2nd International Workshop on Barley Leaf Diseases

(April 5-7, 2017)

Organized by

The International Center for Agricultural Research in the Dry Areas (ICARDA)

Venue

Institut Agronomique et Vétérinaire Hassan-II

Rabat, Morocco

Compiled by

Sajid Rehman

&

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ICARDA, Rabat

Morocco

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Welcome to the 2nd International Workshop on Barley Leaf Diseases

The 2nd International Workshop on Barley Leaf Diseases (2nd IWBLD) is being organized by ICARDA at Rabat, Morocco, April 5-7, 2017. The workshop will provide a forum for the exchange of information and ideas relating to understanding and controlling leaf diseases of barley. The goal of the event is to bring together international experts and young students interested in recent advanced studies in all aspects (theoretical and applied) of barley leaf diseases. Leaf diseases of barley are of economic importance and cause heavy losses in the WANA region. Most of the barley in this region is cultivated under challenging moisture and temperature conditions with minimum inputs and targeted as a food and feed/grazing crop for humans and livestock, respectively. Across the WANA region, foliar diseases result in poor grain yield as well as reduced quality of grain, forage and other components. Chemical control measures are seldom if ever used in this region; thus, to ensure sustainable barley production of the highest quality possible for various end uses, an integrated approach is needed. To produce a healthy barley, crop biotic stress management is an important requirement so that it can withstand the abiotic stresses in the region.
The 2017 Rabat event is a continuation of the 1st IWBLD, held in Salsomaggiore Terme, Italy during June 2014. The 1st IWBLD, replaced the "International Workshop on Barley Leaf Blights", with the ISC's goal to include a wider group of researchers investigating all barley leaf diseases, instead of only leaf blights.

The motto of the workshop is ***"Preventing leaf disease losses of barley for small holder farmers in West Asia and North Africa (WANA)".***

With this background, we cordially invite you to actively participate in and enjoy the 2nd IWBLD in Rabat, Morocco.

M. Baum and Ramesh Verma

On behalf of
The local Organizing Committee,
ICARDA Rabat, Morocco

Scientific Program

Day 1 (5 April, 2017)

Registration & Field Visit

Date	Item	Venue	Time
05-Apr 17	Registration	IAV Hassan-II, Rabat	8.30-9.00
	Visit to ICARDA Labs	Green-ICARDA building	9.00-10.00
	Experimental farm and farmers' fields visit	ICARDA Station, Marchouch	10.00-17.00
	Poster set up	IAV Hassan-II, Rabat	17:30-20:00

Day 2 (6 April, 2017)

Opening Session
Time: 9.00-10.30

Date	Item	Speakers	Time
06-Apr 17	Opening remarks	M. Baum (LOC Chair)	9.00-9.10
	Welcome address	Brian Steffenson (IOC Chair)	9.10-9.20
	Remarks by Director, IAV Hassan-II	Wafae Fassi Fihri	9.20-9.30
	Address by Director, Dryland Cereals	Shoba Sivasankar	9.30-9.40
	Address DG, INRA, Morocco	Mohamad Badraoui	9.40-9.50
	Vote of thanks	Ramesh Verma	9.50-10.00
Coffee Break			10.00- 10.30

Session I: Genetics of Disease Resistance

Chair: Michele Stanca **Time: 10.30-13.30**

Date	Title	Speakers	Time
06-Apr 17	**Keynote Address:** The genetic basis of resistance to *Rhynchosporium commune* in a UK spring barley cultivar collection	Mark Looseley	10.30-11.10
	Identification of QTL for resistance to *Pyrenophora teres* f. *teres* and *Cochliobolus sativus* in Barley employing Genome Wide Association Studies	Fluturë Novakazi	11.10-11.30
	Genome-wide association study of stripe rust (*Puccinia striiformis* f.sp. *hordei*) resistance in a world collection of barley (*Hordeum vulgare* L.)	Ramesh Verma	11.30-11.50
	Towards positional cloning of leaf rust resistance gene RphMBR102 in barley	Frank Ordon	11.50-12.10
Lunch Break			**12.10-13.30**

Session II: Evaluation and breeding for disease resistance

Chair: Ramesh Verma **Time: 13.30-16.00**

Date	Title	Speakers	Time
06-Apr 17	**Keynote Address:** Diversity for resistance to *Pyrenophora graminea* in a European spring barley cultivar collection and association mapping of the involved genes	Alessandro Tondelli	13.30-14.10
	Resistance of Jordanian and Syrian landraces against barley leaf spot diseases	Marja Jalli	14.10-14.30
	Evaluation of ICARDA barley germplasm for resistance to spot blotch (*Bipolaris sorokiniana*) in India	S.S. Vaish	14.30-14.50
	Alternative hosts of yellow dwarf viruses (YDVs) and sources of resistance in barley in Ethiopia	Seid Kemal	14.50-15.10
Coffee Break & Posters View			15.10-16.00

Session III: Molecular plant-pathogen interactions

Chair: Michael Baum **Time: 16.00-18.30**

Date	Title	Speakers	Time
06-Apr 17	**Keynote Address:** The role of *Rhynchosporium commune* effectors in the interaction with barley	Anna Avrova	16.00-16.40
	The mystery of barley spot blotch disease caused by *Bipolaris sorokiniana*	Michael Lyngkjær	16.40-17.00
	The genetics of virulence in the *Pyrenophora teres f. teres* population BB25 × FGOH04Ptt-21	Tim Friesen	17.00-17.20
Poster Session			17.20-18.30
Workshop Dinner			19.30-21.00

Day 3 (7 April, 2017)

Session IV: Structural and functional genomics of barley leaf pathogens

Chair: Brian Steffenson **Time: 9.00-11.30**

Date	Title	Speakers	Time
07-Apr 17	**Keynote Address:** Sequencing of 19 isolates of the fungus *Ramularia collocygni* from multiple origins for improved understanding of the biology using a population genetic approach	Michael Hess	9.00- 9.40
	Pyrenophora teres genome plasticity	Simon Ellwood	9.40-10.10
	Flash-N-Dash	Five minute each presentations	10.10-11.00
Poster Session & Coffee Break			11.00 -11.30

Session V: Epidemiology and integrated management of barley leaf diseases

Chair: Brahim El Yousfi **Time: 11.30-14.00**

Date	Title	Speakers	Time
07-Apr 17	**Keynote Address:** Advances in the integrated management of leaf blotches in Uruguay	Silvia Pereyra	11.30-12.10
	Occurrence of barley leaf disease and control strategies in Denmark	Thies Marten Heick	12.10-12.30
	Sensitivity of *Pyrenophora teres* isolates to propiconazole and pyraclostrobin in the Canadian prairies	Kelly Turkington	12.30-12.50
	Poster session & Lunch Break		**12.50-14.00**

Session VI: Evolution and variation in pathogen populations

Chair: Adrian Newton **Time: 14.00-17.30**

Date	Title	Speakers	Time
07-Apr 17	**Keynote Address:** Diversity in NFNB in South Australia and the detection of durable resistance	Hugh Wallwork	14.00-14.40
	Genetic diversity of *Pyrenophora teres f. teres* in Tunisia using pathotype and AFLP markers	Aida Bouajila	14.40-15.10
	Development of a set of differential barley genotypes and analysis of virulence diversity in the population of *Bipolaris sorokiniana* in China	Ruiming Lin	15.10-15.30
	Evidence of *Pyrenophora teres f. teres* adaptation to barley cultivars with different levels of resistance	Brahim El Yousfi	15.30-15.50
	Coffee Break and Poster Session		**15:50-17:00**

2nd IWBLD - ISC Business Meeting	**16.00-17.00**

Plenary Session

Chair: Brian Steffenson **Time: 17.00-18.00**

Date	Title	Speakers	Time
07-Apr 17	Venue for 3rd IWBLD	All concerned (proposal submission by interested institutions)	17.00-17.15
	Experiences with 2nd IWBLD & Concluding Remarks	Brian Steffenson (ISC Chair)	17.15-17.45
	Vote of thanks	M. Baum (LOC Chair)	17.45-18.00

Contents

Session-I

Genetics of Disease Resistance

The genetic basis of resistance to *Rhynchosporium commune* in a UK spring barley cultivar collection

Mark Looseley[1], Lucie Griffe[1], Bianca Buettner[2], Anna Avrova[1]

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Rhynchosporium commune is a polycyclic fungal pathogen that causes leaf scald; one of the most economically important and destructive diseases of barley (*Hordeum spp*). Agronomic practices in combination with foliar fungicides can provide good level of disease control but represent a significant cost to farmers. Additionally, high levels of fungicide use can induce insensitivity to effective fungicide classes. The most sustainable method of protecting against *R. commune* is through the production of durably resistant barley cultivars, but this requires the identification of tightly linked markers to a number of resistance genes.

To address this demand, a collection of 660 UK spring barley varieties was grown in a field disease nursery at the James Hutton institute in Dundee, Scotland over three growing seasons. Disease severity was recorded at multiple time-points within each growing season and mean AUDPS scores estimated for each line. Multi-environment association mapping methods (treating years as environments) identified a number of QTL in genomic regions previously shown to contain major resistance genes against *R. commune*, in particular *Rrs1* on 3H and *Rrs2* on 7H. In addition, novel QTL on 2H and 5H were detected. These results indicate the importance of major resistance genes in determining resistance in UK spring barley and their continued effectiveness against natural populations of *R. commune*.

Phenotyping with *R. commune* strains expressing the recognised form of NIP1 confirmed that the most significant QTL effect identified was the major resistance gene *Rrs1*. Additional genotyping and phenotyping using a barley landrace collection allowed further refinement of the QTL interval, leading to the identification of diagnostic markers.

These results represent a resource that will allow the efficient production of new, durably resistant, varieties by barley breeders when incorporated into a marker assisted breeding.

Identification of QTL for resistance to *Pyrenophora teres* f. *teres* and *Cochliobolus sativus* in Barley employing Genome Wide Association Studies

Fluturë Novakazi[1], Anna Anisimova[2], Olga Afanasenko[2], Doris Kopahnke[1], Frank Ordon[1]

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Pyrenophora teres f. *teres* (PTT) and *Cochliobolus sativus* (CS) are the causal agents of the net type of net blotch and spot blotch in barley, respectively. Both fungal pathogens are widely spread and cause high yield losses. The most cost effective and environment-friendly way to prevent and control these pathogens is growing resistant cultivars. In order to identify sources of resistance, in a first step more than 10,000 barley accessions including landraces and commercial cultivars were screened for resistance to PTT and CS under greenhouse and field conditions. Out of these, 450 barley accessions derived from the centres of barley diversity, and expressing different levels of resistance to respective pathogens were selected.
Next, greenhouse experiments were conducted with these 450 accessions with two PTT and CS isolates, respectively. Three week old plantlets were inoculated with a spore suspension of 5000 spores/ mL and assessed for symptom expression 14 dpi. Additionally, field trials were conducted in Russia, Belarus and Germany. The disease severity was scored three times during the growing season to calculate the area under disease progress curve (AUDPC).
In parallel respective genotypes were genotyped with the Barley 9k iSelect chip. Markers with a minor allele frequency (MAF) <5%, missing data >10% and heterozygosity >12·5% were removed prior to conducting genome wide association studies (GWAS). The population structure and kinship were calculated based on 508 markers with high PIC-values covering the whole genome at an average distance of about 2 cM. GWAS was carried out using the software TASSEL 5 and a Mixed Linear Model (MLM) including population structure and kinship and a false discovery rate of FDR=0·1. Regions associated with PTT resistance were identified on all chromosomes and two regions associated with CS resistance on chromosomes 2H and 7H.

Genome-wide association study of stripe rust (*Puccinia striiformis* f.sp. *hordei*) resistance in a world collection of barley (*Hordeum vulgare* L.)

Sanjaya Gyawali[1,5], Shiaoman Chao[2], S Mamidi[3], Reda Amezrou[1], A Sreedasyam[2], SC Bhardwaj[4], Sajid Rehman[1] Ramesh PS Verma[1]

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Stripe rust (*Puccicina striiformis* f.sp. *hordei*) (PSH) is one of the major diseases that causes significant yield losses of barley (*Hordeium vulgare* L.) worldwide. Frequent breakdown of resistance genes and emergence of new races have necessitated research for new sources of resistance. The genetic map of PSH resistance in barley is unknown against Indian races. We performed genome-wide association study (GWAS) of stripe rust resistance using 336 barley genotypes collected world-wide. Barley genotypes were evaluated for stripe rust resistance using six races prevalent in India; Q (5S0), 24 (0S0-1), 57 (0S0), M (1S0), G (4S0) and 7S0 (newly reported race). Seedling screening with individual race was carried out in ICAR-IIWBR Regional Station, Shimla, while field screening was carried out at adult stage with mixed inoculum of all the races in Durgapura, Rajasthan, India in 2015. Barley genotypes were genotyped using 9K SNP array based on iSelect Illumina Inifinium Assay in USDA-ARS, Fargo, USA. GWAS was carried out in TASSEL using mixed linear model (MLM) where population structure and Kinship were used as fixed and random effects, respectively. Out of 336 genotypes, 87 (25.9%), 101 (30.1%), 119 (35.4%), 100 (29.8%), 91 (27.1%), 70 (20%) were resistant to races M (1S0), 24 (0S0-1), 57 (0S0), G (4S0), Q (5S0), and 7S0 races, respectively. Both population structure and PCA analyses revealed two sub-populations predominantly based on row types. GWAS revealed 12 race-specific seedling resistance QTL for stripe rust, while seven adult-plant stage QTL were mapped in different chromosomes. Gene annotation using BLAST search revealed genes encoding 1-Phosphatidylinositol-3-phosphate 5-kinase, Glycine-rich domain-containing protein, Purine biosynthesis PurH-like protein, Dof-type zinc finger protein 15 (Dof15), RNA polymerase II transcription subunit 27, NAC transcription factor (NAC012 gene), WUSCHEL-related homeobox 8, Ubiquitin-conjugating enzyme (UBC) and uncharacterized proteins. The QTL mapped in the current study shall be important for PSH resistant barley breeding in India and elsewhere.

Towards positional cloning of leaf rust resistance gene RphMBR102 in barley

Leila Fazlikhani, Frank Ordon, Doris Kopahnke, Dragan Perovic

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The fungal pathogen *Puccinia* hordei Otth is the causal agent of barley leaf rust, an economically important disease of barley in temperate regions, which can considerably reduce yield of susceptible cultivars. Growing of resistant barley cultivars is an efficient way to control this disease, but the deployment of resistance genes is counteracted by the occurrence of new, virulent races of the pathogen. Therefore, there is a constant need for the identification and the deployment of new sources of resistance. Previously the resistance gene RphMBR1012, has been identified in the landrace "MBR1012" and mapped to the short arm of chromosome 1H. In order to get detailed information on this resistance, map-based cloning of the RphMBR1012-locus was initiated. In the course of the development of the high-resolution mapping population (HRMP), 4775 F2-plants were analyzed for recombination in the target interval spanning 8.2 cM by flanking markers and 750 plants turned out to be heterozygous recombinants while 50 plants were homozygous recombinants. Of the heterozygous recombinants, 12 single plants were tested in the F3 generation in order to identify segmental homozygous recombinant inbred lines (RILs). Up to now, a set of 608 F3 recombinants was converted to segmental F4 RILs. This high resolution mapping population (resolution of about 0.01% recombination) is now the back bone for marker saturation. Up to now, 35 markers have been integrated, shortening the interval to 0.12 cM. Further marker saturation will be achieved using the Illumina 50K iSelect array, exome capture, and RenSeq based screening of parental lines. Respective closely linked markers will be the basis for physical map construction and anchoring of the RphMBR1012-locus to the barley genome reference sequence. In addition, to accelerate the gene isolation a non-gridded BAC library with 3x genome coverage was constructed from the landrace "MBR1012", which will be screened with the closest molecular markers. Results of this study will on the one hand lead to a deeper understanding of the function of leaf rust resistance genes and will on the other hand contribute to a more directed use of genetic resources as a prerequisite for breeding resistant cultivars followed by their use in consumer and environmental friendly barley production systems.

Intraspecific population structure of agents causing barley net blotch and barley powdery mildew in Kazakhstan

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Kazakhstan is the main manufacturer of grain of feeding and brewing barley among the countries of Central Asia and Transcaucasia. However, effectiveness of barley cultivation in separate years decreases because of affection by fungal diseases which affect cereals during all vegetation process from sprouting before harvesting. The results of phytosanitary monitoring showed that barley net blotch (*Pyrenophora teres f. teres*) and barley powdery mildew (*Erysiphe graminis DC f. sp. hordei Em. Marchal*) are the most widespread and dangerous among the known fungal diseases of barley in Kazakhstan. In 2012-2016 years the intensive development of barley net blotch is noted in the southern, east and northern regions of the republic which distribution was within 20-70%, and disease development – 30-80%, respectively. In Kazakhstan barley powdery mildew is generally widespread on the production sowings of crops of winter barley in Almatinskiy, Zhambylskiy and Southern-Kazakhstanskiy oblasts. Populations of *P. teres f. teres* with using of the international set of varieties-differentiators of barley net blotch are analyzed *in vitro* (Afanasenko et al., 2009): Harrington, Canadian Lake Shore, Skiff, Prior, c-20019, Harbin, CI9825, CI5791, c-8755. As a result of isolates rate, virulent to Skiff and Harrington varieties were high in all populations. The significant variation on virulence rates to Prior and Harbin varieties with Pt2 gene is detected at isolates from all fungus populations. All tested Kazakhstan isolates were avirulent to CI9825 line with Rptlb, Pt5, Pt11 and Pt12 genes. Differentiation of pathotypes of barley powdery mildew is carried out with use of Pallas isogenic varieties lines (Kølster et al., 1986). There are both weak virulent and strong virulent samples among the isolated pathotypes. The isolated pathotypes of fungus showed high virulence to the isogenic lines P09 and P16 with Mla10, Ml(Du2) and Mlk genes, respectively. Fungus pathotypes affected resistance genes of Mla6 and Mlo-5 are not found, pathotypes with susceptible reaction to Mlg gene are also seldom isolated.

Genome wide association analysis for stripe rust resistance in barley seedlings and adult plant stage in India

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Barley stripe rust (*Puccinia striiformis* f. sp. *hordei,* PSH) occurs worldwide and is a major fungal disease in barley in Southern Asia. In order to identify and the estimate effects of loci underlying quantitative resistance to PSH, an association mapping panel of 261 barley genotypes consisting of released cultivars, advanced lines, differentials, and local landraces from the ICARDA barley breeding program and from North and South America, Europe, and Australia was screened for seedling and adult plant resistances to barley stripe rust. Seedling resistance evaluations were undertaken for 261 barley genotypes with the five prevalent PSH races, 0 (5S0), 24 (0S0-1), 57 (0S0), M (1S0), and G (4S0), individually at ICAR-IIWBR Shimla. The field screening was performed in two different locations, Durgapura (Rajasthan, India) in 2013 and 2014, and at Karnal (Haryana, India) in 2014 under artificial inoculation using a mixture of the five PSH races. The panel was genotyped with the DaRT-Seq high-throughput genotyping platform. The final markers sets used for this work comprises 13,182 PAV and 6,311 SNPs. Results of genome-wide association scans, using the PAV marker set, showed positive associations with resistance to PSH for both seedlings stage and adult plant stages. We identified 49 significant marker-trait associations corresponding to 32 QTL located across the seven barley chromosomes for stripe rust resistance at seedlings stage for the five races. A total of 21 significant marker trait associations resulting in 6 QTL were identified on all chromosomes except 7H for adult plant resistance. Common QTL for resistance to different races of PSH resistance at seedling stage were found on chromosomes 4H and 5H. For adult plant resistance we found a QTL on the long arm chromosome 5H stable across environments and years. Performing GWAS, using the SNP marker set we found 42 significant associations corresponding to 20 QTL, located on the seven barley chromosomes for seedling resistance for the five PSH races. For resistance at field screening, 17 positive associations corresponding to 10 QTL located on chromosomes 1H, 2H, 3H, 5H and 7H were found. The QTL detected in this study will be useful for future PSH resistance breeding efforts in the India and in other countries.

Fine mapping and characterisation of Rrs18, a new resistance gene against *Rhynchosporium* in barley

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Rhynchosporium commune, causing leaf scald, is one of the most destructive pathogens of barley worldwide, leading to yield losses of up to 30-40%. Despite advances in molecular marker technology and the sequencing of the barley genome, little is known about barley resistance to Rhynchosporium at the molecular level. The major resistance gene Rrs13 has been mapped to the short arm of chromosome 6H in a BCline 30 x Clipper population. A large resistance QTL on 6HS has also been identified in CIho3515 x Alexis and Steptoe x Morex double haploid (DH) populations, with both QTL flanking markers sharing very similar physical map positions. The latest version of the barley genome assembly revealed that the large QTL identified in the Steptoe x Morex and Clho3515 x Alexis populations is located at a different locus to that of Rrs13, and has subsequently been named Rrs18. Fine mapping using selected Steptoe x Morex BC1 F3 lines has been combined with exome capture variance calling to narrow down the interval containing Rrs18 to below 1 Mb and to identify candidate genes for Rrs18. Further fine mapping will shorten this list of candidates and enable development of diagnostic molecular markers for use by breeders. Sequencing of RNA from resistant and susceptible lines will be used to identify genes or alleles specific to resistant lines which might be missing in the genome annotation of susceptible cultivar Morex. Candidate genes will be validated by overexpression in susceptible cultivar Golden Promise to identify and clone the first barley resistance gene to *Rhynchosporium*.

Studies on the resistance of barley genotypes to *Pyrenophora teres f. teres* in the seedling stage

Mónika Cséplő, József Bakonyi, Judit Bányai, Ildikó Karsai, Gyula Vida, Klára Mészáros

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Barley (Hordeum vulgare L.) is among the four most substantial cereals in the world and in Hungary. *Pyrenophora teres f. teres*, the causal agent of the net form net blotch disease, is known as one of the main fungal pathogens of barley worldwide. Deployment of resistant cultivars is the most economic and eco-friendly method to control plant diseases. The objective of this study was to identify the resistance of young plants of various barley genotypes after artificial inoculation with *Pyrenophora teres f. teres*. 11 young barley genotypes (C) were inoculated under greenhouse conditions with mycelium suspension of *Pyrenophora teres f. teres* isolate (H-502/1 derived from Hungary). In the study various inoculation methods were used. The seedlings were infected (A) by hand sprayer/ brush, (B) by detached leaf method, with pre-washing/no washing before fertilization. The area under the disease progress curve (AUDPC) was calculated from the lesion types (Tekauz scale) at various times.
Based on the results of analysis of variance, significant differences were found among the genotypes, but no significant differences were found among the various inoculations methods, although the effect of A×B, B×C and A×B×C was significant. This indicates that the infection of some genotypes was affected by the inoculation methods. In average, the most susceptible reaction of all tested cultivars was observed in case of cv 'GK-Judy', while cv 'Hanzi', cv 'Antonella', cv 'Canela' and Mv08-13 lines were significantly more resistant than the experimental mean. The whole seedling resistance (AUDPC values of lesion type) exhibited a significantly positive moderate correlation (r = 0.58 –0.61) with detached leaf technique data by correlation analysis (AUDPC values of the lesion type from point inoculation). Further analysis of barley genotypes could contribute to the complex understanding of net blotch resistance, which could increase the selection efficiency.

Acknowledgements: This work was supported by the NRDI Fund; project number: 119276

Association Mapping of Powdery mildew resistance in barley

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Abstract

Powdery mildew caused by *Blumeria graminis* f sp. *hordei* is an important foliar disease of barley crop worldwide. The disease causes 25 to 30 % yield losses in susceptible cultivars and can reach up to 50 % if the climatic conditions are pertaining to spreading of the disease. Many strategies were improved to in the battle against the disease but the most economic and environmental friendly way to control powdery mildew remains the use of robust varieties with durable resistance. Thus, the objectives of this study were set to identify the genomic regions conditioning resistance to powdery mildew in 336 barley genotypes originating from ICARDA germplasm, as well as to identify markers tightly linked with these loci, to help in better integration of resistance in improved germplasm. An Association Mapping panel (AM panel) was genotyped using 9K iSELECT SNP markers and phenotyped in an alpha-lattice design with two replicates, during two cropping seasons (2015 and 2016) in four hot-spot locations known for their favorable environmental conditions of epidemics development. A mixed linear model (structure and kinship as covariate) was used to identify significant marker-trait associations (MTA) linked with powdery mildew resistance. A total of 39 MTAs were found associated with resistance/susceptibility across all seven chromosomes. Using a cutoff of 5cM, the results show 27 distinct genomic regions conditioning the resistance or susceptibility to the pathogen, including some of the already reported R-genes (Mla6, MlLa, Mlg, Ror2, Mlra). The novel loci identified in the current study may represent a broad spectrum of powdery mildew resistance and will be useful in marker-assisted barley improvement.

Barley -*Magnaporthe oryzae* pathosystem: The Specifity and Genetics of Host Resistance

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Blast disease on cultivated cereals including rice, wheat and barley is caused by the ascomycete fungus *Magnaporthe oryzae*. We studied the host status of wild and cultivated barley to the *tritici, oryzae* and *penniseti* forms of *M. oryzae*. Our screening experiments suggest that both cultivated and wild barley are at seedling stage host to *tritici* and *oryzae* forms. Our results also indicated that the cultivated barley is a nonhost to the *penniseti* form but the wild barley is a near nonhost to this form. Sources of complete and partial resistance to both the *tritici* and *oryzae* forms were identified. We studied the genetic basis of resistance to the *oryzae* and/or *tritici* forms in two RIL mapping populations, the L94 x Vada (LnVa) and Vada x SusPtrit (VaSu), and an association mapping population of West European barley cultivars. In both LnVa and VaSu mapping populations, one large effect QTL (*Rmoq1*) mapped on the short arm of chromosome 7H against the *tritici* isolate and another QTL (*Rmoq3),* was mapped on the long arm of chromosome 5H in the LnVa population against the *oryzae* isolate. In the association mapping study, eighteen markers were associated with resistance against the *tritici* form and twelve markers were associated for resistance against the *oryzae* form. One of the QTLs was associated with resistance against both *oryzae* and *tritici* forms. Seven QTLs mapped in our study coincided with seven already reported QTLs for blast resistance in barley. Our screening and genetic analysis of blast resistance suggests that unlike the wheat, the barley gene pool is reach for sources of resistance against the *Magnaporthe*.

Genome-wide association study for Powdery mildew resistance in Nordic Spring Barley

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This study is a part of the Public Private Partnership (PPP) on pre-breeding initiated by the Nordic Council of Ministers. A main goal for this study is to lay the foundation for effective barley breeding for disease resistance and yield stability that can meet current and future climate challenges in the Nordic region. One of the most important existing world-wide threats to barley (*Hordeum vulgare* L.) production is the powdery mildew disease caused by the fungus, *Blumeria graminis* f.sp *hordei* (Bgh). To develop resistant varieties for the future, it is important to identify existing and new resistance loci and alleles for powdery mildew, preferable with broad-spectrum resistance, to obtain long-term effectiveness against the powdery mildew pathogen. The aim with this study was to identify significant marker-trait associations for powdery mildew resistance in a Nordic spring barley panel using GWAS. For this purpose 169 breeding lines and cultivars, representing the Nordic region and provided by Boreal Plant Breeding, Graminor, Lantmännen Lantbruk, Nordic Seed and Sejet Plant Breeding, were phenotyped for powdery mildew resistance at four different locations in 2012, 2013 and 2014, with total of 24 observations. The barley panel was chosen to represent the available genetic variation in current elite Nordic barley germplasm and was genotyped with the Illumina iSelect 9K SNP barley chip and with 48 microsatellite markers spanning over the seven chromosomes. Several general and mixed linear models were compared and the best model to account for population structure was chosen for the final GWAS analysis. In total four QTLs located on chromosome 4H and 6H, were identified, comprising marker candidates that can be exploited for use in marker-assisted selection for powdery mildew resistance in barley breeding programs. This study has also provided important information about the active and effective resistant QTLs/genes under Nordic condition.

Diallel Analysis of Scald and Net blotch Resistance in barley

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Scald (*Rhynchosporium commune*) and net blotch (*Pyrenophora teres*) are major foliar diseases of barley causing high yield losses in Ethiopia. Development of resistant varieties is a major approach in managing the two diseases. However, the genetics of resistance for the two foliar diseases is not known for food and malt barley cultivars in Ethiopia. The objective of the study was to assess genotype performances, identify superior parents for future breeding and investigate the nature of gene actions governing resistance to scald and net blotch. Twenty eight barley genotypes (21 F_1 crosses and 7 parents) were evaluated in a randomized complete block design with three replications at Holetta Research Center in the 2014/15 cropping season. Highly significant ($P \leq 0.01$) differences were observed among genotypes for initial percent disease severity and final disease severity. Combining ability analysis showed that general combining ability (GCA) and specific combining ability (SCA) was highly significant ($P \leq 0.01$) for initial disease severity, final percent severity and AUDPC indicating the importance of additive and non-additive gene actions controlling resistance for both scald and net blotch. The cultivar HB1307 showed the best general combiner for scald and net blotch resistance while cvHB42 for scald and cv. Miscal-21 and cvAgegnehu parents were the best general combiners for net blotch resistance. The result suggested that resistance is additive for two diseases found and can be fixed through bi-parental or diallel inter-mating of selected segregants followed by selection at late generations. Final disease rating can be useful for evaluating a large number of barley genotypes to both diseases to save time and resources.

Session II

Evaluation and Breeding for Disease Resistance

Diversity for resistance to *Pyrenophora graminea* in a European spring barley cultivar collection and association mapping of the involved genes

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Barley leaf stripe is caused by the seed-transmitted hemi-biotrophic fungus *Pyrenophora graminea*. Race-specific resistance to leaf stripe is controlled by two known *Rdg* (*Resistance to Drechslera graminea*) genes. *Hordeum spontaneum*-derived *Rdg1a* has been mapped to the long arm of chromosome 2H, while *Rdg2a* (chromosome 7HS), has been isolated by positional cloning from the 'Thibaut' winter barley cultivar and functionally characterized. These genes cause hyphal degeneration in the basal part of the coleorhiza and prevent stripe symptoms from appearing on leaves of young plants. Here we describe a survey of the phenotypic diversity for resistance to *P. graminea* in a low-structured collection of about 200 spring 2-rowed European barley cultivars. Artificial inoculation with the highly virulent isolate *Dg5*, against which *Rdg2a* is not effective, was performed using the "sandwich method" technique, showing large phenotypic variation for the incidence of leaf stripe disease. The same cultivars were genotyped with 7,864 gene-based SNPs incorporated into the Illumina Infinium™ iSelect assay, in order to identify the genomic regions involved in resistance to *P. graminea* through a Genome-Wide Association Mapping approach. A highly significant marker-trait association was detected on the short arm of barley chromosome 6H in a region where no leaf stripe resistance genes were previously identified. New molecular markers have been developed to increase the mapping resolution and candidate resistance genes have been proposed, based on the recently available genomic information.

Resistance of Jordanian and Syrian landraces against barley leaf spot diseases

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According to long-term trials, barley diseases decrease the barley yield in Finland on average 650 kg/ha (13 %). The total barley area in Finland has increased by 50% during the past 40 years which has led to shorter crop rotations on farms. At the same time, traditional ploughing has been replaced by reduced and no-tillage systems, which increase the risks for seed- and stubble-borne diseases: net blotch, scald and spot blotch. Breeding barley for improved resistance to leaf spot diseases is a priority in several parts of the world, including the Nordic-Baltic region. The challenge is to find barley genotypes which carry novel resistance sources not yet used in breeding programs.
An experiment was conducted in Luke to study the resistance of 120 Jordanian and 360 Syrian barley landraces against Finnish *Pyrenophora teres* f. *teres*, *P. teres* f. *maculata*, *Cochliobolus sativus* and *Rhynchosporium commune* isolates. The genotypes originated from ICARDA. The resistance was first studied as seedling tests in greenhouse and the most promising candidates were selected for hill plot tests in field. Either single isolates or mixture of Finnish isolates were used as inoculum.
Based on the field tests in 2016 in Jokioinen, both the landraces from Jordan and Syria, carry a lot of interesting resistance against all tested barley diseases.

Evaluation of ICARDA barley germplasm for resistance to spot blotch (*Bipolaris sorokiniana*) in India

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Cultivation of resistant varieties is considered to be the cheapest way to combat plant diseases particularly in developing countries where most of the barley farmers are marginal and unable to afford costly fungicides. Efforts have been made to develop resistant genotypes with good agronomic traits for spot blotch of barley with limited success in India. This necessitated the need to evaluate more germplasm for identifying effective and diverse sources of resistance to check the losses being caused by spot blotch. A total of 605 barley germplasm accessions (340 from association mapping population-16 and 265 from spring barley crossing block-16) received from the International Center for Agricultural Research in the Dry Areas (ICARDA), were screened against *B. sorokiniana* under artificial inoculation at BHU Varanasi, India. The location is also considered as the natural hot spot of the spot blotch in India. The disease scoring at the different growth stages in the double digit scale and area under disease progress curve (AUDPC) were considered to classify the resistant genotypes. Unlike rust resistance, there was no genotype, which could be termed as immune to spot blotch. However, the entries grouped as resistant were able to keep disease level on the plant at very low level with resistant reaction type (very small spots which were not spreading further). The screening of the ICARDA germplasm against spot blotch revealed that out of the total entries 27 entries were found as resistant (R) and 91 as moderately resistant (MR). The findings made clear that ICARDA nurseries had good resistance level in quite enough number that can be exploited for barley improvement program against the disease in South Asia. Though, based on parentage and geographical origin the resistance sources look diverse, however, their diversity at genetic level requires further verification by traditional inheritance studies or the molecular marker methods.

Alternative Hosts of Yellow Dwarf Viruses (YDVs) and Sources of Resistance in Barley in Ethiopia

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Diseases are key biotic constraints facing barley production in many countries. One of the key diseases group is yellow dwarf viruses (YDVs). Alternative hosts play an important role in the epidemiology of YDVs. Studies were made in Ethiopia to identify potential alternative hosts and resistance sources in barley during the period from 2013–2016. Surveys for wild annual and perennial grass hosts were conducted in major cereals growing belts in central, southeast and northwest Ethiopia. Samples were collected following simple random sampling technique, and virus identifications were done by tissue blot immunoassay (TBIA) using virus specific polyclonal antibodies. For host plant resistance screening, around 1500 barley land races were screened for Barley yellow dwarf virus-PAV (BYDV-PAV) resistance under field and artificial inoculation in the greenhouse. Out of 13,604 grass samples tested, YDVs were detected from 392 (2.9%) samples, which consisted of various wild grasses and forage cereals. YDVs were identified from at least 26 grass species, and some of the alternative hosts identified were new records. A large number of alternative hosts reported for the first time in Ethiopia are Andropogon abyssinicus (Fresen.) R.Br. ex Fresen, Avena abyssinica Hochst., Bromus pectinatus Thunb., Eragrostis tef (Zucc.) Trotter, Eragrostis sp. (locally named 'Muriye"), Hyparrhenia anthistrioides Stapf., Panicum coloratum L., Polypogon monspeliensis (L.) Desf., Setaria pumila (Poir.) Roem. & Schult., Setaria australiensis (Scribn. & Merrill) Vickery and Snowdenia polystachya (Fresen.) Pilg. Out of 165 elite barley genotypes selected and tested against Yd2-linked markers (Y1p-CAPS-MF & Y1p-CAPS-MR), 98 (59%) of the selected landraces confirmed to contain the Yd2 resistant gene. In conclusion, the role of alternative hosts can play a major role in initiating YDVs epidemics. Conversely, there are ample sources of resistance that can be deployed using markers assisted selection to combat YDVs.

Session II

Evaluation and Breeding for Disease Resistance

Resistance of barley landraces from the centres of genetic diversity to *P. teres* f. *teres* and *C. sativus*

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Net blotch, caused by *Pyrenophora teres* f. *teres* (PTT) and spot blotch, caused by *Cochliobolus sativus* (CS) are widely spread and harmful foliar diseases of barley. The aim of the study was to characterise the resistance of 242 barley landraces from the centres of genetic diversity from the collection of the N. I. Vavilov All-Russian Institute of Plant Genetic Resources towards three isolates of PTT and CS of different origin, respectively. The inoculation was performed by spraying 14 day old seedlings with single spore isolate suspensions with a concentration of 5,000 conidia/ ml for PTT isolates and 17,000 conidia/ ml for CS isolates. The experiments were set up in four repetitions with two seedlings per repetition. Ten days after inoculation with CS isolates the infection response (IRs) was assessed using the scale by G. Fetch and B. Steffenson (1999). Assessment for PTT resistance was conducted fourteen days after inoculation using the scale of A. Tekauz (1985). The number of resistant accessions towards PTT (IRs 1 – 5.0) was dependent on the isolate used: among landraces from Central Asia 6.1 – 15.8% accessions were resistant, Middle East 3.5 – 7.7%, Mediterranean Region 0.5 – 5.2%, Ethiopia 3.3 – 37.7%, Manchurian Region 2.3 – 7.4%, South America 1.4 – 7.4% and North America 0 – 2.3%. Only 20.2% landraces were resistant (IRs 1 – 4.5) to CS isolate L31, 6.7% to isolate Ch3, and 9.7% to isolate Br13. The highest number of resistant landraces was from Ethiopia, with 1.7 – 9.0% resistant accessions. Genetic diversity of resistance of new sources will be determined by GWAS.

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Exploiting diverse landraces for novel broad spectrum powdery mildew resistance

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Barley Powdery mildew is a significant and widespread threat to barley production with the most cost-effective long term management provided by genetic resistance. The pathogen has evolved to overcome most major race-specific resistance genes deployed in Western Australia and therefore we have screened diverse landraces to discover new broad-spectrum resistance genes. A subtle new broad-spectrum resistance variant based on the mlo resistance in landrace Eth295 has been characterised and other candidates are under investigation. In Eth295, resistance was found to be recessive and genetic and complementation studies indicated the involvement of mlo. However, unlike known mlo alleles, the resistance was developmentally controlled and quantitative without spontaneous cell wall appositions or extensive necrosis. This resistance has two copies of the mlo-11 repeat units, compared to 12 copies in commonly grown cultivars and was designated mlo-11 (cnv2). mlo-11 repeat unit copy number dependent DNA methylation, SiRNA and histone modification corresponded with macroscopic phenotypic, cytological and transcriptional differences between copy number variants. Sequence data indicated mlo-11 (cnv2) formed via recombination between progenitor mlo-11 repeat units and the 3′ end of an adjacent stowaway MITE containing region. mlo-11 (cnv2) is the only example of a moderated mlo variant discovered to date and may have arisen by natural selection against the deleterious effects of the progenitor mlo-11 repeat unit configuration. In summary, Eth295 mlo-11(cnv2) has no deleterious pleiotropic effects as with ‘normal’ mlo’s, so does not need special ‘compensating’ genes bred in. Three additional promising lines are under development for which recent data will be presented.

Evaluation of diverse sets of barley germplasm for resistance to scald disease (*Rhynchosporium Secalis*)

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Barley is one of the most important field crop in the drylands of North Africa and West Asia region. It has multiple uses (food, feed and malt) and is known for its better adaptation to drought and salinity. However, its productivity is affected by a large number of diseases and insect pests. Leaf Blotch, also known as scald, is one of the most destructive diseases of barley (*Hordeum vulgare* L.), caused by the haploid imperfect fungus *Rhynchosporium secalis*. Scald is widely distributed throughout the world and can cause 40% reduction in grain yield. The use of resistant cultivars remains to be the most effective, economical and environmental friendly way to control the disease but the genetic resistance to Moroccan isolates is poorly understood and only few sources of resistance have been identified.

This study aims at the identification of new and effective sources of resistance to scald within various subsets: Best bet subset selected using the Focused Identification of Germplasm Strategy (FIGS; 80 accessions), reference set identified within the Generation Challenge Program (GCP; 204 accessions), and an association mapping panel (284 accessions) developed by ICARDA breeders. All the accessions were sown and evaluated at seedling stage under controlled conditions. We have found that of the 568 barley accessions tested, 78 barley genotypes (14%) were found to be resistant, 86 genotypes (15%) to be moderately resistant, 104 genotypes to be susceptible, and 103 accessions (18%) to be highly susceptible, respectively. Most of the resistant accessions (125; 22%) showed immune reaction. These new sources of resistance will be a useful resource for resistance barley breeding programs around the world.

Streamlining greenhouse and field testing for selection for resistance to net blotch in barley breeding materials

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Net blotch, *Pyrenophora teres* f. *teres*, is a problem for barley production in most barley growing regions and therefore breeding for resistance to this disease is essential. However, efficient selection of resistant genotypes is not trivial. To ensure a high disease pressure it is necessary to artificially raise humidity; and field resistance is manifested in a quantitative way making it difficult to score. To improve the chances for efficient, large-scale resistance evaluations, a combined greenhouse and field phenotyping method was developed; to use as little space, seeds and inoculum as possible, and in both environments expose the plants to more than one fungal genotype.

In the greenhouse, a modified version of the method developed by Arabi and Jawhar (2010) for *Cochliobolus sativus* was used. Five µl of a *P. teres* spore/hyphal solution were applied on a transparent tape, which was then attached to the upper mid-section of a leaf. To facilitate infestation by the fungus, the leaf was punctured with 8 fine insect pins before tape application. The first isolate was inoculated on a fully developed second leaf at seedling stage, ca 2 weeks after sowing. Next isolate was inoculated on a new upper, fully developed leaf on the same plant, after scoring, a couple of weeks later. Lines tested in the greenhouse were subsequently sown in 20-seeds hill plots in lanes bordered by a susceptible cultivar. The 15 x 8 m plot was surrounded by a farmer's field of barley and it accommodated 540 hill plots. Misting with water was applied six times a day for several weeks until scoring.

Single plant greenhouse phenotyping and single hill-plot field phenotyping resulted in significant correlations between infestation scores in the two tests; in those families where the resistance source was highly resistant.

Reference

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Introgression of quantitative resistance to *Rhynchosporium commune* into elite winter barley through marker assisted backcrossing

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Worldwide, *Rhynchosporium commune* is one of the most destructive fungal pathogens of barley. Varietal resistance to *R. commune* has traditionally relied on the incorporation of single major resistance genes, which initially provide high levels of resistance, but are frequently defeated as pathogen populations evolve. The widespread adoption of marker assisted breeding has provided the tools necessary for breeders to produce new varieties with resistance that is based on multiple resistance genes, each of which may have relatively small effects, but combined, can lead to effective as well as durable resistance. The usefulness of this approach for producing broad-based varietal resistance was investigated in UK winter barley

Candidate resistance QTL were mapped from two double haploid populations. These QTL were introgressed into an elite winter variety (KWS Cassia) using marker assisted backcrossing to produce a set of Near-isogenic lines, incorporating single or multiple resistance QTL. Near-isogenic lines were scored for resistance to *R. commune* in a disease nursery. In addition, detached leaf inoculations using characterised strains, along with confocal microscopy using GFP expressing *R. commune* isolates, were used to look at race specificity of the resistances and their effects on *R. commune* growth on the leaf.

Resistant phenotypes were identified from disease nursery trials for a number of candidate QTL whilst alternative contrasting race-specific effects on fungal growth morphology were also identified. High density genotyping of selected NILs provided improved map resolution for a number of candidate QTL. NILs carrying multiple QTL introgressions demonstrated a clear increase in resistance scores compared to single QTL NILs, demonstrating the effectiveness of combing multiple quantitative resistances as a method for designing varietal resistance the *R commune*.

Evaluation of exotic barley germplasm against yellow rust in India

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Barley (*Hordeum vulgare* L.) is an important cereal crop in India currently being cultivated in 0.67 m ha with production of 1.6 m tonnes. The crop suffers from many foliar diseases with yellow rust (*Puccinia striiformis* f.sp. *hordei*) being the most important in the north western plains of India. The disease may cause up to 60 % yield losses in susceptible varieties under severe conditions. Being a low-input crop, host resistance is the cheapest and eco-friendly approach for the management of yellow rust. Resistance breeding in India has been based on limited sources and to find out diverse yellow rust resistance sources from exotic barley, the yellow rust FIGS subset consisting of two hundred ninety-eight genotypes from ICARDA genebank was screened under artificial epiphytotic condition created by using the mixture of four predominant Indian races 24 (0S0-1), 57 (0S0), M (1S0) and G (4S0) at Rajasthan Agricultural Research Institute, Durgapura, Jaipur (India) during 2015-16 cropping season. The inoculum was received from Regional Station, Indian Institute of Wheat & Barley Research (IIWBR) Flowerdale, Shimla. The test material was grown in single rows of 1 m length at 30 cm row distance. One line of infector (Bilara 2) was planted after each set of 10 test lines and also all around the block of the test material. Reaction to yellow rust was recorded by combining severity (per cent leaf area covered by rust) and response (infection type) using modified Cobb's scale. Based on the coefficient of infection (CI) value, ninety-seven lines were found highly resistant to yellow rust (<0.1 CI) and eighty-eight lines were resistant to yellow rust with up to 10 CI as against 100 CI recorded on susceptible check Bilara-2. The identified new sources of resistance from the FIGS sub-set may be utilized in Indian as well as ICARDA global barley breeding program. The FIGS approach is most often used by ICARDA genebank to respond to requests focusing on traits. The results show the relevance the Focused Identification of Germplasm Strategy (FIGS) in identifying large number of sources of resistance to yellow rust.

Identification of sources of resistance to the seed-borne barley leaf stripe disease (*Pyrenophorea graminea*)

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Leaf stripe disease caused by the seed-borne fungal pathogen, *Pyrenophora graminea*, has become increasingly an important disease of barley in the major barley growing regions in Iran during the last decade. The use of disease-resistant cultivars is known as a cost-effective and environmentally friendly method of leaf stripe control. This study was carried out to identify sources of resistance to leaf stripe disease in barley. A set of 90 genotypes including promising lines from the Iranian national barley breeding program (42 lines), Iranian commercial barley cultivars (18 cultivars) and reported sources of leaf stripe resistance (30 cultivars) were evaluated at field conditions in Eastern Iran at Torogh Agricultural Research Station during three consecutive growing seasons (2012-2015). In the first year, each genotype was sown between spreader rows of a highly susceptible cultivar. The genotypes were inoculated via natural infection from spreader rows during the anthesis period. In the second and third year, the disease incidence (proportion of diseased plants) was assessed. Evaluation of resistance was performed according to the Delogu scale. Based on the results, the leaf stripe levels varied greatly across years and the genotypes. In 2015, 24 % of 42 tested promising lines and 39% of 18 tested commercial cultivars were very resistant or resistant (0-12% of disease incidence). The widely grown commercial cultivars, cv. Nik, Yousef, Kavir, Rihan and Nosrat were evaluated as susceptible or very susceptible. Our results also indicated that the characterized genetic sources of resistance including Vada and Thibaut that carrying *Rdg1a* و *Rdg2a* resistance genes respectively and cv. Alabama, Scarlet, Ricarda, Optima,Nizza,Onice,Vega,Aldebaran and Rebelle are very resistant to the local populations of the leaf stripe pathogen and can be used in breeding programs as resistant parent.

Identification of new sources of resistance to spot blotch from *Hordeum spontaneum*

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ICARDA has a global mandate of barley for sustainable production in dry areas through preservation of the genetic diversity and promote its utilization to develop improved and better adapted germplasm. The elite germplasm is shared to national partners across the different regions worldwide. Amongst various biotic yield limiting factors, the economic losses inflicted by spot blotch (SB) are substantial and a yield loss of up to 30% is quite common in barley growing regions which can exceed due to disease conducive environment. The deployment of host plant resistance remains to be the most economical and environmental friendly option to curb the yield losses inflicted by SB. Since the sources of effective resistance in cultivated barley are very few and we need more diversity of the sources to introgress in the elite germplasm, it was considered to explore the resistance from closet wild relative i.e. *Hordeum spontaneum*. In the present study, a subset of 114 *H. spontaneum* accessions from the ICARDA's genebank was screened with a mixture of 14-isolates of SB under controlled conditions. At 10 days' post inoculation, the seedlings were categorized according to the infection responses based on the 0-9 scale developed by Fetch and Steffenson (1999). Of the 114 accessions, 17 accessions were found to be resistant with infection response (IR) between1-3, 66 accessions as moderately resistant (IR 4-5) and 30 accessions were found to be susceptible (IR > 6). In contrary, only 3 accessions were found to be resistant from a random set of cultivated barley exposed to a mixture of 14 SB isolates. Based on our preliminary screening, we have found new SB resistant sources in wild barley accessions which should be exploited through pre-breeding in order to incorporate them into the cultivated barley.

Barley genetic stock conferring multiple field resistance to various leaf diseases in Western Australia

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Epidemics of leaf diseases have devastating effect in barley grown regions of Western Australia which have significant limitation to sustainable barley production. Reducing impacts from diseases is important for reliable production of high quality barley. The lack of resistance to diseases like net blotches, scald, leaf rust and powdery mildew can impact on yield and grain quality and, thus, reduce returns to growers domestically and its marketability in export market. An integrated approach to disease control is recommended, yet genetic resistance underpins effective management strategies in barley production systems to develop varieties with adequate levels of resistance to the range of important biotic stresses. A major objective in barley breeding programs around Australia is to develop varieties with improved combinations of disease resistances without compromising its yield, quality and other agronomic traits. A set of barley lines have been identified showing multiple disease resistance in the field trials in Western Australia. Though some lines show single disease resistance, but many hold resistance to two to four diseases in a particular line. Various lines also show adult plant resistance particularly to net form net blotch, powdery mildew and leaf rust. This is a crucial germplasm resource which is currently evaluated around Australia in the national trials as well to determine its effectiveness against different strains of pathogens. Such lines can provide a major germplasm resource to improve genetic resistances of the existing barley varieties and also can be utilized for the development of the new varieties.

GGE biplot model for environmental delineation and identification of resistant sources for stripe rust and spot blotch in India

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Stripe rust (*Puccinia striiformis f. sp. hordei)* and spot blotch (*Bipolaris sorokiniana*) are major biotic constraints in yield maximization in barley in India. Stripe rust is wide spread in different agro-ecologies of India *i.e.* northern hills and north western plains (NWPZ), whereas, spot blotch is a very serious disease in both NEPZ and NWPZ. Since these are multicycle diseases in one crop season, chemical control with repeated sprays is difficult to practice, hence host resistance is a better option. GGE biplots for environmental interactions were obtained using "R" software for stripe rust and spot blotch on 19 barley genotypes evaluated for stripe rust (at Karnal, Ludhiana and Durgapura) and for spot blotch (at Kanpur, Varanasi and Faizabad) under artificial epiphytotics. Two principal components PC1 and PC2 explained a good amount of variation *i.e.* 51.31 %, 38.87 % for stripe rust and 58.29 % and 33.56 % for spot blotch biplots, respectively. For stripe rust the environments were classified into two groups representing Karnal and Ludhiana in one group, while Durgapura location created separate environment. For spot blotch, all three environments depicted separate niches. Environments Ludhiana and Durgapura for stripe rust and Kanpur and Varanasi for spot blotch evaluation were found discriminative and representative. In mean vs. stability biplots, the genotypes namely BH1013, DWRB147, DWRB149, DWRB150, PL890 and RD2941 showed high resistance for stripe rust, while, for spot blotch, the genotypes viz., BH1011 and DWRB149 were found tolerant with the highest reaction of 47 double digit score. In addition, some promising genotypes viz., Isaria, C9173, Orosus and Emir were also identified from ICARDA material with combined resistance to stripe rust and spot blotch and further utilization in breeding programs.

Session III

Molecular Plant-Pathogen Interactions

The role of *Rhynchosporium commune* effectors in the interaction with barley

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Rhynchosporium commune has remained one of the most destructive and economically important pathogens of barley for over a century. It is a hemibiotroph with an extended asymptomatic phase. Following conidia germination and cuticle penetration, *R. commune* hyphae spread between the barley epidermal cells without directly penetrating them.
Sequencing of the *R. commune* genome and transcriptomes from germinated conidia and an early time point during barley colonisation allowed identification of the putative effectors mediating interaction with the host plant. Some of these effectors, including a secreted chorismate mutase, a putative isochorismate hydrolase and a family of LysM domain proteins may help to explain the asymptomatic infection. While chorismate mutase and isochorismate hydrolase might be involved in manipulation of SA mediated defences in barley SA, LysM domain proteins are likely to prevent the host immune response to chitin. Seven genes coding for proteins containing one or more LysM domains have been identified in the *R. commune* genome. LysM1, LysM5 and LysM7 contain one LysM domain, LysM2 - two domains, LysM3 - three domains, LysM4 and LysM6 - four domains. In addition, two genes code for enzymes that contain LysM domain pairs, a subgroup C chitinase, which also contains a different chitin-binding motif, and a putative peptidoglycan lytic transglycosidase. Upregulation of *R. commune* secreted chorismate mutase, a putative isochorismate hydrolase and several genes coding for LysM domain proteins during the onset of barley colonisation suggests their involvement in early stages of interaction with the host. Targeted gene silencing of candidate effectors will help to determine their importance for pathogenicity.

The mystery of barley spot blotch disease caused by *Bipolaris sorokiniana*

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Bipolaris sorokiniana is described as a classical hemi-biotrophic fungus that performs a short asymptomatic biotrophic stage followed by a symptomatic colonization of the mesophyll tissue. The necrotrophic stage is believed to be the cause of colonization rather than the consequence, implying that host cell death is needed for colonization. However, the infection biology of *B. sorokiniana* seems to be more complex than assumed so far. We have investigated the correlation between severity of disease symptoms and fungal colonization (measured as fungal biomass) in relation to light and photosynthesis. Surprisingly, we found that severity of necrotic disease symptoms are light dependent and associated with LESS fungal colonization and that suppressing the rate of photosynthesis by low light incubation or chemical inhibition (DCMU) make the plants SUPER SUSCEPTIBLE to *B. sorokiniana* colonization. This strongly implies that active photosynthesis is involved in formation of necrotic disease symptom and that necrosis negatively affects fungal colonization. Crude toxin infiltration led to light dependent necrotic symptoms similar to fungal disease symptoms. This indicates that toxins produced by *B. sorokiniana* under colonization affect photosynthesis, leading to a strong local release of ROS, and that this ROS burst is responsible for the necrotic leaf lesion and indirectly leads to an inhibition of fungal colonization. Similar to other hemi-biotrophic fungi *B. sorokiniana* has been implied to colonize barley *mlo* mutants more successful than wildtype *Mlo.* However, by examine several isogenic Mlo/mlo-mutant pairs representing a broad genetic background we observed that the *mlo* mutation did not increase disease susceptibility in general. Instead, there might be a correlation between leaf senescence and increased susceptibility and the *mlo* mutation may promote leaf senescence. The consequences for breeding tolerant spot blotch disease barley will be discussed.

The genetics of virulence in the *Pyrenophora teres* f. *teres* population BB25 × FGOH04Ptt-21

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Pyrenophora teres f. *teres* is a necrotrophic pathogen responsible for causing net form net blotch of barley. In order to characterize the genetics of avirulence/virulence in the *P. teres* f. *teres* pathogen, a fungal population was developed using *P. teres* f. *teres* isolates BB25 (Denmark) and FGOH04Ptt-21 (USA). A mapping population consisting of 109 progeny isolates derived from a cross between BB25 and FGOH04Ptt-21 were utilized for NFNB disease evaluation across ten barley lines including both local cultivars and lines previously used as differential lines for evaluating virulence. BB25 was virulent on three of the barley lines whereas FGOH04Ptt-21 was virulent on all ten barley lines evaluated. A genetic map was generated with SNP markers obtained using a RAD-GBS approach, resulting in the formation of sixteen linkage groups that were used to identify quantitative trait loci (QTL) associated with avirulence/virulence. Nineteen unique QTL were identified on twelve linkage groups, out of which, three QTL had major effects ($R^2 \geq 30\%$) while sixteen QTL were relatively minor. One or two major effect loci were identified for a few of the lines used regularly as differentials, however, virulence on most of the local barley cultivars consisted of several loci that contributed quantitatively to disease. Reference quality genomes of BB25 and FGOH04Ptt-21 developed via Pac-Bio sequencing coupled with *in silico* gene prediction facilitated the identification of candidate necrotrophic effectors underlying two major virulence QTL, *PttNE-Pinn1* (virulence on barley cultivar Pinnacle) and *PttNE-Beecher1* (virulence on barley cultivar Beecher), derived from isolate BB25 and FGOH04Ptt-21, respectively. Analysis of predicted genes underlying *PttNE-Pinn1* and *PttNE-Beecher1* identified six and four small (<50 kD), secreted proteins, respectively, currently being targeted for validation via gene knockout and expression.

Ramularia collo-cygni and barley host-pathogen interactions

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Ramularia collo-cygni is a fungal pathogen of barley, and the causal agent of Ramularia leaf spot (RLS). *R. collo-cygni* has a global distribution and is spread both via the barley seed and through conidiospores. The fungus has a long asymptomatic growth stage, causing necrotic spots only late in the growing season. To uncover the underlying genetics of *R. collo-cygni* infection and disease we have performed RNA-sequencing of both pathogen and host, in a time course experiment over 3, 7 and 12 days after infection with three replicates. The RNA seq reads were used for competitive mapping against the barley and *R. collo-cygni* genomes, a re-annotation of the *R. collo-cygni* genome was performed using Breaker/ GeneMarkET, and differential gene expression analyses were calculated in EdgeR.
The results show 419, 1741 and 4583 genes are up regulated in infected barley compared to control samples at 3, 7 and 12 days post infection respectively, while 87, 364 and 4101 genes are down-regulated for the same time points.
In *R. collo-cygni* more genes are up-regulated at three days after infection compared to both later time points. Five genes annotated as polyketide synthases are differentially expressed. One aflatoxin biosythesis regulatory protein is increasingly up-regulated with time, and one aflatoxin B1 aldehyde reductase is up-regulated at seven days after infection compared to both .three and twelve days post infection.

Determining the role of secondary metabolism in the pathology of *Ramularia collo-cygni*, the fungus responsible for Ramularia leaf spot disease of barley

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Ramularia leaf spot (RLS) is an emerging disease of barley occurring late in the growing season in temperate regions worldwide which can lead to yield losses as high as 70% as well as decrease grain quality. RLS is caused by the Dothideomycete fungus *Ramularia collo-cygni* (Rcc) which produces phytotoxic anthraquinone-derived metabolites called rubellins that have been associated with the development of disease symptoms. Anthraquinone products are known to be synthesised through the action of polyketide synthase (PKS) enzymes. In this study, ten putatively functional PKSs and three hybrids PKSs/non-ribosomal peptide synthases (HPSs) were identified in the genome of Rcc. Using an *in silico* genome walking approach, eleven putative secondary metabolite (SM) gene clusters located near core PKS and HPS genes were identified. The expression of nine of these core genes was investigated in barley seedlings during RLS development and indicated that these SM core genes were most highly expressed during the asymptomatic and early lesion formation stage of the disease. Co-regulation of genes with a predicted function associated with secondary metabolism located in non-SM core gene-containing clusters was also observed. Taken together with the observations that rubellin D when infiltrated in barley leaves failed to reproduce characteristic RLS symptoms and that the presence of Rcc was able to inhibit the growth of other barley pathogen *in vitro*, these results suggest that the role of SMs in Rcc is more complex than previously thought.

Screening of *Pyrenophora teres* f. teres effector candidates by transient expression in barley

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The necrotrophic fungal pathogen Pyrenophora teres f. teres (PTT) is the causal agent of the net form of Net Blotch disease (NFNB) in barley. Although several major host resistance QTLs and genes have been identified, the molecular basis of NFNB pathogenicity remains unclear. Both fungal secondary metabolite and proteinaceous toxins have been investigated, however, the level of complexity of the pathosystem is currently not fully known. The difficulty of genetically manipulating the pathogen has slowed down progress in this area, with standardised protocols for the genetic manipulation of PTT have yet to be developed as stable transformation of different isolates can be extremely difficult. To overcome this bottleneck in the characterization of pathogenicity effectors we are developing an efficient transient expression method to screen effector candidates by heterologous expression in barley leaves using Agrobacterium mediated transformation. The essence of this approach relies on the modification of existing vectors to allow the high throughput seamless cloning of the effector candidates using a type II endonuclease based method (AKA Golden Gate).

Evaluation of candidate genes important in *Pyrenophora teres* f. *teres* virulence on barley

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Net form net blotch is an economically important foliar disease of barley (*Hordeum vulgare*) caused by *Pyrenophora teres* f. *teres*. Yield losses have been reported in the range of 10-40% and complete yield loss has been observed under environmental conditions highly favorable to the pathogen. However, little is known about the molecular mechanism of the *P. teres* f. *teres* – barley interaction. Therefore, a mapping population was developed from a cross between two California *P. teres* f. *teres* isolates that showed a differential response on barley lines Rika and Kombar, with 15A being virulent on Kombar and 6A being virulent on Rika. Two virulence QTL conferred by 6A (*VR1* and *VR2*) that contribute to virulence on Rika and an additional two virulence QTL conferred by 15A (*VK1* and *VK2*) that contribute virulence on Kombar have been identified. To further investigate the genes underlying these loci, we have generated reference-quality genome assemblies of parental isolates 6A and 15A using the PAC-BIO sequencing platform. Additionally, RNA sequencing of culture and infection time points were used to annotate the regions of interest. Previous studies have shown necrotrophic effectors to be small secreted proteins. Current annotation has predicted a total of 15 genes encoding small (<50kDa) secreted proteins (SSP) with close proximity to the most highly associated markers. Two, four, five, and three of the predicted SSP genes are at the *VR1*, *VR2*, *VK1*, and *VK2* loci, respectively. Further validation and characterization of these genes will give valuable insight into the barley *P. teres* f. *teres* host-pathogen interaction.

A genome comparison of five *Pyrenophora teres* f. *teres* isolates differing in virulence

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Net form net blotch (NFNB) caused by the fungal pathogen *Pyrenophora teres* f. *teres* is an important foliar pathogen of barley (*Hordeum vulgare* L.). Historically, yield losses have been recorded in the range of 10 to 40% and up to 100% in extreme environmental conditions favorable to the pathogen. There are both qualitative and quantitative resistance sources to *P. teres* f. *teres*. The qualitative relationship has been proposed to follow a gene-for-gene model requiring the presence of both a dominant resistance gene in the host and a recognized effector in the pathogen. Additionally, an inverse gene-for-gene interaction has been demonstrated where necrotrophic effectors (NEs) interact with dominant susceptibility targets in the host, inducing NE triggered susceptibility (NETS). Little is known about the molecular mechanism of NFNB disease or the constitution of the *P. teres* f. *teres* genome, especially as it relates to the diversity of the genome across the population. Of particular interest, related to pathogen virulence/avirulence, are the set of small secreted proteins defining the effectorome that the pathogen uses to manipulate its host. Within genomes, repetitive sequences are also of interest because they allude to possible mechanisms for evolution as well as syntenic regions alluding to conserved genes required by the pathogen. To investigate the genomic content of *P. teres* f. *teres*, five isolates were sequenced using PacBio sequencing at greater than 100x coverage and reference quality assemblies were obtained. RNAseq was performed with each isolate using both in culture and *in planta* time points for annotation. Comparative analysis of both genomic structure and gene content were evaluated between the five isolates to elucidate the level of diversity between isolates.

Cellular, molecular and biochemical response to *Ramularia collo-cygni* leaf infection in barley

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The formerly hardly recognized pathogen *Ramularia collo-cygni* (*Rcc*) came into focus of scientific research within the last decade in consequence of increasing problems with Ramularia leaf spot disease in Europe, New Zealand and South America. *Rcc* is present in most cultivated barley populations as an asymptomatic endophyte that often shifts into pathogenic growth at the time of flowering, followed by serious negative impact on grain yield and quality. No effective and durable resistance has been identified, and no efficient diagnosis to predict a damaging attack exists. Hence, we are investigating the basic genetic mechanisms and biochemical components underlying recognition and defense of *Rcc* colonization and disease development throughout different time points of the infection process. We have chosen contrasting barley varieties infected with *Rcc* isolates, provoking either low or high levels of disease symptoms. A quantitative leaf inoculation assay has been developed for investigations under artificial but stable conditions. Furthermore, initial histology analysis and qPCR results of relative fungal DNA in the infected leaf samples indicate a complex relationship between fungal colonization and symptom development. On this basis we have established a RNA-seq data set that provides an in-depth look into the complexity of this plant-fungal interaction, and that is used to identify the key molecular components that are involved in the response to the fungus, and subsequently to understand the fine-tuning of gene expression during pathogenic infection. At the same time we are monitoring accumulation of secondary metabolites that may be involved in defense. Interestingly, the defense response on gene level seems to differ strongly between the host varieties and also between the *Rcc* isolates. First combined evidence of both analyses indicates a multi-layered and time-depended defense mechanism involving metabolite synthesis and/or transport, as well as cell wall modification and targeted senescence. The key results from the molecular investigations will be discussed in more detail on the poster.

Molecular mapping of resistance to *Drechslera teres* in Norwegian barley and population structure of a Norwegian *D. teres* population

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Resistance of most current commercial Norwegian barley cultivars against the causal agent of the net blotch disease, Pyrenophora teres, is insufficient. Therefore, we conducted a study to detect QTL associated with resistance and susceptibility in the germplasm most relevant to Norwegian barley breeding. Resistance to three Norwegian D. teres isolates was assessed in a segregating biparental cross of the Norwegian cultivars Arve and Lavrans and an association mapping panel consisting of 209 mostly Nordic barley lines. Inoculation experiments were performed on seedlings in the greenhouse and in adult plants in the field. In the biparental population, a set of 589 SNP markers was used to map a major QTL on chromosome 5H which was stable in all environments and explained up to 48% and 55% of the genetic variation in seedlings and adult plants, respectively. Eight additional QTL explained up to 17% in seedlings and 15% in adult plants, and one of them was isolate-specific. Most resistance alleles originate from the more resistant parent Lavrans. Association mapping in 209 Nordic barley lines genotyped with 5669 SNPs revealed 43 significant marker-trait associations corresponding to 15 QTL, each explaining less than 15%. QTL on 3H and 6H were found both in seedlings and adult plants. These are promising candidates for breeding programs using marker-assisted selection strategies. Additionally, 365 Norwegian D. teres isolates and a selection of globally collected isolates were ddRAD-Seq (double-digest restriction-associated DNA sequencing) genotyped to obtain approximately 2500 SNP markers to study the genetic diversity and population structure of the current Norwegian fungal population. This data will also allow us to perform Genome Wide Association Studies (GWAS) to identify potential novel virulence genes.

Session IV

Structural and functional genomics of barley leaf pathogens

Sequencing of 19 isolates of the fungus *Ramularia collo-cygni* from multiple origins for improved understanding of the biology using a population genetic approach

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Ramularia Leaf Spot (RLS) has emerged to a major threat for barley production in many regions in the world. Due to the late appearance of unspecific symptoms it were especially molecular diagnostics that detected the fungus *Ramularia collo-cygni* (Rcc) throughout the lifecycle of barley and in samples worldwide as the biotic factor of the complex disease.
The economic impact of RLS is depending on the availability and effectiveness of control measures. Since there are no resistant varieties, control is relying on the use of fungicides. In the past years Rcc has quickly adapted to the limited number of effective compounds.
The study of the biology of Rcc as a basis for sustainable control has been complicated by difficulties with traditional approaches by in vitro growth, sporulation, and inoculation.
To address urging questions, especially the uprise to a major disease, the relevance of seed transmission, and quick adaptation to control measures, the genome of Rcc (urug2 isolate) was de-novo sequenced. Additionally fungal RNA from 6 different conditions was sequenced to help the annotation and to uncover putative genes of interest. The finished assembled genome is about 32 Mb and the overall annotation enabled the prediction of 12346 genes. In the analysis of the differentially expressed genes from the RNA-seq data , the functional categories known to be involved in plant-pathogen interactions were highly distributed. Further studies look at homologues to well-studied pathogens, especially the closely related *Zymoseptoria tritici*.
To evaluate the genetic diversity, whole genome sequencing of 19 Rcc isolates from multiple geographic locations and hosts was performed and mapped to the reference genome. Preliminary analysis indicated substantial genetic diversity and a possible population size expansion, which might explain the recent emergence of this fungus. The analysis is ongoing and recent conclusions on the pathogen biology will be presented.

Pyrenophora teres genome plasticity

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High quality genome assemblies are an essential resource for genomics-based gene isolation strategies, investigating genome evolution and gene diversification, and to provide genetic markers for monitoring pathogen populations. We used optical and genetic mapping in combination with long read PacBio DNA sequencing to produce reference genomes for net form of net blotch (*P. teres* f. *teres*) and spot form of net blotch (*P. teres* f. *maculata*).
Despite large differences in genome sizes between the two forms (~10Mbp) and between isolates of each form, *P. teres* does not possess dispensable chromosomes as found in some other fungi. Rather, the two forms possess highly collinear genomes composed of twelve chromosomes. The collinearity extends to *P. tritici-repentis*, punctuated by a two major translocations. The size differences are explained by large repetitive DNA contents, amounting to 40% of the genome in *P. teres* f. *teres*. These regions are largely composed of transposable elements (TEs) encompassing cryptic ancient regions following extensive repeat induced point mutation (RIP). We developed a method to de-RIP such regions to identify false TE pseudogenes which lead to large discrepancies in total gene counts found by current gene prediction programs. This is achieved without having to know where the repeats are initially and operates on the genome sequence directly. This approach enabled the distillation of accurate gene contents for each *P. teres* form and is generally applicable to other RIPed genomes.

A genome comparison of five *Pyrenophora teres* f. *teres* isolates differing in virulence

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Net form net blotch (NFNB) caused by the fungal pathogen *Pyrenophora teres* f. *teres* is an important foliar pathogen of barley (*Hordeum vulgare* L.). Historically, yield losses have been recorded in the range of 10 to 40% and up to 100% in extreme environmental conditions favorable to the pathogen. There are both qualitative and quantitative resistance sources to *P. teres* f. *teres*. The qualitative relationship has been proposed to follow a gene-for-gene model requiring the presence of both a dominant resistance gene in the host and a recognized effector in the pathogen. Additionally, an inverse gene-for-gene interaction has been demonstrated where necrotrophic effectors (NEs) interact with dominant susceptibility targets in the host, inducing NE triggered susceptibility (NETS). Little is known about the molecular mechanism of NFNB disease or the constitution of the *P. teres* f. *teres* genome, especially as it relates to the diversity of the genome across the population. Of particular interest, related to pathogen virulence/avirulence, are the set of small secreted proteins defining the effectorome that the pathogen uses to manipulate its host. Within genomes, repetitive sequences are also of interest because they allude to possible mechanisms for evolution as well as syntenic regions alluding to conserved genes required by the pathogen. To investigate the genomic content of *P. teres* f. *teres*, five isolates were sequenced using PacBio sequencing at greater than 100x coverage and reference quality assemblies were obtained. RNAseq was performed with each isolate using both in culture and *in planta* time points for annotation. Comparative analysis of both genomic structure and gene content were evaluated between the five isolates to elucidate the level of diversity between isolates.

Sessions V

Epidemiology and integrated management of barley leaf diseases

Advances in the integrated management of leaf blotches in Uruguay

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Barley leaf blotches, including net blotches (STNB, NTNB), spot blotch (SB), scald (SC) and Ramularia leaf spot (RLS) limit barley production in Uruguay. Climatic conditions, shifts on agricultural practices such as no-till and shortened crop rotations, area planted to resistant/susceptible cultivars and fungicide management have shaped the prevalence and severity of these diseases in the last two decades. Research findings on the epidemiology, pathogens populations and management strategies, including the coordinated development and characterization of resistant germplasm and commercial cultivars among INIA, Faculty of Agronomy and private programs will be presented and new challenges discussed. Epidemiological studies have quantified the main inoculum sources and determined the most appropriate cultural practices to diminish primary inoculum from seed and crop residues. INIA´s breeding program has emphasized the incorporation of resistance to NTNB, SB, STNB and SC in the last 20 years attaining adequate levels. Germplasm used comprise locally developed cultivars and lines, especially for NTNB, European materials providing acceptable levels of resistance for SC and North Dakota germplasm for SB. Significant marker-trait associations for SB (9 QTL), were identified in germplasm used as resistance sources. Knowledge on disease profiles of sources of resistance, advanced lines and released cultivars are continuously characterized under intermediate to high disease pressure in specific nurseries for each disease and field trials. Optimum fungicide management of the traditional leaf blotch complex (NTNB, STNB, SB, SC), both seed treatments and foliar, had to be adjusted to further achieve an efficient control of RLS. Effective and economical leaf blotches management could be best achieved with multiple strategies.

Occurrence of barley leaf disease and control strategies in Denmark

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Barley (*Hordeum vulgare*) is one of the major crops in Denmark and of special importance for malting and for pig feed. In 2016, the crop was grown covering a total area of 700,000 ha; approximately 25% of arable area in Denmark. To ensure high yield of around 60 dt ha^{-1}, disease-tolerant cultivars and fungicide treatments are required. Each year, barley cultivars are assessed for susceptibility towards leaf diseases in national observation plots. The most predominant fungal leaf diseases in Denmark are barley scald (*Rhynchosporium secalis*), net blotch (*Pyrenophora teres*), brown rust (*Puccinia hordei*), mildew of barley (*Erysiphe graminis f.sp. hordei*) and Ramularia (*Ramularia collo-cygni*). In recent years, brown rust and net blotch have been the most important disease in terms of yield losses. As most cultivars have *mlo* resistance, powdery mildew is today seen as a minor problem. Significant attack of Ramularia has been observed in more recent years, but normally first late in the season, having less impact on yield. Yield responses following fungicide treatments are commonly in the range of 3-10 dt ha^{-1}. One or two fungicide treatments are recommended to minimise the risk of epidemics. The standard application comprises a mixture of strobilurin and triazole fungicides at ca. half field rate around flag leaf emergence (GS 37-51). Both fungicide groups still provide good control – the most effective triazoles being prothioconazole and epoxiconazole. Fungicide resistance of mildew and net blotch have been observed, especially against strobilurins conferred by mutation F129L, affecting mainly azoxystrobin. No resistance has been observed for *P. hordei* and *R. secalis*. Currently, only one SDHI is registered in Denmark, boscalid, used in combination with epoxiconazole and pyraclostrobin. In field trials, SDHI have proven to be quite effective against all leaf diseases, aside from brown rust and mildew. Denmark has a national record system for pesticide usages. All farmers upload their fungicide use by crop, creating a good basis for assessing the differences in use pattern across different regions and cropping systems.

Sensitivity of *Pyrenophora teres* isolates to propiconazole and pyraclostrobin in the Canadian prairies

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Net and spot forms of net blotch caused by Pyrenophora teres f. teres (Ptt) and P. teres f. maculata (Ptm) are important leaf diseases of barley on the Canadian prairies (Alberta, Saskatchewan and Manitoba). Propiconazole and pyraclostrobin are fungicides registered on barley to manage net blotch in this region; however, their routine application might exert a selection pressure on the net blotch pathogen population. Using a microtiter plate bioassay, the effective concentration of propiconazole needed to inhibit fungal growth by 50% (EC50) was determined to be 1.5 mg L-1, for Ptt, and 2.3 mg L-1 for Ptm isolates while the EC50 for pyraclostrobin was 0.015 mg L-1 and 0.024 mg L-1 for Ptt and Ptm, respectively. To identify isolates with increased insensitivity to propiconazole and pyraclostrobin, discriminatory doses of 5 mg L-1, for propiconazole, and 0.15 mg L-1, for pyraclostrobin, were employed to screen 39 Ptt and 27 Ptm isolates collected from across the Canadian prairies. For propiconazole, growth inhibition ranged from 12% to 95%, for Ptt, and 48% to 92% for Ptm isolates, while for pyraclostrobin, growth inhibition values ranged from 40% to 100%, for Ptt, and 24% to 100%, for Ptm. Ptt isolates, AB11 and AB48 from Alberta had fungal growth inhibition of less than 30% and were considered insensitive against propiconazole, while Ptm isolate SK64 from Saskatchewan was considered insensitive against pyraclostrobin. Isolate SK64 also had growth inhibition values of less than 50% in 5, 10 and 20 mg L-1 of propiconazole, which raises the concern of the potential occurrence of dual resistance to two different active ingredients. The identification of pathogen isolates insensitive to propiconazole and pyraclostrobin emphasizes the need for barley farmers to use integrated crop and disease management strategies to avoid the potential for fungicide failure in the Canadian prairies.

Advances in understanding the epidemiology and control of Ramularia leaf spot

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Ramularia leaf spot is a late season disease of barley caused by the fungal pathogen, Ramularia collo-cygni. The fungus has been shown to have a widespread distribution and is now considered a major pathogen of barley both in the UK and other temperate countries. Losses to the disease range from 70 % to 10% depending on variety. The life cycle of the fungus has only slowly been elucidated. A simple life cycle with movement between winter and spring crops via airborne spores has been revised with the discovery of a seed borne stage in the life cycle. Research has been carried out to discover the environmental factors which influence the movement of spores between crops and the relative influence of spore movement on disease severity. Movement of fungal DNA was measured in three countries using 7 day volumetric spore samplers. DNA was extracted from coated melinex tapes and R. collo-cygni DNA levels quantified using a qPCR assay. Figures for daily spore movement were obtained by cutting the tapes into lengths which correspond to a 24 hour time period. Meteorological data was also collected from the sites of the spore samplers. The presentation will discuss the results from Scotland showing how environmental drivers affect spore movement and compare them to spore movement in European countries with different climates. The incorporation of the analysis into a potential risk forecast for this disease will also be discussed. The control of Ramularia leaf spot using integrated crop management methods will also be discussed.

New fungicide chemistry on net type net blotch management – how useful and what are the drawbacks

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Barley is widely grown in Western Australia (WA) for malting or feed use. Net-type net blotch (NTNB) caused by *Pyrenophora teres* f. sp. *teres* is prominent in the southern region of WA. Favourable climate, susceptible cultivars and exposure to infected residues contribute to the high incidence of NTNB. Transmission of seed borne NTNB has been managed by seed dressing fungicides containing combinations of carboxin, thiram, difenoconazole, metalaxyl-M, sedaxane, or flutriafol, however these products provide limited systemic in-crop NTNB control. Fluxapyroxad, a succinate-dehydrogenase inhibitor (SDHI) fungicide was registered as a systemic seed dressing for NTNB in Australia in 2016. Pre-registration experiments to test fluxapyroxad were conducted in a field with NTNB infested stubble. The observed protection on susceptible Oxford barley was up to early-mid stem elongation growth stage (Z32). However, in 2016 at the same test site the period of protection was significantly less; up to mid tillering growth stage (Z25). Several factors could contribute to reduced fungicide control including local weather, disease pressure, application efficiency or changes in pathogen behaviour. To test for changes in fluxapyroxad efficacy, NTNB infected leaf samples collected from the experimental site and two other locations in the region were used for radial growth assays on PDA media amended with 0, 0.15, 0.3, 1.0, 1.5, 2.0µg ml^{-1} of fluxapyroxad (commercial preparation). Preliminary observations revealed significant variation in growth rate among the three isolates tested, with the isolate from the experimental site growing more rapidly than the other two isolates in un-amended media and grew at the highest fluxapyroxad concentration (2.0µg ml^{-1}). Testing on lower nutrient media showed similar isolate variability but greater sensitivity to fungicide. Genetic testing to determine if mutations are present in the SDH-B, SDH-C and SDH-D genes is currently underway, thus no conclusions can be drawn regarding NTNB resistance to SDHI or natural pathogen population variability in growth rate and subsequent fungicide response. This observation demonstrates that in-field control by this fungicide may vary between isolates. Release of a range of SDHI products, including a highly effective single active seed dressing, coupled with disease favourable environment and susceptible varieties suggest significant chance of resistance to SDHI products occurring in NTNB. Therefore, strict fungicide resistance management should be implemented in WA to maintain SDHI as one of the tools for NTNB control.

Strategies to manage Ramularia leaf spot with fungicides

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Ramularia leaf spot (RLS) caused by *Ramularia collo-cygni* represents one major constraint to barley production in Uruguay. It may cause up to 90% grain >2.5mm yield losses. RLS also decreases grain quality, adding up to economic losses. Presently, the main management practice is the use of fungicides. In order to adjust different fungicide application strategies, field experiments were carried out, evaluating different mixtures: Fluxapyroxad + Pyraclostrobin + Epoxyconazole, Pyraclostrobin + Epoxyconazole + Chlorothalonil, Prothioconazole + Trifloxystrobin and Isopyrazam + Azoxystrobin, in three moments: ZGS33, ZGS47 and ZGS33+ZGS47. A healthy control was maintained with applications every 20 days of Fluxapyroxad + Pyraclostrobin + Epoxyconazole and a non-fungicide treatment. Disease incidence (RLSI) and severity (RLSS) at different growth stages, area under disease progress curve (AUDPC), control efficiency, grain yield, percentage of grain >2.5mm and thousand kernel weight (TKW) were determined. Fungicides residues in grains will be analyzed next. The control obtained with early-single applications with different mixtures of fungicides was insufficient to keep the RLSS low over the crop cycle, and did not differ from the untreated control. In contrast, double applications or late applications significantly reduce RLSS ($P<0.05$). All treatments with double applications at ZGS33 and ZGS47 significantly reduced the AUDPC ($P<0.01$). Regardless of timing of application, single applications were not effective reducing the AUDPC. Only Isopyrazam + Azoxystrobin or Pyraclostrobin + Epoxyconazole + Chlorothalonil, sprayed at ZGS47 significantly reduced the AUDPC and showed no differences with double applications. There were significant differences ($P<0.05$) among treatments for grain yield. The highest yield were obtained with application of Prothioconazole +Trifloxystrobin applied at ZGS47 and double application of Prothioconazole + Trifloxystrobin sprayed at ZGS32 and Fluxapyroxad + Pyraclostrobin + Epoxyconazole at ZGS47. No significant differences were observed for TKW or percentage of grains >2.5mm. Further information is needed to confirm these preliminary results.

On the role of natural compounds against pathogens involved in barley leaf diseases (*Blumeria graminis*, *Pyrenophora graminea*)

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In the frame of the Integrated Pest and Disease Management-IPDM, renewed interest is now focused on the antimicrobial potentialities of medicinal plant extracts,as fungicidal agents for crop protection.In our work, we have evaluated the antifungal properties of two different plant extracts: the essential oil (Tea Tree Oil, TTO) derived from the Australian tree*Melaleuca alternifolia* and the aqueous extract of the Mediterranean perennial shrub *Artemisia herba-alba(AEAE).* The choice of the two extracts has been suggested by their effective possible use in agriculture, thanks to their low cost and easy of retrieval or production. TTO, widely used in cosmetic and pharmaceutical preparations, is in fact an example of commercially available essential oil, whereas the *Artemisia* extract is an example of traditional "on farm" preparation. Both TTO and AEAE have been evaluated for the control of two different classes of barley pathogen fungi responsible for leaf diseases. The fungi considered (*Blumeria graminis* and *Pyrenophora graminea*) are characterized by different life cycle, pathogenicity behavior, transmission mode and effects on plants. The antifungal activity of the extractshas been evaluated *in vivo* on barley leaves colonized by *Blumeria graminis* and *in vitro* on *Pyrenophora graminea* mycelium. Both extracts resulted toxic for the fungi at concentrations that are not toxic for the plant. In particular, TTO and AEAE affect *Blumeria graminis*hyphal growth and spore germination,with a drastic reduction of sporulating colonies and conidia. Besides conidia were damaged dramatically and development of germ tubes was inhibited. With the aim to identify the molecular targets of TTO, a whole transcriptome characterization of barley plants, infected with *Blumeria* and treated with sub-lethal concentrations of TTO - alone or in mixture with eugenol and thymol - has been done. The results obtained suggest that these natural compounds can have a double role in crop protection, thanks to their direct antimicrobial activity and to their capacity to stimulate plant response against pathogen attack. The perspective is to move from the *in vitro* and greenhouse evaluations to the open field ones.

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Upsurge of Ramularia leaf spot in South America

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Ramularia leaf spot (RLS) caused by *Ramularia collo-cygni* (Rcc), has become one of the main constraints to barley production in Argentina and Uruguay. Grain yield losses as high as 70% have been reported in susceptible cultivars. The emergence of RLS as a major disease in the last six years has redirected coordinated research efforts to advance on the understanding of Rcc, its epidemiology and best management strategies in our production systems. Based on Rcc-DNA quantification, RLS is widely present in seed lots, especially after epidemic years (*i.e*. 2012). Leaf wetness during elongation associated with stress factors in the crop has an impact on disease onset. Commercial cultivars and advanced lines were characterized under intermediate to high disease pressure in nurseries and field trials. Most of the commonly grown cultivars were intermediate to susceptible to RLS. Best timings for fungicide application for RLS control in epidemic years were at stem elongation (ZGS 32 to 38) and awns-peeping (ZGS 47-49). Fungicides containing SDHI (isopyrazam+azoxystrobin and fluxapyroxad+pyraclostrobin+epoxyconazole) and combinations with prothioconazole were the most effective fungicides in controlling RLS improving yield and grain quality. Yet, the most efficient and economic options may vary in years with moderate epidemics. Results from these studies emphasize the importance of analyzing seedlots for Rcc and suggest that both fungicide and timing are major factors in optimizing RLS control.

Sessions VI

Evolution and Variation in Pathogen Populations

Diversity in NFNB in South Australia and the detection of durable resistance

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In the two decades prior to 1993 NFNB was mostly absent from South Australian crops, controlled largely by effective adult plant resistance (APR) in the main commercial varieties such as Clipper, Schooner and Sloop. NFNB appeared to become a problem only when the variety Franklin was introduced from Tasmania. In 1996 the variety Skiff, which had previously shown seedling resistance to NFNB, also showed infection in a small area of the South-East. In 1998 NFNB spread rapidly across the state and most if not all Skiff crops were heavily infected with many producing 100% screenings at harvest. Since that time, increased virulence has appeared on the varieties Barque, Keel, Maritime, Fleet and other varieties carrying a mix of seedling and adult plant resistances. This pathotype diversity has been studied in the South Australian NFNB population using a set of 24 current and historic South Australian barley varieties grown both as seedlings and as adult plants. Well-defined controlled environment conditions were used including long day lengths to even out variety maturity differences and to speed up the evaluation process. The results showed that most isolates tested were different from each other reflecting a very diverse sexually reproducing pathogen. The data also showed the presence of resistances effective at the seedling stage and others only effective at later growth stages. The data has been invaluable for the selection of isolates suitable for screening of breeding lines carrying specific resistances and also for the genetic study of resistance in specific mapping populations. The testing has supported historical observations of durable resistance in some key historical South Australian varieties. New mapping populations have been developed to study the genetic control of this durable resistance and early results from one of these populations, SloopSA*Fathom will be presented.

Genetic diversity of *Pyrenophora teres* f. *teres* in Tunisia using pathotype and AFLP markers

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The genetic diversity among 78 *Pyrenophora teres* isolates originating from different agro-ecological areas in Tunisia was investigated using host differentials and amplified fragment length polymorphism (AFLP) markers. The Pyrenophora teres f. teres isolates were tested for their pathogenicity against 14 differential barley cultivars. Based on unweighted pair-group method with arithmetic averaging clustering and mean disease rating scores, three virulence groups were identified with low, intermediate and high virulence. Twenty two pathotypes were determined. AFLP markers have been employed for the first time to identify patterns of population structure in 17 Pyrenophora teres f. teres populations from Tunisia. Using 401 amplified polymorphic DNA markers (AFLP), variance analyses indicated that most of the variation is partitioned within rather than between populations. Genotypic diversity (GD), defined as the probability that two individuals taken at random had different genotypes, was high for populations from Rihane, local landraces, and different agro-ecological zones (GD =0.75-0.86). There was high genetic differentiation among pathogen populations from different host populations in Tunisia (Gst = 0.39, ht= 0.263), which may be partly explained by the low gene flow around the areas sampled. AFLP profiling is an effective method for typing the genetically diverse pathogen Pyrenophora teres f. teres. For instance, effective sources of resistance to P. teres in CI09214, TENN61-119, and CI04795 were simply inherited, and incorporated into local barley cultivars that should be feasible. The implications of these findings for Pyrenophora teres f. teres evolutionary potential and net blotch-resistance breeding Tunisia were also discussed.

Development of a set of differential barley genotypes and analysis of virulence diversity in the population of *Bipolaris sorokiniana* in China

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Barley spot blotch, caused by B. sorokiniana (teleomorph Cochliobolus sativus) has been the most destructive disease causing significantly barley yield losses in the northeast China since 1990s. In this area, especially in the east part of Inner Mongolia and Heilongjiang province, barley is one of the major crops. Breeding durable resistance barley cultivars and their application is the most economical strategy for controlling the disease. However, there is no documented evidence of the occurrence of virulence variation in the population of C. sativus on barley in China. Besides, it is not clear that the resistance levels among the cultivars developed in recent years, and resistance evaluation of barley germplasm collections to spot blotch has not ever been conducted systematically. Therefore, it is highly urgent to establish a set of differential genotypes to characterize virulence variation and find out the prevalent pathotypes of B. sorokiniana. Based on the resistance identification of barley cultivars and germplasms to pathogen isolates derived from different barley grown regions in North and Northeast China in seedling stage, a set of differential genotypes was set up, which consisted of ND B112, Kenpimai 11, 10PJ-24, Kenpimai 9, Mengpimai 3, Tradition, Bowman, Kenpimai 7, Varunda, Morex, Zaoshu 3 and ND 5883. All of these genotypes are of significant difference in their genetic backgrounds based on pedigree analysis and in spot blotch resistance spectrums. Among them, ND B112, Bowman and ND 5883 were used as differential hosts for B. sorokiniana in the United States, and the others were used as major cultivars in China for a long time. We also selected the coded triplet nomenclature system developed by Limpert and Müller (1994) for B. sorokiniana. After virulence variation being tested with this set of differential genotypes, pathotypes of B. sorokiniana were classified into several groups. Results indicated the existence in the broader virulence diversity in the pathogen population in North and Northeast China. The prevalent pathotype of C0047 is most widely distributed in the east of Inner Mongolia, Peking, and in the provinces of Heilongjiang, Gansu and Hebei, and its occurrence frequency is as high as 20.0%. The occurrence frequency of another two pathotypes of C0007 and C0067 is nearly 12.7%, which may possibly become prevalent ones in the future. So we should avoid using the susceptible barley genotypes in the areas in which pathotypes of C0007, C0047 and C0067 are prevalent. The development and application of this set of differential genotypes will greatly facilitate the deployment of resistance breeding of barley to *C.sativus* and the study about its virulence variation in China.

Evidence of *Pyrenophora teres* f. *teres* adaptation to barley cultivars with different levels of resistance

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Net blotch disease is one of the major production constraints of barley worldwide. Efficient protection methods, mainly through plant breeding and cultivars deployment, require sufficient knowledge of the effect of host selection pressure on the pathogen population for a durable resistance management. The purpose of this study was to investigate the evolution of Pyrenophora teres virulence with consecutive passages on barley cultivars with various resistance levels, and to evaluate changes in quantitative components of pathogenicity of the recovered isolates. Experiments, carried out in the greenhouse, aimed to reproduce ten consecutive passages with artificial inoculations of a single isolate of P. teres f. teres on three susceptible and two resistant barley cultivars. The virulence of the derived isolates was assessed and detached leaves method was used to reveal changes in pathogenicity of isolates that reached the tenth generation (G10). Results showed a significant effect of the host genotype on the virulence of P. teres isolates. Whether with infection types or disease severity, recovered isolates from each host cultivar coevolved into adapted pathotypes that depend on host genetic diversity. Virulence quantification confirmed that virulent strains derived from isolates inoculated to resistant hosts. Serial passages on resistant cultivars Heartland and Taffa increased disease severity by 58% and 66% respectively. However on susceptible hosts (Annaceur, Arig8 and Massine), isolates evolved into avirulent to moderately virulent strains. Detached leaves assays revealed that the increased virulence of Heartland G10 isolate was observed within a short incubation period and a high severity. However, less virulent isolates deriving from susceptible hosts induced changes in seven components of pathogenicity. In conclusion, this work highlights the evidence of P. teres adaptation to barley host genotypes and the dynamic character of virulence. Considering selection pressure and emergence of new pathotypes, that could overcome resistant cultivars, is crucial for an accurate disease management practices.

Virulence attenuation by successive transfer of *Pyrenophora teres* f. *teres* on a synthetic medium

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This current study aimed to investigate changes in virulence of *Pyrenophora teres* initiated by successive conidial transfer on synthetic medium. Three isolates of *Pyrenophora teres* f. *teres* labeled as Tam, Rabat071 and Merchouch were chosen for their different virulence levels and were subjected to successive transfers on homemade V8 juice agar medium. Successive transfers ended when we reached a total of 70, 40 and 15 transfers for Tam, Rabat071 and Merchouch isolates respectively and also when isolates' sporulation was sparse and rare. Along with their original isolates, virulence of 14 intermediate generations of Tam, 4 of Rabat071 and 2 of Merchouch were evaluated on a set of 22 differentials for their induction of disease severity and infection types. Results pointed out that isolates' virulence evaluated as disease severity and infection types was evolving with serial transfers. These transfers gradually attenuated virulence of all original isolates of *P. teres.* f. *teres* until converting them into avirulent ones. This drastic change in virulence was coupled with considerable phenotypic changes such as a decrease in conidial size, low intensity of sporulation and a fade color of the cultures. Furthermore, this virulence decline was dependant on initial virulence of the original isolates; the more an isolate is virulent the greater the number of successive transfers is required to invert its virulence. Virulence attenuation also differed according to cultivars resistance level. Isolate Tam virulence decreased by 57% on susceptible and moderately susceptible cultivars and by 73% on moderately resistant to resistant ones. This epigenetic effect on virulence should draw scientist attention to this change in virulence for reliable results in research activities that deal with virulence evaluation and/or selection of resistant lines or cultivars. And opens more research on how virulent genes are silenced.

Study of the dynamics of the Moroccan population of *Pyrenophora teres* f. *teres* collections separated by 4 years against five Moroccan varieties of barley

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In Morocco, Pyrenophora teres f. teres (PTT) pose a serious risk to barley cultivation. This situation is due to different causes as monoculture that contributes to spread the pathogen that is able to evolve and overcome the deployed resistance. In order to verify the possible evolution of PTT, it is proposed to check the population dynamics in time (two crop years apart by 4 years) and space (6 regions) for the type of infection studied in 5 Moroccan varieties that was considered as a National differential set and check the stability of the resistance of each level. Selected varieties are Acsad 176, Rabat 071, Aglou, Arig 8 and Tamellalt. The results showed that the behavior of the isolates of P. teres to the host plants was not always stable and the variation is seen in the same region and between regions. This can be justified by the variation of the weather. There is also the exchange of genetic material between regions that would have influenced the response of the isolates; knowing that the majority of Moroccan farmers are supplied in the seeds of the souk or even constitute their own seed stock. Therefore the genetic power of this material wears out and increases the pathogen\'s potential to evolve under selection pressure.

Investigation of Morphological diversity of Net Blotch (*Pyrenophora teres* f. *teres*) infecting barley (*Hordeum vulgare*) in Morocco

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Net blotch caused by *Pyrenophora teres* net form is the most destructive foliar disease of barley (Hordeum vulgare) in Morocco. Pyrenophora teres is genetically and morphologically diverse pathogen of all the barley growing areas of Morocco. After survey of different growing areas of barley representing different climatic conditions, infected leaf samples were collected for the study. Isolates were identified by the comparison of cultural type refered to literature. Criteria considered in this study are importance of mycelial growth, thall colour, colony texture, formation of concentric ring on nutrient media and sporulation. Based on the analysis of the dissimilarity for \"Weighted Neighbor-Joining\" the results of the overall classification of the population showed that there is a divergence between isolates. The combination of cultural characters showed that there are 7 distinct groups or morphotypes with different levels of similarity to identified isolates. The diversity indicates the presence of many strains of *P. teres* f. *teres* in Morocco.

Responses of differential barley genotypes to Moroccan isolates of *Pyrenophora teres* f. *teres*

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Barley net blotch, caused by *Pyrenophora teres* f. *teres (Ptt)*, is one of the most important foliar diseases in Morocco. The disease causes appreciable yield losses under favorable environmental conditions. To identify effective sources of resistance to net blotch, the understanding of virulence spectrum of *Ptt* is essential.
Sixteen barley genotypes were inoculated at seedling stage with 15 *Ptt* isolates that were collected from different agro-ecological zones of Morocco during the disease survey of 2009-2010. The experiment was conducted in factorial arrangement of treatments in randomized complete block design with three replicates. ANOVA and GGE biplot were employed to understand the barley-*Ptt* interactions. The ANOVA revealed highly significant effects of genotype (G), isolate (I) and G×I interactions. Furthermore, the results indicated that race nonspecific interaction and aggressiveness of *Ptt* isolates dominated over gene-for-gene interaction (race specific/virulence). The study of virulence of *Ptt* isolates revealed highly diverse virulence pattern of Moroccan isolates. In addition, GGE biplot revealed that Moroccan cultivars, Taffa and Aglou, showed increased level of stable resistance to *Ptt* comparatively while Coast and Rabat071 were the most susceptible genotypes. The emergence of new *Ptt* pathotypes which are highly virulent to durable resistance in Rabat071 poses a greater risk of breaking down currently deployed resistance to net blotch in Morocco. GGE Biplot also discriminated virulence of *Ptt* isolates of which Pt2, Pt7, Pt8, and Pt4 being the most virulent isolates, while Pt10 and Pt11 were found the least virulent isolates. A careful evaluation and selection of *Ptt* isolates based on virulence pattern to barley genotypes is essential for successful barley breeding for resistance to net blotch in Morocco.

Application of PCR assay for detection of *Ramularia collo-cygni* in the asymptomatic phase of infection in winter barley (*Hordeum vulgare*)

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Over the last years Ramularia leaf spot (RLS) caused by Ramularia collo-cygni (Rcc) has beome recognized as an important disease of barley. Rcc field screening has some limitations; it is slow, time-consuming and depends strongly on the presence of the proper environmental conditions such as humidity, temperature, sun-light and simultaneous presence of other fungal and bacterial pathogens. The main objective of the present study was to develop a reliable PCR detection of Rcc in leaf of winter barley varieties â€œFredericusâ€ and â€œCinderellaâ€ that helps in the development of a successful disease management in the early phase of infection, including symptomless Rcc infection from vegetative to reproductive stages of the host plants. For a sensitive experiment, the desired PCR primers must be highly specific as there may be a number of other fungal or bacterial saprophytes or pathogens present on the surface of the leaves which will be extracted as well as Rcc. A relationship between Rcc DNA levels and disease symptoms was established in winter barley under natural infection conditions. Pathogen extracted from barley leaves could be quantified to the picogram level in both leaves showing symptoms of infection and symptomless barley leaves. Analysis of seeds from infected plants did not reveal the presence of Rcc DNA. Thus, it seems that fungus seems capable of colonization of different grass species and can be transmitted to winter barley.

Monitoring the population of barley powdery mildew pathogen, *Blumeria graminis* f. sp. *hordei*, in North and North-Eastern regions of Iran

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Powdery mildew of barley caused by the Ascomycetous fungus, *Blumeria graminis* f.sp. *hordei*, is one of the most important foliar diseases of barley worldwide including the major barley production regions in Iran. Assessment of the virulence spectrum of the pathogen population help to determine effective sources of resistance to be incorporated in breeding programs. In order to monitor the population of powdery mildew pathogen in North and North-East regions of the country, trap nurseries were established at four powdery mildew conductive locations in Northeast (Mashhad), North-central (Karaj) and North (Sari and Gorgan) during three cropping seasons (2013-2016). At each site, 87 lines and cultivars including 19 Pallas near-isogenic lines, a supplementary set including 33 cultivars carrying known or unknown resistance gene (s) and 35 Iranian commercial barley cultivars and promising breeding lines were evaluated against powdery mildew under the natural disease development. Based on the results there was a significant variation in virulence spectrum of the pathogen in different locations. The recessive *mlo* resistance gene in different genetic background, *Mla7*, *Ml(No3)*, *Mla12*, *Mla13, Ml(Ru3), Ml(Em2) R* genes, were highly effective in all locations. Virulences for *Mlat*, *Mlh, Mlk, Mlp, Mlg* and *Mlcp* appear to be common in most locations. The local population of the pathogen in Southwest (Dezful) tended to be more aggressive than other locations since only *Mla7, Ml(No3), Mla12, Ml(Em2), Mla13, Ml(Ru3)* and *mlo* were effective. In addition to the *mlo* cultivars, a number of European barley cultivars that carrying a combination of different resistance genes including cv. Punto (*Mla3*, *Ml(Tu2*), *Ml(Im9*), *Ml(Hu4*)), Tofta (*Mla13*,*Ml*(*Im9*)), Meltan(*Mla13*,*Ml*(*Im9*), *Ml*(*Hu4*)), Jarek (*MlLa*, *Ml*(*Kr*)) and Escort (*Mlg*, *Mla7*, *Mlk*,*MlLa*)) were fully resistant in all locations.

Impact of climate change on susceptibility to stripe disease in barley

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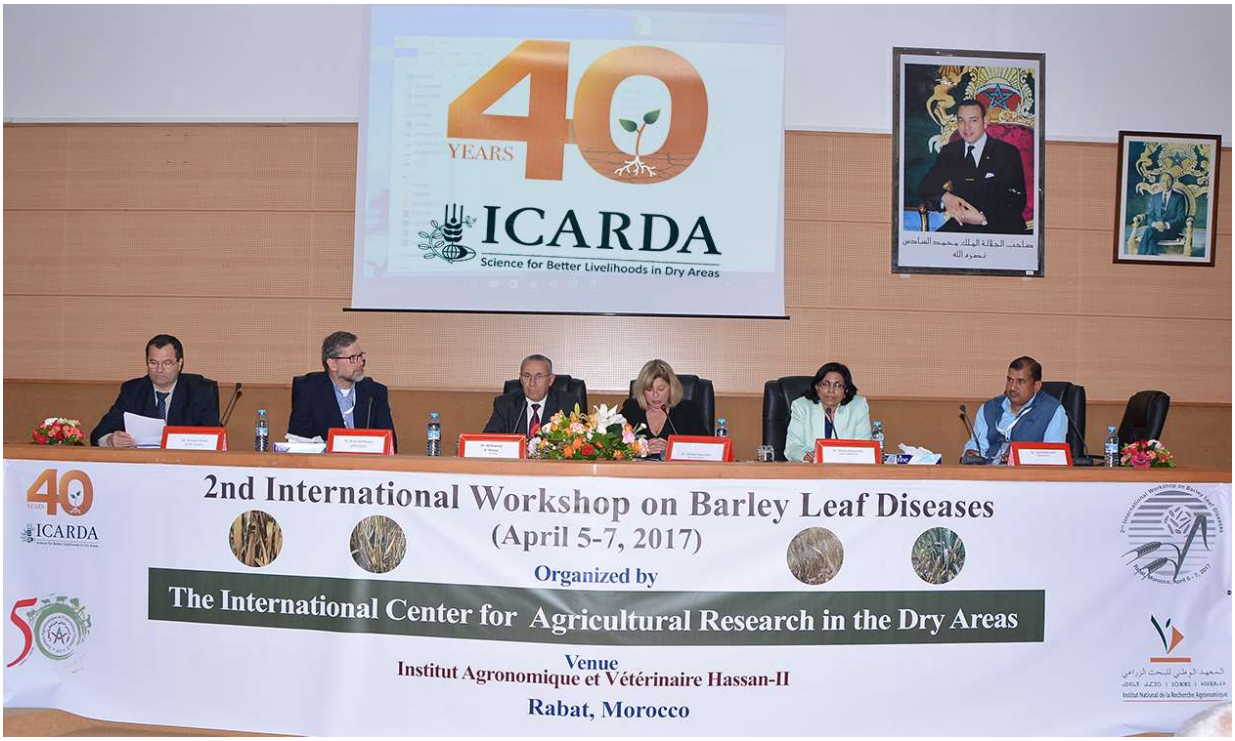
The weather plays a big role in the development of the disease on plant crops and increases the risk of severe epidemics. In fact, increased frequency of heat stress, droughts and floods negatively affect crop yields. Changes to any of these climatic factors may influence the distribution and biology of plant pathogens with positive, negative or neutral effects. Even if research in the effects of climate change on plant disease continues to be limited, some striking progress has been made in Tunisia. In fact, researchers suggest that climate change will increase the risk of serious diseases on cereals in Northern Tunisia by the middle of this century where climate change models are predicting warmer, wetter winters for the country. The purpose of our study was to establish the influence of climate change on stripe barley disease based on data gathered from field varieties/accessions trials conducted in the Béja region of north western Tunisia under rainfed conditions. Data were extracted for the years 2009 to 2015. Every year, there were seven local accessions, one introduced line and two standard varieties (the most cultivated in this region). The options other than varietals deployment are to identify and incorporate new genetic diversity in barley, produce stable resistant genetic stocks and eventually transfer these new genes into some leading, presently resistant or even susceptible varieties. Results showed that in this region, global minimum temperature and precipitation have increased and summers rains have become more frequent and stormy. Results revealed also that year by year, there is resurgence of Stripe barley diseases but intensity of pathogenicity depends upon the resistance level of the germplasm and the concerned year. Intensity of disease infection during the year 2011/2012 was higher as compared to the year of 2010/2011.This severity may be attributed to wet weather, which prevailed during this year. More rainfalls during 2011/2012 favored the intensity of disease in almost materials. Plant material tested demonstrated that varieties/line/accessions are infected and most of them are susceptible but one autochthonous accession and introduced line are partially resistant and had a great potential to be used as a resistant germplasm source against Stripe.



The 2nd International Workshop on Barley Leaf Diseases was inaugurated by Prof. Mohamed Badraoui, DG, INRA Morocco and member ICARDA Board of Trustees. The other dignitaries present included Dr. Shoba Sivasankar, Director Dryland Cereals, Dr. Brian Steffenson, IOC Chair, IWBLD, Dr. Wafae Fihiri, Director, IAV Hassan-II, Dr. Michael Baum, Director BIGMP, ICARDA and Ramesh Verma, organizing sectary, 2nd IWBLD

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(April 5-7, 2017)

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