Phytopathologia Mediterranea (2013) 52, 1, 222–241

Abstracts

Abstracts of oral and poster presentations given at the Ascochyta 2012 Workshop, Córdoba, Spain, 22–26 April 2012

The third International Ascochyta Workshop was held in Córdoba, Spain 22–26 April 2012. This meeting was attended by 70 participants, and 33 oral presentations, 17 posters and 4 invited speeches were presented dealing with Ascochyta blights of the cool season food legumes (peas, lentils, chickpeas and faba beans). In addition, a special session was held on "Food legumes Research in North Africa". Abstracts of the oral presentations and the posters of the congress are presented in this issue.

Invited speakers

RNAi silencing technology in cereals for durable resistance to fungal pathogens. P. SCHWEIZER. *Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Corrensstrasse 3, D-06466 Gatersleben, Germany. E-mail: schweiz@ipk-gatersleben.de*

Durable resistance of crop plants to major pathogens is a research goal of high priority for phytopathologists and breeders. In addition to durability it should also be broadly efficient against many races of a given pathogen species. This goal can be achieved by either introgressing quantitative trait loci for race-nonspecific basal resistance from (exotic) donors into elite germplasm or by transgene technology. Here I introduce two approaches to generate durable pathogen resistance into barley and wheat by RNAi-transgene technology. The first approach focuses on the silencing of potential susceptibility factors of barley during the interaction with the powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (Bgh). In order to identify genes that affect race-nonspecific resistance of barley to the powdery mildew fungus *Blumeria graminis* f.sp. *hordei* we combined a functional-genomics approach based on genomewide transcript profiling and transient-induced gene silencing (TIGS, 1400 genes) with association-genetic (re-sequencing) and meta-QTL mapping approaches. This guided us to a shortlist of approximately 40 candidates with converging evidence for an important role in race-nonspecific resistance of barley. Several of those candidates enhanced resistance upon TIGS and thus might function as susceptibility factors. The second approach focuses on host-induced gene silencing (HIGS) in fungal pathogens attacking transgenic plants that carry RNAi constructs directed against transcripts of the pathogen. Proof of concept was obtained in the barley/Bgh system and in wheat attacked by the *Fusarium* head blight fungus *F. culmorum*. The prospect and risks of both approaches will be discussed based on recent data and on planned work.

Overview of world-wide importance of grain legume crops. A. RAMOS. *Centro para la Calidad de los Alimentos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Ministerio de Economía y Competitividad. José Tudela s/n 42004 Soria, Spain. E-mail: alvaro.ramos@inia.es*

Grain legumes were the main source of protein for human beings and together with cereals have been present in agriculture since its beginning ten thousand years ago. Different cereals and legumes were used along the different parts of the world. Since the fifties-sixties of the twentieth century the increased standard of living in certain parts of the world produced a dramatic change in the use of pulses that were quickly replaced by animal protein. This situation continues at present and, as a result, the production and the surface of pulses have reached a stand still for many years at world level. The growing population needs growing amounts of different food: cereals, meat, milk and derivates, coming from animals that require bigger amounts of cereals and proteins. Besides cereals, only grain legumes which are considered protein crops and used preferably for, animal consumption, through compounding industry, such as peas and faba beans, or even further, as oil producers for food and energy industries, are increasing their yields and land occupied thanks to the research attention received due to its economical interest.

Reference: Cubero J.I., 2013. Chickpea in History. Legume Perspectives-Chickpea special issue. In print.

ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm

Pathogen biology and epidemiology

First insights into the genomes of *Didymellaceae* species. J. LICHTENZVEIG[1*], F. KESSIE[1,2], R. MOHD-SHAH[1,2], A.H. WILLIAMS[1,2] and R.P. OLIVER[1]. [1]*Australian Centre for Necrotrophic Fungal Pathogens, Department of Environment and Agriculture, Curtin University, Western Australia.* [2]*Murdoch University, Western Australia.* **E-mail: Judith.Lichtenzveig@curtin.edu.au*

The *Didymellaceae* family includes some of the most important pathogens of legume crops: the causal agents of Ascochyta blight in chickpea, pea, lentil, and faba beans among others. Despite their substantial economic impact, little is known about the molecular aspects of pathogenicity in these closely related species. At this meeting, the first genome assemblies of *Ascochyta rabiei*, *Peyronellaea pinodes* (syn. *A. pinodes*), and *Phoma medicaginis* (pathogen of the model legume *Medicago truncatula*) will be presented. Untrained *in silico* annotation resulted in the identification of 9,000–12,000 genes per species, of which 91–97% are complete gene models. The sequence data served to: pinpoint areas of synteny and clusters of genes associated with reproduction and adaptation; identify pathogenicity-related gene-candidates through proteomic, transcriptomic and *in silico* comparative analyses; and design DNA makers for diagnostics and studies in population structure. Among the pathogenicity related genes, we have identified potential necrotrophic effectors that seem to operate similarly to those found in *Stagonospora nodorum*. Detailed understanding of the molecular mechanism involved in fungal adaptation in general, and pathogenicity in particular, is facilitating the development of novel tools and strategies in crop protection.

Evolution of pathogenic fitness of *Dydimella pinodes* and *Phoma medicaginis* var *pinodella* on pea (*Pisum sativum* L.) after several cycles grown on leguminous alternative hosts. C. LE MAY* and M. GUIBERT. *INRA-AGROCAMPUS Ouest, TGU IGEPP, Domaine de la Motte, 35693, Le Rheu, France.*
**E-mail: lemay@agrocampus-ouest.fr*

Many studies have been undertaken to know how the fungi responsible for plant diseases can survive year after year. Fungi display different strategies to survive and colonise the crops. These strategies seem to have different importance, depending on the fungi species, cropping practices and climatic factors. A passage through alternative hosts can modify the pathogenic fitness of fungi. The knowledge of ecology of alternative hosts and the way they affect the pathogen fitness would be precious to allow a management over many years and on a larger scale than the field itself. Ascochyta blight is a disease complex involving two main separate pathogens, *Dydimella pinodes* and *Phoma medicaginis* var *pinodella*. Four main primary sources of inoculum have been described: seeds, stubble, soil and volunteer plants. The purpose of this study is to evaluate the evolution of the fitness components of *D. pinodes* and *P. pinodella* after being grown for ten generations on a pea cultivar (Lumina) or on an alternative host (common vetch, alfalfa, clover, horse bean). Plants were maintained in growth chambers at 18-20°C and 12h photoperiod for three weeks before the inoculation of the two fungi. Seven days after the inoculation, the fungi were isolated. Necrosis area was measured to estimate the aggressiveness of these isolates on pea plants by using the ASSESS software (7 days after inoculation), and the production of pycnidiospores was estimated with a Malassez cell (14 days after inoculation). No adaptation on the different legume species was observed for both pathogens. Main results showed differences in the effect of the passage according to the alternative host-plant. Average aggressiveness of *D. pinodes* was found higher than the one of *P. pinodella*. Concerning the reproductive fitness, no difference was observed between the control isolates of the two fungi and the other isolates. This study showed that some behaviour modifications could occur between pathogen agents and its host. The possible application of such studies could be a help to estimate the risk of cropping pea according to cultivated and wild potential host-plant.

Applying RNA Sequencing to investigate pathogenic mechanisms of *Ascochyta rabiei*. D. QIU[1], G. VANDEMARK[1,2] and W. CHEN[1,2*]. [1]*Washington State University, Pullman, WA 99164, USA.* [2]*USDA-ARS, Washington State University, Pullman, WA 99164, USA.*
**E-mail: w-chen@wsu.edu*

Ascochyta rabiei causes Ascochyta blight of chickpea. To study the pathogenic mechanisms of *A. rabiei*, total mRNAs were isolated from isolates AR19 of pathotype I and AR628 of pathotype II of *A. rabiei*, and also from diseased tissues of chickpea 'Spanish White' inoculated with these two isolates at times from 6 to 96 hours post inoculation (hpi), and were sequenced with the 454 Titanium RNA sequencing technology. The transcripts in the interacting transcriptomes were separated into either plant RNA or pathogen RNA based on BLAST searches and the pathogen transcripts were compared with the transcripts of the pathogen from pure culture. The pathogen transcripts that were not found in the transcripts of pure culture were considered as induced transcripts (in response to infecting chickpea). A total of 21,226 and 28,061 unique transcripts (unigenes) were obtained from AR19 and AR628, respectively, and about 70% of them marched annotated genes in NCBI database. An average of 29,725 unigenes for each library was assembled from a total of 867,855 raw

reads in the interacting transcriptomes. About 10% of unigenes were from the pathogen. There were 132 induced unigenes of isolate AR19 in the first 12 hpi, nine of them were highly expressed during 24 to 96 hpi, and 42 of them were consistently expressed up to 96 hpi. There were 178 induced unigenes of isolate AR628, six of them were highly expressed during 24 to 96 hpi, and 69 of them were consistently expressed up to 96 hpi. Analysis showed a strong induction for the expression of pathogen genes that belonged to different functional categories. The functions of these induced genes and their roles in pathogenesis remain to be investigated.

Characterization of *Ascochyta rabiei* for population structure, mating type and pathogenic variability from Pakistan and United States. H. ALI[1], S.S. ALAM[1], R.N. ATTANAYAKE[2], M. RAHMAN[3] and W. CHEN[2]. [1]*Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.* [2]*USDA-ARS, Washington State University, Pullman, Washington, USA.* [3]*National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan.*
** E-mail: hinali991@hotmail.com*

Chickpea production is greatly hampered by blight causing fungal pathogen *Ascochyta rabiei* (AR) in chickpea growing regions of the world. Genetic variability and mating type frequency of thirty two AR isolates from six geographical regions of Pakistan were compared with a US-AR population. Pakistani AR (PAR) population had an apparent skewed (3 Mat1-2: 1 Mat1-1) distribution, although Chi-square tests showed non-significant deviation from equal distribution due to small sample sizes and the US-population showed a 1:1 distribution. The results showed that sexual reproduction is rare in PAR due to either unavailability of both mating types or lack of conductive environment but statistical analysis showed panmixia which may be due to past recombinational events. Genetic variation at six microsatellite loci was assessed and each isolate was assigned to a microsatellite haplotype. Population structure using Bayesian analyses differentiated isolates into three distinct clusters, two clusters of PAR and one of the US isolates. However, few isolates from US shared same genetic background with one cluster of the PAR isolates, providing a link of inter-continental migration of the pathogen due to import of seeds. Additionally, the two clusters of Pak-isolates are not strictly linked to the geographic locations in Pakistan, suggesting frequent gene flow of AR among different locations. Pathogenic variability of nineteen PAR collected from two different provinces was assessed. The results based on the reaction of isolates with differential lines showed that aggressive and highly aggressive pathotypes II and III respectively are prevalent in Pakistan as compared to least aggressive pathotype I. It is interesting to note that highly aggressive pathotypes III and IV have only been reported from Syria and Pakistan where we assume less frequency of sexual reproduction due to predominance of one mating type, in contrast to other countries where both mating types are present in equal ratio hence, this issue needs further investigations.

Note: The complete study has been published in *Journal of Plant Pathology*, 2012, 94(1), 99–108.

Effects of temperature on virulence of *Didymella rabiei* pathotypes affecting chickpea. S. AHMED* and M. IMTIAZ. *International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.*
**E-mail: s.a.kemal@cgiar.org*

Chickpea (*Cicer aritienum*) is one of the most important food legumes grown around the world in more than 50 countries. The yield gap of chickpea is high due to abiotic and biotic constraints where Ascochyta blight (*Didymella rabiei*) causes yield instability in different regions. High yielding chickpea varieties were developed and released in many countries; however some varieties were taken out of production due to susceptibility to new virulent pathotypes of the pathogen. Knowledge of the interactions of *Didymella-Cicer* with the environment has practical significance because the environment may alter the performance of cultivars and pathogen populations in the field. Little is known on how temperature may affect the virulence of different pathotypes of *D. rabiei*. The effects of temperature (10, 15, 20 and 25°C) on the virulence of four Pathotypes (P-I, P-II, P-III and P-IV) were studied using six chickpea genotypes (Ghab-1,Ghab-2, Ghab-3, Ghab-4, Ghab-5 and ICC-12004) with varying levels of blight resistance under controlled conditions. The results showed that the interactions of temperature with pathotypes as measured by virulence index were statistically significant but without changes in the ranking of the pathotype virulence on the host genotypes. The mean virulence index of the four pathotypes ranged from 3.3 in P-I to 7.2 in P-IV. P-4 showed high mean virulence index under all temperature regimes followed by P-3 compared with the other two. The least virulent P-1 among the tested pathotypes showed high average virulence index at 10°C on the test genotypes. Similar results were obtained for the chickpea genotypes where significant genotype-temperature interactions were detected but no changes in the ranking of resistance to Ascochyta blight. Except for Ghab-1, which is the most susceptible genotype, the remaining genotypes showed high disease severity at temperature 10 and 15°C. This study showed that *D. rabiei* population has developed pathotypes that can cause disease epidemics irrespective of temperature regimes in Syria and there is a need to further investigate the response of pathogen populations to temperature from similar Mediterranean environments.

Toxin production and DNA sequence analysis of Turkish isolates of *Ascochyta rabiei*. F.S. DOLAR
Ankara University, Faculty of Agriculture, Department of Plant Protection, Ankara, Turkey.
E-mail: dolar@agri.ankara.edu.tr

Chickpea is the most produced legume crop of Turkey. One of the greatest biotic stresses reducing potential yield in chickpea is ascochyta blight caused by *Ascochyta rabiei* (Pass.) Lab. It is known that many toxins are responsible for pathogenicity of fungus. *A.rabiei* synthesizes the four toxic compounds solanapyrone A, B, C and cytochalasin D. The symptoms caused by the solanapyrone, epinasty, chlorosis and necrosis, are consistent with the disease. The taxonomy of Ascochyta species has been based on morphology and host plant association. In some cases, morphology has been unsuccessful in characterising fungi. Recently, significant advances in fungal taxonomy and identification have come about through DNA analysis. The objective of this research was to determine production of toxin by Turkish isolates of *A. rabiei* and demonstrate DNA sequence of *A. rabiei* isolates which produced different amount of toxin or not. In order to determine of solanapyrone production of the twenty two isolates, the fungus was grown on Czapek Dox liquid culture medium (CDLCM) for 12 day. Quantitation of solanapyrones was determined with HPLC analyses. The results demonstrated that all of the isolates produced solanapyrone A in CDLCM at 20°C but not at 30°C. Confirmation of the identity of the pathogen was sought by sequence analysis of rDNA. These experiments showed that the sequences of the internal transcribed spacers and 5.8S gene of the seven Turkish isolates, which were identical to each other, were also identical to that of a Pakistan isolate of *A.rabiei*. Two interpretations of the perfect match of the DNA sequences of the Turkish and the Pakistan isolates are that either these regions are particularly conserved within in *A.rabiei* or that the Pakistan and Turkish isolates are of common origin. rDNA sequences of the PCR products of isolates of *A.rabiei* which were produced different amount toxin were same.

***Mycosphaerella pinodes* isolates morphological and pathogenic variation.** L. BOROS. *Institute of Plant Breeding and Acclimatization–PIB, Radzikow, Poland.*
E-mail: l.boros@ihar.edu.pl

Ascochyta blight caused by *Mycosphaerella pinodes* is a serious pea disease worldwide. Its common occurrence also in Polish condition was demonstrated in earlier studies. Many studies have shown pathogenic diversity among isolates *M. pinodes*. Knowledge of pathogenic diversity is important in choosing appropriate isolates to screen for resistance. Variability of 20 Polish isolates was investigated on the basis of cultural, morphological and pathogenicity. Significant variation was recorded among tested isolates for colony colour, linear growth, abundance of mycelium, abundance and size of pycnidia and size of pycnidiospores. Significant differences occurred between pea genotypes, pathogen isolates and their interaction for days from inoculation to symptoms appearance (DISA) and for disease severity on leaves and stems. Although genotype-isolate interactions were significant for disease severity, their contribution to total variation was very low comparing to that of isolates and genotypes respectively. The mean squares for isolates were higher than that for genotypes, indicating that there were more variation between *M. pinodes* isolates than between pea genotypes. All isolate produced symptoms but ranking of genotypes was similar irrespective to isolate. Obtained results do not provide clear evidence for existence of pathotypes among *M. pinodes* isolates. The isolates aggressive on one genotype were generally aggressive across all genotypes and conversely. Isolates grouping on the basis of their aggressiveness on host genotypes with cluster analysis revealed that some isolates pathogenically similar were isolated from different cultivars and from different location.

Using detached-leaf technique for assessment of pathogenicity of *Ascochyta fabae* Speg. isolates to faba bean (*Vicia faba* L.). T. GÓRAL*, D. WALENTYN-GÓRAL. *Department of Plant Pathology, Plant Breeding and Acclimatization Institute-National Research Institute, Radzików, 05-870 Blonie, Poland.*
**E-mail: t.goral@ihar.edu.pl*

Ascochyta blight caused by *Ascochyta fabae* is the most important faba bean disease in Poland. It is very destructive and often accompanied by chocolate spot, other important faba bean disease. Both diseases can be devastating and cause substatntial loses, especially in years with high rainfall, e.g 2009. Pathogenicity of *Ascochyta fabae* isolates to faba bean was assessed. Isolates were collected in Poland in the years 2008–2011 from infected leaves and seeds of faba bean. One isolate was derived from population used in research on faba bean resistance to Ascochyta blight in Radzików before 2004. Detached-leaf technique was used. Surface sterilized leaves of 7 varieties of faba bean ('Albus', 'Amulet', 'Kasztelan', 'Leo', 'Granit', 'Olga', 'Titus') were placed on the Petri dishes with a filter paper moistened with sterile water. Each leaflet was inoculated with a drop (50 μL) of *A. fabae* spore suspension at concentration of 10^5 spores mL^{-1}. The diameter of necrotic lesions was measured every 3–4 days. The most pathogenic were isolates AF4 and AF10-1 from 2009. The most susceptible were cultivars 'Albus' and 'Kasztelan'. Cultivar 'Granit' was the most resistant.

Pathogenicity of Australian *Ascochyta rabiei* isolates. V.L. ELLIOTT, P.W.J. TAYLOR and R. FORD*. [1]*Department of Agriculture and Food Systems, Melbourne School of Land and Environment, the University of Melbourne, Australia. *E-mail: rebeccaf@unimelb.edu.au*

Ascochyta blight is the most important foliar disease of chickpea worldwide. With the commercialisation of new resistant cultivars in Australia, durability of this resistance has become a priority. Previously, low genetic diversity has been found within the Australian *A. rabiei* population, however much less information is available on the pathogenic diversity. Forty two *A. rabiei* isolates, collected from chickpea growing regions throughout Australia, were screened over a set of 12 chickpea cultivars at the seedling stage, to increase the understanding of the pathogenicity within the Australian population. Principal components analysis of the area under the disease progress curve (AUDPC) found no evidence of discrete pathotypes, with the population displaying a continuum of pathogenicity. A detached leaf assay, using six of the same isolates, was completed at plant maturity, using the same 12 cultivars, to assess the effect of plant age on resistance. The ranking of mean AUDPC was very similar to the seedling screening test for the six isolates, suggesting that isolates that are highly pathogenic at the seedling stage are also highly pathogenic at plant maturity. The ranking of the mean AUDPC for cultivar was significantly different due to plant age suggesting that there may be different defence mechanisms of resistance occurring at different growth stages. These isolates can now be utilised in breeding programs to provide greater knowledge on the likely longevity of future cultivars.

Genetics and Breeding for resistance. Fast track development of ascochyta blight resistance in chickpea through marker-assisted backcrossing. B. TAR'AN*, T. WARKENTIN and A. VANDENBERG. *Crop Development Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A8.*
**E-mail: bunyamin.taran@usask.ca*

Ascochyta blight (AB) caused by the fungus *Ascochyta rabiei* is one of the major diseases of chickpea worldwide. The use of varieties with high levels of resistance is considered the most economical solution for long-term disease management. Resistance to AB is complex; many genes with minor to moderate effects control the resistance. Previous reports demonstrated a common set of AB resistance QTL distributed over several linkage groups (LG) in different crosses and genetic backgrounds. Although, each QTL appeared to explain small to medium amounts of the phenotypic variation in disease reaction, the effects appeared to be additive. Thus, it is essential to select for multiple genes to provide a sufficient level of resistance to AB in chickpea. The main goal of this research was to convert the adapted varieties, which otherwise susceptible to AB, within a 2–3 years time frame through a targeted marker-assisted backcrossing approach for the QTL associated with AB resistance. Simple sequence repeat (SSR) markers linked to the QTL for AB resistance and those unlinked to the resistance were used in foreground and background selection, respectively, in backcrosses between the adapted susceptible varieties (CDC-Xena, FLIP98-135C and CDC-Leader) and the moderately resistant donors (CDC-Frontier and CDC425-14). The strategy included two backcrosses and selection for three major QTL for AB resistance. By the BC_2 generation improved ascochyta blight resistant were recovered. The selected plants possessed the majority of recurrent parental type SSR alleles on all fragments analyzed, except the segment of the LG3, LG4, and LG8 that possessed the target QTL. The results showed that the adapted variety which otherwise susceptible to ascochyta blight could be effectively converted into an improved resistance variety in two backcross generations within a time frame of two to three years.

Mapping of *Ascochyta rabiei* resistance QTL in bi-parental chickpea populations. G.K. KISHORE[2], A.G. SHARPE[2], H.M. BOOKER[3], B. TAR'AN[3], E. MADRID[4,5], T. MILLAN[4], J. GIL[4], J. RUBIO[5] and L. BUCHWALDT[1*]. [1]*AAFC, 107 Science Place, Saskatoon, SK, Canada.* [2]*NRC-PBI, 110 Gymnasium Place, Saskatoon, SK, Canada.* [3]*CDC, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada.* [4]*Dpt of Genetics, Córdoba University, Campus Rabanales, Edif. C5, 14071 Córdoba, Spain.* [5]*Área de Mejora y Biotecnología, IFAPA-Alameda del Obispo, Apdo 3092, 14080 Córdoba, Spain. *E-mail: lone.buchwaldt@agr.gc.ca*

Our research aim at identifying QTL conferring resistance to *Ascochyta rabiei* in diverse chickpea (*Cicer*) lines that when combined in future cultivars by marker-assisted-selection (MAS) could improve field resistance. Bi-parental mapping populations of either F_2 or RIL were developed from crosses with a susceptible parent and each of resistant *C. arietinum* lines ICC4475, ICC6328, ICC3996, ICC4200 (desi), ILC72, ILC3279, ILC195, Amit (kabuli) and *C. reticulatum* PI489777. DNA of parents and progenies was genotyped with already published SSR markers and linkage maps were generated for each population using MapMaker. Progenies were phenotyped for ascochyta blight resistance by inoculating detached leaves with a conidial suspension of a single virulent *A. rabiei* isolate (3279a). The composite interval function in QTL Cartographer identified QTL (LOD 2.4-8.9) explaining 8–24% of resistance to *A. rabiei* and named Ar followed by the linkage group number and a letter. All QTL in *C. reticulatum* (Ar2b, Ar4b, Ar6a) also occurred in *C. arietinum*. Ar4b was common in re-

sistant germplasm, breeding lines and cultivars, while some QTL were present only in kabuli lines (Ar2a, Ar2c, Ar4a, Ar5a) and others only in desi lines (Ar1a, Ar3c, Ar7a). Selected F_2 populations are being advanced to F_7 for fine mapping using single nucleotide polymorphism markers needed for effective pyramiding of QTL with complementary effect on the pathogen.

Genetic enhancement of resistance to ascochyta blight in chickpea. P.M. GAUR[1*], S. PANDE[1], T. KHAN[2,3], S. TRIPATHI[1,4], M. SHARMA[1], L. KAUR[5], J.S. SANDHU[5,6], S. SINGH[5], A. BASANDRAI[7], D. BASANDRAI[7], A.K. JUKANTI[1], C.L.L. GOWDA[1] and K.H.M. SIDDIQUE[2]. *[1]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad 502 324, AP, India. [2]The UWA Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia.[3]Formerly at Department of Agriculture and Food Western Australia, 3 Baron-Hay Court, South Perth, WA 6151, Australia. [4]Presently at Indian Agricultural Research Institute, New Delhi 110 012, India. [5]Punjab Agricultural University, Ludhiana 141 004, Punjab, India. [6]Presently at Indian Council of Agricultural Research, New Delhi 110 001, India. [7]Hill Agricultural Research and Extension Centre of CSKHPKV, Dhaulakuan 173 001, HP, India. *E-mail: p.gaur@cgiar.org*

Chickpea (*Cicer arietinum* L.) suffers significant yield losses from ascochyta blight (AB), caused by *Ascochyta rabiei,* in areas where the crop growing season is cool and wet. Developing chickpea cultivars with high and stable resistance to AB has been challenging because the resistance is controlled by several major and minor genes and the pathogen is known to be highly variable. Concerted efforts were made to develop breeding lines with enhanced resistance to AB in an international project that involved partnership between ICRISAT and Universities/Research Institutions in Western Australia and Northern India. The breeding strategy included use of diverse sources of AB resistance and multiple crosses for enhancing level of AB resistance. Over 250 crosses were made involving several sources of AB resistance (GL 90135, ICC 3996, ICC 12004, ICC 12965, ICC 14917, ICC 12964, ICC 18965, ICCV 98502, ICCV 98503, ICCV 04512, ICCV 04516, ICCV 04538, ICCV 04539, ICCV 05529, ICCV 05530, PBG 5) and popular cultivars/elite lines from Western Australia (Sona, Sonali, Rupali, WACPE 2098, WACPE 2099, WACPE 2113, WACPE 2114, WACPE 2125, WACPE 2131, WACPE 2152, WACPE 2162) and India (ICCV 10, ICCC 37, JG 11, JAKI 9218, JG 130). Top AB resistant lines derived from different crosses were intercrossed to further accumulate AB resistance genes from a number of sources. F_4 populations from bi-parental and multi-parental crosses were screened for AB resistance at seedling stage under artificial epiphytotic conditions in controlled environment growth room at ICRISAT-Patancheru. The AB resistant plants were transplanted to greenhouse and their progenies (>13,000) were subjected to preliminary evaluation for phenology, plant type and seed traits. Over 5,000 progenies were selected and evaluated for adult plant resistance to AB in the field at Ludhiana in northern India. Over 2,600 progenies were evaluated for AB resistance and agronomic traits under field conditions in Western Australia. A large number of breeding lines that show high levels of AB resistance (score 2–3 on 1–9 scale) in Western Australia and northern India have been developed. Majority of these lines also have desirable agronomic and adaptation traits and good seed quality. Some of the AB resistant lines also combine resistance to botrytis grey mould and fusarium wilt.

QTL identification for Ascochyta blight resistance and its application in chickpea breeding. M. IMTIAZ[1*], A. HAMWIEH A.[1], S. AHMED[1], R. MALHOTRA[1], A. KHALIFEH[1], N.A. DOUBA[2] and S. SHEET[2]. *[1]International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. [2]Aleppo University, Aleppo, Syria. *E-mail: m.imtiaz@cgiar.org*

Ascochyta blight (AB), caused by *Didymella rabiei* regularly occurs in epidemic form causing heavy yield and quality losses, and thus is a major biotic constraint to chickpea production, particularly in the cool and wet areas. Various chemical and cultural practices have been reported to control the disease, however, their usage is neither eco-friendly nor economical where the cultivated varieties possess low level of resistance. Therefore, development and deployment of resistant cultivars is the most viable alternative to overcome the impact of disease particularly in winter planted chickpea in West Asia and North Africa. ICARDA, being the hub for AB research, has made tremendous progress in the development of AB resistant germplasm through phenotypic selection, but significant difficulties are often encountered in phenotypic selection such as genotype by environment interactions, and expensive and unreliable screening methodologies. Therefore, to increase genetic gain and generate comprehensive knowledge, the chickpea breeding program at ICARDA started to map QTLs for AB resistance in different genetic backgrounds and use marker-assisted selection in early generations (F_2) as proof of concept. Recombinant inbred (RI) populations viz. FLIP98-1065 X ILC3279, FLIP98-1065 X ILC482, FLIP98-1065 X ILC1929, ILC3279 X ILC482, ILC3279 X ILC1929, and ILC482 X ILC1929 were developed to map and validate SSR markers associated with different putative sources of resistance in these populations. RI population derived from FLIP97-1065C x ILC1929 was phenotyped for three years and genotyped with 110 SSR markers. SSR markers GA-16, H5H-02, TA-194 and H1A-10b were found linked with

resistance under field and control conditions with phenotypic variation explained ranging from 10 to 20%. These markers and other publically reported markers were validated in the remaining 5 RI populations. The marker validation results showed that none of the markers alone differentiated resistance sources against different pathotypes in these populations. Thus a set of closely linked markers could be utilized to select for AB resistant genotypes until the availability of diagnostic markers for maker- assisted selection in future.

Validation of allele specific markers for detection of ascochyta blight resistance loci in chickpea. E. MADRID[1,4*], W. CHEN[2], P.N. RAJESH[2, 3], J. RUBIO[4], P. CASTRO[4], T. MILLAN[1] and J. GIL[1]. [1]*Dept of Genetic, Córdoba University, Campus Rabanales, Edif. C5, 14071 Córdoba, Spain.* [2]*Grain Legume Genetics and Physiology Research Unit, USDA-ARS, Washington State University, Pullman, WA 99164, USA.* [3]*Trait Genetics and Technologies, Dow AgroSciences, Indianapolis, Indiana, 46268, USA.* [4]Área de Mejora y Biotecnología, IFAPA Centro "Alameda del Obispo", Apdo 3092, 14080 Córdoba, Spain.
**E-mail: b62mahee@uco.es*

Although various candidate genes have been hypothesized to be responsible for blight resistance in chickpea, none have previously been found to be associated with QTLs for resistance to this disease so far. Recently a chickpea ethylene receptor-like sequence (*CaETR-1*), homologue to the *Arabidopsis thaliana* ethylene insensitive 4 gene (*EIN4*), has been reported to be associated with resistance and susceptibility to blight. The *CaETR-1a/b* locus was mapped in LGIV tightly linked to QTL_{AR1}, explaining up to 33.8% of the total phenotypic variation. Using this information an allele-specific associated primer (ASAP) was designed. With the aim of monitor the presence of blight resistance associated QTLs to avoid escapes and ambiguity in phenotype evaluation, more than 50 genotypes, including collection of landraces, cultivars, advanced breeding lines and wild relatives, were genotyped using the ASAP primer and the previously described SCAR marker $SCY17_{590}$, tightly linked to QTL_{AR2}. According with our results, in very few cases resistant genotypes was incorrectly predicted. Therefore, chickpea breeders could confidently use the markers to carry out selection of germplasm with high levels of resistance to blight in their breeding programmes, discarding genotypes with the susceptible allele for both QTLs.

Association Genetics Mapping of Referred QTLs Markers conferring resistant to ascochyta blight, earliness and seed size in chickpea. M. MARWAN[1*], B. TARAN[1], A. SHARPE[2], T. WARKENTIN[1] and A. VANDENBERG[1]. [1]*Crop Development Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A8.* [2]*National Research Council-Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada.*
**E-mail: m.marwan@usask.ca*

Understanding genetic diversity and population structure in target populations is a prerequisite for association mapping. The current study used association mapping approach to identify molecular markers associated with resistance to ascochyta blight, earliness and seed size, three of important characteristics in chickpea breeding. A total of 136 genome-wide simple sequence repeat (SSR) markers were used to assess the genetic diversity and population structure of a set of 94 chickpea genotypes including cultivars, elite breeding lines and their progenitors and several landraces. These genetic materials have been used in chickpea breeding program at the University of Saskatchewan in the past 20 years. Analysis of population structure in combination with pedigree and breeding history grouped the chickpea genotypes into six subpopulations. Two subpopulations consisted of genotypes of different origins used mainly as sources for ascochyta blight resistance. The next three subpopulations were mostly genotypes used as sources for earliness. The last subpopulation consisted of genotypes used to improve seed quality. The association analysis using population admixture approach identified two new SSR markers associated with ascochyta blight resistance: H4G11 ($P<0.001$) and TR20 ($P<0.05$) markers accounted for 47% and 22%, respectively, of the variations for reaction to ascochyta blight. The current analysis also confirmed the association of previously mapped SSR markers such as TA2, TA8, TS19 and TA144 (1, 2) with ascochyta blight resistance. In addition, we identified two SSRs (TA2 and GA20) associated with seed size and another two (H3C11 and TA25) associated with earliness, which have not been detected in previous QTL studies. The results suggested that the current population may be useful for genome-wide marker–trait association study that has enabled the identification of new QTL for ascochyta blight resistance, earliness and seed size, three of most important characteristics in chickpea.

Combining Ascochyta blight resistance and high yield in lentil cultivars. S. SINGH[1*], S. KUMAR[2], R.K. GILL[1], L. KAUR[1], S. AHMED[2], J. KUMAR[3] and A. SARKER[2]. [1]*Department of Plant Breeding & Genetics, Punjab Agricultural University, Ludhiana 141004, India.* [2]*International Center for Agricultural Research in the Dry Areas, Aleppo, Syria.* [3]*Indian Institute of Pulses Research, Kanpur, India.*
**E-mail: sarvjeetm@rediffmail.com*

Ascochyta blight (AB), caused by *Ascochyta lentis* Bond and Vassil, is a major disease of lentil (*Lens culinaris* L. Medik.) causing 30–70% yield losses in Canada, USA, and Australia and northern parts of India. The aim of the present study was to combine AB resistance with

high yielding potential. The F_3 and F_4 populations from crosses involving AB resistant donors with ICARDA elite breeding lines, were advanced at Punjab Agricultural University, Ludhiana, India, respectively during main and off-seasons. The single plant F_5 progenies were exposed to AB infections under field conditions during 2006–2007 using disease rating scale of 1-9. Thirty two promising lines, bulked in F_7 generation, were evaluated for seed yield in multi-location trials (2009–2010 and 2010–2011) and for their reactions to AB under artificial inoculations along with susceptible check, LL147 (8 score). Six elite lines namely LL1187 (1.70 t ha^{-1}), LL1210 (1.75 t ha^{-1}), LL1197 (1.76 t ha^{-1}), LL1186 (1.76 t ha^{-1}), LL1201 (1.80 t ha^{-1}) and LL1205 (1.80 t ha^{-1}) recorded 9.6–16.1% yield advantage over the best check variety LL931 (1.55 t ha^{-1}). The level of resistance (≤ 4 score) of these lines to AB was also higher compared to check variety LL931 (5 score). Inheritance of AB resistance is simple and controlled by major genes; hence it is easy to incorporate stable resistance in high yielding background by selecting parents carefully for hybridization program.

Breeding faba bean for resistance to Ascochyta blight. F. MAALOUF[1*], S. AHMED[1], S. KHALIL[2] and B. BAYAA[3]. [1]*International Center for Agricultural Research in the Dry Areas (ICARDA), P.O Box 5466, Aleppo, Syria.* [2]*Field Crop Research Institute, Agriculture Research Center, Giza, Cairo, Egypt.* [3]*Faculty of Agriculture, Aleppo University, Aleppo Syria. *E-mail: F.maalouf@cgiar.org*

Ascochyta blight (*Didymella fabae* Jellis and *Punithalingam*) is one of the most important foliar diseases that affect the quality and seed yield of faba bean (*Vicia faba* L.) worldwide. Breeding for resistance to Ascochyta blight is a major objective in many faba bean breeding programs. At ICARDA, systematic screening of germplasm accessions and elite breeding lines for resistance to Ascochyta blight is carried out at Tel Hadya Aleppo and Lattakia under artificial field inoculation with mixtures of aggressive isolates of the pathogen, since 1977. This has resulted in the identification of 120 resistance sources with origins mainly from Ecuador, Egypt, Ethiopia, Canada China, Greece, Italy, Lebanon, Morocco, Peru, Spain, Syria and Tunisia. The resistance sources were inter-crossed with landraces adapted to diverse environments. More than 2000 breeding lines (F_4 to F_7 generations) emanating from the crosses involving resistant donors with landraces are screened for their reaction to Ascochyta blight on yearly basis. Only the identified resistant lines in advanced generations are tested under natural infestation in different locations under platform of international nurseries. From 2008 to 2011, 65 lines were tested in different locations in North Africa and west Asia. 28 lines maintained their resistance to ascochyta blight in all locations.

Allele diversity analysis for improvement of mycosphaerella blight resistance in pea. A.B. JHA*, G.C. ARGANOSA, B. TAR'AN and T.D. WARKENTIN. *Crop Development Centre/Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada.*
**E-mail: ambuj.jha@usask.ca*

The narrow available gene pool has slowed the development of pea cultivars with improved disease resistance. The objective of this study was to identify single nucleotide polymorphisms (SNPs) within the candidate genes associated with resistance to mycosphaerella blight as well as intrinsic seed compositions such as carbohydrate metabolism and protein deposition that can be used to aid selection in breeding program. A total of 169 diverse *Pisum sativum* accessions from eastern Europe, western Europe, Australia and Canada including two check cultivars, 'CDC Striker' (fair resistance) and 'Alfetta' (poor resistance) were initially screened for mycosphaerella blight resistance at mid-flowering and late pod filling stages. From the initial screening, 56 accessions with relatively higher resistance were selected. Additional four wild accessions of *P. sativum* ssp. *elatius* and *P. fulvum* with promising resistance were included for sequence analysis. Primer pairs of candidate genes associated with mycosphaerella blight resistance, carbohydrate metabolism and protein deposition were used to amplify DNA fragments from these accessions. Overall, 228 SNPs were detected within these candidate genes. Nine SNP loci were significantly associated with mycosphaerella blight score. Marker loci SS-273 and RGA-G3A-103 were significantly associated ($P \leq 0.01$) with mycosphaerella blight score at mid-flowering stage. At late pod filling stage, the association was significant ($P \leq 0.01$) for marker loci Gpt-116, Gpt-164, Agpl1-29 and highly significant ($P \leq 0.001$) for Agpl1-238, Gbsts1-137, Gbsts1-208 and Gbsts1-250. These marker loci explained 7 to 17% of the variation. The markers developed in this study have the potential to aid selection for development of pea cultivars with improved disease resistance.

Improving mycosphaerella blight resistance in pea. T.D. WARKENTIN[1*], Y. LIU[1], A. JHA[1], B. TAR'AN[1], A. SINDHU[1], M. MARWAN[1], A. SHARPE[2] and S. BANNIZA[1]. [1]*Crop Development Centre/Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada.* [2]*Plant Biotechnology Institute, National Research Council, Saskatoon, Canada. *E-mail: tom.warkentin@usask.ca*

Mycosphaerella blight is the most important pea disease in Canada, thus breeding for resistance is a major objective. Lack of strong resistance sources and the quantitative nature of resistance have resulted in slow breeding progress, however, cultivar ratings for resistance have generally improved from 'poor' to 'fair'

between 1995 and 2012. Most current cultivars have mycosphaerella blight resistance similar to cv. Radley which was identified by as one among those with the greatest resistance in an extensive screening study. Breeding for resistance is primarily conducted in an indirect manner by annually exposing a large and diverse set of populations to the native pathogen population in field trials conducted in a rotation that promotes moderate disease severity. Advanced lines are systematically rated for resistance twice per season at the local nursery location. QTLs associated with resistance have been identified by several researchers. A current study based on a recombinant inbred line population derived from Carrera/CDC Striker has identified a potentially new QTL on LGIII, explaining 8–20% of the variation. This population, as well as a population derived from Orb/CDC Striker and an association mapping panel consisting of 94 diverse cultivars and breeding lines will be genotyped using a 1536 single nucleotide polymorphism assay in 2012. Together with multi-location phenotyping the potential exists for detection of more robust QTLs. We have developed populations based on 4 wild pea accessions displaying improved resistance to western Canadian isolates of *M. pinodes* to determine the inheritance of resistance, as well as mapping and pyramiding resistance. *Ascochyta pisi* has secondary importance in the ascochyta complex of pea in western Canada. We have initiated studies to characterize germplasm for resistance to *A. pisi*.

Investigation of the resistance to Mycosphaerella blight of pea germplasm lines PI404221 and PI413691. D. BING[1*] and R. BOWNESS[2]. [1]*Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C and E Trail, Lacombe AB. Canada T41 1W1.* [2]*Alberta Agriculture and Rural Development, 6000 C and E Trail, Lacombe AB. Canada T41 1W1.* **E-mail: Dengjin.bing@agr.gc.ca*

Mycosphaerella blight (caused by *Mycosphaerella pinodes* Berk. & Blox. Vestergr.) is the most prevalent disease of field pea in Canada. Yield losses of more than 30% have been reported. No strong resistance to this disease has been identified in the *Pisum* germplasm pool despite extensive screening and investigations. Therefore, searching for strong resistance to this pathogen is a research priority of field pea breeding. At the Ascochyta 2009 workshop, two germplasm lines, PI404221 and PI413691, were reported to have a high level of resistance to Mycosphaerella blight under South American conditions, where the warm temperature and high humidity were more favorable for Mycosphaerella blight epidemics of pea than most other pea growing regions in the world. We were very interested in the resistance level of PI404221 and PI413691 under Central Alberta growing conditions. Dr. Clarice Coyne in Pullman, WA, USA provided the germplasm lines and we grew them in replicated field tests at Lacombe, AB, a field pea production area in Canada, in 2010 and 2011. Commercial field varieties and elite breeding material were grown for comparison. The plots were not inoculated in 2010, but in 2011 Mycosphaerella blight infected pea straw, harvested from the previous year, was spread evenly across the plots. The studies in both years showed that PI404221 and PI413691 were, indeed, more vigorous in growth, and had more green leaves than the other varieties and breeding lines. Unfortunately, both were highly susceptible to Mycosphaerella blight, similar to the other field pea varieties and breeding lines. Results indicated that the more green leaves of PI404221 and PI413691 were simply the effect of their taller plants and later maturity. Therefore, it is important to evaluate Mycosphaerella blight severity at the same plant growth stage or level of maturity. Such a rating system should be established and adapted by all researchers in this area. It remains a priority of field pea breeding to identify high level and usable resistance to Mycosphaerella blight.

Marker assisted selection for pathotype I of Ascochyta blight in chickpea. P. CASTRO[1], J. RUBIO[1], E. MADRID[2], T. MILLAN[2] and J. GIL[2*]. [1]*Área de Mejora y Biotecnología, IFAPA, Córdoba, Spain.* [2]*Dpto. de Genética, University of Córdoba, Córdoba, Spain.*
**E-mail: ge1gilij@uco.es*

Ascochyta blight is one of the most devastating diseases of chickpea. Breeding for Ascochyta blight resistance is one of the aims in most of the chickpea breeding programs. Marker assisted selection (MAS) would greatly accelerate the development of new cultivars. In a previous study carried out in our group, 600 F_2 plants derived from the cross ILC3279 × WR315, resistant and susceptible to blight respectively, were used for MAS and phenotypic selection. This population was evaluated in the field and genotyped with markers linked to QTLs for blight resistance (STMS GAA47 linked to QTL_{AR1}, STMS TA72 and the SCAR SCY17 to QTL_{AR2} and the STMS TA194, TS82 and TR58 to QTL_{AR3}), showing that markers linked to QTL_{AR3} were not associated to resistance. QTL_{AR3} has been reported to be associated with resistance to pathotype I. In order to study if the lack of association could be related to the presence of other pathotypes in the field, 58 $F_{2:3}$ families selected as resistant during 2006–2007 were evaluated under controlled conditions with pathotype I (AR19) using a 1 to 9 rating scale. There were significant differences among families (P<0.001), indicating genotypic variation to pathotype I. 41 out of the 58 families were resistant, with values lower than 3. Markers linked to QTL_{AR3} and QTL_{AR1} were significantly associated with disease reaction to pathotype I. However, markers linked to QTL_{AR2} were not associated. Marker TA194 explained

the highest percentage of the total phenotypic variation (R^2=29.6%) followed by GAA47 (9.5%). Interaction analysis between GAA47 and TA194 showed that resistance to pathotype I could be conferred by either QTL_{AR1} or QTL_{AR3}. Therefore QTL_{AR1} is very important because it could be involved in the resistance to different pathotypes. Recently, a marker (CaETR) linked to QTL_{AR1} has been developed. This marker was genotyped in F_2 population explaining 31% of the total phenotypic variation. Therefore GAA47, CaETR and TA194 could be reliable markers to predict resistant phenotype and they would be useful in MAS for Ascochyta blight.
This research was supported by the Spanish Ministerio de Ciencia e Innovación (MICINN; project RTA2010-00059), co-financed by Fondo Europeo de Desarrollo Regional (FEDER).

Improving resistance to *Mycosphaerella pinodes* in field pea by utilizing wild pea germplasm. A.B. JHA*, T.D. WARKENTIN, V. GURUSAMY, B. TAR'AN and S. BANNIZA. *Crop Development Centre/Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada. *E-mail: ambuj.jha@usask.ca*

Mycosphaerella blight caused by *Mycosphaerella pinodes* is the most important disease of pea (*Pisum sativum*). Thus far, high levels of resistance to mycosphaerella blight have not been identified in field pea; however, some studies have shown higher levels of resistance in *P. fulvum*, a wild relative of field pea. The objective of this study was to identify mycosphaerella blight resistant wild pea accessions for use in introgression breeding in Canada. Forty-four *P. fulvum* accessions, and several accessions from sub-species *elatius, transcaucasicum*, *asiaticum*, *arvense*, and *abyssinicum* obtained from USDA, Pullman, WA, and IFAPA, Spain were evaluated for reaction to mycosphaerella blight, along with check cultivars, Radley and CDC Striker (fair resistance) and Alfetta (poor resistance). Based on greenhouse and field experiments, 4 wild accessions namely, PI 344538 (*P. sativum* ssp. *elatius*), PI 560061 (*P. fulvum*), W6 15017 (*P. fulvum*) and P 651 (*P. fulvum*) were considered the most promising sources for resistance breeding. These lines were then used in crossings with the susceptible check cultivar Alfetta. Progeny from successful crosses are currently in the F_5 generation. Recombinant inbred lines derived from these populations will be evaluated for mycosphaerella blight resistance and for quantitative trait locus (QTL) analysis to identify markers for resistance. Selected accessions are being evaluated to determine the inheritance of resistance. This study thus far has identified promising wild pea accessions for potential use in the breeding program to improve mycosphaerella blight resistance.

Candidate genes, translational genomics and mapping approaches to identify *Ascochyta, Mycosphaerella* and *Fusarium* resistance QTLs/genes. P. SMÝKAL[1,3*], C.J. COYNE[2]. [1]*Agritec Plant Research Ltd., Biotechnology Department, Zemědělská 2520/16, 787 01 Šumperk, Czech Republic.* [2]*USDA-Agricultural Research Service, Washington State University, Pullman, 59 Johnson Hall, WA, USA.* [3]*Department of Botany, Faculty of Science, Palacký University, Šlechtitelu 11, 783 71 Olomouc, Czech Republic. *E-mail: petr.smykal@upol.cz and smykal@agritec.cz*

Ascochyta blights are the most important foliar diseases of cool season food legumes and are found in nearly all production regions. The ascochyta blight complex of pea involves three pathogens: *Ascochyta pisi, Mycosphaerella pinodes* and *Phoma medicaginis var. pinodella.* Similarly Fusarium root rot, caused by soil-borne pathogen complex species *Fusarium solani* (teleomorf: *Nectria haematococca*) affects many agricultural crops, including legumes. Crop rotation is the only mean of maintaining safe levels of inoculum. Some control can be achieved with fungicides but the use of resistant cultivars of plants is the preferred approach. We have used translational genomics and candidate gene approaches to identify QTLs for Fusarium root rot resistance on DSP × 90_2131 RILs. We mapped the cluster of highly homologoues disease response pI39 (DRR230A, B, C) genes to *FRR2* region. These genes, members of defensin family, map to *Asc3.1* locus confering pea resistance to *Ascochyta pisi* and also to *mpIII-4* QTL responsible for 29% of resistance to *Mycosphaerella.* It has 87% identity on protein level to *Medicago MtDef2.1* on chromosome 2, shown to have antifungal activities. Moreover, its expression is induced by *A. pisi, M. pinodes* or *F. solani* infections and differentially expressed in contrasting genotypes. Another candidate DRR206C (dirigent protein family involved in lignin biosynthesis pathway) we mapped to LGVII region of *Asc*7.1 QTL. Similarly, we refined the position of *FRR1* on LG II and *FFR3* on LG VI by *Medicago,* pea syntenic markers which enable the use of *Medicago truncatula* genome knowledge to identify potential candidates in syntenic region and/or offer these markers as probes for BAC library screening. The progress on further mapping will be presented and the relationship in resistance response to ascochyta blight and fusarium root rot will be discussed.

The work was supported by KONTAKT ME10006 project.

Response of faba bean varieties to different *Ascochyta fabae* isolates. M.M. ROJAS-MOLINA, A.M. TORRES and J.C. SILLERO*. [1]*Área de Mejora y Biotecnología, IFAPA-Alameda del Obispo, Apdo 3092, 14080 Córdoba, Spain. *E-mail: josefinac.sillero@juntadeandalucia.es*

Ascochyta blight caused by *Ascochyta fabae* Speg. is a common disease on faba bean (*Vicia faba* L.) and is dis-

tributed world-wide. Symptoms caused by *A. fabae* occur on leaves, stems and pods of infected plants. The firm definition of true races of *A. fabae* is still controversial and only few varieties with incomplete resistance have been registered in recent times. The purpose of the present work was to determine the response of three commercial varieties against different isolates of *A. fabae*. Fifteen-days-old faba bean seedlings of three commercial varieties (Baraca, Histal and Erika) and the resistant lines L-831818 and 29-H were studied under growth chamber conditions. Four *A. fabae* isolates with different geographical origins (three Spanish, from Córdoba, Santa Susana and Cabrera, and one from France) were used. Fifteen days after inoculation, Infection Type (IT) was scored according to the 0 to 9 scale where values 0–3 are considered resistant and 6–9 susceptible. Disease Severity was also estimated separately in leaves (DSL) and stem (DSS). Line L-831818 was the most resistant one, displaying immune response (IT=0) against all the isolates. 29-H showed immune response with the isolate from Cabrera and moderately resistant reaction with the other isolates, displaying well-formed lesions (IT=4), but in a very low amount (DSL<1%). All varieties displayed susceptible response against all the ascochyta blight isolates. Baraca was the less susceptible, allowing the development of lesions in the stem but no stems constrictions (IT=6). Varieties Erika and Histal were highly susceptible to ascochyta blight infections, with 30% of their leaves area covered with well-developed lesions and even defoliation. The isolate from France seemed to be the least aggressive one, as DSS values were the lowest, and no stem constriction was observed in any line. Due to the complexity of the resistance and the lack of good sources of resistance available, line L-831818 appears as a good source of *A. fabae* resistance accessible for faba bean breeding. New approaches for a more efficient targeted marker development are being followed by our group to introduce the L-831818 resistance to these varieties. Gene expression analysis of the faba bean-*Ascochyta fabae* by SuperSage are being combined with comparative mapping and QTL analysis. These approaches will uncover candidate genes to refine the position of QTLs controlling the resistance and thus facilitating the development of markers highly efficient in resistance breeding programs.

The saturation of genetic maps Carneval x MP1401 and P665 × Messire with morphological, isozymic and sequence-defined markers. M. GAWŁOWSKA*, M. KNOPKIEWICZ and W. ŚWIĘCICKI. *Institute of Plant Genetics PAS, Strzeszyńska 34, 60-479 Poznań, Poland.*
**E-mail: mgaw@igr.poznan.pl, mkno@igr.poznan.pl*

Saturated genetic maps are necessary for QTL analyses, comparison of their localization in different genetic backgrounds and identification of molecular markers linked to chosen genomic regions. Today, localization of quantitative trait loci (QTL) has become a standard approach for finding genomic regions responsible for agriculturally important traits. Tar'an *et al*. (2003) conducted the experiment to identify loci responsible for lodging resistance, plant height and resistance to mycosphaerella blight. Fondevilla *et al*. (2008) detected several QTLs associated with resistance to *M. pinodes*. However, a precise comparison of these locations was not possible because of a lack of common markers in these maps. Our aim was a supplementation of the both maps by new sequence-defined markers or other markers related to common *Pisum* map. The sequence-defined markers came from the Grain Legumes Integrated Project. 131 STS markers were used to test parental lines Carneval and MP1401. Single nucleotide polymorphisms were found in 10 PCR products. Five sequence-defined markers were studied in the Carneval × MP1401 population. Six morphological and isozymic markers were analysed in the P665 x Messire population. The localization of sequence-defined markers can be compared in the genetic map of *Medicago truncatula* and narrow-leafed lupin (*Lupinus angustifolius*).

Disease assessment and management

Management of ascochyta blight in field pea related to spore release patterns. J.A. DAVIDSON[1,2,*], C.J. WILMSHURST[1], E.S. SCOTT[2] and M.U. SALAM[3]. *[1]South Australian Research and Development Institute (SARDI), Adelaide, Australia. [2]School of Agriculture, Food and Wine, University of Adelaide, Glen Osmond, Australia. [3]Department of Agriculture and Food Western Australia (DAFWA), Perth, Australia.*
**E-mail: davidson.jenny@saugov.sa.gov.au*

Ascochyta blight of field pea, a disease complex caused by *Didymella pinodes*, *Phoma medicaginis* var. *pinodella*, *Phoma koolunga* and *Didymella pisi*, is controlled through manipulating sowing dates to avoid *D. pinodes* ascospores and by strategic fungicides. A forecasting system for ascospore release (G1Blackspot-Manager), based on daily temperature and rainfall, was developed to identify sowing dates that minimise ascochyta blight risk. The forecasting system was validated for South Australia by incubating field pea stubble naturally infested with ascochyta blight in the field, and periodically counting ascospores released from the stubble captured on sticky tape in a wind tunnel. Forecasts from G1Blackspot-Manager are available on DAFWA website prior to the sowing period. Secondary inoculum in field pea disease management trials was estimated using field pea seedlings as trap plants in canopies originating from three sowing dates and external to field pea canopies, over three seasons. Lin-

ear regressions between secondary inoculum and rainfall or disease severity were significant ($P<0.001$); the interaction between rainfall and disease severity was not significant. Timing of foliar fungicide applications was compared with peaks of secondary inoculum; only eight of seventeen sprays were applied immediately before spore dispersal.

Integrated management of Ascochyta blight (*Didymella fabae*) on faba bean in northern Syria. S. AHMED[1,*], M.M. ABANG[2] and F. MAALOUF[1]. [1]*International Center for Agricultural Research in the Dry Areas, Aleppo, Syria.* [2]*International Center for Tropical Agriculture (CIAT) I˙c/o Kawanda Agricultural Research Institute, 13 Km Bombo Road P.O. Box 6247, Kampala, Uganda.*
**E-mail: s.a.kemal@cgiar.org*

Faba bean (*Vicia faba*) is one of the most important food legume crops in West Asia and North Africa, East Africa and China. The average yield loss due to Ascochyta blight (*Didymella fabae*) is estimated to be 15–35%. The disease spreads most rapidly in the early spring before flowering starts. Although resistance breeding is key to tackle Ascochyta blight, the integration of different disease management options can even better manage the disease by delaying the appearance of more aggressive pathogen pathotypes. Integrated disease management options (sowing dates, fungicides and faba bean genotypes) were evaluated for three cropping seasons (2006–2009) for their effects on disease parameters and seed yield at ICARDA Tel Hadya Research Station in northern Syria. Significant differences ($P\leq0.05$) were observed between sowing dates in all seasons and among genotypes in 2006/2007 and 2008/2009 cropping seasons, where mean disease severity was highest on early-planted faba bean genotypes. Fungicides significantly ($P\leq0.05$) reduced disease severity in the first two seasons, with the best protection observed with chlorothalonil and azoxystrobin. Significant differences ($P\leq0.05$) were observed among genotypes in their yielding ability in the first two seasons. No significant interaction effects among treatments were observed for disease severity, percent seed infection and yield. However, significant sowing date by genotype and sowing date by fungicide interactions were observed for rate of disease development. Averaged over the three seasons, single applications of azoxystrobin and chlorothalonil were effective in reducing disease severity, rate of disease development and increase yield. In conclusion, the traditional early November planting date and a single application of chlorothalonil or azoxystrobin significantly reduced disease severity and percent seed infection and increased yield, and can be used under northern Syria conditions by famers and seed growers, where the epidemiological period for disease development is short.

Impact of foliar fungal pathogens on pulse crops in the US Northern Plains region. K.E. MCPHEE[1*] and M. WUNSCH[2]. [1]*Department of Plant Sciences, North Dakota State University, Fargo, ND, USA.* [2]*Carrington Research Extension Center, North Dakota State University, Carrington, ND, USA.* [*]*E-mail: kevin.mcphee@ndsu.edu*

Foliar pathogens cause significant disease in pea, lentil and chickpea in the US Northern Plains region. Research trials established within North Dakota for agronomic adaptation and yield evaluation served as an indirect source of disease ratings due to natural infection. Several pathogens affect pea and lentil, while *Ascochyta rabiei* was the primary pathogen affecting chickpea. *Mycosphaerella pinodes* was the primary foliar pathogen of pea, while incidence of *Ascochyta lentis*, *Colletotrichum truncatum*, *Stemphylium botryosum*, and *Botrytis cinerea* were noted on lentil in 2011 field trials. Pea, lentil and chickpea production in the US Northern Plains accounts for greater than 80% of all US production; therefore, identification of resistant germplasm and development of improved varieties is a high priority of the NDSU Pulse Crop Breeding program. Breeding lines and check cultivars for all three crops demonstrated significant variation for resistance to each of the pathogens indicating genetic improvement through breeding will be possible. Resistance to Ascochyta blight of chickpea has the greatest priority in the breeding program and requires careful management of natural infection in field trials. Fungicide applications are necessary to manage the disease to the benefit of the breeding program. Field trials at four NDSU Research Extension Centers in 2010 and 2011 demonstrated the value of both sprayed and unsprayed treatments for identification of resistant germplasm. Additional screening and development of disease management practices will benefit growers in the region and the NDSU Pulse Crop Breeding program.

Ascochyta blight of field pea caused by *Ascochyta pisi* in the Czech Republic. R._DOSTALOVA*, E. ONDRACKOVA and M. ONDREJ. *Agritec Plant Research Ltd., Sumperk, Czech Republic.*
**E-mail: dostalova@agritec.cz*

The occurrence of Ascochyta blight on harvested seeds of field pea has been evaluated in the Czech Republic (CR) from 50th the last century. Changes in varietal composition, susceptibility of cultivated pea varieties to viral diseases (PEMV) and changing weather conditions, they all affect the frequency of Ascochyta blights occurence on field peas. Until 1966 *Ascochyta pisi* predominantly occurred on harvested seed of field pea. Since 1966 highly productive and short stature pea cultivars replaced previously grown high stature cultivars with low yield. However, the new cultivars were very

susceptible to *Mycosphaerella pinodes* and *Phoma pinodella* complex. *A. pisi* has stopped occuring in harvested pea seed samples. Until the late 70 years the occurrence of *M. pinodes* dominated. The gradual introduction of semi-leafless types of pea in practice resulted in decreasing occurrence of *M. pinodes* and *P. pinodella* complex while an occurrence of *A. pisi* increased again. In 2007 both, *A. pisi* and complex *M. pinodes* together with *P. pinodella* attacked 56 and 44% of pea cultivars respectively, grown in the CR. Progressive return of *A. pisi* to pea growing areas in the CR was also observed in other countries. In field trials in 2007 and 2008 pea growths were severely attacked with PEMV at the locality Sumperk. A very close relationship between intensity of PEMV infestation and Ascochyta blight was found. The inoculation tests with PEMV confirmed reduction of plant immunity. Pea genotypes resistant to PEMV were attacked with *A. pisi* up to 3% compared to susceptible genotypes which the harvested seeds were attacked up to 24–36% in. Reductions in the yield and TWS of susceptible genotypes were 4 to 5 times higher compared to resistant genotypes. An effective protection against *A. pisi* is difficult. Treatment of infected seed with fungicides or biological products has little or partial efficiency only. An introduction of pea cultivars with higher resistance against *A. pisi* and full resistance against PEMV can be asserted in plant protection. Intercropping pea with cereals can also reduce Ascochyta blight on pea.

Host-Pathogen interactions-Screening techniques

Cell wall-associated apyrase, a key player for conditioning susceptibility/resistance in plant-pathogen interactions. K. TOYODA*, Y. SHIOBARA, H. NAGAI, E. KAWAKAMI, M. AMANO, K. TANAKA, Y. INAGAKI, Y. ICHINOSE and T. SHIRAISHI. *Graduate School of Natural Science and Technology, Okayama University, Japan. *E-mail: pisatin@cc.okayama-u.ac.jp*

Apyrases (EC3.6.1.15) are enzymes that efficiently hydrolyze ATP and ADP and function intracellularly and extracellularly. In plants, genes encoding an apyrase (NTP/NDPase, NTP diphosphohydrolase) are known to comprise a multigene family. Our recent studies have shown that an extracellular apyrase (PsAPY1) from pea binds and strictly responds to pathogen-derived molecules such as an elicitor and a suppressor from a pea pathogen, *Mycosphaerella pinodes*. The elicitor enhances the ATP-hydrolyzing activity in cell walls of all plants tested, but the suppressor inhibits the activity in a strictly species-specific manner. To assess the roles of apyrases in plant-pathogen interactions, we elaborated to screen the apyrase genes from a model legume *Medicago truncatula*. DNA sequencing and the subsequent phylogenetic analysis showed that *M. truncatula* contained at least seven apyrase genes, five of which (*MtAPY1;1*, *MtAPY1;2*, *MtAPY1;3*, *MtAPY1;4* and *MtAPY1;5*) are members of a legume-specific family, whereas two genes (*MtAPY2* and the newly identified *MtAPY2;2*) are close to those of non-leguminous plants. Using an *Agrobacterium*-based *in vivo* transient expression in *Nicotiana benthamiana*, combined with a triple c-myc epitope tag technology to define the cellular location, the MtAPY1;1 was found to be a secreted protein. Indeed, the MtAPY1;1 without the putative *N*-terminal signal sequence resided intracellularly. Transient expression of *MtAPY1;1* in *N. benthamiana* leaves restricted symptom development caused by a virulent pathogen, *Colletotrichum orbiculare*. In our separate study, we have shown that an extracellular apyrase likely links with the peroxidase-catalyzed superoxide generation through the hydrolysis of adenine nucleotides (see a Tanaka *et al.*, this issue). Given the roles of apyrase in recognition and modulation for the cell wall-based defenses; i.e. production of superoxides, it is no wonder that some pathogens have evolved mechanisms to target apyrase activity to condition susceptibility of the host cells.

Characterization of the chitin-binding PR4a gene from lentil and implications for resistance to *Ascochyta lentis*. R. FORD[1], J. SELBY[1] and J. DAVIDSON[2]. [1]*Department of Agriculture and Food Systems, Melbourne School of Land and Environment, the University of Melbourne, Australia.* [2]*Waite Campus Agri-Science Precinct, South Australian Research and Development Institute, Australia.* **E-mail: rebeccaf@unimelb.edu.au*

Lentil is grown as high protein seed and is a significant commodity on the global grain market. The fungal disease ascochyta blight, caused by *Ascochyta lentis*, reduces seed quality and quantity, resulting in large potential yield losses. Recent evidence of breakdown in moderately resistant cultivars (Northfield and Nipper) in South Australia highlights the need to identify the mechanisms behind sustainably resistant sources for potential future selective breeding strategies. Previous investigations of genes involved in resistance to *A. lentis* have identified a PR4a family member *DY396388* to be highly differentially expressed between high and low resistant genotypes in response to inoculation. Further investigation determined its role in the defence response via predicted protein sequence, regulation of transcription and antifungal activity. The study confirmed *DY396388* encoded the lentil PR4a protein, and transcript analysis correlated with sustained *DY396388* upregulation in response to inoculation in the most resistant genotype (ILL7537). Antifungal activity was confirmed via growth inhibition of *A.*

lentis, and dependent on PR4a protein concentration. We therefore propose that *DY396388* activation is an important response in sustainable defence to *A. lentis*. The responsive *DY396388* gene from ILL7537 may be introduced into current elite cultivars that encode low expression or non-functioning *DY396388* genes to improve resistance.

Microscopic studies of *Ascochyta rabiei* infection in *Cicer* species. C.L. ARMSTRONG-CHO and S. BANNIZA*. *Crop Development Centre, University of Saskatchewan, Saskatoon, Canada. *E-mail: sabine.banniza@usask.ca*

Ascochyta blight is the most important biotic constraint to chickpea production in Canada. Current sources of resistance in *Cicer arietinum* come from a narrow background and no accessions with resistance better than Amit (B90) have been identified, despite large-scale screening of international germplasm. Microscopic studies three, five and seven days postinoculation revealed a delay in the infection process on the resistant cultivar ILC 195 compared to the susceptible Canitez 87. Intense autofluorescence, indicative of the hypersensitive reaction, was observed in the resistant cultivar ILC3279, but not the susceptible ILC1929, within 24 to 48 h postinoculation, followed by necrotic spots representing single dead cells. Wild relatives of *C. arietinum* potentially represent a novel source of resistance to Ascochyta blight, but require a significant investment for gene transfer using conventional breeding. To better inform inter-specific breeding efforts, resistance responses at the cellular level compared four highly resistant *Cicer* species accessions to 'CDC Frontier' as the resistant and 'CDC Xena' as the susceptible cultivated controls. *Cicer* accessions chosen were perennials *C. anatolicum* (PI 383626) and *C. oxyodon* (PI 561103), and annuals *C. judaicum* (ILWC 165) and *C. bijugum* (ILWC 260). Plants were inoculated in the vegetative stage and tissues examined from 2 to 6 days postinoculation. Quantitative and qualitative differences in the growth of *A. rabiei* were noted on the different genotypes. Obvious localized responses of *C. anatolicum* and *C. oxyodon* accessions and the role of active oxygen species in the host defense response as revealed by treatment with diaminobenzidine are under investigation.

DeepSuperSAGE transcription profiling reveals significant changes of the lentil (*Lens culinaris* Medik.) transcriptome in response to *A. lentis* infection. P. GARCÍA[1*], L. SÁENZ DE MIERA[1], F. VAQUERO[1], F.J. VENCE[1], R. JÜNGLING[2], A. FRANK[2], R. HORRES[2], N. KREZDORN[3], B. ROTTER[3], P. WINTER[3], G. KAHL[2] and M. PÉREZ DE LA VEGA[1]. [1]*Area de Genética, Dpto. de Biología Molecular, Univ. de León, 24071 León, Spain.* [2]*Molecular BioSciences, Biocenter, Johann Wolfgang Goethe Univ., Max-von-Laue-Str.9, D-60438 Frankfurt, Germany.* [3]*GenXPro GmbH, Altenhöferallee 3, 60438, Frankfurt am Main, Germany. *E-mail: pgarg@unileon.es*

A genome-wide deepSuperSAGE transcription profile of the response of lentil seedlings to *Ascochyta lentis* is presented. Fifteen days old lentil seedlings of the cv. ILL5588 (resistant to Ascochyta) were infected with *A. lentis* isolate AL-84 supplied by Dr. T. Peever. After 24 h a.i. total RNA was extracted from aerial parts of infected and uninfected (control) seedlings by phenol/chloroform-LiCl. DeepSuperSage delivered a total of 1,126,438 defined SuperTags of 26 nucleotides each representing a certain transcript (353,397 in control and 773,041 in infected) representing 52,070 unique transcripts (unitags). In control and infected plants 8,161 and 14,061 exclusive unitags were founded respectively, whereas 29,848 were shared by both. A total of 177 and 613 unitags were significantly up- and down-regulated (≥3-fold and $P \leq 0.001$), respectively. To assign putative functions to the SuperTags, these were annotated by "BLASTing" to three collections of lentil cDNAs (two already published and one comprising more than 27,000 contigs obtained during the LEGRESIST project) and other nucleotide sequences from closely related legumes species. Approximately 35% of the 26 pb tags matched with one or more of the lentil cDNA sequences obtained in this project. Searches in plant databases allowed the identification of related sequences/genes for the 51% of the unitags. Significantly (≥2-fold, $P \leq 0.05$p) differentially expressed SuperTags matched to many known genes potentially involved in disease reactions including 31 genes coding for known disease-resistance proteins, 66 transcription factors, 197 kinases, etc. A Gene Ontology (GO) analysis including 19% of all SuperTags identified the GO category "signaling" as most enriched Biological Process. A selection of the differential expression of SuperTags showing the most striking quantitative differences between infected and control seedlings (clearly upregulated or downregulated in infected seedlings) is being confirmed by qPCR. This pilot project demonstrates that NGS-based technologies are efficient means to unravel the complex interaction of plant hosts with their pathogens and may pave the way to identification novel lentil R- and pathogenesis-related genes responsible for quantitative resistance to *A. lentis* and other pathogens.

This research was supported by grants from the EU ERA-NET Plant Genome project LEGRESIST, Micin AGL2009-07853 and Castilla y León GR113.

Screening techniques for resistance to Ascochyta blight disease of chickpea. S. PANDE[1*], M. SHARMA[1], A.K. BASANDRAI[2], L. KAUR[2], P.M. GAUR[1], D. BASANDRAI[2] and C.L.L. GOWDA[1]. [1]*International Crops*

*Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, 502324, Andhra Pradesh, India. [2]CSK, Himachal Pradesh Krishi Vishawavidyalaya (HPKV), Regional Research Station, Dhaulakuan, Sirmour, 173001, H P, India. [3]Punjab Agricultural University, Ludhiana 141 004, Punjab, India. *E-mail: s.pande @cgiar.org*

Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labr. (anamorph), is an important disease of chickpea (*Cicer arietinum* L.) in areas where cool (15–25°C) and humid weather (>150 mm rainfall) prevails during the crop season. Host plant resistance (HPR) is the most effective either alone or as a major component of integrated AB management. The preliminary step for exploiting HPR is the development of reliable and repeatable techniques for large-scale screening of germplasm. The present investigations were carried out to understand the biology of the pathogen, epidemiology of AB, and identification of reliable screening techniques. Components of controlled environment resistance screening techniques (CESTs) using detached - leaf (cut-twig) and seedling resistance (10-day-old seedlings) were standardized in a controlled environment facility at ICRISAT, Patancheru. Field screening techniques (FST) were standardized for adult plant resistance at Dhaulakuan and Ludhiana in India, where environmental conditions are more favorable for AB development. Correlation coefficient between disease severity rating in CESTs and FST was calculated. The results of the CEST using cut-twig (detached-leaf) were found to be highly correlated with the 10-day-old seedlings (r=0.94) and adult plants in FST (r=0.88). Similarly results between seedling (CEST) and adult plants (FST) resistance were also highly correlated (r=0.89). Using these techniques, high levels of stable resistance in breeding and germplasm lines were identified, and shared worldwide.

Food Legumes Research in North Africa

Grain Legume Research Progress in Tunisia: History and Perspectives. M. KHARRAT*, M.H. HALILA, H. BEN SALAH, B. SIFI, R. SAYAR, F.S. BEN AYED, M. AMRI, N.O. BENYOUSSEF, M. BOUHADIDA, Z. ABBES, D.S. TARRES, S. BOUKHRIS-BOUHACHEM, M. BÉCHIR ALLAGUI, A. BEN ABDERRABEH-NAJAR, H. BEN SALEM. *Field Crops Laboratory, Crop Protection Laboratory & Animal and Forage Production Laboratory, University of Carthage, INRAT, Rue Hédi Karray, 2080 Ariana, Tunisia. *E-mail: moha.kharrat@gmail.com*

In Tunisia, grain legumes are growing on 70,000 ha, more than 85% are located in the north of the country in areas receiving more than 400 mm of average rainfall. The main grain legume crop is faba bean (large and small seeded) occupying about 65% of total area followed by pea and chickpea. Small areas (less than 2,000 ha) of lentil are mainly growing in the south of the country. Although there have been slight increases in the national average yields of the main grain legume crops recorded during the last years, they are still remaining lower than the average global yields. Research on grain legume has been intensified in Tunisia with the creation of the Grain Legume Laboratory at INRAT (National Agricultural Research Institute of Tunisia) in 1981 with the kind support of ICARDA. The main missions of this programme were to select high yielding genotypes of faba beans, chickpea, lentil and field pea locally adapted to Tunisian grown areas and to develop, in different agro-ecosystem, appropriate technical production packages for different grain legume crops. Support from national funds has increased during the last ten years with the Field Crop Laboratory creation in 2000 which includes cereal, grain legume and industrial crops programmes. The grain legume programme, which has the main tasks to select improved varieties with high yield potential and tolerant to main biotic and abiotic stresses, has registered in the national catalogue of plant varieties; 7 chickpea varieties mostly for winter sowing, 5 faba beans including three small seeded, 4 lentils, 2 beans and one field pea. Out of the 19 varieties already included in the national catalogue, 14 were registered during the last decade. The programme has recently concluded 10 contracts with national seed production companies (public and private) for commercial exploitation. Two faba bean varieties (Badï and Bachaar) with two chickpea varieties (Béja 1 and Nayer) from the recently released ones, are now largely cultivated in different grain legume production areas. In addition, optimization of nitrogen fixation is continued and great progress is achieved in identifying high potential fixation rhizobia strains. Our research activities are now focussed on the development of new high yielding varieties with higher level of tolerance/resistance to main diseases and pests and well adapted to climate changes, basically drought and high temperature. Actually, the reinforcement of our research team can be considered as a great opportunity for us to diversify our research fields within the grain legume and focus on phytopathology and physiology aspects using biochemical and molecular tools. Conducting and updating the studies on pathogen and pest population variability have been initiated as well epidemiological studies of some pathogens and pests on main grain legume crops. Exploring the different mechanisms of resistance and the possible use of induced resistance products or biological control agents (bacteria, fungi, etc.) are additional tasks that had been included. The programme is also focused on selecting genotypes with high nutritional values and technological quality particularly in faba bean and chickpea. Recently, molecular markers were included in the exploration of the degree of diversity of our advanced lines of chickpea and faba bean and to confirm the effectiveness of markers closely

linked to interesting genes or QTLs in the marker assisted selection. In this way studies on the identification of new markers linked to different biotic and abiotic stresses should be undertaken in the near future. A great collaboration with University of Cordoba (Spain) and ICARDA was undertaken in this way.

Status and prospects of food legumes improvement in Morrocco. O. IDRISSI[1,*], C. HOUASLI[1], Z. FATEMI[1], K.S. BENCHEKROUN[1], S.K. AGRAWAL[2], M. IMTIAZ[2] and F. MAALOUF[2]. [1]*National Institute of Agronomic Research of Morocco (INRA).* [2]*International Center for Agricultural Research in the Dry Areas (ICARDA)*
**E-mail: o.idrissi@yahoo.fr*

In Morocco, faba bean, chickpea and lentil are being grown under rainfed conditions and play an important role in cereal-based cropping systems. Food legumes improve soil fertility through biological nitrogen fixation and have a special role in human nutrition. In Morocco, while food legumes exports represented 45 to 60% by volume in the 1970's, the failure of production to keep pace with the national overall demand means thatMorocco now relies on importing to supplement local production capacity. Productivity levels of food legumes have remained low and variable, mainly due to susceptibility of cultivars to environmental stresses, diseases, insect pests and parasites, poor seed multiplication systems, limited use of inputs, access to market, labor cost and availability and low degree of mechanization. Food legume improvement team at INRA, Morocco works in collaboration with ICARDA to address these constrains and develops improved varieties suitable for Moroccan conditions. In lentils, drought tolerance, earliness, ability for mechanization, seed quality, resistance to rust, ascochyta blight and fusarium wilt, as well as high and stable seed yields are the most important traits being considered for improvement. Eight lentil cultivars with beneficial characteristics have been released and some other promising lines are included in yield trials and few others are ready to submit for registration. In chickpea, several lines with good level of resistance to blight and fusarium wilt have been identified and released for winter cultivation. Further efforts are in progress to develop drought tolerant and bold seeded type of chickpea lines. In faba bean, the improvement activities are focused on developing *Orobanche*, chocolate spot, ascochyta blight, rust, drought, heat and cold tolerant germplasm. Screening of ICARDA Faba Bean International Nurseries during 2009–2010 and 2010–2011 resulted in identification of 18 and 11 lines moderate to highly resistant respectively for *Botrytis* and *Ascochyta* blight diseases at Douyet station. Currently, molecular markers are being used for characterization of faba bean germplasm and ascochyta blight pathogens of chickpea. Further integration of molecular markers in breeding programs would lead to improve the efficiency and effectiveness of genetic enhancement.

Prospects for accelerating faba bean genetic improvement in a genome-enabled era. D.M. O'SULLIVAN. *Crop & Pathogen Genomics, NIAB, Huntingdon Road, Cambridge CB3 0LE UK. E-mail: donal.osullivan@niab.com*

Vicia faba, despite its importance as a productive grain legume and staple food across North Africa and the Middle East, has until recently not enjoyed extensive sets of sequence-based molecular markers and sequence databases that would enable routine genetic dissection of agronomic traits of interest. Affordable sequencing technology has changed everything by allowing us and others to begin to access the transcriptome from contrasting genotypes, and to derive substantial numbers of molecular markers. Our intial sequencing of ICARDA inbred BPL10 and a Polish white-flowered line *Albus* quickly generated alignments of 14,000 transcripts shared between the two transcriptomes from which over 40,000 predicted SNPs were mined. A conservative set of filters was employed to prioritise 930 high quality predicted SNPs for validation in the form of KBioscience KASPar assays of which 850 were validated as working assays. Together with previously developed gene-based markers, the community now disposes of more than 1,000 informative SNP markers which can be flexibly deployed as a full set in association and linkage mapping studies or as individual locus assays in marker-assisted selection mode. The parents of pre-existing populations developed by various community members for the study of biotic and abiotic stress tolerance, seed size and quality characters and plant architecture traits have already been screened with these new marker sets and the maps which will result from the application of just the polymorphic markers will comprise between 250 and 700 SNP loci and it will be possible in most cases to target additional markers to SNP-defined intervals using synteny with fully sequenced legumes. Thus we can foresee molecular markers and possibly even identified genes for a number of new traits emerging in the coming years. A number of challenges remain to be overcome to maximise the potential opportunities. The *Vicia faba* community needs a recognised genetic and genomic data repository which should act as a focal point for drawing together fragmented genomic resources. Secondly, it needs to plan for development of a new generation of genetic resources better placed to take advantage of growing marker density. And last but not least, in the context of the present workshop, effective north-south partnerships that can mobilise significant resources to developing genetic solutions to key constraints on faba bean production and adapting them to local needs are needed to ensure timely impacts of the new technologies across North Africa.

North-Africa-ICARDA Partnership on Food Legumes Research for Development: Present Status and Future Strategy. F. MAALOUF[1,*], S. KUMAR[1], M. IMTIAZ[1], S. AHMED[1], M. NAWAR[1], M. KHARRAT[2] and Z. EL ABIDINE-FATEMI[3]. *[1]International Center for Agricultural Research in the Dry Areas (ICARDA), P.O Box 5466, Aleppo, Syria. [2]National Institute for Agricultural Research in Tunisia (INRAT), Ariana, Tunisia. [3]National Institute of Agronomic Research (INRA), BP. 578, VN 50000, Meknes, Morocco. *E-mail: F.maalouf@cgiar.org*

Food legumes are important crops for human food, animal feed and services for sustainable agriculture. They are the rich sources of protein and micronutrients, thus contributing significantly to the health and nutritional security of low-income consumers. Cool season food legumes (e.g. faba bean, chickpea, pea, lentil) are cultivated on 1.1 m ha area in North Africa (NA), out of which faba bean is grown on 442,000 ha, chickpea on 128,000 ha and lentils on 48,000 ha. These crops suffer significant yield losses due to various biotic and abiotic stresses in the region, making them less remunerative to small-holder farmers. Some of the major biotic and abiotic stresses in the region are *Orobanche spp.* infestation, weeds, fungal diseases, and severe and recurrent drought. In addition, non-availability of quality seed of improved varieties, lack of improved varieties suitable for machine harvest, high production costs, and government priorities are other key constraints to higher productivity. Research conducted by ICARDA in collaboration with National Research System (NARS) of NA countries has resulted in the development of improved germplasm, which combines high yield with resistance to key stresses and other desirable traits like suitability to machine harvest and improved seed quality. Winter or early spring sown chickpea technology, which clearly demonstrated its yield superiority in the region, could not be adopted by farmers because of a lack of timely availability of seed of appropriate varieties and limited technology transfer efforts. Collaborative research has also resulted in the development of faba bean varieties with tolerance to *Orobanche*. Breeding for wilt resistance has been successful in releasing improved lentil varieties. ICARDA's relations with national programs have strengthened through bilateral and regional collaborative research projects, and through support to specific food legume research networks. The regional networks in the Nile Valley program and with NA-NARS are good examples of such collaboration. However, collaboration on food legumes research for development needs further strengthening to respond to the needs of the small holder farmers, especially under climate change.

Papers presented at the Student competition

Identification of quantitative trait loci for specific mechanisms of resistance to *Mycosphaerella pinodes* in pea. E. CARRILLO*, D. RUBIALES and S. FONDEVILLA. *CSIC, Institute for Sustainable Agriculture, Córdoba, Spain. *E-mail: ecarrillo@ias.csic.es*

Resistance to *Mycosphaerella pinodes* in pea is a polygenic trait, with a number of quantitative trait loci (QTLs) identified. However, the position of these QTLs should be further refined in order to facilitate the identification of the molecular markers most closely linked to the resistance genes. Resistance is a multi-component event, being the observed symptoms the consequence of a battery of resistance mechanisms acting at different phases of the infection process. However, screenings are usually based on resistance indexes that consider only final symptoms. We have previously histologically identified several mechanisms of resistance acting against *M. pinodes* in *Pisum* spp., including *P. sativum* ssp. *syriacum* accession P665. Here we evaluated these mechanisms in the RIL population P665 × Messire, previously used to identify QTLs associated with a resistance index to this pathogen. This approach allows a more accurate assessment of the resistance and is expected, after a new QTL analysis, to result in a better definition of the genomics region involved in the resistance and to enable the association of the QTLs identified with specific mechanisms of resistance.

Genetic control of architectural traits and partial resistance likely to reduce ascochyta blight epidemics on pea (*Pisum sativum* L.). C. GIORGETTI*, G. DENIOT, H. MITEUL, F. MOHAMADI, G. MORIN, C. ONFROY, M.L. PILET-NAYEL, J.P. RIVIERE, B. TIVOLI and A. BARANGER. *INRA, UMR 1349 IGEPP (Institute of Genetics, Environment and Plant Protection), BP 35327, 35653 Le Rheu cedex, France.*
**E-mail: carole.giorgetti@rennes.inra.fr*

Mycosphaerella pinodes is the causal agent of ascochyta blight, one of the most damaging foliar diseases in pea. Only partial resistance is available which underlying mechanisms are still unknown. Previous work has shown the effect of plant and canopy architectural traits on epidemics development, as plant height and leaf area index. Moreover QTL mapping studies showed co-localizations between QTL of partial resistance and QTL controlling earliness, plant height and aerial biomass. Our main objective is to consider the potential link between architectural traits and partial resistance genetic control. Our strategy was to (i) conduct QTL analysis in a recombinant inbred line population derived from a cross between JI296 (susceptible) and FP

(partially resistant) for several architectural traits (plant height, stipule size, number of branches and number of internodes) under controlled and field conditions; (ii) to select and map genes known to control earliness, ramification, plant height, senescence and foliar characteristics in pea; (iii) to screen architectural pea mutants for their ability to reduce ascochyta blight epidemics phenotyped in controlled conditions. Our results showed two major genomic regions of co-localizations on linkage groups V and VI between QTL controlling partial resistance to *M. pinodes* and QTL involved in stipule size that could correspond to the ones observed previously. In addition 11 genes controlling architectural traits were mapped. Among them, *Ago1* was mapped on linkage group VI close to one of the two genomic regions of QTL co-localizations identified. At last architectural mutants for ramification, wax production and earliness showed significantly different disease levels.

Differentiating lentil resistance genes for ascochyta blight by phenotyping recombinant inbred lines of resistant by resistant crosses. E. SARI, A. TULLU, S. BANNIZA* and A. VANDENBERG*. *Department of Plant Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. *E-mail: sabine.banniza@usask.ca/bert.vandenberg@usask.ca*

Resistance breeding is important for developing a durable and sustainable strategy for managing problems with lentil ascochyta blight. Germplasm sources with genes for resistance to ascochyta blight have been identified both in the cultivated and the wild *Lens* species. However, large scale and long-term cultivation of lentil cultivars with single resistance genes (R-genes) may enable the pathogen to overcome the resistance. To deal with this problem, the widely accepted genetic improvement strategy is to pyramid resistance genes. In order to increase the efficiency of pyramiding genes for resistance, efforts were made to gain insight into the distinctiveness of R-genes available in varieties and breeding lines. The first hypothesis was that the recombinant inbred lines (RILs) developed from resistant by resistant crosses should not segregate for the levels of resistance if there was only one R-gene shared by the resistant parents. To test this, reciprocal crosses were made between resistant parents including CDC Robin, 964a-46, ILL 1704 and ILL 7537, and a single seed descent strategy was adopted to develop RILs advanced to F9-generation. The populations then were subjected to pathogenicity testing using the virulent isolate of *Ascochyta lentis* AL57. Results showed low amount of segregation for populations from ILL 1704 × 964a-46 and CDC Robin × ILL 7537. This was well supported by the initial parental studies as there were no significant differences between these parents in the level of disease established by the *Ascochyta lentis* isolate. However there was a considerable amount of segregation in the RILs developed from CDC Robin × 964a-46 (LR18) as well as ILL 7537 × ILL 1704 (LR3) for which chi-square tests supported a fit with a 13R:3S ratio for both the LR18 and LR3 populations (P=0.05 and P>0.1, respectively). These results suggested the presence of one dominant gene in 964a-46 and ILL 7537 and one recessive gene in CDC Robin and ILL 1704. There was no difference between resistant genes in ILL 1704 and 964a-46, or between those in CDC Robin and ILL 7537 as there were no transgressive segregation in the corresponding RIL populations. In conclusion, there are only two different major resistant genes to Ascochyta blight of lentil in the breeding lines tested.

Identification of solanapyrone biosynthesis genes and generation of solanapyrone-deficient mutants in *Ascochyta rabiei*. W. KIM[1,*], H.O. AKAMATSU[1], T.L. PEEVER[1], G.J. VANDEMARK[1,2] and W. CHEN[1,2]. [1]*Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA.* [2]*USDA-ARS, Grain Legume Genetic and Physiology Research Unit, Washington State University, Pullman, WA 99164, USA.* **E-mail: wonyong.kim@wsu.edu*

Ascochyta rabiei, the causal agent of Ascochyta blight of chickpea, produces solanapyrone toxins which are toxic to chickpea. However, very little is known about the genetics of toxin production and the role of the toxins in pathogenesis. In the present study, solanapyrone biosynthesis genes in *A. rabiei* were identified using PCR primers designed to solanapyrone biosynthesis genes from *Alternaria solani*, a fungus that produces the same toxins. Six genes (*sol1* - *sol6*) form the solanapyrone biosynthesis gene cluster in *Al. solani*. The *sol5* gene, encoding solanapyrone synthase catalyzing the final step of solanapyrone biosynthesis, was targeted in *A. rabiei* to generate toxin-deficient mutants. Gene replacement mutants of four *A. rabiei* isolates, AR628, AR20, AR19, and GFP-expressing AR628 (AR628G), produced no solanapyrones in liquid culture. The precursor of solanapyrones, prosolanapyrone III, accumulated in culture media of the gene replacement mutants, but was not toxic to chickpea. The toxin-deficient mutants showed increased radial growth and reduced colony pigmentation relative to wild-type on solid agar media. Virulence of the toxin-deficient mutants was not significantly different from wild-type progenitor isolates in chickpea inoculation assays. Using the GFP-strain AR628G and its toxin-deficient mutant, colonization and reproduction of the fungus on chickpea plants was visualized under a fluorescent microscope. No significant differences in pre-penetration behavior, penetration and colonization ability were found between toxin-producing and toxin-deficient strains. These results demonstrate that solanapyrone is not essential for the infection processes such as penetration and colonization of host tissues.

Extracellular apyrase participates in elicitor-induced superoxide generation and impacts on non-host resistance of cowpea. K. TANAKA*, K. TOYODA, Y. INAGAKI, Y. ICHINOSE and T. SHIRAISHI. *Graduate School of Natural Science and Technology, Okayama University, Japan. *E-mail: gag422131@s.okayama-u.ac.jp*

Supprescins A and B from *Mycosphaerella pinodes* are glycopeptide suppressors for defenses, but they act as elicitors on non-host plants, indicating that the suppressor production essentially decides to condition susceptibility or resistance of plant cells. Recently, one target for the suppressins is proposed to be cell wall-bound apyrases, which efficiently hydrolyze ATP to form ADP and AMP. Indeed, they can inhibit the ATP-hydrolyzing activity in cell walls of pea, but rather stimulate the activity of non-host plants such as cowpea. In this study, cowpea was used to analyze the role of ATP hydrolysis in non-host responses. Purified suppressins induced K^+ efflux from cowpea leaves within a few minutes, followed by a biphasic generation of SOD-sensitive superoxides ($O_2^{\cdot-}$). Pharmacological studies with inhibitors and AOS-scavenging enzymes showed that the suppressin-induced $O_2^{\cdot-}$ generation largely depends on an extracellular peroxidase(s) rather than a membrane-bound NADPH oxidase, because it was sensitive to salicylhydroxamic acid (SHAM). Since NADH inhibitor I-1 completely reduced the $O_2^{\cdot-}$ generation, the oxidation of apoplast NADH (as an electron donor) is likely involved in the peroxidase-catalyzed $O_2^{\cdot-}$ generation. Interestingly, the $O_2^{\cdot-}$ generation was accompanied by a production of a low molecular weight anti-fungal (yet-unidentified) compound(s), which suppresses fungal penetration from appressoria. Silencing of *VsNTPase1* encoding a cowpea cell wall apyrase attenuated the $O_2^{\cdot-}$ generation, allowing *M. pinodes* (non-pathogenic to cowpea) to infect cowpea leaves. Similarly, the suppressin-induced $O_2^{\cdot-}$ generation in cowpea leaves was markedly reduced by a specific inhibitor of apyrase, NGXT191. Experiments with adenine nucleotide analogues revealed that ADP enhanced $O_2^{\cdot-}$ generation induced by the suppressins. Moreover, a non-hydrolysable ADP[b]S alone evoked SHAM-sensitive $O_2^{\cdot-}$ generation. Taken together, these results indicate that cell wall-associated apyrase spatially regulates the peroxidase-catalyzed superoxide generation through the hydrolysis of adenine nucleotides, substantially sustaining non-host resistance of cowpea.

The Australian *Ascochyta rabiei* population structure and implications for resistance durability. A.E. LEO[1], R. FORD[1] and C.C. LINDE[2]. *[1]Department of Agriculture and Food Systems, Melbourne School of Land and Environment, the University of Melbourne, Australia. [2]Evolution, Ecology and Genectis, Research School of Biology, 116 Daley Rd, The Australian National University, Canberra, ACT 0200, Australia. *E-mail: aeleo@student.unimelb.edu.au*

Populations of *Ascochyta rabiei* from Australia, a fungal pathogen of chickpea, was characterised using 20 polymorphic microsatellite markers. The overall Australian genetic diversity analysis included 241 isolates derived from six major chickpea growing regions collected from 1999 to 2010. Additionally, the genetic relationships among 206 *A. rabiei* isolates sampled in 2010 from three Australian states from cultivars with differing levels of resistance were also assessed. The overall gene (H=0.094) and genotypic ($\hat{G}$=22.91%) diversities among the Australian isolates were relatively low, indicating that the Australian *A. rabiei* population is a recent founder population that was likely introduced at Kingsford, South Australia in 1973, which is where the disease was first noted in Australia. Little genetic differentiation was also detected among sets of isolates originating from different geographical regions (ϕpt=0.040, P>0.05), suggesting that the pathogen has migrated around Australia, most likely on seed with anthropogenic dispersal. These results coupled with the presence of a single mating type, MAT1-2, observed within all isolates examined, resulted in the low overall diversity with a high proportion of clones. However, a small number of isolates were found to be genetically distinct and with no detected clones, signifying the probable occurrence of local adaptation and/or recent and multiple independent introductions of the pathogen in Australia. Although no correlation was detected between fungal haplotype and chickpea genotype, several of the clonal isolates were highly pathogenic. These were detected on resistant cultivars Genesis090 and HatTrick™, and thus they should be monitored closely for occurrence in subsequent seasons and used for selection in resistance breeding programs.

Spatial and temporal dynamics of Ascochyta blight caused by *Ascochyta fabae* in faba beans in Tunisia. N. OMRI BENYOUSSEF[1*], H. CHAAR[2], M. KHARRAT[1] and C. LE MAY[3]. *[1]INRAT Tunisia. [2]INAT Tunisia. [3]Agrocampus Ouest, Rennes France.*E-mail: noura.mori@gmail.com*

Disease dynamics and temporal and spatial progress of Ascochyta blight caused by *Ascochyta fabae* Speg. were studied in a faba bean field (cv. Badi) in 2011 in Oued Mliz, Tunisia (36°28'46"N, 8°29'40"E, altitude: 178 m) using theoretical models. Mass disease index (MDI) was assessed every two weeks on leaves and stems at different distances (0, 1, 2, 6, 12 and 18 m) from the inoculum source (inoculated central square). From several models tested, the Gompertz function best fitted the disease's progress at varying distances from the inoculum, thus confirming the polycyclic character of the disease and allowing different parameters to be estimated such as Increase Rate Specific to Gompertz model (r_g), final MDI (y_f), Epidemic Onset Time (t_{5p}) and Area Un-

der Disease Progress Curve (AUDPC). The r_g parameter remained relatively constant on leaves (almost 0.07 d^{-1}), except at the farthest distance (18 m) where r_g averaged 0.14 d^{-1}. On stems, it decreased slightly from 0.07 d^{-1} to around 0.04 d^{-1}. Final MDI (y_f) decreased progressively from 0.97 to 0.57 between 0 and 18 m on leaves and from 1 to 0.17 on stems. The t_{5p} parameter increased from 10 to 44 days between 0 and 44 m for leaves and from 14 to 51 days for stems. Estimated AUDPC was highly and significantly correlated with the calculated one. It decreased with distance from 58 to 20 and from 54 to 4 for leaves and stems, respectively. These results show the disease's aggregative distribution and confirm its splash dispersal. Therefore, the Gompertz model, which integrates distance from the inoculum source as an independent variable, could adequately describe the spatio-temporal dynamics of the disease.

Relative effects of mesoclimate and microclimate on ascochyta blight development in pea canopies with contrasting architectures under field conditions. B. RICHARD[1,*], C. LANGRUME[1], F. ROUAULT[1], S. JUMEL[1], R. FAIVRE[2] and B. TIVOLI[1]. *[1]INRA, UMR 1349 IGEPP (Institute of Genetics, Environment and Plant Protection), BP 35327, 35653 Le Rheu cedex, France. [2]INRA, UR 875 BIA (Biometry and Artificial Intelligence), BP 52627, 31326 Castanet-Tolosan cedex, France.*
**E-mail: benjamin.richard@rennes.inra.fr*

Ascochyta blight (*Mycosphaerella pinodes*) development on pea is affected by leaf wetness duration (LWD) and temperature. These climatic conditions, particularly LWD, are reported to be dependent on canopy architecture, and as a result canopy architecture may modify epidemic development. However, few investigations have been conducted to discriminate between the specific contributions of the microclimate versus the mesoclimate. Consequently, the aim of this study was to investigate the relative effects of mesoclimate and microclimate on ascochyta blight development in pea canopies with contrasting architectures under field conditions. A split-plot experiment was conducted at Le Rheu, France, in the spring of 2009 and 2010 with three pea cultivars sown at two and three densities respectively (30, 40 and 80 seeds m^{2-1}). LWD was recorded with leaf wetness sensors at the base and mid-level of each canopy, and air temperature (Ta) was recorded with thermocouples at the base (2009) or mid-level (2010) of each canopy. Mesoclimate (LWD and Ta) was recorded with sensors placed above the canopy at 1.50 m height from the soil level. Regarding the impact of the canopy architecture on the microclimate during rainfall periods, no general leaf wetness distribution patterns were observed inside the canopy with an average daily LWD of around 15h. However, LWD was greater inside the canopy than outside (more than 3 to 10h daily). During dry periods, LWD, due to dew was longer at the middle than at the base, with an average daily LWD of around 4h that decreased with canopy development. No canopy effect was observed on Ta. Regarding the effect of the microclimate on disease development, we observed that during dry periods, temperatures were too low during LWD to favour disease development and correspondingly, a prediction model showed that infection periods occurred only during/after rainfall periods. During these periods, because LWD was longer inside than above the canopy, microclimatic data were more useful to explain the infection periods than mesoclimatic data.