ABSTRACTS OF POSTER PRESENTATIONS
October 28-30, 2001 Fargo, North Dakota

Interrelationship of factors influencing yield of field pea (*Pisum sativum* L.) in western Canada

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The interrelationships of yield, days to flowering (DTF), days to maturity (DTM), vine length (VL), late-flowering lodging score (L1), pre-harvest lodging score (L2), and seed weight (SW) of field pea (*Pisum sativum*) were investigated using 18 lines tested in western Canada in 1999-2000. These 18 lines were tested together with the other 18 lines in a 6 x 6 lattice experiment at 10 locations in Saskatchewan, Alberta, and Manitoba. The test locations were classified into Zone 1 (Z1) or Zone 2 (Z2) on the basis of the annual rainfall and temperature at each location, where Z1 was cool and moist whereas Z2 was warm and dry. In general, yield was negatively associated with DTF and positively associated with SW in both years. The highest yielding entries had DTF of 57-61, and SW of about 240 mg. All traits but L1 showed a high positive correlation between the two climatic zones each year, suggesting that these traits could be evaluated in either zone, and dividing the test locations into two zones in a given year may not be necessary.

Induction of embryogenesis from isolated microspore cultures of pea (*Pisum sativum* L.)

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Isolated microspore culture has become an important tool for the induction of instant homozygosity in breeding lines of many crops. This project investigated the application of haploid technology to pea (*Pisum sativum* L). A protocol is being developed in which embryos can be obtained directly from microspores without an intervening callus phase. The switch from a gametophytic to a sporophytic pathway was induced by either heat stress and/or auxin depending on the genotype. This resulted in symmetrical microspore nuclei divisions during mitosis. Regeneration followed the normal stages of embryo development from pro-embryo through globular to the mature form. A plantlet was regenerated from one cultivar. Efforts continue to identify key factors to improve this protocol and to achieve complete regeneration.

Effect of variety and environment on

the composition of field pea (*Pisum sativum* L.)

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Protein content was used as an indicator of environmental effect for a study on the effect of variety and environment on the composition of field peas. Four field pea varieties, each with three levels of protein content, were selected from farm grown samples from the 1999 crop and tested for their proximate composition, oligosaccharides and mineral contents. Protein content overall ranged from 20.2 to 26.7%. Analysis of variance showed that both variety and environment had a significant effect on starch, acid detergent fibre (ADF), neutral detergent fibre (NDF) and ash content. Significant differences in sucrose and raffinose were found among varieties. Environment had a little effect on the content of oligosaccharides. Significant varietal and environmental differences in K, Mn and P were noted. Ca, and Cu showed significant varietal differences while Fe, Mg and Zn had significant environmental differences. An interaction effect of variety and environment on NDF and Mn was noted. Protein and starch content were inversely correlated. ADF and NDF were directly correlated with protein and inversely correlated with starch. Also, ADF and NDF directly correlated with each other. There were no significant correlations between proximate composition and oligosaccharides. However, raffinose was positively correlated with sucrose while negatively correlated with verbascose. There were significant correlations between mineral contents and some of the proximate components and oligosaccharides.

Genetic dissection of tolerance to common root rot, *Aphanomyces euteiches*

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Recombinant inbred populations derived from crossing the line MN313 (tolerant to common root rot) with several other lines, were subjected to field and greenhouse trials to identify and analyze the genes involved in the tolerant response. RIL populations were grown in Le Sueur, MN in fields known to be infested with common root rot. Linkage maps were generated for each of the RIL populations and segregation of tolerance for common root rot was compared to the segregation of markers on these linkage maps. One RIL was generated from a cross with another tolerant line with an independent pedigree. All of the resulting lines displayed reasonable levels of tolerance, indicating that the two original parents possessed the same genes for tolerance. Three other RIL populations (from crosses with susceptible lines) gave results suggesting the presence of tolerance genes on linkage groups IV, VI and VII. No indication of a role of other disease resistance genes (virus resistance, mildew resistance, wilt resistance) was observed.

STS markers for comparative mapping in legumes

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PCR primers for approximately 40 genes have been developed in pea (*Pisum sativum* L.). Most of these primers also amplify homologous sequences in other legume genera, including *Lens*, *Phaseolus* and *Lupinus*. The primers were designed to be complementary to highly conserved sequences in exons of known genes. In addition, the priming sequences were selected to be 1000 to 5000 bp apart on the genomic DNA and to amplify a fragment that contained at least one intron. Sequence polymorphism was observed by restriction of the amplified fragment with an endonuclease with a 4-base recognition site. Mapping of these genes in lentil and pea indicate that the primers should have general utility for comparative mapping in legumes.

Genetic study of resistance to Ascochyta blight in lentil (*Lens culinaris* Medik)

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Ascochyta lentis is one of the leading factors responsible for severe crop losses in most lentil production areas around the world. Several sources of resistance genes have been identified. Although breeding efforts have developed some moderate resistant lines, there are still gaps in understanding the basic genetics of the disease resistance in lentil. The study was conducted to enhance our understanding of the genetic basis for ascochyta resistance in lentil by means of QTL analysis using the existing linkage map and PCR-based approaches to isolate resistance gene analogues (RGA) sequences from the lentil genome. Identification of QTL and disease-resistance related sequences in lentil would provide plant breeders with new tools, which will enhance their ability to select for disease resistant lentil a breeding program. For the study, 80 recombinant inbred lines derived from a cross between ILL5588 and L692-16-1(s) were used. The lines were tested for the reaction to two isolates (RM 389-1 and PC-1) of *A. lentis* in a growth chamber at the Saskatoon Research Centre. Over 800 RAPD and ISSR primers including those that are listed in Ford et al. (1999) were screened for polymorphisms between the two parents and the resistance and susceptible bulks. The marker data generated in this analysis were integrated into the existing linkage map developed from the same population (Eujayl et al., 1998) followed by QTL analysis for the reaction to two isolates using QTL Cartographer (Version 1.13) program. Two QTL (LOD > 3.0) each on LG 2 and LG 4 were identified to control the resistance to ascochyta blight in this population. These QTL explained 36 % (LG 2) and 29 % (LG 4) of the disease reaction variability. The QTL on LG 2 at the $OPB18_{680}$ locus controls the resistance to at least the two independent isolates (RM 389-1 and PC-1). The $OPB18_{680}$ marker was converted into a SCAR marker to simplify the analysis. More than 48 RGA clones were generated from the amplification of lentil ILL5588 DNA using degenerate primers targeting leucine rich repeats (LRR), nucleotide binding-sites (NBS) and kinase domain-containing sequences. Preliminary analysis indicated several of these genomic fragments were homologous to each other (64 to 92 % identity). Database search results indicated some significant sequence similarities to known resistance genes such as RPS2 (from *Arabidopsis*) and L6 (from flax). We are currently in progress to map these RGAs into the lentil genome and to determine the contribution of these RGAs to the reaction to ascochyta blight.

Impact of seeding date on performance of early maturing lentil varieties in different agro-regions of Saskatchewan

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The large green market class (Laird type lentil) dominates lentil production in Canada. This market class has accounted for more than 50% of total lentil production in the past 25 years. About 90% of Canadian lentil production is in Saskatchewan where production is centered in the Dark Brown and moist Dark Brown soil zones. Lentil production is usually not successful in the northeast part of the province (moist Black or the Grey soil zones) where the soils are often too wet for production of high quality seed or the growing season is too short for the late maturing and highly indeterminate lentil varieties. The lush canopy under cool moist conditions also provides an excellent microclimate for development of diseases.

Recently, the breeding program at Crop Development Centre has released early maturing lentil cultivars (in comparison to Laird). Early maturing lentil germplasm from ICARDA is also available. These new cultivars have improved canopy aeration due to a lower degree of branching. The new lentil cultivars offer the potential to shift lentil production in the province northwards to include the Thin Black and Thick Black soil zones. In these new areas, seeding dates may have to be adjusted so that crop maturity does not occur early in the season when precipitation is still high. A study was conducted with the objective of determining the optimum seeding date for the early maturing lentil cultivars in three different crop production areas of Saskatchewan. Fifteen lentil genotypes, eight from ICARDA, two from Australia and five local cultivars were seeded at five seeding dates starting the second week of May and continuing at weekly intervals till the second week of June. The results showed a large decrease in seed yield when seeding is delayed beyond the third week of May in drier environments but yield was maintained up to the first week of June in wetter environments. In general, germplasm from ICARDA performed poorly in comparison to local checks implying poor adaptability of the exotic germplasm.

Lentil canopy modification through architecture and stem stiffness

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Current large green lentil cultivars similar to the cultivar Laird are susceptible to lodging in the Saskatchewan environment. The goal of the study was to identify characteristics of lentil canopy architecture that will lead to reduced lodging and improved yield and quality. Four stiff-stem ICARDA (FLIP) genotypes varying in leaf size and canopy openness were compared to large green and other locally adapted cultivars at three population densities in the field in 2001. Data were recorded for parameters that influence canopy structure. Measurements included stand establishment at seedling emergence, biomass and light interception at weekly intervals, and stem stiffness. Stem stiffness measurements were based on the degree of stem bending and on the ability of the canopy to regain height after crushing. Cultivars adapted to Saskatchewan had greater biomass, but large green cultivars with canopy development similar to Laird had weak stems and were prone to lodging. Some FLIP genotypes had stiffer stems and less branching, resulting in less biomass and zero lodging compared to locally adapted cultivars. Other FLIP genotypes had intermediate canopy characteristics. Crimson and CDC Milestone responded similarly to FLIP genotypes, but were more similar to the large green cultivars for lodging and biomass. CDC Milestone and Crimson had improved canopy architecture compared to large green cultivars. Stem stiffness was superior in FLIP genotypes. Reduced branching may be useful trait to help reduce lodging.

Development of microspore culture technology for the production of doubled-haploid lines of chickpea (*Cicer arietinum* L.)

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Isolated microspore culture is a technique employed in a number of important field crops for the induction of instant homozygosity in breeding lines. This project investigated the application of this technology to chickpea (*Cicer arietinum* L.). At this point in our research, embryos can be induced directly from microspores without an intervening callus phase. Embryo development followed the usual stages from pro-embryo through globular to 'heart-shaped' stages and sometimes to the cotyledonary stage. Our research is now focused on obtaining mature embryos and their conversion into plantlets.

Assessing diversity in a Canadian population of *Ascochyta rabiei* from chickpea

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Forty isolates of *Ascochyta rabiei* of chickpea from Canada were assessed for variability using virulence tests and randomly amplified polymorphic DNA (RAPD). Differential lines were selected from an initial pool of 62 lines/cultivars. Several groups of isolates were virulent only on certain lines of the eight differentials that were used. Isolates from Canada were highly variable in virulence, ranging from those which were weakly virulent on all 8 differentials to those which were highly virulent on 6 of 8 differentials. Three of the 7 RAPD banding patterns observed among isolates from Canada, accounted for 77% of the Canadian population. All five isolates from Australia, 3 of 6 from Syria, 3 of 5 from the USA, and 1 of 2 from India had a banding pattern similar to the most common pattern among Canadian isolates, but both isolates from Turkey were different from any Canadian isolate. There was no association between RAPD and pathotype groups. Based on RAPD banding patterns, *A. rabiei* (teleomorph *Didymella rabiei*) was more closely related to *A. lentis* (teleomorph *Didymella lentis*) than to *A. pinodes* (teleomorph *Mycosphaerella pinodes*).