Ascochyta 2016

Fourth International Ascochyta Workshop, Portugal

IV International Ascochyta Workshop

10-11th October, 2016
Tróia, Portugal



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Bob Henson Student Award supported by the Spanish Legume Association

PROGRAM
AND
BOOK OF ABSTRACTS

PROGRAM

DAY ONE - MONDAY 10th October

9.00-9.45 **Registration and coffee (atrium)**

9.45 Welcome and Opening session: Jenny Davidson

Session 1 **Host resistance breeding and Marker Assisted Selection**

Chairs: Sara Fondevilla, Sukhjiwan Kaur

Oral presentations (12 mins plus 3 mins questions)

10.15 **Ambuj Jha**: Fine mapping of QTLs to identify closely linked markers for ascochyta blight resistance in pea.

10.30 **Teresa Millan**: Breeding large seed size kabuli chickpea for resistance to Ascochyta blight and Fusarium wilt.

10.45 **Pooran Gaur**: Breeding for combined resistance to Ascochyta blight and Fusarium wilt in chickpea.

11.00 **Pedro García**: Analysis of transcriptome changes in lentil (*Lens culinaris*) after the infection of *Ascochyta lentis.*

11.15 **Amit Deokar**: Transcriptome analysis of partial resistant and susceptible chickpea genotypes during early stages of *Ascochyta rabiei* infection.

11.30 **Canan Can**: Tolerance evaluation of registered chickpea varieties *against Didymella rabiei* in the Cukurova region of Turkey.

11.45 **Mamta Sharma**: Genotype × environment interactions and an update on host plant resistance to Ascochyta blight in chickpea (*Cicer arietinum* L.).

Lightning show: (3 mins - title slide plus one slide)

12.00 **Mariem Bouhadida**: Breeding chickpea for resistance to Ascochyta blight in Tunisia.

12.03 **Aladdin Hamwieh**: Multi-environment QTL analyses for Ascochyta blight resistance in a recombinant inbred population of chickpea (*Cicer arientinum* L.).

12.06 **Magdalena Gawłowska**: Identification of quantitative trait loci associated with resistance to ascochyta blight disease in [P665×Messire] and [Wt10245×wt11238] pea *(Pisum sativum* L.) mapping populations.

12.09 **Anna Torres**: Transcriptome analysis and mapping to identify candidate genes controlling *Ascochyta fabae* resistance in faba bean (*Vicia faba* L.).

12.12 Question panel

12.30 **LUNCH (hotel restaurant)**

Session 2 Epidemiology and host pathogen interactions

Chairs: Dani Shtienberg, Kevin McPhee

Oral presentations (12 mins plus 3 mins questions)

1.30 **Salman Ahmad**: Development of meteorological model to give prediction of chickpea blight (*Ascochyta rabiei*) in semi-arid zones.

1.45 **Canan Can**: Correlation of Ascochyta blight severity in chickpea with pathotypes, mating type, altitude, nodulation and weed density in Turkey.

2.00 ***Yasir Mehmood**: Differences in isolate behaviour during the early *Phoma rabiei* – chickpea interaction.

2.15 **Weidong Chen**: Toxin solanapyrone production in *Ascochyta rabiei*: Genetic control and ecological roles.

2.30 **Praveen Verma**: Secreted effectors of *Ascochyta rabiei*: Molecular analysis of a novel effector necessary for establishing pathogenesis and possible host target.

Lightning show: (3 mins - title slide plus one slide)

2.45 **Seid Ahmed**: Effects of low temperature on the susceptibility of chickpea genotypes to different pathotypes of *Didymella rabiei.*

2.48 **Mohamed Bencheikh**: Response surface methodology approach to determine the Influence of some environmental factors on mycelial growth and spore production of *Didymella pinodes.*

2.51 **Anne Moussart**: Plant and canopy architecture to control ascochyta blight epidemics in pea fields.

2.54 **Sabine Banniza**: Effect of pea seed infection with *Ascochyta pisi* on plant establishment and ascochyta blight development.

2.57 **Alessandro Infantino**: *Ascochyta lentis* var. *lathyri*, causing a new disease of grasspea (*Lathyrus sativus*) in Italy.

3.00 ***Momiji Miki**: Extracellular apyrase (PsAPY1) modulates the PAMP-induced oxidative burst and accumulation of PR10-1-mRNA in pea.

3.03 **Kazuhiro Toyoda**: The plant cell wall as a site for molecular contacts in fungal pathogenesis.

3.06 **Getinet Desalegn**: Microbial associations with legume plant trigger systemic resistance against *Didymella pinodes* infection.

3.09 ***Shiori Yamasaki**: Extracellular apyrase (ecto-ATPase) impacts on the non-host resistance to fungal and bacterial pathogens.

3.12 Question panel

3.30 CLOSE with COFFEE (atrium)

DAY TWO - TUESDAY 11th October

Session 3 Germplasm resources and plant breeding

Chairs: Teresa Millan, Tom Warkentin

Oral presentations (12 mins plus 3 mins questions)

9.00 **Sukhjiwan Kaur**: Identification and validation of molecular markers for resistance to *Ascochyta lentis* in Australian lentil breeding germplasm.

9.15 **Alain Baranger**: Association mapping of partial resistance to *Didymella pinodes* and architectural traits in pea.

9.30 **Bunyamin Tar'an**: Genome wide analysis of NBS-LRR genes in chickpea and their potential as candidate genes for ascochyta blight resistance.

Lightning show: (3 mins - title slide plus one slide)

9.45 **Ibrahim Elkhalil Benzohra**: Evaluation of wild *Cicer* species accessions for resistance to *Ascochyta rabiei* (Pass.) Labr., the agent of ascochyta blight on chickpea (*Cicer arietinum* L.) in Algeria.

9.48 ***Rama Harinath Reddy Dadu**: Screening of wild lentil germplasm to identify novel *Ascochyta lentis* resistance sources.

9.51 **Diego Rubiales**: Use of *Pisum* spp. in pea breeding for ascochyta blight resistance.

9.54 ***Ester Murube Torcida**: Development of resistance sources to ascochyta blight caused by *Phoma exigua* var. *diversispora* in common bean germplasm

9.57 **Juan Osorno**: Climbing beans affected by *Ascochyta* spp. in the Guatemala Highlands

10.00 **Garry Rosewarne**: Breeding strategies for ascochyta resistance in field peas.

10.03 Panel questions

10.30 COFFEE (atrium)

Session 4 Pathogen genetics, genomics and populations;

Chairs Tim Sutton, Seid Kemal

Oral presentations (12 mins plus 3 mins questions)

11.00 **Rohan Kimber**: Virulence dynamics within *Ascochyta fabae* populations in Australia.

11.15 **Noura Omri Benyoussef**: Genetic and pathogenic diversity of *Ascochyta fabae* Speg. Population.

11.30 **Christophe Le May**: Patterns determining of population structure of *Didymella pinodes* in the Mediterranean Basin.

11.45 **Dani Shtienberg**: Epidemiology of *Peyronellaea pinodes* isolates originating from wild and domesticated *Pisum* sp. in Israel.

12.00 **Sara Blake**: Diversity of aggressiveness of the *Ascochyta lentis* population in Australia.

12.15 **Sara Fondevilla**: *De novo* genome and transcriptome sequencing combined with differential expression analysis identify putative pathogenicity factors from *Ascochyta rabiei*.

Lightning show: (3 mins- title slide plus one slide)

12.30 **Sanae Krimi Bencheqroun**: Determination of genetic and pathogenic diversity within Ascochyta bight pathogen of chickpea in Morocco.

12.33 **Julie Pasche**: Characterization of Ascochyta blight pathogens of chickpea, field pea and lentils in Montana.

12.36 **Christophe Le May**: Characterization of fungal species complex of Ascochyta blight developing on wild and cultivated legumes and their host spectrum evaluation.

12.39 Question panel

1.00 LUNCH (hotel restaurant)

Session 5 Group Discussions

Chairs: Rohan Kimber, Christophe Le May

2.00 Group Discussions

3.00 Report back Group Discussions

3.30 Business Meeting and Closing Session

4.00 CLOSE AND COFFEE (atrium)

SESSION 1

Host resistance breeding and Marker Assisted Selection

Fine mapping of QTLs to identify closely linked markers for ascochyta blight resistance in pea

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Ascochyta blight is the most destructive disease of pea which may cause serious yield losses under wet conditions. A recombinant inbred line population (PR-19) was developed from a cross between ascochyta blight resistant *Pisum fulvum* accession P651 and cultivar Alfetta. Nine quantitative trait loci (QTLs) were identified for ascochyta blight resistance in PR-19 under field and greenhouse conditions. QTLs abI-IV-2 and abIII-1 were consistent across locations and/or years and were selected for fine mapping to identify closely linked markers. With this objective, heterogeneous inbred families (HIFs) populations HIF-173 and HIF-224 were developed from lines PR-19-173 and PR-19-224 to fine map abIII-1 and abI-IV-2, respectively. Phenotyping of HIF-173 and HIF-224 conducted under field conditions in 2015 showed a wide range of variation for reaction to ascochyta blight resistance. Phenotyping of HIFs in 2016 at multiple locations and genotyping using markers generated from an Illumina GoldenGate array are in progress. Genotyping will also be conducted using markers from genotyping by sequencing method to identify closely linked molecular markers.

Breeding large seed size kabuli chickpea for resistance to Ascochyta blight and Fusarium wilt

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Chickpea is a traditional crop in the Mediterranean basin being the most appreciated kabuli type with large seeds (>0.45 g). Therefore, this study has been focused in the development of large seeds chickpea genotypes resistant to the two major fungal diseases in the crop, Ascochyta blight and Fusarium wilt. Sixteen parental lines with large seed size and resistance to Fusarium race 5 were crossed with six advanced lines with large seed and resistance to blight. A selection of 29 F_1 seeds was used to generate F_2 populations. The best F_2 seeds, based on size, colour and shape of the seeds, were sown in an infected field with Fusarium wilt race 5. Young leaves in F_2 plants were collected for DNA extraction and genotyped with markers associated with resistance to both blight (SCY17, CaETR) and Fusarium wilt (TA59) (1, 2, 3). Data from phenotypic and genotypic evaluation allowed selecting 34 F_3 lines combining resistance to both diseases and large seed size. Advanced generations derived from these plant materials will be evaluated for other agronomic traits (flowering time and yield).

References

1. Winter P, Benko-Iseppon AM, Hüttel B, Ratnaparkhe M, et al (2000) A linkage map of the chickpea (*Cicer arietinum L.*) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: Localization of resistance genes for *fusarium* wilt races 4 and 5.*Theor Appl Genet* 101: 1155–1163.
2. Iruela M, Rubio J, Barro F, et al (2006) Detection of two quantitative trait loci for resistance to ascochyta blight in an intra-specifi c cross of chickpea (*Cicer arietinum* L.): development of SCAR markers associated with resistance. *Theor Appl Genet* 112: 278–287
3. Madrid E, Chen W, Rajesh PN et al (2013) Allele-specific amplification for the detection of Ascochyta blight resistance in chickpea. *Euphytica* 189:183–190

Acknowledgments INIA RTA2013-00025, project co-financed by the European Union through the ERDF 2014-2010 "Programa Operativo de Crecimiento Inteligente" and Contract 77863.

Breeding for combined resistance to Ascochyta blight and Fusarium wilt in chickpea

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Among various diseases of chickpea (*Cicer arietinum* L.), Ascochyta blight (AB), a foliar disease caused by *Ascochyta rabiei* (Pass.) Labr., is the most important disease in cool and wet areas, while Fusarium wilt (FW), a root disease caused by *Fusarium oxysporum* f. sp. *ciceris,* is the most important disease in dry and warm areas. AB affects chickpea production in more number of countries as compared to FW, but the total chickpea area affected by FW is much higher than that affected by AB. In some chickpea growing areas (e.g. northern India, Pakistan, Ethiopia), both these diseases occur together. Thus, chickpea varieties with combined resistance to AB and FW are required for these areas. For combining AB and FW resistance, a large number of crosses were made between known donors of AB resistance and the popular cultivars/elite lines with FW resistance. The segregating populations (F_4 or F_5) were first screened for AB resistance at seedling stage under artificial epiphytotic conditions in controlled environmental conditions at ICRISAT to select AB resistant plants. The progenies developed from these plants were further screened for AB resistance under epiphytotic conditions in field in northern India. The selected AB resistant lines were then screened for FW resistance in wilt sick field at ICRISAT. Several breeding lines with combined resistance to AB (score 3 to 4, on 1 to 9 scale) and FW (<10% plant mortality) have been developed and these can be obtained from ICRISAT.

Analysis of transcriptome changes in lentil (*Lens culinaris*) after the infection of *Ascochyta lentis*

García P[1], Vaquero F[1], Vences FJ[1], Sáenz de Miera L[1], Polanco de la Puente C[1], González AI[1], Horres R[2], Krezdorn N[2], Rotter B[2], Winter P[2], Pérez de la Vega M[1]

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Transcriptome changes of lentil seedlings in response to *A. lentis* infection have been studied by the Massive Analysis of cDNA Ends (MACE) technology, considered one the most cost-effective methodologies for the studies in gene expression (Zajac et al., 2015). Three samples of *Lens culinaris* were analyzed, two of them belong to the cultivated species *L. c.* subsp. *culinaris* (ILL5588, considered as moderately susceptible to the pathogen, and cv. Lupa, highly susceptible) and one to the wild ancestor *L. c.* subsp. *orientalis* (BG16880, resistant to the fungus). Plants were grown in sterile conditions until inoculation (15 days after germination), and total RNA was extracted 24 hours after the infection. A total of 1,1416,403 reads were assembled in 43,970 contigs ranging between 100 bp and 2000 bp (mean 300 bp). The contigs were blasted against the lentil, legume and the non-redundant nucleotide NCBI databases, allowing the identification of the 43% of them. Further, a functional classification of the contigs was carried out by the assignation of GO categories using the software Blast2GO. In the three samples, the infection with *Ascochyta* resulted in significant changes for some transcripts, being more frequent the up-regulation (ILL5588 showed 504 up-regulated and 155 down-regulated sequences; in Lupa 419 were up- and 99 down-regulated; in *L. orientalis* 225 were up- and 86 were down-regulated). One hundred and seventy-seven contigs showed a statistically significant up-regulation in all samples when infected and they were supposed to be involved in general mechanisms of defense against the pathogen. In fact, many of them are related with genes coding for pathogenesis related proteins (PR) or disease resistance response proteins. When analyzing the common genes exclusively up regulated in resistant genotypes, some coding for WRKY transcription factors and RING finger proteins, besides other disease resistance response proteins are detected. The development of molecular markers for these genes, and their genetic mapping in a RIL population obtained from the cross Lupa x *L. c. orientalis* will allow us to identify which of these candidate genes could be related with the QTL involved in the resistance to *Ascochyta* in this population.

References

1. Zajac BK, Amendt J, Horres R, Verhoff MA, Zehner R (2015) De novo transcriptome analysis and highly sensitive digital gene expression profiling of *Calliphora vicina* (Diptera: Calliphoridae) pupae using MACE (Massive Analysis of cDNA Ends). *Forensic Science International: Genetics* 15: 137-146.

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Transcriptome analysis of partial resistant and susceptible chickpea genotypes during early stages of *Ascochyta rabiei* infection

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Several sources of resistance to Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr., have been identified in chickpea, however none showed complete resistance to Ascochyta blight and, hence, categorized as partial resistance. These partial resistant genotypes have been extensively used in many breeding programs to improve AB disease resistance. However, precise genetic mechanisms or gene network that contributes to partial resistance is unknown in chickpea.
We used RNA sequencing (RNA-seq) approach to compare transcriptional changes in chickpea upon infection of *A. rabiei* local isolate AR170. We used two AB resistant genotypes CDC Corinne and CDC Luna and a susceptible genotype ICCV 96029. All genotypes were inoculated with AR-170 at 8-10 node growth stage and samples were collected at 24, 48 and 72 hr post inoculations.
A total of 789.7 million high quality (Q30) pair-end reads were generated, yielding an average of 65.8 million reads per sample. More than 90% of chickpea transcriptome reads were mapped to the CDC Frontier chickpea reference genome sequence, representing transcriptome of around 20,261 chickpea annotated genes. The differential gene expression (DEG) analysis discovered that some of the common and unique genes were significantly differentially expressed in the resistant and susceptible genotype in response to *A. rabiei* infection. The DEG includes pathogenesis-related genes, cell wall-mediated pathogen resistance genes, enzymes involved in defense mechanisms and different classes of stress-responsive transcription factors. The genes that are located in the genomic regions associated with QTL for AB resistance were selected and their expression was validated using quantitative real-time PCR technique. These results added important information in understanding the molecular mechanism underlying partial disease resistance to Ascochyta blight in chickpea.

Tolerance evaluation of registered chickpea varieties against *Didymella rabiei* in the Cukurova region of Turkey

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This research was conducted to evaluate disease tolerance/resistance of 34 registered varieties at Cukurova region of Turkey during winter sowing conditions at disease gardens of Eastern Mediterranean Research Institute trial area. During 2014-2015 sowing season, 4 different disease gardens for 4 different pathotypes have been planted from registered chickepa varieties and tolerance of the varieties was studied through observations and readings. During trials, 34 registered chickpea varieties were evaluated and cv Canitez, which is known to be sensitive against disease was used as control. During this study, artificial inoculation of 4 pathotypes that were identified at Turkish legume plantation areas was done at disease gardens of Adana trial location, and evaluations were made on suitability of the registered varieties to the region for winter sowings, through disease readings made on day 7, day 14 and day 21 based on 1-9 scale. From disease garden trials conducted by artificial inoculation of 4 pathotypes, lowest scores were obtained from Pathotype 1 applications and highest scores were obtained from Pathotype 4 applications.

Acknowledgements
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Genotype × environment interactions and an update on host plant resistance to Ascochyta blight in chickpea (*Cicer arietinum* L.)

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Ascochyta blight (AB) caused by *Ascochyta rabiei* (Pass.) Labr. is an important disease of chickpea (*Cicer arietinum* L.) particularly in regions where cool and humid weather persists during the crop season. The disease is reported to be extending to new niches in past few years due to climate variability. Breeding for host resistance is an important component of management means to combat this disease. However, absence of high levels of stable resistant sources to AB has necessitated the continued search for identification of new sources of resistance. In this paper, attempts have been made to summarize the progress made in identifying resistance sources, genetics and breeding for resistance, and genetic variation among the pathogen population in India. The search for resistance to AB in chickpea germplasm, breeding lines and land races using optimized screening methods has been updated. Genotype × environment (G×E) interactions in elucidating the aggressiveness among isolates from different locations and stability of the breeding lines through multi-location testing have also been discussed.

A collection of 424 elite Chickpea genotypes were evaluated for AB resistance under controlled environmental conditions at ICRISAT. Ascochyta Blight Nursery (ABN) was constituted to evaluate the 29 resistant chickpea genotypes to AB at hot spot locations in India over three crop seasons (2007–2008 to 2009–2010). Genotype and genotype × environment (GGE) biplot analyses of the multi-environment data revealed not only significant genotypic effects but also significant effects of the environment and the G × E interaction for AB severity. Five genotypes were identified resistant (ICCV 04537, ICCV 98818, EC 516934, EC 516850 and EC 516971) with mean disease severity ≤3.0 on the 1–9 scale and the reactions were consistent across the environments. A significant positive correlation was found between the performance of the genotypes under controlled environment and field screening conditions ($r=0.70$; $P<0.01$). The resistant genotypes identified in the present study would be useful in breeding programs as stable resistant donors to evolve agronomically desirable AB resistant varieties.

References

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2. Sharma M, Ghosh R (2016). An update on genetic resistance of chickpea to Ascochyta Blight. Agronomy 6 (1), 18.

Breeding chickpea for resistance to Ascochyta blight in Tunisia

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Ascochyta blight (AB) caused by *Ascochyta rabiei* is one of the most important foliar diseases affecting chickpea yield worldwide. Breeding for resistance to AB is one of the major objectives of the Tunisian chickpea breeding program. Screening of germplasm accessions and elite lines for resistance to AB is carried out in Tunisia at Morneg and Kef experimental stations in 2015 under artificial field inoculations with mixtures of aggressive isolates of the pathogen. Isolates of both pathotype I (AR19) and pathotype II (AR628) of *A. rabiei* (Chen et al. 2004) were used under controlled conditions in WSU-Pullman to test the levels of resistance of chickpea advanced lines previously selected for AB resistance in the field in both locations (Morneg and Kef). Some of the studied chickpea lines showed good level of resistance to both pathotypes. The virulence of 7 Tunisian isolates collected from the main chickpea growing area was compared with isolates AR19 and AR628 using 2 chickpea genotypes 'Spanish White' (susceptible for both pathotypes) and 'Dwelley' (only susceptible to pathotype II), under controlled conditions using a 1-9 rating scale of Singh et al. (1981). The virulence of the 7 Tunisian isolates of *A. rabiei* was very similar among themselves and resembled that of pathotype II isolate AR628. The 64 chickpea lines evaluated in the two field locations for AB in Tunisia and under controlled condition (AR19 and AR628) in WSU-Pullman were genotyped with markers linked to QTLs for blight resistance (CaETR linked to QTL_{AR1} and SCAR SCY17 linked to QTL_{AR2}). Genotyping and phenotyping results are generally in concordance and confirm usefulness of the markers in assisting chickpea breeding program.

References

1. Chen W. et al. (2004). Plant Pathology, 53:759-769.
2. Singh K.B., G.C. Hawtin, Y.L. Nene and M.V. Reddy, 1981. Plant Disease 65, 586-587.

Multi-environment QTL analyses for Ascochyta blight resistance in a recombinant inbred population of chickpea (*Cicer arientinum* L.)

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Chickpea (*Cicer arietinum* L.) is widely grown around the world and occupies the third position among food legumes in terms of cultivated areas. Ascochyta blight (AB) caused by *Ascochyta rabiei* (Pass.) Labr. is one of the destructive foliar diseases that can cause complete loss of the crop in many chickpea growing regions around the world. A recombinant inbred line (RIL) population, comprising 165 lines derived from FLIP98-1065 (R) x ILC1929 (S) have been evaluated in 8 environments across three years (2008-2011) and three locations in Syria (Tel Hadya "TH", Lattakia "Lat" and the greenhouse). Field screening was conducted using a randomized complete block design with three replications. The greenhouse experiments were conducted against two pathotypes I and II. A total of 1398 (134 SSR, 652 DArTseq and 612 SNP) markers have been produced to develop a genetic map. Significant differences for AB resistance have been observed between the RILs, and the QTL analysis indicated two major and conserved QTLs on Chromosome 1 and 4 explaining maximum 25% of the total variation. This study produced a high resolution map for two major QTLs conferring Ascochyta blight resistance in chickpea. Concentrating on LG1 and LG4 in molecular breeding programs for Ascochyta blight speed up improvement for these traits.

Identification of quantitative trait loci associated with resistance to ascochyta blight disease in [P665xMessire] and [Wt10245xWt11238] pea (*Pisum sativum* L.) mapping populations

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Ascochyta blight disease is one of the main constraints for pea cultivation. QTL analysis were performed in [P665×Messire] and [Wt10245×Wt11238] pea mapping populations to identify the genomic regions controlling resistance to ascochyta blight. Disease assessments were performed under field conditions during 5 years at Radzików, Poland, using the scale reported by Xue et al. (1996) (0 – resistant, 9 – susceptible). Average ascochyta disease (AAD) scoring was **2.09** for [P665×Messire] RIL population, 2.5 for Messire and 1.5 for P665 in 2008. In 2009 (AAD) scoring for this population was **4.14**, 5 for Messire and 3.5 for P665. In 2013 (AAD) scoring was **4.18**, 5.5 for Messire and 2.7 for P665. In 2014 (AAD) scoring was **4.05** and 5.0 for Messire. In 2015 (AAD) rating was **3.98** and 4.8 for Messire. Therefore P665 was more resistant to ascochyta blight than Messire under our field conditions as reported previously by Fondevilla et al. (2008). Average ascochyta disease scoring value was higher for [P665×Messire] than for [Wt10245×Wt11238] population [(AAD) for population **3.6**, 3 for Wt10245 line and 4 for Wt11238 line in 2011, **4.36** for the RIL population, 3.3 for Wt10245 line and 4.9 for Wt11238 line in 2014]. Three QTLs associated with resistance to ascochyta blight were identified on [P665×Messire] linkage map. QTLs were not conserved across the years what may be due to the strong influence of environmental conditions on the measured traits. Seven QTLs associated with resistance in 2011 and four associated with resistance in 2014 were detected in the [Wt10245×Wt11238] population. Two QTLs were found in the same genomic region in both populations (in LGIIIB, near AA170 and in LGVB, near Pis_GEN_27_2_1 and AD280 marker). QTLs in similar intervals were detected by Carrillo et al. (2014) in Spanish conditions, suggesting the existence of genetic factors controlling resistance effective in different genetic backgrounds and environments: one in LGIII (near AA170 marker) and two in LGV (near AD280 marker0. According to these authors some genes co-localizing with QTLs may have an interesting role in defense and therefore, they could be candidate genes involved in resistance to *D. pinodes* in P665.

Acknowledgements
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Transcriptome analysis and mapping to identify candidate genes controlling *Ascochyta fabae* resistance in faba bean (*Vicia faba* L.)

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Faba bean (*Vicia faba* L.) is the third most important food legume in the world being used as a protein source in human diets and animal feeding. Among biotic stresses, diseases are the key limiting factors for faba bean cultivation. One of the most important is ascochyta blight (*Ascochyta fabae* Speg.) which causes yield losses ranging from 35 to 90%. In this work, we aimed to saturate the main quantitative trait loci (QTLs) for *A. fabae* resistance identified in the 29H×Vf136 RIP (Recombinant Inbred Population).

We have exploited a recent transcriptome database obtained from lines 29H (one of the most resistant accessions) and Vf136 after their infection with *A. fabae* (Ocaña et al. 2015), to identify candidate genes related to pathogen resistance. From the 39.060 SNP found after the transcriptome assembling, 229 representing differentially expressed transcripts were selected to be genotyped using the Kaspar and the IPLEX-Sequenom platforms. Finally, 92 SNPs were genotyped in the 29H×Vf136 RIP and used to improve the last genetic linkage map (Atienza et al. 2016), saturate the regions bearing QTLs and refine their position.

Linkage analysis included 307 markers (215 genotyped in previous studies). Using a LOD score of 4, 287 markers mapped into 18 linkage groups. Eighty six of the 92 SNPs, were added in the new map being distributed in the 6 faba bean chromosomes. QTLs located on chromosome II, III, and VI reported previously were confirmed and flanked with new SNPs markers. These gene markers are a good starting point for expression studies and development of diagnostic markers. Apart of information to understand the pathways involved in the mechanism of resistance to *A. fabae*, this study provides the most comprehensive genetic map described in this RIP that will be used for marker-assisted selection in this crop.

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SESSION 2

Epidemiology and host pathogen interactions

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Development of Meteorological Model to give Prediction of Chickpea Blight (*Ascochyta rabiei*) in Semi-arid Zone

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More than five foliar sprays are applied on chickpea (*Cicer arietinum* L.) to control chickpea blight in semi-arid zone of Pakistan. This excessive use of fungicides can be curtailed by predicting early onset of disease. Current research was designed for the development of model to predict chickpea blight based on meteorological variables i.e. maximum and minimum temperatures, rainfall, relative humidity (RH) and wind speed. Relationship of meteorological variables with disease severity was determined through correlation analysis, and stepwise regression was used for the development of model. For this purpose, data of two years i.e. 2011-12 of meteorological variables and chickpea blight disease severity were used. Significant correlation was found between all environmental parameters and blight severity. A model based on weekly all meteorological variables fit the data well ($R^2 = 0.82$). Predictions of the model were evaluated on two statistical indices i.e. root mean square error (RMSE) and error (%). Overall, RMSE and error between observed and predicted data points were $\leq \pm 20$ indicating the model as a good model. Model was validated with five years (2006-10) independent data set. Homogeneity of regression equations of two models i.e. two year (2011-12) and five year (2006-10) showed that two models validated each other. Predictive model i.e. multiple regression model developed during this study is first time attempt in semi-arid zone of Pakistan and would help in judicious use of fungicides by giving correct predictions of blight disease on chickpea crop.

***Keywords*:** chickpea blight model, regression, validation

Correlation of Ascochyta blight severity in chickpea with pathotypes, mating type, altitude, nodulation and weed density in Turkey

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Chickpea (*Cicer arietinum* L.) is one of the main food resources in human nutrition and used in crop rotation to enrich the soil structure. Turkey is an important chickpea producing county with 3.885,175 ha cultivation area and 450.000 tons of production (TUIK, 2014). Ascochyta blight caused by *Didymella rabiei* (Kovachevski) von Arx [anamorph: *Ascochyta rabiei* (Passerini) severely reduce chickpea yield as well as seed quality in Turkey. This study was conducted to explore Ascochyta blight severity in correlation with pathotype and mating type distribution, altitude, nodulation and weed density in the chickpea growing areas of Turkey.

Survey studies to chickpea fields were conducted in chickpea growing areas covering seven regions (Bosporus, Black Sea, Aegean, Central Anatolia, Mediterranean, Southeastern and Eastern Anatolia) of Turkey in 2014-2016 growing seasons. Altitudes, GPS locations, Fusarium wilt occurrence, weed coverage, nodulation and vegetation data were also collected for each filed. Over 1000 chickpea fields were evaluated and disease severity was calculated according to Reddy and Singh (1984). Mating types and pathotypes of *D. rabiei* isolates were determined and their distribution in Turkey was investigated.

Ascochyta blight disease severity exhibited difference among years depending on mean rain fall and regions in Turkey. The disease was prevalent at flowering and capsule filling stages, but chickpea fields of Aegean, Mediterranean and Bosporus regions were severely affected from the disease before flowering. Distribution of pathotype, mating type and Ascochyta blight disease severity in correlation with altitude, weed density and nodulation were discussed through the data of 3 years survey studies in Turkey. The data obtained is the first large scale analyses of Ascochyta blight of chickpea in Turkey.

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Differences in isolate behaviour during the early *Phoma rabiei* – chickpea interaction

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This study was conducted to determine if significant differences exist among previously characterised isolates of *P. rabiei* in their abilities to germinate and grow on hosts with varying levels of resistance. Microscopy observations revealed differences in early behaviour among four isolates representative of four pathotype groups detected within the Australian *Phoma rabiei* population based on the severity of gross disease symptoms produced on ICC3996 (R), Genesis090 (R), PBA HatTrick (MR) and Kyabra (S) (1). As an initial measure of aggressiveness, spore germination percentages and germ tube lengths were assessed.

The highest germination percentages for all isolates at 36 hpi were observed on Kyabra (the most susceptible host), with significant differences among isolates (ranging from 43.98% for isolate TR6421 to 100% for isolate FT13092-6). Isolate FT13092-6 (of pathotype group II) germinated the fastest (by 8 hpi) and with the highest percentage (98.17%) on PBA HatTrick (moderately resistant). The slowest isolate on PBA HatTrick was TR6421 (of pathotype group I), which germinated 4.22% by 16 hpi. Similarly, isolate TR6421 took until 36 hpi to germinate just 14.04% on ICC3996 (the most resistant host). Whereas, isolate FT13092-6 took just 8 hpi to germinate 0.88%, and 36 hpi to germinate 49.27% on ICC3996, potentially indicating a faster mutual recognition and compatible interaction among this host-isolate combination. Significantly shorter germ tube lengths were observed for all isolates on ICC3996 (ranging from 15.61µm to 25.08µm) than on PBA HatTrick (ranging from 20.78µm to 76.01µm Also, isolates FT13092-4 (of pathotype group III) and FT13092-6 both had significantly shorter germ tubes on ICC3996 than on Genesis090 (also a resistant host).

Although the germination percentages and germ tube lengths did not show the same ranking of isolate pathogenicity as previous gross disease symptomology, significant differences were detected in early isolate behaviour among isolates and on different hosts. This initial finding requires validation through assessment with a larger number of spores. Assessments of post germination and germ tube growth processes are required to more fully discriminate the biological evidence behind the observed differences among isolate pathogenicity.

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Toxin Solanapyrone Production in *Ascochyta rabiei*: Genetic Control and Ecological Roles

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Many fungi produce secondary metabolites including those that are toxic to plants (phytotoxins). The Ascochyta blight pathogens produce an array of non host-selective toxins. And these phytotoxins have often been proposed to be virulence/pathogenicity factors, but conclusive evidence was elusive. This study was aimed at investigating the genetic control and roles of the solanapyrone toxins produced by the chickpea pathogen *Ascochyta rabiei*. A novel type of cluster transcription factor was found controlling expression of the solalapyrone biosynthesis genes. Full genome sequencing of *A. rabiei* revealed a solanapyrone gene cluster in an AT-rich region proximal to a telomere end and surrounded by *Tc1/Mariner*-type transposable elements. Among the six solanapyrone cluster genes (*sol1*-*sol6*), *sol4* encodes a novel type of Zn(II)2Cys6 zinc cluster transcription factor. Deletion of *sol4* resulted in complete loss of solanapyrone production, but did not affect growth, sporulation or virulence. Gene expression studies with the *sol4*-deletion and *sol4*-overexpression mutants delimited the boundaries of the solanapyrone gene cluster and revealed that *sol4* is likely a specific regulator for solanapyrone biosynthesis, and appears to be necessary and sufficient for induction of the solanapyrone genes.

The phytotoxin solanapyrones are not required for pathogenicity by *A. rabiei*. Using targeted gene replacement techniques, the solanapyrone synthase gene in *A. rabiei* was disrupted. The resulting mutants do not produce solanapyrones, but accumulate the precursor presolanopyrone which is not toxic to plants. Surprisingly, these solanapyrone-deficient mutants are equally pathogenic to chickpea as the wild-type strains.

Solanapyrone A plays an important role for competition and presumably survival of the fungus. Solanapyrone A was specifically produced during its saprobic growth, but not during the parasitic growth of *A. rabiei*. Expression of the gene encoding the last step enzyme for solanapyrone biosynthesis was specifically associated with development of the asexual fruiting bodies. In confrontation assays with saprobic fungi that were commonly found in chickpea debris, *A. rabiei* effectively suppressed the growth of the competing fungi. Solanapyrone A was directly detected in the inhibitory zone, and showed significant antifungal activities.

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Secreted effectors of *Ascochyta rabiei*: Molecular analysis of a novel effector necessary for establishing pathogenesis and possible host target

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Recent studies on genomics of plants and fungus are beginning to unravel new portrait of the plant-fungus interactions with respect to the survival of both organisms. The discovery of pathogen secreted effector proteins which involved in the suppression of host generated immune responses, are the important findings to understand pathogenesis and virulence. Most of the effectors have been characterized in biotrophic fungi and oomycetes. Nevertheless, the knowledge regarding necrotrophic effectors and the mechanisms by which they manipulate the host cell machinery remains limited. However, only a few studies have explored for necrotrophic fungal pathogen effectors. Chickpea-*Ascochyta rabiei* system provides an excellent model for studying necrotrophy. Despite extensive pathological and molecular studies, it is still unclear how *A. rabiei* suppresses early defenses or subsequently trigger defenses to support the activation of host cell death. We have sequenced 34.6 megabase draft genome sequence of *Ascochyta rabiei* and predicted 10,596 protein-coding genes that encode large and diverse inventories of secretory proteins, carbohydrate-active enzymes, transporters and primary and secondary metabolism enzymes, reflecting its necrotrophic lifestyle. Comprehensive analysis predicted a set of 758 secretory proteins with different conserved motifs. Further analysis of the secretome revealed a high abundance of host nuclear localized effector candidates. We characterized a novel effector, PEC25, and found that this effector is indispensable for *A. rabiei* virulence. PEC25 translocate into the host nucleus during infection, which is critical for its functionality. To identify the host target, we explored the candidate genes of major QTLs of chickpea against *A. rabiei* resistance for PEC-25 interaction. Interestingly, we showed that PEC25 is interacting with transcription factor in the nucleus of chickpea. The knowledge obtained by functional characterization of such effectors would provide comprehensive understanding of different *Ascochyta* species as well as other necrotrophic fungi for pathogenesis and virulence, which can be utilized to design powerful strategies to control such fungal diseases.

Effects of low temperature on the susceptibility of chickpea genotypes to different pathotypes of *Didymella rabiei*

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Winter and spring planted chickpea are affected by Ascochyta blight (*Didymella rabiei*) in different parts of the world. Several management practices including resistance breeding are used to manage Ascochyta blight in many countries. Since the expansion/adoption of winter planted chickpea technology requires cold tolerance and Ascochyta blight resistance, they are key traits in ICARDA Kabuli chickpea breeding program. In Syria some of the cultivars released for winter cultivation were out of production due to emergence of new aggressive pathotypes. Though studies have been made on the drivers of increase pathogen aggressiveness, the role of low temperature exposure of winter planted chickpea in relation to Ascochyta blight development is not known but known in other host-pathogen pathosystems. Two experiments were conducted (In first experiment seedlings seven chickpea genotypes were exposed to 2, 4 and 6 days at 10^0C and inoculated with Pathotype-3 and the second experiment three chickpea genotypes were exposed at 5^OC for five and ten days and inoculated seedlings with four pathotypes (Pathotypes 1-4)with varying levels of aggressiveness. Seedlings grew at 20-22^0C in plastic house and inoculated with the different pathotypes were used as checks. Data on incubation and latent periods and disease severity (1-9 rating scales) were recorded. The results of the two experiments showed that Incubation and latent periods were decreased when genotypes were exposed to low temperature for longer days. Moreover, Ascochyta blight severity was increased on all genotypes where exposure time was long to cold temperature. This preliminary finding showed that low temperature exposure of winter planted chickpea in late December and January could pre-disposed Ascochyta blight and the level of resistance will not enough to provide the necessary crop protection and requires 1-2 fungicide sprays. Since this experiment used 5-10^OC, lower temperatures need to be explored to have more conclusive results.

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Response surface methodology approach to determine the influence of some environmental factors on mycelial growth and spore production of *Didymella pinodes*

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Ascochyta blight caused by *Didymella pinodes* (Berk. et Blox.) Vestergr. Can cause severe damage in pea. The objectives of the present study was to determine the optimum conditions for both the growth and spore production of *D. pinodes* in vitro on PDA medium using the response surface methodology. Overall, both the growth and sporulation were significantly influenced by the three environmental factors tested. Mycelial growth was optimal at 20C and decreased at 30C. Similarly, the spore production was maximum at 20C and ceased at 30C. The fungus grew at three pH levels with no sporulation at 8.1. The pH of 6.8 best for both growth and sporulation. RH% of 85% and 95% was favorable for both sporulation and growth with optimal response at 95%. There was positive interaction between the pH and temperature, and between temperature and RH. The quadratic regression model was checked using the coefficient of determination R^2, the adjusted R^2_{adj} and the multiple correlation coefficient R. All the three parameters were revealed high(R^2 = 0.933; R=0.966) which indicates high significance of the model and the correlation between the experimental data and the predicted values.

Keywords:
Response surface methodology, pH, temperature, *Didemylla pinodes*, relative humidity, growth, sporulation

Plant and canopy architecture to control ascochyta blight epidemics in pea fields

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The influence of plant and canopy architecture for the control of ascochyta blight, the main aerial disease in pea cropping, was studied using 5 spring pea cultivars susceptible to the disease and presenting various architectural traits. They were sown using a split-plot design with 3 levels of fungicide protection, and therefore levels of epidemics, as subplots and randomization of cultivars into subplots. Plots were screened both for plant and canopy architectural traits (canopy closure, plant and canopy height, leaf area index) and for the dynamics of disease severity over time. Besides, temperature and leaf wetness sensors were settled at three levels (bottom, middle and top) of the canopy for two cultivars with contrasted architectures. Time periods conducive for infection were calculated using the Magarey et al (2005) model.
Chosen spring pea cultivars distinguished themselves mainly by their height at blooming. Cultivars differences for canopy closure speed were weak, and all canopies closed before the beginning of the epidemics. Leaf area indexes did not vary much between varieties but leaf area densities did due to the significant height differences. Microclimatic conditions differed both within each canopy between bottom, medium and top sensors, and between canopies with contrasted architectures. They were more conducive to the disease at the bottom of the canopies, and in canopies showing higher leaf surface densities. Ascochyta blight epidemics varied significantly between cultivars according to canopy architectures, with lower disease severity within canopies showing lower leaf area densities and higher height.
These results suggest that higher canopies with lower leaf densities are likely to generate microclimatic conditions less conducive to the disease.

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Effect of pea seed infection with *Ascochyta pisi* on plant establishment and ascochyta blight development

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Ascochyta pisi is one among four pathogens that have been associated with ascochyta blight of pea. This species has been considered less virulent compared to *Peyronellaea pinodes*, which is considered the most dominant and damaging pathogen in this complex, including in Canada. For reasons unknown, *A. pisi* has been observed more commonly than *P. pinodes* in the southern and south-western parts of the Canadian province of Saskatchewan, in particular on seed samples tested for seed infections. Commercial pea seeds of cultivar CDC Patrick with very low *P. pinodes* infection based on commercial seed testing results, and 0.5, 5, 10 and 14.5% *A. pisi* infection were compared in field experiments to assess the impact of pea seed infection with *A. pisi* on seedling establishment and *A. pisi* development on pea. Although seedling emergence was lower in treatments with 10 and 14.5% seed infection, this had no effect on *A. pisi* development, seed yields or *A. pisi* infection of harvested pea seeds, indicating that seed infection up to 14.5% does not pose a risk to field pea production. To further explore seed infection by *A. pisi*, naturally infected seeds from field experiments were classified into categories based on the level of surface staining, and seed components were plated onto PDA after surface sterilization as described for *P. pinodes* by Moussart et al. (1998). Irrespective of the level of seed coat staining, *A. pisi* was present in all components of the seed, which is different from *P. pinodes* where only the seed coat was shown to be infected in seeds with staining up to 25% (Moussart et al., 1998).

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Ascochyta lentis var. *lathyri*, causing a new disease of grasspea (*Lathyrus sativus*) in Italy

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Grasspea (*Lathyrus sativus* L.) cultivation in Italy has gained renewed interest for low input and organic agricultural systems of central and southern Italy for the preservation of crop biodiversity. During field surveys in experimental plots, grasspea plants showing necrotic lesions on leaves and stems were observed. Identification of fungal isolates obtained from these lesions was performed by morphology using elliptic fourier analysis and MANOVA and by amplification with specific primers of the nuclear ribosomal internal transcribed spacer, and of fast-evolving protein-coding loci chitin synthase, translation elongation factor 1-alpha, glyceraldehyde-3-phosphate dehydrogenase. Under controlled environmental conditions, isolates from grasspea were able to infect and to induce symptoms similar to those observed in the field only on grasspea, but not on seedlings of nine other leguminous species including lentil. Elliptical Fourier analysis of conventional morphometric measures and 25 EFA coefficients demonstrated that conidial dimensions of the grasspea fungus were significantly different from those of *A. lentis*. Similarity ranging from 99.6% to 100% to sequences from *Ascochyta lentis* Vassiljevsky were obtained. Crosses between the grasspea fungus and *A. lentis*, were successful and produced progeny with normal cultural morphology and growth rates. Hybrid status of the progeny was confirmed by segregation of mating type and microsatellite markers, suggesting a recent common ancestor of these taxa. The overall results do not support separation of these taxa as either biological or phylogenetic species. On the basis of the obtained results, we considered that the fungus infecting grasspea is a pathogenic and morphological variant of *Ascochyta lentis* and should be named *Ascochyta lentis* var. *lathyri.*

Extracellular apyrase (PsAPY1) modulates the PAMP-induced oxidative burst and accumulation of *PR10-1*-mRNA in pea

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The plant cell wall, the most external layer of the plant surface, is the site where most pathogenic fungi first make contact with host cells. Our current research focusing on the plant cell wall have discovered the ecto-type ATP-hydrolyzing enzyme (apyrase; EC3.6.1.15) as a key player of extracellular defense. In response to elicitor preparation from *Mycosphaerella pinodes*, the catalytic activity in extracts from the cell walls of pea is enhanced *in vitro*, with the consequent increase of O_2^- generation by peroxidase(s). In contrast, the suppressins A and B, virulence factors secreted by the same fungus, potently inhibit the apyrase-dependent ATP-hydrolyzing activity in a host-specific manner. In this study we operationally silenced *PsAPY1* gene encodings the ecto-ATPase in pea, using an *Apple latent spherical virus* (ALSV)-based virus-induced gene silencing. The *PsAPY1*-silenced peas (*PsAPY1*) exhibited enhanced disease susceptibility phenotype against infection by pycnospores of *M. pinodes*, which was accompanied by a significant increase in the successful penetration. We also observed reduced responsibility of *PsAPY1* to chitosan and lipopolysaccharides (LPS), which is derived from fungal cell wall and glycolipid components of the outer membrane of the Gram-negative bacteria, respectively. Indeed, our *in vitro* study using extracts from the pea cell walls revealed that, in the presence of NADH, *p*-coumaric acid and Mn^{2+}, the SHAM (salicylhydroxamic acid)-sensitive O_2^- generating activity was enhanced in response to chitosan or LPS. Moreover, the chitosan-induced expression of *PR10*-mRNA was impaired in *PsAPY1*-silenced pea. On the basis of these results, it seems likely that PsAPY1 spatially regulates the apoplastic oxidative burst as well as the downstream signaling leading to expression of defense-related genes in pea. Considering the role of the apyrase (ecto-ATPase) in modulation of the cell wall-based defenses (see a presentation by Yamasaki *et al*.), it is also conceivable that *M. pinodes* targets the apyrase (ecto-ATPase) through secretion of the suppressors, to counter the extracellular defense of host cells.

The plant cell wall as a site for molecular contacts in fungal pathogenesis

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Establishment of the basic compatibility in plant-microbe interactions is recognized as the result of a sophisticated process, in which an adapted pathogen gained the ability to prevent or overcome preformed and induced defense mechanisms in a given plant species. This form of resistance is referred to a non-host resistance to resist infection by potential pathogens and is considered to be mounted upon the perception of pathogen-derived elicitors, also known as pathogen-associated molecular patterns (PAMPs), by cell surface pattern recognition receptors. Successful colonization by an appropriate pathogen can be therefore achieved by the suppression or circumvention of PAMP-triggered immunity through the secretion of pathogen-derived effectors. In *Mycosphaerella pinodes*, which causes leaf spots (blights) of pea, two structurally-related glycopeptide suppressors named Supprescins A and B are secreted in the pycnospore germination fluid. Pure suppressors potently inhibit the ecto-ATPase (apyrase; EC3.6.1.15) of host cell wall, temporarily reducing the ability of the host cells to defend itself. The catalytic activity in extracts from the cell walls of pea was enhanced *in vitro*, with the consequent increase of ROS generation by extracellular peroxidase(s), when exposed to the fungal elicitor. Interestingly, the Blue-Native PAGE analysis for cell wall proteins from pea epicotyls demonstrated that cell wall-associated, ecto- ATPase(s) formed a large protein complex(es) ranging from 450 to 900 kDa, one of components was the hydrogen peroxide-producing copper amine oxidase (CuAO). The CuAO activity was coordinately regulated with ATP-hydrolyzing activity *in vitro*, by an elicitor and a suppressor from *M. pinodes*. Moreover, *in vitro* treatment of cell wall proteins with the suppressor caused an appearance of the apyrase monomer. It is thus likely that *M. pinodes* targetss the host ecto-ATPase-containing protein complex(es) to attenuate cell wall-based, extracellular defense(s).

Microbial Associations with Legume Plant Trigger Systemic Resistance against *Didymella pinodes* Infection

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Species of ascochyta blight such as *Didymella pinodes* is one of the most damaging aerial pathogen of field pea (*Pisum sativum*. L) (Fondevilla et al., 2013; Tivoli and Banniza, 2007). It sometimes results in the loss of the entire crop, especially in monoculture and whenever there is a favourable environmental condition for its infection and further development. In a pot experiment, with and without *D. pinodes* infection tests were conducted to study the effects of three symbionts (*Rhizobium,* arbuscular mycorrhiza fungi and co-inoculation of the two) and a non-symbiotic control treatments on pea in a completely randomized design with four replicates. After infection with this pathogen, leaf metabolites and proteome, disease severity and shoot biomass and green areas were analysed. We found significantly highest biomass and green areas in pea plants inoculated with *Rhizobium*. Furthermore, the overall regulation of the citric acid cycle, amino acid and secondary metabolism including the pisatin pathway were most pronounced in rhizobia associated plants which had also the lowest infection rate and the slowest disease progression. The co-inoculation increased the synthesis of stress related proteins, while mycorrhizal treatments were involved in metal ion homeostasis and dampening of reactive oxygen species. Overall, we conclude that microbial associations, *Rhizobium* bacteria in particular, modify the interaction between host plant and the aerial pathogen.

Key words: biomass, field pea, inoculation, metabolome, mycorrhiza, rhizobia

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Extracellular apyrase (ecto-ATPase) impacts on the non-host resistance to fungal and bacterial pathogens

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In *Mycosphaerella pinodes*, which causes leaf spots (blights) of pea, two structurally-related glycopeptide suppressors named Supprescins A and B are secreted in the pycnospore germination fluid. Pure suppressors inhibit the ecto-ATPase activity of host cell wall as well as the peroxidase-catalyzed superoxide (O_2^-) generation, temporarily reducing the ability of the host cells to defend itself. Interestingly, the suppressors directly binds to and inhibit the ATPase, indicating that *M. pinodes* likely targets host's ATPase to counter the extracellular defense(s). In this study we silenced *PsAPY1* gene that encodes the ecto-ATPase in pea, using an *Apple latent spherical virus* (ALSV)-based virus-induced gene silencing. The *PsAPY1*-silenced peas (*||PsAPY1*) exhibited enhanced disease susceptibility even against infection by a non-adapted fungal pathogen, *Colletotrichum higginsianum*. Notably, on the surface of *PsAPY1*-silenced pea, the frequency of a hyphal tip-based entry (HTE), a recently discovered alternative way to enter the plant cells without formation of appressoria (Hiruma *et al*., 2010), significantly increased, eventually causing necrotic spots. This phenotype is quite similar to that in the *pen2* and *pen3* Arabidopsis mutants challenged with non-adapted *C. gleosporiodesm* (Hiruma *et al*., 2010). A similar phenomenon was also observed with inoculation with *Pseudomonas syringae* pv. *glycinea* (non- pathogenic to pea), that the non-adapted bacteria were able to propagate in the apoplatic space of *PsAPY1*-silenced pea. In our separate study (see a presentation by Miki *et al*.), we showed that *PsAPY1*-silenced pea attenuated the peroxidase-dependent oxidative burst induced by PAMP treatment. Taken together, it is likely that the ecto-ATPase (apyrase) play a role in extracellular defense(s) during the early stage of infection, especially before the initiation of PTI.

SESSION 3

Germplasm resources and plant breeding

Identification and validation of molecular markers for resistance to *Ascochyta lentis* in Australian lentil breeding germplasm

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Ascochyta blight is one of two economically significant fungal diseases in Australian lentil. Early in the introduction of lentil to Australia, resistance to *Ascochyta lentis* was set as a high breeding priority and there has been an ongoing effort to incorporate multiple sources of resistance in Australian lentil breeding germplasm. The main resistance sources used in the breeding program have originated from ICARDA (via ILL5588, also known as cultivar Northfield (NF)), and from the Canadian breeding program (via the resistant cultivar Indianhead (IH), or CDC Matador, derived in part from Indianhead). Using biparental mapping populations with Indianhead, Northfield and an Australian cultivar Digger (Dig) (IH x NF, IH x Dig, NF x Dig), we have generated molecular maps and identified three QTLs for resistance to *A. lentis*. Two QTLs, with associated SNP markers, were derived from resistant parent Indianhead, and a third QTL was derived from Northfield. These three QTLs have been validated in a panel of germplasm including Australian breeding material as well as foreign accessions. As a result of the ability of new *A. lentis* isolates to overcome one of the resistance genes derived from cv Northfield, the two QTLs from Indianhead were found to be the most effective in determining field resistance to ascochyta blight and recent field-derived isolates of *A. lentis*. The major QTL identified in two independent populations (IH x NF and IH x Dig) had the strongest effect (Vp = 47%), by itself correctly predicting resistant phenotype in 84% of lines in the validation panel of lentil germplasm. Phenotyping of Australian breeding lines with *A. lentis* isolates of alternative virulence patterns has also enabled the effect of secondary QTLs to be seen more clearly, with the effectiveness of two resistance QTL often seen across isolates.

Association mapping of partial resistance to *Didymella pinodes* and architectural traits in pea

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Ascochyta blight is the most damaging aerial disease of pea worldwide. Linkage mapping studies identified numerous quantitative trait loci (QTL) controlling partial resistance to the most detrimental pathogen of the ascochyta complex, i.e *Didymella pinodes*. These QTL often colocalise with loci controlling major architectural traits, which raises both hypothesis on involvement of linkage or pleiotropy effects in these colocalisation regions, and issues on breeding applications when partial resistance is linked to architectural undesirable alleles (Giorgetti, 2013). We started a Genome Wide Association approach to identify new loci, refine confidence intervals of previously reported QTLs, and confirm colocalisations through using both (i) the larger variability of a 192 pea accessions panel, comprising mainly winter field pea varieties from France and foreign countries (ii) the new molecular marker SNP resources recently made available (Tayeh et al, 2015; Boutet et al, 2016). The 192 pea accessions panel was screened both for partial resistance components in controlled conditions (using two single-spore isolates) and in the field (two environments). It was also genotyped using the SNP from the recently developed GenoPea 13.2K array (Tayeh et al, 2015).
Using the Multi Locus Mixed Model (Segura et al, 2012), new loci were detected for the control of partial resistance to *D. pinodes* and of architectural traits. Some QTL already identified in linkage studies were confirmed. The co-localisation region CLRVI.1 (Giorgetti, 2013) on LG VI between partial resistance components and plant ramification and stipule size was confirmed, but we could not conclude on linkage breakage between resistance and architectural traits in that region.
These results provide both new insights into comparative genetics of disease resistance and plant architecture and tools (SNP, haplotypes) to support breeding strategies for an improved control of ascochyta blight epidemics.

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Genome wide analysis of NBS-LRR genes in chickpea and their potential as candidate genes for ascochyta blight resistance

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Plant disease resistance genes are key components of genetic interaction between plant and fungal pathogen and among them a sub-class-Nucleotide binding site and leucine rich repeat (NBS-LRR) is the most common domain involved in governing resistance against the pathogen. This study tested the hypothesis that NBS-LRR genes are involved in resistance against ascochyta blight in chickpea. Genome wide analysis identified 115 NBS-LRR genes in the chickpea genome that comprises of 0.4 % of the total annotated genes. The NBS-LRR genes are not evenly distributed across the chickpea genome, and inclined to form clusters. Chromosome 5 has the highest number of the NBS-LRR genes (27% of mapped genes) while chromosome 8 has the lowest number of NBS-LRR genes (4%). A total of 26 NBS-LRR genes were co-localized with the previously reported QTLs for ascochyta blight resistance. Real-time PCR was used to measure relative expression of these 26 genes in four chickpea cultivars (three resistant and one susceptible) at different time points (6, 12, 24, 48 and 72 hours) after inoculation with isolate *AR170*. Differential expression as early as 6 h post inoculation between the moderately resistant (Amit) and the susceptible (ICCV 96029) cultivars was observed. Differential expression was also observed among the different resistant cultivars (CDC Luna, CDC Corinne and Amit) at different time points indicating the potential of these cultivars as different sources of resistance. Further efforts to examine the association between NBS-LRR genes with reaction to ascochyta blight infection were done using four recombinant inbred line (RIL) populations derived from crosses between resistant by susceptible and resistant by resistant genotypes under field and greenhouse conditions.

Evaluation of wild *Cicer* species accessions for resistance to *Ascochyta rabiei* (Pass.) Labr., the agent of ascochyta blight on chickpea (*Cicer arietinum* L.) in Algeria

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Twenty-five accessions of 5 wild *Cicer* species (*Cicer judaicum, C. bijugum, C. cuneatum, C. echinospermum* et *C. reticulatum*) were screened for resistance to ascochyta blight disease caused by *Ascochyta rabiei*, by artificially inoculating the germplasm under glasshouse at temperature ranged from 20±2 °c and relative humidity was maintained above 80% by sprinkling fresh water. Highly significant effect ($P<0.01$) was observed on their reaction to three pathotypes of *Ascochyta rabiei* (Mos02 'pathotype III: highly aggressive', At02 'pathotype II: moderate aggressive', and Sba02 'pathotype I: least aggressive'), there is a difference in accessions reaction to *A. rabiei* isolates but very important resistance was observed (>50% of accessions collection). All five *Cicer judaicum* accessions are resistant to *Ascochyta rabiei* isolates, two resistant accessions in the wild species *C. echinospermum* (ILWC0 and ILWC246) and 3 accessions in *C. reticulatum* (ILWC81, ILWC104 and ILWC247), *C. cuneatum* (ILWC37, ILWC40 and ILWC232) and *C. bijugum* (ILWC195, ILWC285 and ILWC286).

Key words: *Ascochyta rabiei*, *Cicer arietinum*, *Cicer* sp., aggressiveness, resistance.

Screening of wild lentil germplasm to identify novel *Ascochyta lentis* resistance sources

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Ascochyta blight (*Ascochyta lentis* Vassilievsky) in lentil is a devastating fungal disease, causing substantial yield losses and poor seed quality worldwide. Favourable climatic conditions and frequent evolution of aggressive isolates of *A. lentis* have increased disease severity in lentil and consequently led to susceptibility of previously released resistant cultivars such as Northfield and Nipper in Australia. Furthermore, the narrow genetic base of the current breeding program poses significant risk to further selective pathogen evolution against the currently used resistance genes. Introduction of diverse germplasm that contains potentially novel resistance genes into the resistance breeding program is proposed to improve stability of elite future cultivars. Accordingly, 30 accessions, previously reported to contain some level of resistance in the literature and sourced from five wild relative species (*Lens orientalis, Lens odomensis, Lens ervoides, Lens nigricans* and *Lens lamottei*) were screened for disease reaction to two highly virulent *A. lentis* isolates (FT13037 & FT13038) from South Australia. The disease reactions were determined using complete randomised and replicated bioassays conducted within controlled environment growth chambers and with accessions ILL 6002 (susceptible) and ILL 7537 (resistant) as controls. Disease symptoms were assessed using a qualitative scoring system (1-9) at 14 and 21 dpi (days post inoculation).

Distribution of disease reaction across the 30 accessions showed significant variation for disease reaction to both isolates. Interestingly, three accessions previously reported to be resistant to Canadian isolates were susceptible to Australian isolates. Among the accessions that were moderately resistant, six were of *L. nigricans* and two each were of *L. ervoides* and *L. orientalis* with one of *L. odomensis*. Of these, accession IG 72703 of *L. orientalis* was most resistant against both isolates and more resistant than ILL 7537. The moderately resistant *L. orientalis* accession potentially can be used in breeding program as it is from primary gene pool and a wild progenitor of the cultigen.

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Use of *Pisum* spp. in pea breeding for ascochyta blight resistance

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There is consensus that only incomplete resistance to ascochyta blight us available in pea germplasm and that the inheritance of resistance is largely polygenic with additive gene action playing a major role. Breeding strategies consisted in intercombining the available levels of incomplete resistant from different parents and selecting for greater resistance through progeny testing of segregating plants (Fondevilla et al. 2007; Warkentin et al. 2012). As a result, many current cultivars now have resistance similar to cv. Radley but not more than that. However, higher levels of resistance are available in wild germplasm.

In an attempt to exploit this resistance in pea breeding we throroughtly screened germplasm collections of *Pisum* yielding the identification of valuable sources of resistance (Fondevilla et al. 2005) that are being introduced in our breeding program. Inheritance has been studied identifying QTLs associated with resistance from *P. sativum* subsp. s*yriacum* (Fondevilla et al. 2008). More recently resistance mechanisms characterized and mapped (Carrillo et al. 2014). In spite of this advances in the characterization of the resistance responses at the histochemical, proteomic and genomic level (Carrillo et al. 2013; Castillejo et al. 2010; Fondevilla et al. 2011, 2014), actual progess in the breeding program has been slow. Althought we have broadened the array of resistances available, this did not yet result in the release of a cultivar more resistance than Radley. This contrasts with the successes that we experienced accumulating resistances to broomrape and powdery mildew. Perspectives will be discussed.

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Development of resistance sources to ascochyta blight caused by *Phoma exigua* var *diversispora* in common bean germplasm

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Ascochyta blights are major problems of many legumes and an emerging disease in many areas in which common bean (*Phaseolus vulgaris* L.) is grown. Symptoms in bean can be caused by three fungus species. We investigated the species that caused ascochyta blight symptoms in bean crops from northern Spain using nucleotide sequences of species-specific molecular markers. Results suggested that the two local isolates analyzed may correspond to *Phoma exigua* var *diversispora*. A total of 289 bean accessions and 11 *Phaseolus coccineus* accessions were screened in controlled conditions to identify potential sources of resistance. Reactions were scored using a 1 - 9 severity scale in five tests with 8-10 seedlings per test. Five lines were obtained by self-crossing from each of the 17 selected accessions showing higher levels of resistance. Evaluation of these lines allowed verification of the high level of resistance in lines derived from three accessions. The lines UI465, BGE04435-22 and BGE04453-4 revealed resistance levels not significantly different from the most resistant *P. coccineus* accession evaluated (score < 3.5). The identified resistance sources could be used in the short term to increase the level of resistance to ascochyta blight in specific bean genotypes or for development of new resistant genotypes by pyramiding of genes.

Climbing Beans Affected by Ascochyta spp. in the Guatemala Highlands

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Common bean (*Phaseolus vulgaris* L.) is the most important food legume and both bush-type beans and climbing beans are grown worldwide. However, climbing bean production is only present in few regions within specific countries in Africa (e.g. Rwanda, Uganda) as well as Central/South America (Guatemala, Southern Mexico, Ecuador, Peru, and Colombia). Historically, climbing beans have received less attention and breeding efforts worldwide in comparison with the bush-type beans commonly grown in the lowlands. Maize and beans are the main staple food in most poor households in Guatemala. Per capita bean consumption is approximately 12kg per year. Intercropping (locally known as Milpa) is the main production system in the highlands, where maize-bean is the most common. Unfortunately, on-farm productivity of these climbing beans is approximately 1/4 of their genetic yield potential mostly due to the lack of improved cultivars that are able to withstand biotic and abiotic stresses. Fungal and bacterial diseases as well as pests are the main cause for yield reductions. In addition, production is made with almost no inputs of fertilizers and/or other chemicals. Ascochyta leaf spot is one of the most yield-limiting diseases in this region. At least two species (*A. boltshauseri sacc*. and *A. phaseolorum sacc*.) have been identified in the region. The cold/wet conditions of the Guatemalan highlands favor the development and spread of this pathogen. In addition, faba beans (*Vicia faba* L.) are usually grown in conjunction with maize and beans, which can increase disease pressure. A field evaluation of the 600 accessions of the Guatemalan climbing bean collection allowed the identification of at 2 and 12 accessions with high and intermediate levels of resistance, respectively. In addition, 7 breeding lines with high levels of resistance were identified from a set of 91 lines from CIAT that combined Ascochyta resistance and high mineral content. All these resistant material is currently being used in crosses for the improvement of genetic resistance to this disease in the region.

Breeding Strategies for Ascochyta Resistance in Field Peas

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The multiple causal organisms of ascochyta blight in field peas complicates breeding for resistance. Furthermore, the partial nature of resistance indicates that multiple resistance genes may need to be combined for adequate control to each of the causal organisms. The Australian field pea breeding program conducts annual germplasm screening at Medina in WA, where there are frequent, naturally occurring epidemics. Adventitious disease notes are taken elsewhere in the country whenever disease outbreaks occur. A statistical, Multi-Environment Trial (MET) analysis has shown that the germplasm responds differentially to disease in different agro-ecological regions, a likely indication that different combinations of the ascochyta complex are prevalent. This disease information, along with field pea pedigree information, has allowed the development of transgressive segregation breeding strategies to pyramid loci in an effort to increase the level and stability of resistance.

SESSION 4

Pathogen genetics, genomics and populations

Virulence dynamics within *Ascochyta fabae* populations in Australia

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Didymella fabae and its anamorph (*Ascochyta fabae*) are both present in Australia, increasing the likelihood of genetic variability in the population (1). Monitoring of pathogen populations of *A. fabae* is a critical part of integrated disease management to identify shifts in pathogenicity and inform breeding programs to help mitigate potential resistance breakdown within faba bean germplasm. A series of controlled environment studies were used to examine pathogenic variation amongst *A. fabae* isolates collected in Australia and evaluate genetic diversity of faba bean accessions and durable sources of resistance to the pathogen. Seventeen *A. fabae* isolates collected from commercial crops from 1999-2012 from major growing regions within Australia were first tested on three *Vicia faba* cultivars, Icarus (susceptible), PBA Rana (resistant) and PBA Zahra (resistant). Disease severity varied from 5-57% LAD on susceptible plants. Whilst no isolate collected prior to 2012 caused symptoms on resistant plants, one isolate collected from a remote site in 2012 caused symptoms on the two resistant genotypes. Subsequent testing of isolates collected from 2012-2015 identified similar virulence patterns in several isolates collected in South Australia and Victoria indicating distinct virulence groups and the potential for loss of resistance. Of 31 isolates tested, 3 were highly aggressive on PBA Rana whilst 5 isolates collected in 2015 were highly aggressive on the resistant cultivar Farah and of these, 2 were also aggressive on PBA Rana. During this time severe disease in cv Farah crops grown in the lower- to mid-north districts of South Australia confirmed the resistance breakdown. Further testing of two isolates from these distinct virulence groups were used to screen 190 faba bean accessions and breeding lines representing eight different international origins and over 100 resistant lines from the Australian breeding program. Resistance to both virulent groups were identified within collections originating from the Middle-East and Mediterranean regions. Sustainable management of resistance to ascochyta blight will require constant monitoring of pathogen variability and development of lines with distinct sources of durable resistance to a variable and dynamic pathogen such as *A. fabae*.

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Genetic and pathogenic diversity of *Ascochyta fabae* Speg. population

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Simple sequence repeats and mating type markers were used to estimate the genetic diversity of 122 *Ascochyta fabae* (*Didymella fabae*) isolates collected from five regions of Tunisia in 2011, 2012 and 2013. Moreover, these isolates were phenotyped on Badï faba bean cultivar by measuring incubation period and necrotic leaf area percentage at different dates after inoculation and by calculating their AUDPC.

The two mating types were detected in Tunisia with a predominance of mating type MAT1-2. SSR analysis revealed 66 multilocus genotypes (MLG) among all isolates. Genetic diversity of *D. fabae* populations was low with inter-population variability accounting for only 14.5% of the total variation, whereas the genetic diversity within populations was high (74.3%). Principal Component Analysis (PCA) using phenotyping data revealed that these isolates could be grouped into 5 groups, according to their aggressiveness (incubation period, necrotic leaf area and AUDPC). Factorial Analysis of Multiple Correspondence considering mating type, MLG and aggressiveness groups, revealed no correlation between mating type and aggressiveness groups. However some MLGs are associated with aggressiveness groups particularly MLG68, MLG72 and MLG100 that are associated to groups 3, 4 and 5 characterized by a short incubation period.

Keywords: *Ascochyta fabae*, mating type, genetic diversity, aggressiveness, incubation

Patterns determining of population structure of *Didymella pinodes* in the Mediterranean Basin

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Plant diseases are caused by pathogen populations, which are continually exposed to evolutionary forces. Measurement of genetic and phenotypic variability is therefore essential to estimate speed adaptation of these populations and assess viability of control methods. Ascochyta blight, caused by *Dydimella pinodes* fungus is the most devastating foliar disease on pea crops. Our study assessed genetic and phenotypic variability of *D. pinodes* populations among three countries (France, Tunisia, and Algeria) of Mediterranean basin, close to the center of origin of pea crop. Genetic analysis, investigated with 13 microsatellites markers, shows that 88% of the genetic variability (148 haplotypes in 201samples) is most explain by intra-population variability. A genetic isolation by distance is found. Our study shows that the sexual reproduction is not implicated (Psex<0,00001 and IA=2,62) and that the variability is explain by the origin of primary inoculum, and by the individuals exchanges between populations. The phenotypic characterization, which was realized with a detached stipule assay on a set of four genotypes differing in their levels of susceptibility to *D. pinodes* (two with a partial resistance and two sensitive genotypes), shows the high level of aggressiveness variability of *D. pinodes* strain. This genetic and phenotypic variability shows the high adaptation potential of *D. pinodes*, and so the difficulty of establishing a management strategy.

Epidemiology of *Peyronellaea pinodes* isolates originating from wild and domesticated *Pisum* sp. in Israel

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Domesticated pea fields are grown in relatively close proximity to wild pea species in Israel. Despite the major role attributed to ascochyta blight in causing yield losses in domesticated pea, very limited information is available on the pathogens prevailing in natural ecosystems. The objectives of this study were (i) to identify the species causing ascochyta blight symptoms on leaves, stems, and petioles of domesticated pea and wild *Pisum* sp. plants in Israel, and (ii) to determine the factors governing disease development over time on individual plants. Eighteen fungal isolates were examined and identified; three of them were sampled from *P. sativum*, 11 from *P. fulvum*, and 4 from *P. elatius*. All isolates were identified as *Peyronellaea pinodes*. Analyses of the data revealed that temperature responses, spore germination rates, and aggressiveness of isolates sampled from domesticated pea plants did not differ from those of isolates sampled from adjacent or distant wild populations. Host specificity was not observed.
In a comprehensive survey that was conducted in the winters of 2007/8 and 2008/9 at two sites in northern Israel it was found that ascochyta blight was ubiquitous in *P. elatius* populations. Based on analyses of the survey data it was concluded that in natural ecosystems the teleomorph stage of *P. pinodes* serve as the main source of the primary and the secondary inoculum of the disease. These observations suggest that Israel may be inhabited by a single metapopulation of *P. pinodes*.

Diversity of aggressiveness of the *Ascochyta lentis* population in Australia

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The loss of resistance to *Ascochyta lentis* in Australia by the previously resistant lentil cv Nipper just 4 years after commercialisation was recently reported[1.] Field reactions of lentil cultivars against *A. lentis* have been studied since 2005, whilst pathogenic variability of the *A. lentis* population on commonly grown cultivars and on parental germplasm used in the Australian lentil breeding program have been more recently investigated in a number of controlled environment (CE) experiments. The change in response on cv Nipper was confirmed using isolates collected over different years inoculated onto differential host sets. Specific isolate/cultivar interactions produced a range of low to high aggressiveness disease reactions with isolates increasingly aggressive on the cvs Nipper and Northfield. This reaction was again verified when, of 40 isolates collected in 2015 and tested, 38 were aggressive on Nipper. Diversity in the aggressiveness of the *A. lentis* population has been previously reported[2,3]. A small percentage of isolates collected prior to the commercial release of cv Nipper can infect this cultivar[1] and may have subsequently been selected for in response to high intensity cropping. Spore release studies from naturally infested lentil stubbles from commercial crops also resulted in a high percentage of infection on cvs Nipper and Northfield whilst a very low level of disease developed on the resistant differentials ILL7537 and cv Indianhead. Eleven of 40 isolates collected in 2015 caused very low levels of disease in CE experiments on the moderately resistant cv PBA Hurricane XT. This poses a further risk of loss of effective disease resistance as the current Australian lentil crop is widely planted to this cultivar. Monitoring of pathogen populations is in progress including collection of new isolates from growing regions as well as a suite of field trials, controlled environment experiments and spore release studies.

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De novo genome and transcriptome sequencing combined with differential expression analysis identify putative pathogenicity factors from *Ascochyta rabiei*

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Efforts to control ascochyta blight in chickpea have mostly been based on the identification of resistance genes in the host. The identification of *A. rabiei* pathogenicity determinants will open a wide range of new alternatives to control ascochyta blight disease and increase our knowledge about Ascochyta-legume interactions. However, very little is known about the pathogenicity factor of *A. rabiei*. Equally, little information about *A. rabiei* is available at the molecular level. We have used Illumina and PacBio RS II platforms for sequencing the genome and transcriptome of an isolate of *A. rabiei* belonging to pathotype IV. *A. rabiei* secretome was also predicted. In addition, an accurate transcriptomic analysis using Massive Analysis of cDNA Ends (MACE) was performed to compare the gene expression profile of the fungus infecting chickpea with that of the fungus growing in absence of its host (1). A detailed analysis of the genes up-regulated during infection identified a collection of candidate pathogenicity factors and unravelled the strategies that allow the pathogen to cause disease: *A. rabiei* produces a battery of cell wall-degrading enzymes, used by the pathogen to penetrate its host, secretes toxins to kill the host cells, and is able to detoxify the fungitoxic compounds produced by the plant as defense.

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Determination of genetic and pathogenic diversity within Ascochyta bight pathogen of chickpea in Morocco

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Ascochyta blight caused by *Ascochyta rabiei* Lab. is the most destructive foliar disease on chickpea in Morocco and worldwide. Genetic variability among a population of *A. rabiei* isolates, collected from different chickpea growing regions in Morocco was assessed at 9 microsatellite loci and compared to other overseas populations from Syria and Turkey. Based on 47 amplified alleles, a high level of genetic diversity was observed among Moroccan population with the majority attributed to diversity within subpopulations (Gst = 0,16). Cluster analysis based on genetic similarity differentiated isolates into 10 genotypes with no geographical distribution pattern. A significant gene flow (Nm =2.53) were detected among pathogen populations originated from Morocco, Turkey and Syria suggesting a high pathogen migration probably due to seed exchange. The pathogenic variability among the Moroccan population of *A. rabiei* was assessed by screening over a set of chickpea differential genotypes. Isolates were classified into three pathotypes groups according to their level of virulence. Pathotypes PI and PII were the most prevalent; however the most aggressive pathotype PIII was present in the majority of surveyed regions. The results of this study revealed a significant potential risk for the spread of novel alleles or genotypes of pathogen that might contribute to fungicide resistance or the breakdown of resistance genes. The classification of isolates in to pathotypes groups will be useful to improved screening strategies of resistant germplasm in Morocco for more durable resistance.

Characterization of Ascochyta blight pathogens of chickpea, field pea and lentils in Montana

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Ascochyta blight (AB) causes yield losses in Montana, where nearly 450 thousand hectares were planted to cool season pulse crops (chickpea, field pea and lentils) in 2016. Seed-borne AB can result in early foliar infections and increased risk of losses due to the disease. The Regional Pulse Crop Diagnostic Laboratory detected high frequencies of seed infection by AB-causing fungi. *Ascochyta pisi* was detected in 57% of field pea seed lots (n=256), while Ascochyta rabiei was detected in 29% (n=35) of chickpea seed lots and Ascochyta lentis was detected in 18% (n=170) of lentil seed lots sent from 18 counties in Montana. Quinone outside inhibitor (QoI) fungicides were the choice of farmers for management of AB in chickpeas until a mutation on the cytochrome b gene was determined to confer resistance. Fungicide resistant Ascochyta rabiei isolates were found in one 2015 chickpea seed lot sent from Daniels County, MT. The G143A was responsible for resistance in A. rabiei isolates from this seed-lot. The AB complex in field pea is caused by a combination of pathogens either in single or multiple infection. Molecular characterization of fungi recovered from field pea seed lots (n=100) conducted using ITS gene and SSR markers revealed 100% infection by *A. pisi*. Additionally, 13% and 7% were co-infected by *Didymella pisi* and *Phoma* spp., respectively. These results suggest diversity in the pathogens causing AB in pea with likely variations in pathogenicity and aggressiveness.

Characterization of fungal species complex of Ascochyta blight developing on wild and cultivated legumes and their host spectrum evaluation

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Spatial and temporal dynamic of host plants, and life history traits associated to pathogens are crucial parameters to explain how plant-pathogens interact and evolve. Ascochyta blight due to Ascochyta genus fungi, is a serious disease infected cultivated and wild legume species worldwide. Co-occurence of these fungal species on both legume species supports the idea that this pathosystem is an appropriate system to address evolutionary questions about the role of host specificity in the fungal speciation. Genetic analysis performed using 3 genes of interest (ITS, EF, CHS) on isolates sampled on different wild and cultivated legume species associated a lot of isolates to Ascochyta genus, with a particular genetic proximity from several isolates to *A. pinodes, A. viciae* and *Phoma spp*. Phenotyping of 28 isolates selected according their host (wild or cultivated) on 12 legume species (5 cultivated and 7 wild) revealed different levels of host specialization. Results showed that origin of isolates (wild or cultivated host) did not contribute to the behavior of these isolates toward the different wild and cultivate legume species tested. Finally, genetic and phenotyping data comparison showed that genetic proximity between isolates and/or host did not play a role on isolate specialization. These results are indicative of the adaptation potential of Ascochyta blight pathogen complex and underline the importance to integrate this knowledge for improving the control of this disease.

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