique has potential as a useful tool in lentil breeding, as a means of improving seed multiplication rate of rare genetic resources of wild lentil and as a way to reduce the costs of germplasm multiplication of wild lentil species.

Variety Adaptability and Yield Stability Analysis for a State-wide Variety Testing Study in Montana

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Introduction

Dry pea *(PisumsativumL.)* and lentil *(Lens culinaris* Medik) production in Montana has been rapidly increasing in recent years. Planted acreage of dry pea has been over 200,000 acres since 2006 placing Montana as second largest producer in the nation. Lentil production reached to 260,000 acres in 2010 doubled from the planted acreage in 2009. Pea and lentil fit well in the wheat-based cropping systems as rotation crops to replace fallow, providing many agronomic and economic benefits. However, the most suitable cultivars and agronomic practices have not been well understood in Montana where considerable variation in geography, soil, and the climate exist. Multi-location testing is important to the development and sustainability of the pea and lentil industry in Montana. This paper reports a coordinated statewide dry pea and lentil variety testing from 2008 to 2011. Our objective is to show adaptable varieties with stable yields using additive main effects and multiplicative interactions (AMMI) biplot analysis.

Materials and methods

Selected commercial varieties and breeding lines of dry pea and lentil were planted at nine locations across Montana from 2008 to 2011. Dry pea evaluation consisted of 22 smooth green and yellow cultivars, of which, nine are commercially available, and four are experimental lines from the USDA-ARS Grain Legume Genetics and Physiology program at Pullman, Washington. Lentil evaluation consisted of 13 different classes cultivars, of which, ten are commercially available, and three are experimental lines from the USDA-ARS Grain Legume Genetics and Physiology program. The experiments were randomized complete block design with three to four replications. The grain yield data were subjected to analysis of variance within each year and for combined four years respectively to determine interaction between variety (G) and location (E). Subsequent PCA analysis followed using the same data where each combination between nine locations and four years generating 31 environments for the dry pea variety evaluation and 28 environments for lentil variety evaluation. Genotype plus genotype x environment interaction biplots were generated from the first two principal components (PC1 and PC2). Correlation among the environment was calculated from the angle between two environment vectors.

Results and discussion

Grain yield of both dry pea and lentil varied greatly among the locations across Montana. Significant interactions between variety and location were also observed. The interaction effects (genotype over environment or *vice versa)* can be described as distance from the plot origin. Thus, the most responsive varieties can be visualized in the biplot at the corner or vertex. Majoret, Medora, Stirling, PS0010836, and PS9910140 were identified as extremes. Aragorn, Bridger, Meadow, Patrick and Spider were the most stable varieties among all environments. Among the test sites, Moccasin in all test years was relatively clustered and close to the plot origin indicating less interactive, hence, stable and repeatable location for testing general adaptability in Montana.

The biplot was analyzed for lentil in the same manner as that of dry peas. Brewer, Crimson, Essex, Meteor, Vantage, LC01602245P, and LC01602300R were the most responsive lentil cultivars to the environments while Pennel was the least responsive. Bozeman, Moccasin, and Richland were close to the plot origin indicating these locations were relatively stable. Bozeman and Moccasin were relatively clustered and hence could be good repeatable locations.

Biplot analysis allowed us to identify the most or the least responsive varieties in the various environments. This information is a useful tool to evaluate variety adaptability and yield stability in a particular environment.

Table 1. Dry pea and lentil varieties evaluated in Montana statewide trials, 2008

Pea variety			Lentil variety		
Entry	Variety	Type	Entry	Variety	Type
1	Admiral	Yellow	1	Brewer	Medium Green
2	Aragorn	Green	2	Crimson	Small Red
3	Arcadia	Green	3	Essex	Small Green
4	Bridger	Yellow	4	LC01602062T	Small Red
5	Montech 4152	Yellow	5	LC01602245P	Spanish Brown
6	Cruiser	Green	6	LC01602300R	Medium Green
7	Delta	Yellow	7	Merrit	Large Green
8	Golden	Yellow	8	Meteor	Medium Green
9	K2	Green	9	Pennell	Large Green
10	Majoret	Green	10	Redberry	Small Red
11	Meadow	Yellow	11	Richlea	Medium Green
12	Medora	Green	12	Riveland	Large Green
13	Midas	Yellow	13	Vantage	Medium Green
14	Mozart	Yellow			
15	Patrick	Green			
16	PS0010836	Yellow			
17	PS011102958	Yellow			
18	PS03101822	Yellow			
19	PS9910140	Yellow			
20	Spider	Yellow			
21	Stirling	Green			
22	Striker	Green			

Characterization of mycosphaerella blight resistance, lodging resistance, and micronutrient concentration in a pea recombinant inbred line population

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Abstract
Moderate variation for adult plant resistance to mycosphaerella blight, pre-harvest lodging and selenium accumulation have been observed in field pea. In order to map the quantitative trait loci (QTLs)

associated with these traits, a population of 142 F_9 and F_{10} recombinant inbred lines (RILs) derived from a cross between Carrera (susceptible to mycosphaerella blight and lodging) and CDC Striker (moderately resistant to mycosphaerella blight and lodging) were phenotyped in Saskatoon and Rosthern, SK, Canada in 2010 and 2011. Based on phenotypic data collected from 2010 and 2011, area under the disease progress curve (AUDPC) of mycosphaerella blight ranged from 131 to 205 and 144 to 235 for Saskatoon and Rosthern, respectively. At physiological maturity, lodging ratings of the RILs ranged from 3.8 to 8.3 at Saskatoon and 4.5 to 8.5 at Rosthern. Micronutrient (Se, Zn and Fe) concentrations in the seeds of each RIL were determined using an atomic absorption spectrophotometer for the 2010 samples. A genetic linkage map was generated using 65 SSR (microsatellite) markers resulting in 13 linkage groups that cover 290.3cM of the pea genome. A region between AA491 and AA278 on linkage group 13 was identified as putative QTLs associated with mycosphaerella blight, lodging, Zn and Fe concentrations. All QTLs were derived from CDC Striker, except the one associated with higher Zn concentration which was derived from Carrera.

Introduction and objective

Field pea production in western Canada is negatively affected by several fungal diseases and lodging. The major fungal disease is mycosphaerella blight. Complete resistance to mycosphaerella blight is lacking in field pea germplasm. Pea cultivars with weak stems show severe lodging after flowering, causing reductions in forage and seed yield (Stelling, 1994). Lodging resistance enhances harvest and is associated with reduced severity of mycosphaerella blight (Banniza et al. 2005). Soils in Saskatchewan are rich in Se. Field pea cultivars grown in Saskatchewan displayed a moderate level of variation in Se accumulation (Thavarajah et al. 2010), whereas large regions of Asia and Europe have soils deficient in Se (Gawalko et al. 2009), thus a good opportunity is available to market Canadian peas to these regions for nutritional benefits. The objective of this study is to determine the genetic control of several traits in field pea including mycosphaerella blight resistance, lodging resistance and selenium concentration by genotyping and phenotyping a recombinant inbred line population segregating for these traits.

Methods

A pea population consisting of 142 RILs was developed from a cross between Carrera and CDC Striker. In 2010 and 2011 field trials, pea seeds were planted in microplots (1 m^2) with two replications in each of two locations (Saskatoon and Rosthern, SK). Fertility and management practices were provided sufficient for pea production in these regions. From two weeks after flowering until maturity, assessments were made to record mycosphaerella blight severity and lodging. Mycosphaerella blight was rated under natural infection on the basis of all plants in a plot using 0-9 scale where 0 = no disease and 9 = whole plant severely blighted. Four assessments of mycosphaerella blight were done and calculated into area under disease progress curve (AUDPC). Lodging was assessed 4 times during the season and one time at physiological maturity on a 1-9 scale where 1 = completely upright and 9 = completely lodged.

Regarding genotyping, young fresh leaves were randomly sampled from 5 plants from each RIL grown in a greenhouse for genomic DNA extraction by hexadecyltrimethyl ammonium bromide (CTAB) method. A total of 330 Simple Sequence Repeat (SSR) primers derived from the Agrogene consortium (France) were screened on the parents using polyacrylamide gel electrophoresis (PAGE). Polymorphic markers between parents were screened among RILs by using an Applied Biosystem 3730 DNA analyzer.

Results and discussion

Significant effects of genotype, location and year were detected for AUDPC (mycosphaerella blight) and lodging in 2010 and 2011. At Saskatoon, AUDPC ranged from 131 to 205 among RILs, while at Rosthern it ranged from 144 to 235. At physiological maturity, lodging ratings of the RILs ranged from 3.8 to 8.3 at Saskatoon and 4.5 to 8.5 at Rosthern. Lodging was significantly correlated with mycosphaerella blight score ($r = 0.35$; $P < 0.001$), which suggests that RILs that are less susceptible to lodging may escape disease to some extent. A total of 112 out of 330 SSR markers were polymorphic between Carrera and CDC

Striker and 65 out of 112 SSRs were polymorphic among the RILs. A total of 13 linkage groups were generated consisting of 56 SSRs with 9 unlinked by using Carthagene 1.2.2 (De Keyser et al. 2010). QTL analysis was conducted by composite interval mapping (CIM) using the Qgene program to detect associations between phenotype and genotype. A region between AA491 and AA278 on linkage group 13 was identified as putative QTLs associated with mycosphaerella blight, lodging, Zn and Fe concentrations. All QTLs were derived from CDC Striker, except the one associated with higher Zn concentration which was derived from Carrera. Phenotypic variation explained by these QTLs associated with mycosphaerella blight resistance, lodging resistance, Zn and Fe concentrations were 28.5%, 10.1%, 7.4% and 13.1%, respectively.

Acknowledgments

We would like to thank Ms. Reshma Rizvi, Mr. Brent Barlow, Dr. Aziz Rehman, and Ms. Carmen Breitkreutz for their great help during this project. The technical support from the field lab crew was also appreciated. Funding for this research was supplied by Saskatchewan Pulse Growers and Saskatchewan Ministry of Agriculture.

References

1. Banniza, S., Hashemi, P., Warkentin, T.D., Vandenberg, A. and Davis, A.R. 2005. The relationships among lodging, stem anatomy, degree of lignification and resistance to mycosphaerella blight in field pea *(Pisumsativum).* Can. J. Bot. 83: 954-967.
2. De Keyser, E. D., Shu, Q. Y., Bockstaele, E.V., and Riek, J.D. 2010. Multipoint-likelihood maximization mapping on 4 segregating populations to achieve an integrated framework map for QTL analysis in pot azalea *(Rhododendron* simsiihybrids). BMC MolBiol, 11 (1): 1-20.
3. Gawalko, E., Garrett, R.G., Warkentin, T., Wang, N., and Richter, A. 2009. Trace elements in Canadian field peas: a grain safety assurance perspective. Food Additives and Contaminants.26 (7): 1002-1012.
4. Joehanes, R., and Nelson, J.C. 2008. Qgene 4.0, an extensible Java QTL-analysis platform. Bioinformatics Applications Note, 24: 2788-2789.
5. Semagn, K., Bjornstad, A., and Ndjiondjop, M.N. 2006. An overview of molecular marker methods for plants.African Journal of Biotechnology.5 (25): 2540-2568.
6. Thavarajah, D., Warkentin, T. and Vandenberg, A. 2010. Natural enrichment of selenium in Saskatchewan field peas *(Pisumsativum* L.).Can. J. Plant Sci. 90: 38 3-389.

Comparison of transcriptomes between *Sclerotinia sclerotiorum* and *S. trifoliorum* using 454 Titanium RNA sequencing

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Both *Sclerotinia sclerotiorum* and *S. trifoliorum* cause Sclerotinia stem and crown rot of chickpea and white mold on many economically important crops. The host range of *S. trifoliorum* is mainly on cool season forage and grain legumes of about 40 plant species, whereas the host range of *S. sclerotiorum* encompasses more than 400 plant species including all the host plant species of *S. trifoliorum.* Despite of morphological and ecological differences between the two species, both species are equally pathogenic on chickpea. Extensive research has been conducted on *S. sclerotiorum* and its genome sequences are available. However, relatively very little is known about *S. trifoliorum.* To take advantages of the genomic

information of *S. sclerotiorum,* we compared the transcriptome of *S. trifoliorum* with that of *S. sclerotiorum* in order to gain a better understanding of the biology of both species. Total mRNAs of both species during vegetative growth were extracted and sequenced using the latest 454 Titanium RNA sequencing technology. A total of 23325 unique transcripts with average length of 534 nt (12.5 mb genome coverage) were obtained from *S. sclerotiorum,* whereas 21214 unique transcripts with average length of 509 nt (10.8 mb genome coverage) were obtained from *S. trifoliorum.* Comparison of the transcripts between the two species will be presented and their implications will be discussed.

Insertional mutation at the Cu-Zn-superoxide dismutase gene reduces virulence of *Sclerotinia sclerotiorum* on pea *(Pisum sativum)*

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Sclerotinia sclerotiorum causes white mold disease on pea and on many other economically important pulse, vegetable and field crops, demonstrating a non-host-specific pathogenic mechanism. Despite extensive studies on this pathogen, its pathogenic mechanisms are still incompletely understood. In order to gain insight in understanding its non-specific host-pathogen interactions, Agrobacterium-mediated transformation (AMT) was used to generate random mutations and to identify potential virulence/pathogenicity factors in *S. sclerotiorum.* Among several hundreds of AMT transformants screened, two stable mutants showed significantly less virulence in comparison with the wild type strain as measured by colonizing pea leaves in detached leaf assays. Southern hybridization and inverse PCR analyses showed that the mutation was due to a single T-DNA insertion at the gene Cu-Zn-superoxide dismutase (SsSOD1, SS1G 00699) of *S. sclerotiorum.* In addition to reduced virulence, the mutant had reduced tolerance to heavy metal toxicity and oxidative stress. The SsSOD1 gene was able to functionally complement SOD in a yeast strain defective of the SOD gene. There was more accumulation of superoxide in disease lesions caused by the mutant than that caused by the wild type strain. Evidence showed that the SsSOD1 gene is an important virulence factor of *S. sclerotiorum.*

Rhizoctonia root rot of lentil caused by *Rhizoctonia solani*AG 2-1

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Lentil root rot symptoms were observed in commercial fields in the US Pacific Northwest during the unusually cool and moist spring weather of 2010. Symptoms included sunken lesions on root and stem with brown discoloration, resembling diseases caused by *Rhizoctonia solani. Rhizoctonia solani* was isolated from diseased plants and from surrounding soils and were identified to be AG 2-1 based on ITS sequences. Pathogenicity tests were conducted at 16C 12 h day and 10 C night temperature conditions using three isolates of *R. solani* AG 2-1 from lentil. Isolates were severely pathogenic to lentil cvs. Pardina

and Merrit, as well as to spring canola cv Sunrise, and the pathogen was reisolated from the inoculated plants. *Rhizoctonia solani* AG 2-1 is known to be a pathogen of canola in Australia, Canada and US Pacific Northwest, but not previously isolated from lentil. This is the first report of *Rhizoctonia solani* AG 2-1 infecting lentil.

Field evaluation of fungicides for control of ascochyta blight of chickpeas in North Dakota

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Registered and experimental fungicides, including several products with anticipated registration in 2012 and 2013, were evaluated for their control of ascochyta blight caused by *Ascochtya rabiei* in Carrington, Minot, and Williston, ND in 2011. Significant differences in fungicide efficacy were observed among products, and at least one experimental chemistry gave excellent disease control. Disease efficacy, seed yield, and seed quality results will be reported.

Field evaluation of fungicides for control of anthracnose, botrytis gray mold, and sclerotinia stem rot of lentil in North Dakota

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Registered and experimental fungicides, including several products with anticipated registration in 2012 and 2013, were evaluated for their control of anthracnose, caused by *Colletotrichum truncatum,* and sclerotinia stem rot, caused by *Sclerotinia sclerotiorum,* in Carrington and Williston, ND in 2011. Fungicide timing was also evaluated in Carrington and Minot, ND for the control of anthracnose and botrytis gray mold, caused by *Botrytis cinerea.* Significant differences were observed among treatments; disease efficacy, seed yield, and seed quality results will be reported.

Field evaluation of fungicides for control of ascochyta and mycosphaerella blights of field peas in North Dakota

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Registered and experimental fungicides, including several products with anticipated registration in 2012 and 2013, were evaluated for their control of Ascochyta and Mycosphaerella blights caused by *Ascochtya pisi, A. pinodes,* and/or *Phoma pinodella* in Carrington and Newburg, ND in 2010 and 2011. Significant differences in fungicide efficacy were observed among products, and several experimental chemistries gave excellent disease control. Disease efficacy, seed yield, and seed quality results will be reported.

Natural outcrossing rate of faba bean under Pullman field conditions and its implication to germplasm management and enhancement

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Abstract

Knowledge of the natural outcrossing rate of faba bean *(Viciafaba* L.) under Pullman field conditions will enable us to refine strategies for both germplasm management and enhancement. We used the white flower phenotype governed by a recessive gene in the investigation of the natural outcrossing rate of faba bean. Seeds from white flowered plants grown in Pullman, 2010 were harvested and planted plant-to-row in spring 2011. During flowering, the number of plants with white or regular colored flowers was recorded for each row. The percentage of plants with regular colored flowers was used as an estimate of natural outcrossing rate, which averaged 30.8% with a range from 0 to 82.6 % among 50 rows. This observed outcrossing rate is within the range of previous reports for faba bean grown in various locations. The high outcrossing rate is likely the result of abundant bumble bees and honey bees which visited the faba bean flowers frequently during bloom. Therefore, for germplasm management it is necessary to regenerate faba bean accessions using insect-proof cages to maintain the genetic integrity of individual accessions. For germplasm enhancement using phenotypic selection, it is also crucial to physically isolate the selected plants with insect-proof bags to prevent unwanted cross-pollinations and produce self-pollinated seeds for subsequent generations.

Introduction

Faba bean *(Viciafaba* L.) is a partially allogamous crop and the outcrossing rate has been investigated by many researchers in various locations. These research results reported prior to 1980s were summarized by Bond and Poulsen (1) and ranged 4 to 84%. More recent reports were within this range (2; 3 and 4). It has been reported that the outcrossing rate for faba bean is strongly influenced by local environmental and climate conditions as well as the presence of pollinating insects such as species of bumble-bees, honey bees, and solitary bees.

The U.S. National Plant Germplasm System (NPGS) is one of the world's largest national genebank networks focusing on preserving the genetic resources of crops and wild relative species for the continuing improvement of agricultural productivity. The USDA faba bean germplasm collection is maintained by the Western Regional Plant Introduction Station (WRPIS) in Pullman, Washington. Knowledge of the natural outcrossing rate of this crop under Pullman field conditions will enable us to refine strategies for both germplasm management and enhancement.

Materials and methods

Plants with pure white flowers were found in 13 among 466 accessions that were planted in 2010 for evaluation. Two of the 13 accessions consisted of only white flowered plants while the remaining 11 accessions were segregating. Open-pollinated seeds from these white flowered plants were harvested in fall 2010 and planted plant-to-row in spring 2011. During flowering, plants with white and plants with regular colored flowers were counted and recorded for each row. The percentage of plants with regular colored flowers was calculated with the formula of (number of plants with regular colored flower/total number of plants)*100 and used as an estimate of natural outcrossing rate.

Results and discussion

There were a total of 810 plants scored for flower color in the 50 rows, each of which was derived from the open-pollinated seeds of a single white-flowered plant grown in Pullman in 2010. The number of plants per row varied from two to 25. A wide range of outcrossing rate from 0 to 82.6 % was observed among the 50 rows. The average was 30.8% and the standard error was 2.98 (Figure 1). The high outcrossing rate is likely the result of abundant bumble bees and honey bees which visited the faba bean flowers

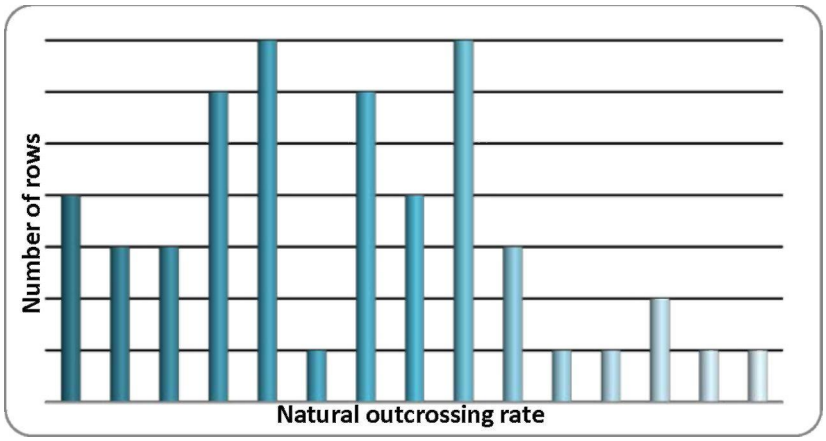

Figure 1. Wide range of natural outcrossing rates observed among the 50 single-plant-derived rows.

frequently during bloom. Therefore, for germplasm management it is necessary to regenerate faba bean accessions using insect-proof cages to maintain the genetic integrity of individual accessions. For germplasm enhancement using phenotypic selection, it is also crucial to physically isolate the selected plants with insect-proof bags to prevent unwanted cross-pollinations and produce self-pollinated seeds for subsequent generations.

Acknowledgements

Assistance from Landon Charlo, Wayne Olson, Kurt Tetrick, Sean Vail and Leslie Elberson is gratefully appreciated. Funding includes a Germplasm Evaluation grant from the USDA Cool Season Food Legume Crop Germplasm Committee and the USDA-ARS CRIS Project 5348-21 000-026-00D.

References

1. Bond, D. A., Poulsen, M. H. 1983. In: Hebblethwaite PD, editor. The Faba Bean *(Viciafaba* L.). Butterworths, London , pp. 77-101.
2. Cartujo, F., Suzo, M. J., Pierre. J., Moreno, M. T., Le Guen, J. 1998. Proc. International Symposium on Breeding of Protein and Oil crops. Eucarpia, Pontevedra, Spain. p. 49-50
3. Suso, M. J., Moreno, M. T. 1999. Plant Breeding 118:347-350.
4. Suso, M. J., Pierre J., Moreno, M.T., Esnault, R. and Le Guen, J. 2001. J. Agric. Sci. Camb. 136: 399¬ 405.

Genome mapping and molecular markers for Ascochyta Blight resistance in pea *(Pisum sativum* L.)

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Ascochyta blight is the most common disease of economic importance in peas *(Pisum sativum* L.) in North Dakota. It is caused by three pathogens: *Ascochyta pisi* Lib., and *Mycosphaerellapinodes* (Berk & Bloxam) Vestergr, which causes leaf and pod spot; and *Ascochytapinodela,* recently designated *Phoma medicaginis* var. *pinodella* (L. K. Jones) Boerema, which causes foot rot. The ultimate goal of this research is to reduce the economic impact of Ascochyta blight on the pea crop. Objectives of this research are: 1) to implement marker-assisted selection (MAS) in the breeding program to enhance plant selection for cultivar development, 2) to develop pea varieties that are resistant to the Ascochyta blight that occurs in ND, and 3) integration of field and laboratory technologies to provide sustainable disease management that is compatible with overall crop production in the region. An F7-derived recombinant inbred line population of 394 lines was developed from the cross 'Lifter'/'Radley' and DNA was extracted from each RIL for PCR-based marker analysis. Phenotypic evaluations were conducted in the greenhouse during the summer of 2011. Five replicate plants were scored using 0 to 5 scale, where 0 = no disease and 5 = high incidence of disease or plant death. Disease ratings were taken on 5 different dates, starting 21 days after planting and every 3 days subsequently. Forty-three lines showed a high level of resistance and RIL-369 and RIL-387 showed the greatest level of resistance. QTL analysis was conducted to identify DNA markers associated with Ascochyta blight resistance genes in the Lifter/Radley population that can be applied in the pea breeding program.

Developing a method to scale up production of solanapyrone toxins by *Ascochyta rabiei*

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Ascochyta rabiei, the causal agent of Ascochyta blight of chickpea, produces solanapyrone toxins. The toxins may play a role in pathogenesis, and a toxin assay using chickpea plant tissues may enable

screening breeding materials at early generations for resistance to blight. In order to develop toxin assays for screening chickpea genotypes and to investigate the role of the toxins in developing the disease Ascochyta blight, sufficient quantity of the toxins is needed for replicated and repeated experiments. A new method with solid medium using natural substrates is being developed to replace the previously published method with liquid medium in static culture. The new method significantly improved production in larger quantity of the solaynapyrone toxins with much reduced requirement of laboratory space, and will facilitate future research employing and exploiting the solanapyrone toxins.

First report of stemphylium blight of lentil in Montana and North Dakota: evidence, distribution, and prospects for management.

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Stemphylium blight, caused by the fungal pathogen *Stemphylium botryosum*, was observed for the first time in Montana and North Dakota in 2010 and 2011. The disease occurred in all production regions surveyed, sometimes at moderate to high severity. Stemphylium blight severity data collected in a fungicide timing trial and variety performance trials suggest that commonly employed fungicide application strategies may not effective against the disease but that several lentil varieties adapted to the region may show some resistance.

Response of chickpea varieties to foliar fungicides

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Ascochyta blight *(Ascochyta rabiei)* can be devastating to chickpea *(Cicer arietinum* L.) production in North Dakota. Ascochyta susceptible varieties, as well as resistance in the pathogen to strobilurin fungicides, have impacted production. In order to determine an effective screening method for the chickpea breeding program, an evaluation of the response of varieties from different market classes to foliar fungicides was essential. Eight chickpea varieties were grown at four North Dakota locations. Agronomic performance and quality of each variety was compared in a fungicide/no fungicide management system. In 2010, seed yield, canopy height, 1000KWT, disease ratings and seed sizing between fungicide treatment and the untreated control at two locations were significant. Preliminary results from 2011 indicate a similar trend. Significant yield differences of each variety in response to foliar fungicide were more evident at higher yielding locations. Other production constraints may have impacted this response at lower yielding locations. Based on two years of evaluations, it is clear that fungicide treatment of breeding materials is necessary in North Dakota environments to ensure seed production and prevent loss of valuable germplasm. Even in the presence of fungicide treatment, significant disease is evident and sufficient to allow differential selection among germplasm lines. This research will allow breeding programs to

effectively manage Ascochyta blight to an advantage and avoid complete loss and setbacks in breeding and selection of improved varieties.

Pea germplasm with partial resistance to *sclerotinia sclerotiorum* that extends the time required by the pathogen to infect host tissue

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White mold, caused by the fungus *S. sclerotiorum* can be a serious disease on pea. Currently there are no pea genotypes with complete resistance to this pathogen. Selected wild pea genotypes from the *Pisum* Core Collection and cultivars were assessed for the time required by *S. sclerotiorum* to severely infect these genotypes at all combinations of five temperatures (15.6, 18.3, 21.1, 23.9, 29.4°C) and four (12, 24, 48 and 72 h) periods of high relative humidity (PHRH). Commercial genotypes did not prevent severe infection at any temperature/PHRH combination. Three wild pea genotypes were capable of preventing severe infection for up to 24 hours. PI169603 and PI240515 are recommended to pea breeders as the best germplasm to extend the time required for serious infection by *S. sclerotiorum.*

Use of resistant-susceptible cultivar mixture to preserve *er-1*gene rendering resistance to powdery mildew of field pea *(pisum sativum* L.)

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Powdery mildew (caused by *Erysiphepisi* DC. var pisi.) resistance of field pea *(Pisumsativum* L.) cultivars is rendered by a recessive gene *er-1.* Cultivation of resistant cultivars over large geographic areas creates a typical gene monoculture, which encourages the pathogen evolution for more virulent races and breakdown of the resistance. We investigated the use of mixtures made up of resistant and susceptible cultivars to limit the pathogen evolution and to preserve the resistance gene. Since severe powdery mildew infection can cause significant yield reduction, it is necessary to define a proper ratio for such cultivar mixtures. Seed of three powdery mildew resistant cultivars was mixed with 10, 20, or 30% of a susceptible cultivar, and grown in replicated field trials. The yield reduction in cultivar mixtures depended on the yield potential and performance of component cultivars and disease severity. When disease severity was high and the resistant cultivar yielded well, the susceptible cultivar could comprise 10-30% of the mixture without significant yield reduction compared with the resistant cultivars in pure stand. The results suggest that such cultivar mixtures may be used in field pea production by providing more substrate to the pathogen so that the breakdown of resistance gene *er-1* may be delayed.

Powdery mildew is a common disease of field pea, and can cause significant yield losses. The majority of powdery mildew resistant field pea cultivars carry a single recessive gene erJ. Cultivation of such resistant cultivars over large geographic areas could press the pathogen to mutate for more virulent race(s), accompanied by a sudden breakdown of the resistance. We propose to use cultivar mixtures consisting of resistant and susceptible cultivars in an appropriate ratio to provide more substrate to the pathogen, and therefore to reduce selection pressure on the pathogen, so that the life time of the erJ-rendered resistance may be extended. The main objective of this study was to define proper ratios of resistant-susceptible cultivar mixtures with no or little yield reduction compared to the resistant cultivars in pure stands.

Materials and methods

Three powdery mildew resistant field pea cultivars, Agassiz, Cutlass, and Reward were mixed with the powdery mildew susceptible cultivar, CDC Striker, in 10, 20 or 30% by number of seeds to make resistant-susceptible cultivar mixtures. Such mixtures were grown in replicated field trials along with the four cultivars in pure stands. Also included in the study was CDC Striker sprayed with the fungicide Kumulus (denoted as CDC Striker + Kumulus), where powdery mildew was controlled by spaying Kumulus on the plants when the disease symptoms occurred on the plants. The seeding rate was adjusted to 85 viable seeds m^{-2}. The yield difference between CDC Striker+Kumulus and CDC Striker was the measure of yield reduction caused by powdery mildew, and the yield of each cultivar in pure stand was compared with the yield of each of its mixtures with CDC Striker to determine the yield reduction of each cultivar mixture. The experimental design was a randomized complete block with four replications. The field trials were grown in four locations: Lacombe, AB; Saskatoon and Swift Current, SK; and Morden, MB in 2006 and 2007. However, the disease severity at other three locations in both years was much less than the disease severity in Lacombe, which may lead to biased estimations of the impact of the disease on the yield. Therefore, only the results from Lacombe were reported here.

Table 1. Yield difference of cultivars in pure stands and cultivar mixtures

	Year	Difference [a]
Agassiz – (Agassiz70+CDC Striker30)	2006	130
	2007	799 (p=0.053)
Agassiz – (Agassiz80+CDC Striker20)	2006	-521
	2007	956 *
Agassiz – (Agassiz90+CDC Striker10)	2006	-223
	2007	440
Cutlass – (Cutlass70+CDC Striker30)	2006	34
	2007	994 *
Cutlass – (Cutlass80+CDC Striker20)	2006	-86
	2007	501
Cutlass – (Cutlass90+CDC Striker10)	2006	-266
	2007	330
Reward – (Reward70+CDC Striker30)	2006	460
	2007	-246
Reward – (Reward80+CDC Striker20)	2006	-226
	2007	-53
Reward – (Reward90+CDC Striker10)	2006	-92
	2007	-122

[a] Yield difference (kg ha^{-1}) between resistant cultivar in pure stands and cultivar mixture. * = $p < 0.05$.

Results and discussion

Yield reduction caused by powdery mildew. CDC Striker+Kumulus yielded 3766 kg ha^{-1} in 2006 and 3760 kg ha^{-1} in 2007. In comparison, CDC Striker yield 3033 kg ha^{-1} and 2483 kg ha^{-1} in 2006 and 2007, respectively. Thus, CDS Striker yielded 24% and 32% less than CDC Striker+Kumulus, This yield reduction was attributed to the powdery mildew infection. The higher yield reduction in 2007 than in 2006 was attributed to the higher disease severity in 2007. This signifies the importance to preserve the resistance conferred by gene erJ.

Proper ratio of resistant vs. susceptible cultivar in a cultivar mixture. No significant

difference was found between any of the three cultivars in pure stands and their mixtures with CDC Striker in 2006 (Table 1), whereas in 2007, Agassiz had significantly higher yield than Agassiz80+CDC Striker20, and nearly significant higher yield than Agassiz70+CDC Striker30. Cutlass had significantly higher yield than Cutlass70 + CDC Striker30 in 2007. There was no significant difference between Reward and its mixtures with CDC Striker. The difference between 2006 and 2007 was attributed to the combination of two factors. In general yield and powdery mildew severity were lower in 2006 than in 2007. Thus, the yield potential of the resistant cultivars was not fully expressed in 2006, and the yield reduction caused by powdery mildew was less in 2006 than in 2007.

Conclusion

The proper ratio of resistant vs. susceptible cultivar in a cultivar mixture depends on yield potential of component cultivars and powdery mildew severity. When disease severity was high and the resistant cultivar yielded well, the susceptible cultivar could comprise 10-30% of the mixture without significant yield reduction compared with the resistant cultivar in pure stand. Such cultivar mixtures may be used in field pea production by providing more substrate to the pathogen so that the breakdown of resistance gene *erJ* may be delayed.

Variability for micronutrient composition of pea and lentil grown in Montana

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Pulse crop production in North Dakota and the Midwest region including eastern Montana and South Dakota has increased over the past 15 years to become the primary production region for these crops in the US. Pea and lentil are well regarded as highly nutritious foods and serve as the primary protein source in diets for many populations worldwide. In addition to serving as an excellent source of protein, starch and fiber, pea and lentil seed contain many additional micronutrients that contribute to the daily nutritional requirements. This research was conducted to explore the environmental effects on micronutrient accumulation in pea and lentil and to provide a base line for future variety development. Seed harvested from genotypes comprising the statewide yield trials conducted at eight locations in Montana were analyzed for micronutrient composition using high performance liquid chromatogrphy (HPLC) and inductively coupled plasma emission spectroscopy (ICP-Emission) methodology. Iron (Fe) concentration ranged from 46.3 to 68.0 mg/kg among 21 lentil genotypes and from 50.5 to 69.1 mg/kg across the eight locations. Fe concentration in pea ranged from 37.3 to 51.2 mg/kg among 27 genotypes and from 38.2 to 50.5 mg/kg across nine locations. Zinc (Zn) concentration ranged from 31.2 to 43.7 mg/kg among 21 lentil genotypes and from 27.6 to 50.0 mg/kg across eight locations. Zn content among 27 pea genotypes ranged from 31.9 to 129.0 mg/kg and from 29.4 to 124.1 mg/kg across nine locations. Additional micronutrients and antinutrients including caroteinoids and phytic acids were also analyzed. Proximity of locations appeared to show a trend in seed micronutrient content. These data indicate that environmental factors, i.e. soil type, may have a significant role in accumulation of micronutrients in pea and lentil. This research serves as the basis for future variety development focused on increasing the micronutrient composition of pea and lentil seed with intent of providing greater nutrition to undernourished populations worldwide.

Chemical composition and mineral content of flours from various varieties of mature and immature yellow pea.

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The present study was undertaken to investigate how immature seeds of yellow pea affect chemical composition and mineral content of flours from various varieties of yellow peas. Three yellow pea varieties were used in this study. Mature and immature seeds in the samples were manually picked, dehulled and then ground into flours. Chemical composition, sugar and mineral content of the pea flours were analysed according to published methods. Preliminary results demonstrated that immature seeds displayed a significant effect on chemical composition, sugar and mineral content of flours from yellow peas.