5th International Symposium on Fusarium Head Blight
2nd International Workshop on Wheat Blast

COSTÃO DO SANTINHO • FLORIANÓPOLIS SC • **2016**

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Book of Abstracts

5th International Symposium on Fusarium Head Blight
2nd International Workshop on Wheat Blast

April 6 to 9, 2016 - Florianópolis, SC, Brazil

scabandblastofwheat.org

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Ed. Universidade de Passo Fundo
Passo Fundo, RS
2016

Proceedings compiled and edited by:

Universidade de Passo Fundo
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99052-900Passo Fundo, RS
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36570-900 Viçosa, MG
Telefone: (31) 3899-2200
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Editorial supervision: *Fátima Maria De Marchi*

Cover**:** *Logo by Vide Design*

Typesetting: *Emerson M. Del Ponte and*

Fátima Maria De Marchi

Bibliographic standardization: *Jucelei Rodrigues Domingues - CRB 10/1569*

1st edition

Publicação digitalizada (2016)

Cataloguing in publication record (CIP)
UPF

International Symposium on Fusarium Head Blight (5. : 2016 : Florianópolis, SC, Brazil).
Book of Abstracts [electronic resource] / Emerson Medeiros Del Ponte ... [et al.] editors. – Passo Fundo : Ed. Universidade de Passo Fundo, 2016.

Access: < http://mosaico.upf.br/~events/scabandblastofwheat-book.pdf >
Quadrennial: 2000-
Other editors: Gary Carlton Bergstrom, Willingthon Pavan,
Alexandre Lazzaretti, José Maurício Cunha Fernandes.

1. Wheat - Disease - Symposium. 2. Fusarium - Blight - Symposium. I. Del Ponte, Emerson Medeiros, ed. II. Bergstrom, Gary Carlton, ed. III. Pavan, Willingthon, ed. IV. Lazzaretti, Alexandre, ed. V. Fernandes, José Maurício Cunha, ed. VI. International Workshop on Wheat Blast (2. : 2016 : Florianópolis, SC, Brazil). VII. Title. VIII. Title: Annals of V International Symposium on Fusarium Head Blight. VI. Title: Annals of II International Workshop on Wheat Blast.

CDU: 633.11

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Co-organization, planning and logistic support
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Welcome

It is with pleasure that we welcome you to the 5th International Symposium on Fusarium Head Blight (ISFHB) and the 2nd International Workshop on Wheat Blast (IWWB). This will be the first joint meeting on two of the most important fungal diseases with devastating impact on wheat, both of which have emerged as an increasing threat to global food security and human health.

The joint meeting provides an opportunity for two complementary research communities to come together and learn cutting-edge advancements in these exciting research areas. We hope it will help to promote cross-community interactions and facilitate the development of joint research - all of this within a single meeting.

The overwhelming interest in participation in these conferences has exceeded earlier expectations. From a total of 141 submitted abstracts, it was possible to develop what we, the Organizing Committee, believe is an exciting scientific program that includes 20 keynote lectures by internationally recognized keynote speakers, 24 invited talks and 97 poster presentations by speakers from all over the world.

We will open our meeting with a plenary talk that will provide an overview on the risk status of mycotoxins in wheat and barley in South America. Then, we will proceed with five sessions of the 5th ISFHB over three days and four sessions of the 2nd IWWB on the last day. In the 5th ISFHB meeting, oral and poster presentations will follow the keynote talks for each session.

The organization of both conferences was only possible due to the help of several people. Special thanks go to the Session Chairs and the Scientific Committee Members for supporting the development of the scientific program. We are particularly grateful for the effort of the local hosts Embrapa, Federal University of Viçosa, University of Passo Fundo, Epagri, and IFSUL for organizing the venue, registration, website and logistics of the meeting. The financial and in-kind support from CAPES, Biotrigo, Bayer and Coodetec are likewise gratefully acknowledged. Special thanks to FB Eventos for helping us with the organization.

We wish all participants a very fruitful and inspiring conference and we look forward to the interesting keynote lectures, oral and poster presentations. We hope to have the chance to interact with many of you during the course of the conference. We also hope that the conference will support ongoing collaborations and initiate new collaborations to further advance research on Fusarium head blight and wheat blast.

José Mauricio Cunha Fernandes
Chair of the Joint Meeting

Barbara Valent
Co-Chair of the Joint Meeting

Emerson Del Ponte
Scientific Committee Chair

Table of contents

5th International Symposium on Fusarium Head Blight

5th ISFHB: Plenary talk

5th ISFHB: Session 1 - Germplasm Development and Breeding for Scab Resistance

5th ISFHB: Special Session – Fhb1 gene

5th ISFHB: Session 1 - Germplasm Development and Breeding for Scab Resistance

5th ISFHB: Session 2 - Genetics and Genomics of Scab Resistance

5th ISFHB: Session 3 - Genetics and Genomics of Fusarium Species

5th ISFHB: Session 4 - Epidemiology and Management

5th ISFHB: Session 5 - Toxicology and Food safety

2nd International Workshop on Wheat Blast

5th ISFHB

Keynotes, Oral and Poster Presentations

K1 *5th ISFHB: Plenary talk*

TRICHOTHECENES CONTAMINATION IN WHEAT AND BARLEY: A PROBLEM FOR THE PLANT SCIENCE COMMUNITY

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Deoxynivalenol (DON) is a type B trichothecene produced by *Fusarium graminearum* and *Fusarium culmorum*. DON has important toxicological relevance and its synthesis is stimulated under conditions of high humidity and moderate temperatures. More than 200 trichothecenes have been reported and DON is the most frequently detected in cereals such as wheat and barley. DON affects human and animal health, causing temporary acute nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever. DON is also known as vomitoxin due to its strong emetic effects post-consumption, especially when ingested by swine. In a study conducted by the Laboratório de Análises Micotoxicológicas (LAMIC) of the Universidade Federal de Santa Maria, over the last 10 years 69% of 8,444 analyzed samples of Brazilian wheat and its byproducts were contaminated by DON, with an avarege level of 760 ppb. During the same period, 89% of 8,703 barley samples analysed were contaminated with an average level of 846 ppb. Wheat and barley are winter crops highly sensitive to climate changes, which justifies the variations in DON prevalence and contamination in these two cereals between different crops and regions. Wheat and barley are classified by quality standards, and the grain of lower quality is destined to animal feed industry. Over the last 10 years DON was analyzed in more than 8,500 samples of feed intended for different species of animals. The mycotoxin prevalence was 28% of the samples with an average concentration of 102 ppb. It is important to highlight that in Brazil corn is the basis for the feed formulation. Since the prevalence of DON is about 19% in corn (with an average level of 97 ppb), the higher prevalence of DON in feed, comparing to corn, may be related to the inclusion of wheat and barley in feed composition. DON's high prevalence and its high concentration levels in wheat and barley signify a great risk to both human and animal health. Therefore, it is advisable that a continuous monitoring program should be adopted in order to avoid the use of very contaminated grains in feeding. Therefore, it is imperative to use reliable contamination control methods such as implementation of good practice in the agricultural management, production systems and genetics.

Keywords: DON; wheat; barley; surveillance

5th ISFHB

Session 1

Germplasm Development and Breeding for Scab Resistance

NOVEL FINDINGS IN BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE

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Fusarium head blight (FHB) is a devastating disease of worldwide significance affecting all small grain cereals. The development of resistant varieties is certainly the most effective approach to control the disease from ecological and economical point of view. Genetic mapping of FHB resistance in wheat resulted in the discovery of numerous quantitative trait loci (QTL). The most prominent among these is *Fhb1*, located on chromosome 3B, and mainly involved in resistance to fungal spread. Using marker-assisted backcrossing we introgressed *Fhb1* into susceptible durum and triticale elite background and generated segregating mapping populations. These populations have been genotyped and phenotyped in field trials. Notably, in these populations markers linked with *Fhb1* were clearly associated with FHB severity, confirming for the first time its successful introgression into durum wheat and triticale. Novel and highly breeding relevant germplasm has been generated. Our previous data revealed the association of *Fhb1* with toxin metabolization of DON to DON-3-Glucoside (D3G). The effect of FHB resistance breeding on the amount of masked mycotoxin D3G was investigated, showing that increasing FHB resistance reduced both DON and D3G levels in the grain. The reduction was lower for the masked toxin resulting in higher D3G/DON ratios in resistant lines. This phenomenon is not *Fhb1* specific, but rather depending on the resistance level. The second major QTL *Qfhs.ifa-5A* is mainly associated with resistance to initial infection. Fine-mapping of this QTL is hampered due to its location in the low-recombining pericentromeric region of chromosome 5A. Recombination and radiation mapping allowed characterizing the QTL region in much higher resolution. Several other resistance QTL are associated with phenological traits e.g. plant height and the extent of retained anthers after flowering. We analysed the effect of the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* on FHB resistance and anther retention. Generally, reduced plant height was associated with increased FHB severity and higher number of retained anthers. The effect of *Rht-D1b* was significantly stronger for both traits than the one of *Rht-B1b*. Whether the *Rht* effects on increased anther retention is due to pleiotropy or linkage remains to be clarified. For FHB resistance breeding the semi-dwarfing allele *Rht-B1b* should be preferred over *Rht-D1b*. In several regionally adapted sources of resistance e.g. in the European winter wheat line 'Arina' where FHB resistance is governed by multiple small effect QTL, resistance QTL partly overlap with QTL for anther extrusion. We have frequently observed association of FHB resistance and anther extrusion also in modern breeding material. Anther extrusion is a highly heritable trait and selection for rapid and complete anther extrusion appears promising as an indirect selection criterion for enhancing FHB resistance. In addition we explore the suitability of genome-wide predictions for FHB resistance improvement.

Keywords: resistance-breeding; Fhb1; Qfhs.ifa-5A; anther-extrusion; Rht-genes

IMPROVEMENT OF WHEAT FUSARIUM HEAD BLIGHT (FHB) RESISTANCE IN CHINA

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Wheat scab, Fusarium head blight (FHB), is a serious disease in the Southern winter wheat and Northeast spring wheat growing area in China. In the last decade, wheat scab was expanded from the Middle and Lower Valley of the Yangtze Rive to the Yellow Rive and Huai Rive winter wheat growing region. Epidemic of wheat scab in 2010, 2012 and 2015 occurred even more seriously in the Southern Yellow Rive and Huai Rive area due to the humid and warm conditions around the flowering stage. However, the lack of cultivars resistant to wheat scab in these areas and widely using non-tillage technique are two of the most important reasons. Improvement of the scab resistant level of the cultivars in this area is an urgent task for wheat breeders. Significant progress has been achieved in the improvement of wheat scab resistance in China through the exploration and pyramiding of resistance genes (QTL's) in wheat and relative species. The resistance genes, which are different from those of Sumai 3 and Wangshuibai, have been transferred from wheat relative species, such as *Leymus racemosus*, *Rogneria kamoji* and *Elytrigia elongata*, into common wheat by the development of alien addition and translocation lines. New genetic resources with high FHB resistance and high yield have been obtained by using somaclonal variation in tissue culture combined with addition of mycotoxin selection pressure in the medium. Backcross, multiple crosses and recurrent selection combine with molecular assistant selection and multiple years and multiple locations disease evaluation using single floret inoculation, soil-face wheat diseased seeds inoculation and natural condition in heavy epidemic area have been successfully used in wheat scab resistance breeding program. Most of the wheat cultivars developed and released in the Southern winter wheat and Northeast spring wheat growing area, such as Yangmai, Ningmai, Shengxuan, E-mai and Longfumai series, were with moderate scab resistance as well as high yield, good quality and agronomical trains, and occupied about 80% of these wheat growing area and have reduced the severity. The isolates of FHB pathogens collected from different ecological regions in China were used to study the aggressiveness, genotype, chemotype and sensitivity to fungicides. *F. asiaticum* and *F. graminearum* were two predominant species and co-existed in China. *F. asiaticum* seemly distributes in southern wormer region and *F. graminearum* in northern cooler region. Benzimidazole fungicide resistance has occurred in the population of *F. asiaticum* and subsequently aggravated the FHB in southern China. To counter with the resistance of *Fusarium* pathogenes to benzimidazole fungicide, a novel myosin inhibitor phenamacril has been developed. Several genes related to FHB resistance have been cloned, such as antibody and RNAi targeted to specific sequences of *Fusarium* pathogens were isolated and used in wheat transformation. Wheat transgenic plants with FHB resistance genes have been obtained.

Keywords: wheat; Fusarium head blight; China

GENOMIC SELECTION FOR FHB RESISTANCE IN WINTER WHEAT ADAPTED TO THE EASTERN USA

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Fusarium Head Blight (FHB, Fusarium head scab) is a major disease caused by-*F. graminearum* that infects wheat (*Triticum aestivum* L.) and other cereals. One major aspect of managing FHB is breeding for resistant varieties. However, breeding for resistance requires considerable time and phenotypic resources due to low heritability, environment effects and genotype x environment interactions. Marker assisted selection (MAS) has been effective for a few QTL, but most of the genes controlling resistance have a small effect and are not amenable to MAS. Genomic selection (GS) is a new form of MAS and can facilitate breeding for complex traits by estimating all marker effects simultaneously and predicting the genomic estimated breeding values (GEBVs). GS has the potential to increase the genetic gain per year by decreasing the duration of a breeding cycle. Effective GS requires high GS prediction accuracy and that may require optimization of data set used to train the GS model. We evaluated the prediction accuracy of GS in a population of 640 soft winter wheat lines. The population was evaluated in inoculated FHB nurseries in multiple environments for incidence (INC), severity (SEV), index (IND), Fusarium damaged kernel (FDK), kernel damage index (ISK), and deoxynivalenol concentration (DON). Across all traits we observed high entry-mean heritability (0.88 to 0.93). Principal component and Fst analysis indicated the population had three subgroups. GS accuracy estimated by cross-validation using all data ranged from 0.45 (INC) to 0.57 (SEV). Similar prediction accuracies were obtained within clusters but were much lower when data from one cluster was used to predict another. Eliminating the top 10-15% less predictable individuals increased prediction accuracy by up to 58%. Accuracies similar to those obtained using elimination approach could be obtained with smaller sample sizes using two optimization approaches (coefficient of determination and predicted error variance). The results indicate that GS should be very effective for FHB resistance in this population and significantly improve gain per year. Also systematic reduction of the model training data set can significantly improve GS accuracy.

Keywords: breeding; genomic selection; wheat

THE GRAIN YIELD AND QUALITY OF WINTER WHEAT CULTIVARS UNDER FHB PRESSURE, CONTENT OF *Fusarium* MYCOTOXINS - UNDERSTANDING THE RESISTANCE BY ANTIOXIDANT PATHWAYS

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Fusarium head blight (FHB) is a significant wheat disease severely limiting crop productivity, which is caused by fungi that can synthesize toxic components. Human or animal exposure to mycotoxins can harm their health and in some cases cause death, because of carcinogenic or cytotoxic activity or impairment of the immune system. Because this is also widespread disease in Croatia in three year project we want to analyse the impact of *Fusarium* spp. infection on yield components and protein quality parameters, showing the degradation of wheat proteins by reversed-phase high-performance liquid chromatography (HPLC) and the effect on processing quality parameters. The LC-MS/MS method in this study will be applied for the determination of mycotoxins in four different treatments (seed and malt in control and in inoculated treatment). Also we will check some antioxidant compounds (guaiacol peroxidase, ascorbate peroxidase, superoxide dismutase, catalase, free proline and malondialdehyde content) which can influence the fungal redox status playing an important role during trichothecene biosynthesis. In preliminary results the genotypes Srpanjka and Olimpija showed the highest resistance level for resistance component I. *Fusarium*-colonized kernels were ranking from 3 to 45% in inoculated treatment, which was in correlation with AUDPC for general resistance. The best yields in the inoculated treatment were retained by genotypes Vulkan and Srpanjka which didn't had any relative grain yield loss. Differences for grain yield between the two treatments (control and inoculation) have exceeded 40%. Lower yields were accompanied with lower test weights and higher 1,000 kernel weight. The technological quality parameters, such as gluten index and falling number, were less affected by the *Fusarium* than grain yield, but anyway decrease of protein content, wet gluten content and sedimentation value was obvious. Gliadins were influenced by FHB- in one wheat cultivar by decreasing more then 25%. On the contrary, we observed a slight increase in total gliadin content and gliadin subfractions in severely *Fusarium*-infected kernels. In contrast, the content of total glutenins was significantly reduced, except in one cultivar. Consequently, gliadin-to-glutenin ratio was increased in the infected samples, while an increase in a albumins and globulins (up to 50%) was observed. In control group deoxynivalenol (DON) was present in 1 of 6 wheat seed samples (51.64 ng/ml) and 2 of 6 wheat malt samples. In inoculated treatment all samples were positive with DON (51.2 to 3,246.53 ng/ml). The most susceptible cultivar had the highest 3-ADON accumulation in kernels. Zearalenone was present in 5 of 6 malt samples in inoculated treatment (17.80-232.57 ng/ml). HT-2 toxin occurred in two samples in control (31.7 ng/ml and 33.42 ng/ml), and in one sample in inoculated treatment (30.94 ng/ml). This work has been supported in part by Croatian Science Foundation.

Keywords: wheat; grain yield; grain quality; mycotoxins; antioxidant compounds

DEVELOPMENT OF WHEAT-*Thinopyrum* TRANSLOCATIONS WITH SHORT ALIEN SEGMENT CARRYING FHB RESISTANCE GENE FHB7 AND ITS APPLICATION IN WHEAT BREEDING

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Fusarium head blight is a major wheat disease globally. A major FHB resistance gene, designated as *Fhb7*, derived from *Thinopyrum ponticum*, was earlier transferred to common wheat, but was not used in wheat breeding due to linkage drag. The aims of this study were to (1) saturate this FHB-resistance gene region; (2) develop and characterize secondary translocation lines with shortened *Thinopyrum* segments carrying *Fhb7* using *ph1b*; (3) pyramid *Fhb7* and *Fhb1* by marker-assisted selection. *Fhb7* was mapped in a 1.7 cM interval that was flanked by molecular markers *XsdauK66* and *Xcfa2240* with SSR, diversity arrays technology (DArT), EST-derived and conserved markers. KS24-2 carrying *Fhb7* was analyzed with molecular markers and genomic in *situ* hybridization, confirming it was a 7DS.7el$_2$L Robertsonian translocation. To reduce the *Thinopyrum* chromatin segments carrying *Fhb7*, a BC$_1$F$_2$ population (Chinese Spring *ph1bph1b**2/KS24-2) was developed and genotyped with the markers linked to *Fhb7*. Two new translocation lines (SDAU1881 and SDAU1886) carrying *Fhb7* on shortened alien segments (approximately 16.1% and 17.3% of the translocation chromosome, respectively) were developed. Furthermore, four wheat lines (SDAU1902, SDAU1903, SDAU1904 and SDAU1906) with the pyramided markers flanking *Fhb1* and *Fhb7* were developed and the FHB responses indicated lines with mean NDS ranging from 1.3 to 1.6 had successfully combined *Fhb7* and *Fhb1*. Three new molecular markers associated with *Fhb7* were identified and validated in 35 common wheat varieties. The translocation lines with shortened alien segments carrying *Fhb7* (and *Fhb1*) and the markers closely linked to *Fhb7* will be useful for improving wheat scab resistance.

Keywords: *T. aestivum*; *T. ponticum*; wheat scab; Fhb7

THE ROLE OF PHYTOHORMONES IN RESISTANCE TO FUSARIUM HEAD BLIGHT AND IMPLICATIONS FOR BREEDING

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The role of the salicylic acid (SA) and jasmonic acid (JA) pathways in disease resistance responses to biotrophic and necrotrophic pathogens has been well documented. It has become clear that other phytohormone pathways are also involved in disease resistance and that some pathogens exploit these pathways to compromise host defences. We have investigated the role of gibberellic acid (GA), brassinosteroid (BR), ethylene (ET) and auxin (AUX) signalling on resistance to Fusarium head blight and diseases caused by other cereal pathogens. Our data suggest that many of these pathways are exploited by Fusarium and other pathogens to aid infection and colonisation. In the case of GA, the influence appears to differ between resistance to initial infection (Type 1) and resistance to spread (Type 2) with GA signalling promoting resistance to initial infection but compromising resistance to spread of Fusarium within the wheat head. Our findings suggest that it is important to appreciate how selecting for particular plant traits that depend upon responsiveness to phytohormones may influence resistance to FHB.

Keywords: giberellic acid; brassinosteroid; auxin; ethylene

A CANDIDATE GENE REGULATES *Fhb1* RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT

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Many FHB-resistant sources have been reported in wheat with about 50 unique quantitative trait loci (QTLs) identified. Among those QTLs, *Fhb1* from Chinese sources is the only QTL that consistently shows a major effect on FHB resistance and low DON content in diverse genetic backgrounds. Using marker-assisted backcrossing, we transferred *Fhb1* into more than a dozen of US winter wheat cultivars. Those near-isogenic lines (NILs) harboring *Fhb1* had a reduction of about 40% in FHB severity compared with non-*Fhb1* NILs in most backgrounds. Cloning of the gene underlying *Fhb1* has important implications for long-term control of the disease. We developed a fine mapping population of (Clark*8/Ning7840) F_2 using marker-assisted backcrossing, screened about 15,000 F2 plants for fine mapping using the flanking markers, Sts3B-163 and Sts3B-142, and identified 22 recombinants. Sequence alignment of the two flanking markers with the Chinese Spring 3B survey sequences located *Fhb1* on the contig Ctg0954. Fine mapping narrowed *Fhb1* to a region of ~300kb and identified 10 putative genes in the region. Gene expression analysis of the candidate genes found only one gene, designated as *TaFhb1*, that was differentially expressed between the two *Fusarium*-inoculated NILs. We also conducted an RNA-Seq experiment and identified *TaFhb1* as the only differentially expressed gene located in the *Fhb1* region. To confirm its function in FHB resistance, we sequenced the gene in two NILs and an association mapping population of 143 germplasm lines with different levels of FHB responses, and found a large deletion in the start codon region of the open reading frame (ORF) in the resistant genotype. This deletion results in a loss-of-function of *TaFhb1* and causes at least 30% reduction in FHB susceptibility. Haplotype and Candidate gene association analysis confirmed that the start codon deletion is the causal mutation for *TaFhb1*. We used RNA interference knock down the expression of *TaFhb1* in the susceptible cultivar Bobwhite, the T1 and T2 plants with lower *TaFhb1* expression show significantly slower spread of FHB symptoms in the inoculated spikes than in the non-transgenic control. Cloning *TaFhb1* reveals a new type of genes for plant resistance, provides a diagnostic marker for breeding, and may open a new door to defeating wheat FHB by manipulating *TaFhb1* levels in susceptible cultivars using bioengineering approaches.

Keywords: Fusarium head blight; *Fhb1*; susceptible gene, wheat

DETECTION OF RESISTANCE TO FUSARIUM HEAD BLIGHT (FHB) AMONG THE ELITE WHEAT GENOTYPES IN IRAN USING PHENOTYPIC CHARACTERISTICS AND MOLECULAR MARKERS

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Fusarium head blight (FHB), caused by various species of *Fusarium* particularly *Fusarium graminearum*, is one of the most destructive diseases of wheat in Iran especially in northern parts of the country. In addition to yield loss, FHB may be a threat for human and animal health and food safety due to mycotoxin production by the fungal pathogens causing the disease. In the present study, reaction of 20 lines of the Elite Regional Wheat Yield Trials from the north warm and humid zone in 2012 (ERWYT-N-2012) in Iran were determined to FHB under field conditions in two locations for two years and in the greenhouse. In addition, 43 microsatellite (SSR) molecular markers associated with resistance to FHB were examined to the genotypes to detect FHB resistance. Results of field combined data analysis of variance of disease incidence, disease severity and disease index showed that while the effect of either of year or location was significant on disease severity and index, the interaction effect of year x location was significant on disease severity and incidence (α=5%). Significant differences among the genotypes were also observed for disease incidence and index at α=5%. In addition, the interaction effects of year x genotype or location x genotype was significant on all variables, i.e. disease incidence, severity and index (α=5%). Moreover, the interaction effect of year x location x genotype was also significant on disease incidence and index (α=5%). Based on the results of field evaluations, the genotypes N-91-1 (cv. Morvarid), N-91-15 and N-91-3 were determined as the most resistant materials. Results of greenhouse simple data analysis of disease spread within the inoculated spikes (disease severity) showed significant differences among the genotypes at α=5%. Based on the greenhouse experiments, three lines of N-91-9, N-91-6 and N-91-17 were recognized as the most resistant genotypes. Therefore, wheat genotypes reacted differently under field and greenhouse conditions. It may be concluded that different mechanisms of resistance are conferring FHB resistance to wheat in field conditions and in the greenhouse. Results of marker analysis showed that four out of 43 SSR markers including *Xbarc117*, *Xgwm304*, *Xgwm261*, and *Xgwm282* were associated to FHB resistance genes/QTLs in the elite wheat genotypes. These markers were used for selection of FHB-resistant wheat genotypes. Phenotypic data obtained from the present study supported effectiveness of these SSR markers for marker-assisted selection. Findings from the present investigation may be used for providing wheat cultivars/lines identification information and in releasing new wheat cultivars.

Keywords: FHB; resistance; SSR markers

TRITICALE BREEDING PROGRAM FOR FUSARIUM HEAD BLIGHT RESISTANCE

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Since the late seventies triticale (X *Triticosecale* Wittmack) breeding has been conducted at Embrapa Trigo. Triticale is cultivated in southern Brazil with 135,000 hectares in 2005, but decreased to the lowest value in 2015, with 35,000 hectares. Factors that have contributed to this decline included less available seeds, diseases and weather damage. Fusarium Head Blight (FHB), induced by *Gibberella zeae* (*Fusarium graminearum*), is one of the most important diseases and a challenge to researchers. Here we report methods used to evaluate, at field conditions, the reaction of triticale genotypes to FHB. In the first method, three sowing times, have been used to screen genotypes. At full flowering stage, the central spikelet of 30 spikes in each plot is inoculated with 0.02 mL of a suspension containing $5x10^5$ propagules of *F. graminearum*. At the soft dough stage, FHB severity at spikes is evaluated using the following scale: 10= disease symptoms not spread beyond the infected spikelet; 30= symptoms spread to no more than three spikelets; 50= symptoms spread to less than half of the spike; 70= symptoms spread to less than three quarters of the spike; and 90 = symptoms spread all over the spike also peduncle. The disease indexes are represented by the average scores over sowing times. The highest index has been used to rank the genotypes for FHB reaction. The second method used fungicide for foliar diseases control until the booting stage. To induce infection, wheat grains with *Giberella zeae* perithecia are spread over the ground after the inflorescence emergence. The experimental area is submitted to irrigation in a daily-basis with fog formation during five minutes at every 20-30 minutes, except in rainy days. Reaction of triticale genotypes to FHB is based on the infection severity of kernels collected at ripening. Some released cultivars are moderately susceptible to scab: BRS 203 with the five types of resistance; BRS Minotauro classified as type II, but with higher levels of deoxynivalenol (DON), and the newest cultivar BRS Saturno has high yield grain is type II and III. The best results were obtained with lines PFT 1304, PFT 1402 and PFT 1404, moderately resistant to FHB.

Keywords: field trials; resistance; Triticosecale

MOLECULAR GENETIC ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANCE IN TETRAPLOID WHEAT

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Fusarium head blight (FHB) affects wheat production worldwide reducing yield and quality of harvest due notably to accumulation of mycotoxins in contaminated grains. Development of resistant cultivars is pivotal in FHB disease management. Breeding for FHB resistance in durum wheat (*Triticum durum*) is complicated by the lack of variation available in its gene pool, most durum lines being susceptible and efforts have been targeted at finding resistance sources in related wheat species. To date no successful use of FHB resistance from hexaploid wheat (*Triticum aestivum*) has been reported in durum wheat. In our institute, the major *T. aestivum* resistance QTL *Fhb1* has been introgressed in durum wheat through recurrent back-crosses (BC5) and a genotype (DBC-480-1) presenting stable and improved resistance has been identified. Three mapping populations from crosses of DBC-480-1 with durum cultivars were developed to characterize FHB resistance and evaluate the effect of *Fhb1* in agronomically adapted durum background. About 100 F7 RIL per population were evaluated during three seasons in field experiments at IFA-Tulln, Austria, using artificial spray-inoculation of *Fusarium culmorum* conidia. The lines were genotyped with DArTseq markers and analyzed by linkage mapping. A total of seven resistance-QTL was detected across experiments. QTL for FHB resistance was repeatedly detected at the locus of *Fhb1* on 3B, providing stable and improved resistance. The major effect resistance-QTL overlapped with QTL for plant height notably at the semi-dwarf loci *RhtB1* on 4B. Morphological characters play an important role in modulating response to FHB disease and strong negative correlation between plant height and FHB resistance was evident. Among short plants, resistance was enhanced in lines carrying *Fhb1*. The results are promising for future resistance breeding since, for the first time, improvement of FHB resistance in *T. durum* could be achieved by introgression of resistance derived from *T. aestivum*. Selected short lines harboring *Fhb1* represent a step forward in enhancing FHB resistance and are readily incorporated in durum wheat breeding programs. We gratefully acknowledge financial support from the Austrian Science Fund (FWF), projects P17310-B05; and from the French Ministry of Higher Education and Research, CIFRE funding 2012/1405.

Keywords: durum wheat; *Fhb1*; tetraploid wheat

RESISTANCE ANALYSIS AND UTILIZATION RESEARCH ON SCAB-RESISTANT WINTER WHEAT SHENGXUAN 6

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Shengxuan 6, which was certified by the National Wheat Professional Committee in 2009, is the first new wheat variety approved in China for high yield and scab resistance in winter wheat region in the mid-lower Yangtze River plain. During the evaluation period (2007-2010), the scab-resistance level was identified as R by Agriculture Ministry designated organizations in four consecutive years. DNA molecular markers were utilized to study the resistance-associated markers in Shengxuan 6, Sumai 3, and Wangshuibai. The results showed that Shengxuan 6 not only carried resistance markers presented both in Sumai 3 and Wangshuibai, such as Gwm149, Gwm533, Umn10, Barc075, Jaas01on Chromosome 3B; Gwm518 on chromosome 6B; Gwm88 on chromosome 7A; but also markers presented in only one of them. Examples of Sumai 3 specific markers are Gwm533 and Gwm493 on chromosome 3B; Wmc491 on chromosome 4A/4B, and Gwm304 on chromosome 5A. Examples of Wangshuibai specific markers are Wmc291 and Barc133 on Chromosome. It was demonstrated that Shengxuan 6 possesses abundant genetic background of scab resistance. In order to further expand its scab resistance breeding space, dozens of hybrid combinations were made between Shengxuan 6 and other varieties, namely Zhoumai and Lankao series in Henan, Jimai series in Shandong, and Huaimai series in Jiangsu. A group of stable strains with *moderate to high resistance level*, high yield potential, and acceptable agronomic traits were obtained which are adapted to the Huanghuai winter wheat area. Some of them have participated in the national or provincial variety assessments trial. In spring 2015, the *artificial inoculation* by single flower droletp method was conducted on the two F2 populations: AiKang 58 (highly susceptible varieties)/Shengxuan 6 and Zhoumai25 (highly susceptible varieties)/Shengxuan 6, in three locations (Henan Lankao, Anhui Chuzhou, and Shanghai Chongming). After 22 days inoculation, the disease spikelet rate was investigated. The results showed that the individual plants with less than 35% scab spikelet rate are over 75% of the total population in all three locations, i.e. 3/4 of the total plants are mid or highly resistant to scab. It's demonstrated that Shengxuan 6 is a good resistant source to improve winter wheat cultivars for scab resistance in Huanghuai region, and can be used as a genetic breeding material with abundant resources for breeders.

Keywords: wheat scab; resistant resource; Shengxuan 6; resistance analysis

A SYSTEMIC APPROACH TO INCORPORATE THE HIGHEST LEVELS OF FHB RESISTANCE WITH AGRONOMIC MERIT AND QUALITY IN WHEAT

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To create FHB resistant germplasm (and cultivars), the systemic approach described by Comeau *et al.* (2010) is significantly more successful than other approaches used by Canadian breeding programs, with probability levels ranging from about $p<0.05$ to $p<0.00006$ for many key traits. In Canada, the systemic approach was used to create complex crosses that were selected with complex stress for a few generations (F_1 to F_5-F_7). Stresses included BYDV, *Fusarium* and rust inoculations. Then, the resistant germplasm selected was shared with public and private breeding projects for further crosses. Breeders also isolated candidate lines for cultivar registration directly from the systemic germplasm. The result is clear: for disease resistance traits at large, and especially for FHB, the systemic-derived lines significantly surpass other candidate lines. Frequently the systemic approach delivered better protein and sometimes higher yields. High protein correlates to low yield; the systemic approach slightly reduced this undesirable effect. We got a protein increase (significant, $p<0.006$) with fewer drawbacks. The method effect on yield is not significant: some systemic lines are high yielders, and others, low yielders. Yet some good yielders have the FHB resistance with very good agronomics, and quality getting close to what would make a cultivar popular. Putting high FHB resistance in a short statured plant remains a challenge, not impossible, but it is a difficult goal which has not yet been achieved. We have developed medium-height genotypes with proper straw properties-for lodging resistance; this compromise works best in Eastern Canada. Other methods exist to create FHB resistant lines, but so far the systemic way is the best. The method has a parallel in systemic methods used by EMBRAPA in Brazil, where the overall emphasis is stronger on quality and agronomics than on FHB and BYDV. Success also crowned the EMBRAPA endeavour. Therefore the systemic approach had success in two different countries. We conclude that wheat breeders should pay attention to such new ideas, in which the focus is on globally better phenotypes rather than on specific genes. The method is adaptable to other species, once the basics are understood.

Keywords: breeding; systemic approach; stresses; diseases

EVALUATION OF IMPORTED SCAB ENTRIES UNDER FIELD AND GLASSHOUSE CONDITIONS

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Fusarium head blight (FHB) is one of the most problematic diseases in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) worldwide. South Africa (SA) is not exempted from this disease and it occurs frequently in the expanding irrigation areas. Currently there are no resistant cultivars available in SA and no chemicals registered to control the disease. Therefore a project was initiated to improve the resistance of the current susceptible commercial cultivars. Resistant donors/entries were imported and evaluated in the field and glasshouse to confirm their respective FHB resistance against local isolates. The project imported 13 different resistant donors from the United States Department of Agriculture (USDA) and 73 entries from the 13th -15th Fusarium Head Blight screening nurseries (FHBSN) from the International Maize and Wheat Improvement Center (CIMMYT), Mexico. These donors/entries were phenotypically screened in a field and glasshouse at Small Grain Institute near Bethlehem. The donors/entries were planted in a honeycomb design, together with Gamenya as a very susceptible control and Sumai #3 as a resistant control. The entries were inoculated by spray inoculation during flowering with a cocktail of different single spore *Fusarium* isolates. Inoculated heads were covered with plastic bags to increase the humidity and were screened 28 days post inoculation to test their resistance. Entries were evaluated by using a CIMMYT scale to determine resistance. The 13 USDA resistant donors were evaluated over two years (2013 and 2014). Five donors displayed resistance, five donors displayed moderate resistance (including Sumai #3), three donors were susceptible and one entry was the susceptible control (Gamenya). The 73 CIMMYT FHBSN entries displayed the following reactions: 12 entries were resistant, 14 entries were moderately resistant, 14 entries were moderately susceptible, 14 entries were susceptible and 19 entries were very susceptible. The 13th FHBSN entries were evaluated in the glasshouse and the results showed that eight entries were resistant, three entries were moderately resistant, two entries were moderately susceptible, four entries were susceptible and one entry was very susceptible (Gamenya). Nine of the resistant and moderately resistant donors from the USDA were incorporated in a pre-breeding crossing block. The reason being to stack a number of different FHB resistance genes from diverse donor sources into SA wheat cultivars that are well-adapted to the irrigation production areas. The 14th and 15th FHBSN will also be evaluated in the glasshouse to confirm resistance. Testing of different FHB donors/lines are imperative to the development of resistant cultivars. Host plant resistance remains the most efficient and environmentally responsible method to control FHB.

Keywords: screening; CIMMYT; USDA

DEVELOPMENT OF WHEAT VARIETIES WITH FUSARIUM HEAD BLIGHT RESISTANCE IN HUANGHUAI WHEAT REGION OF CHINA

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In recent years, Fusarium head blight (FHB) was gradually expanded to HuangHuai wheat region, main wheat producing zone in China, and made great damage in epidemical year of 2012 and 2015. Unfortunately, most of the varieties from this region are susceptible to FHB due to the lack of resistant germplasms in breeding programs and neglecting to develop resistant varieties. Developing varieties with resistance to FHB is now an urgent task for wheat breeders in HuangHuai wheat region. At present, the resistant germplasms are mainly from Yangtze River region and with spring growing habit. It is very hard to utilize these germplasms directly in traditional breeding program. To accelerate the pace of developing resistant varieties to FHB, molecular marker assistant selection (MAS) backcrossing and doubled haploid breeding was integrated and utilized in a breeding program, beginning at 2011 in Xuzhou Institute of Agricultural Sciences. In this study, four resistant spring varieties (Sumai3, Wangshuibai, H35 and N553), which carrying different major QTLs were used as the donor. A susceptible winter wheat variety Aikang 58, with a high general combining ability and the greatest planting area in HuangHuai wheat region, was used as the recurrent parent. The special marker JAAS01, GWM493 and GWM533 were used for the detection of *FHB1* in the progeny of backcrossing at first, and then markers for other FHB QTLs were used in the positive samples. Several BC_2F_1 plants from the cross of H35/Aikang58 were chose as female parent and crossed with Xumai 1108 and Xumai 2023 respectively to produce the F_1 plants, which were used as the materials in maize pollen induced doubled haploid. Up to present, hundreds of BC_4F_1 and BC_3F_2 plants were obtained by MAS backcrossing, which carrying *FHB1* and 0-3 other FHB QTL (*FHB2, FHB4,* and *FHB5*). The plants with good agronomic traits will be selected to genotype and used in the breeding for FHB resistant varieties. More than 400 DH plants were obtained, and the genotyping and phenotyping will be conducted on these DH plants in next plant season. MAS backcrossing is an efficient way to transfer target genes from germplasm to adaptive wheat variety. However, the selection of agronomical traits is very important during the backcross. Application of DH breeding in this study can greatly shorten the time to develop advanced lines with *FHB1*. Our results provide a good example of breeding for FHB resistance in the HuangHuai wheat region.

Keywords: wheat; FHB; MAS; doubled haploid

EVALUATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT GENOTYPES

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Fusarium head blight (FHB), mainly caused by *Fusarium graminearum* and *F. culmorum*, is one of the most serious diseases affecting cereal crops, with the potential contamination of grain mycotoxins. The best approach to control FHB and to reduce mycotoxin contamination is to create wheat genotypes which are carrying effective resistance genes. Epidemic conditions (high humidity and moderate temperatures) can lead to severe outbreaks. Contamination of wheat with the mycotoxin deoxynivalenol (DON), at levels exceeding the permitted levels, results in rejection of sale or severe price dockage in countries that have adopted DON regulation. The Food and Agriculture Organization (FAO) compiled these guidelines and limits in various countries around the world. Croatia, as new member of EU, will need to adjust to the EU regulative. The macroconidia treatment was carried out of 50 winter wheat genotypes from different origins in two replications at the time of flowering. In inoculated treatment the lowest grain yield had genotype Golubica (39.24 dt ha^{-1}) and the highest grain yield had Graindor (130.99 dt ha^{-1}). The relative yield loss between the two treatments (control and inoculation) was the highest in genotype Golubica (>40%), which is already known as susceptible genotype. The smallest relative ear weight loss had more resistant genotypes and more late ripening genotypes, which are usually too late in regards to growing conditions in Croatia. However, these late ones could escape disease pressure. The highest 1,000 kernel weight in inoculated treatment had genotype Renan (48.35 g). Most of the genotypes had higher 1,000 kernel weight in inoculated treatment. The lowest percentage of Fusarium damaged kernels had genotypes Renan, Ludwig, MV Palotas, Sirban Prolifik and Vulkan. Further examinations of 25 selected genotypes should check differences in mycotoxin accumulation and quality traits in the grains among genotypes. This work is supported in part by Croatian Science Foundation.

Keywords: *Fusarium*; agronomic traits

ENHANCING FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT BY MOLECULAR MARKER ASSISTED BREEDING

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Shannong20 is well adapted and widely grown in China, but it is susceptible to the Fusarium head blight (FHB) disease because of not having resistance QTL/gene. The production and quality are highly affected, thereby influencing the livelihood and health of the people. One donor parent You2S004 (a NIL of Shannongyoumai 2) transferred by Nanjing Agricultural University with the major QTLs on 5A and 3B chromosomes was used to transfer resistance against FHB disease in Shannong20. One program targeted the locus on 5A using SSR marker *Xbarc180*, while the second focused on the locus on 3B using the *Xbarc147* marker. In F1 population, 96 lines from 100 single plants at seedling were selected having these two loci. In F2 population, 633 plants were detected containing the two QTLs by using Xbarc180 and Xbarc147 markers. There were 205 and 217 plants for anti-infection and anti-expansion, respectively. In F3 population, 212 lines containing both the two QTLs for anti-infection and anti-expansion were detected from 663 lines. The resistance level was from 6.85 to 9.21. These molecular results were corresponded with the field investigation results. These selections showed improved FHB resistance in the field, and also yielded better than the recipient parent in presence of the disease.

Keywords: wheat; FHB; QTL; marker-assisted selection

WHEAT SPIKE INOCULATION METHODOLOGY FOR TESTING FUSARIUM HEAD BLIGHT TYPE II RESISTANCE REACTION

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Genetic resistance has been considered one of the most important strategies for managing Fusarium head blight (FHB) and mycotoxins in wheat. Among the active resistance mechanisms, type II has been commonly used by breeders due to its practicality and better control of environmental factors. The type II mechanism measures the resistance to pathogen spreading from infected tissue (measured by the spread from central florets inoculated by the single floret inoculation). However, a question was raised whether it is possible to group the inoculated main spike along with inoculated tillers´ spikes of the same plant for mycotoxins content determination. Thus, the objective of this research was to check if there was difference in FHB resistance and mycotoxins accumulation between inoculated spikes of the main stem and to FHB resistance and mycotoxins accumulation in the tillers´ spikes, within the same pot. The experiment was conducted in greenhouse during 2013 growing season. The experimental design was nested and the factors studied were genotypes (n=3), pots (3), site of inoculation (1=main stem spike and 2= tillers´ spikes) and aliquots (3). It was established an average of six plants per pot. Within the same pot, main stem spikes, with similar growth stage, were artificially inoculated by single floret inoculation technique using a conidial suspension of *Fusarium graminearum* species complex (FGSC) at mid-anthesis. Later, pots were appropriately mist irrigated. Within each pot, an average of six days later, tiller spikes with similar growth stage were also inoculated by the single floret inoculation technique and the pots were also mist irrigated. An average, six main stem spikes and eight tillers´ spikes were collected separately within the same pot. Grains from the main stem spike and from tillers´ spikes were milled separately into whole wheat grain flour (WWGF) with a laboratory mill. WWGF mycotoxin content was determined with UPLC-MS/MS. DON, ADON (3-ADON plus 15-ADON) and ZEA contents were above the level of detection, whereas HT-2, T-2, DAS, OTA, AFLA B1, AFLA B2, AFLA G1, AFLA G2, FUMO B1 and FUMO B2 contents were below the level of detection. There was no difference in FHB resistance between inoculated spikes of the main stem and inoculated tillers´ spikes. However significant differences in DON, ADON and ZEA contents were observed between the two sites of inoculation. These results indicate if a breeder is selecting wheat genotypes using type II mechanism to assess FHB resistance, he/she could use main stem spikes and/or inoculated tillers´ spikes. Nonetheless, if a breeder wants to additionally evaluate resistance to mycotoxins accumulation, he/she should collect the main stem spike (preferably) or the tillers´ spikes.

Keywords: screening genotypes; inoculation methodology

P11 *5th ISFHB: Session 1 - Germplasm Development and Breeding for Scab Resistance*

IMPROVING RESISTANCE TESTING BY MULTIPLE PHENOTYPING OF WHEAT CULTIVARS TO FUSARIUM HEAD BLIGHT, SEPTORIA AND RUST DISEASES

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Fusarium head blight (FHB) resistance is an important trait in European winter wheat breeding. However, wheat is threatened by many other diseases with stripe or yellow rust (YR), *Puccinia striiformis*, and Septoria tritici blotch (STB), *Zymoseptoria tritici*, being the most important. Additionally, stem rust (SR), *Puccinia graminis*, might become an emerging disease due to global climate change. For breeding wheat cultivars competitive in European markets, resistances to multiple diseases are indispensible. Our objectives were (1) to develop efficient methods for evaluating resistances to a multitude of diseases on one field plot by inoculating different plant parts (leaf, ear, stem) with different fungi and (2) to analyze ranking of wheat cultivars in their resistance to one disease when challenged with other diseases. We tested 25 and 36 winter wheat cultivars, respectively, for their resistances to FHB and STB (experiment 1) or FHB, YR and SR (experiment 2) in individual plots ('solo') and all fungi combined in one plot ('mix'). Inoculations were carried out at the optimal plant stage in multi-locational trials. As resistance trait, disease severity was rated as percentage affected leaf, head, and stem area, respectively, at several assessment dates. In experiment 1, mean FHB severities ranged from 0.3% to 67% and mean STB severities from 12% to 70% across wheat cultivars. Resistances to FHB and STB were not correlated. Correlations between both inoculation variants were extremely high (*r*-=-0.98). In experiment 2, FHB and YR inoculations were successful at four locations, SR at two of them. Mean YR severities were low due to a majority of resistant cultivars, FHB ratings ranged from 7 to 56%. Wheat cultivars reacted similarly to the solo *vs.* mix variants for all three pathogens (*r*-=-0.93-0.99). One wheat cultivar was highly resistant (Pamier), some other cultivars moderately to highly resistant (Anapolis, Arnold, Opal, Wiwa) to FHB, YR and SR. In conclusion, phenotyping of wheat cultivars to multiple diseases can be performed on the same plot without ranking differences of the respective resistances when different plant organs are used. This saves plots when selecting for multiple resistances and allows testing of more genotypes with the same budget. Screening larger wheat populations should result in a higher probability to select rare progenies with resistances to FHB and other diseases.

Keywords: Resistance; wheat; multiple pathosystem

BREEDING AND IMPROVEMENT OF WHEAT SCAB RESISTANCE IN YELLOW AND HUAI VALLEYS WINTER WHEAT ZONE, CHINA

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In China, the largest wheat-producing region is in Yellow and Huai Valleys Winter Wheat Zone (YHVWWZ), where 14.12 million hectares of wheat are planted annually, accounting for more than 45% of the country's total wheat area, with the total production of 64.17 million tons, accounting for more than 50 percent of Chinese wheat production. Since the 20th century, rapidly expanded maize growing areas, global warming, and early occurrence of rainy seasons, result in high frequencies of serious wheat scab epidemics in this region. Thus, improvement of wheat scab resistance in commercial cultivars is crucial to ensure food security in China. 1.Approaches for wheat scab resistance breeding: 1) Induce scab resistance genes to new cultivars by making single crosses between the genotypes with good quality and high yielding from YHVWWZ as female parent and winter wheat genotypes with high or moderate scab resistance from the YRWZ as male parent; 2). Further improve scab resistance and plant architecture of locally adapted scab resistant lines by repeated backcrosses. 3) Accelerated breeding process by quickly obtaining homozygous breeding lines carrying scab resistance genes using double haploid technique. 2.Identification and screening of wheat scab resistant materials 1) Screening wheat resistant genotypes growing in YHVWWZ, and materials containing resistance genes from alien fragments that stably expressed scab resistance in wheat. 2) The half of harvested seeds from F1 were planted in Jianyang of Fujian province where severe- natural scab infection occurs every year for scab evaluation of F2 plants. Another half of the seeds from each F1 were sent to Jianyang to phenotype for scab and other agronomic traits in natural conditions. In next April, the genotypes (plant and/or lines) with at least moderate scab resistance, short plant status, tight plant type and upright flag leaf, were selected as idiotype" of YHVWWZ. The selected F2 seeds were sent to Kunming City in June for generation advancement using double haploid technique. 3) The plants of F3 or higher generations were inoculated with a local *F. graminearum* isolate using single floret inoculation to determine their scab resistance. 3.The advances of wheat-scab resistance 1). A total of 213 wheat accessions that were widely distributed in YHVWWZ, and 1356 accessions derived from alien species were evaluated for scab resistance using single floret inoculation for three years, and 68 accessions were selected to have at least moderate scab resistance. 2).By the end of 2015, 31 advanced lines were developed with the "idiotype" of agronomic traits and moderate scab susceptibility. Among them, 11 genotypes showed moderate resistance to scab and were further sent to national YRWZ yield trials for yield test and accession Tianjin 008 was ranked top second in the 2015 yield trials, and recommended to 2016 yield trial again. Moreover, a new line, Tianmin13E009, will also participate 2016 national YHVWWZ yield trials.

Keywords: wheat; scab resistance; breeding; double Haploid

FLOWCHART FOR THE PRODUCTION OF PERITHECIA OF *Gibberella zeae* IN WHEAT GRAINS

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The flowchart is one of the enabling tools to understand the analytically sequence of work, by characterizing the operations, the officials and/or organizational units involved in the process. At Embrapa Wheat, in a survey conducted through interviews, literature and personal observation, was detected the absence and the need to elaborate flowcharts of the protocols used in the access of Fusarium Head Blight disease (FHB). The objective of this study was to develop a routine work flowchart for the production of perithecia, reproductive structures of the fungus causing FHB (*Gibberella zeae*), based on scientific publication (Lima, 2007). For perithecia production the following materials and/or equipment are needed: good quality wheat grains; tap water; sterile distilled water; cotton; thick sand sifted; autoclave; wooden boxes (40 cm wide x 42 cm long x 10 cm high) with a false bottom; aseptic chamber; micro-organism growth chamber (22 ± 2 ° C and 12 hour photoperiod); Erlenmeyer of 500 ml with wide mouth; disposable plastic gloves; disposable protection mask; plastic sieve; beaker of 100 mL; pipette of 10 mL; Petri dishes with *Fusarium graminearum* on PDA culture with 5 - 10 days; watering can or garden hose and a shade net. Sixteen steps were defined in the flowchart and were represented in pictures: 1 - wheat grains; 2 - soaking in H_2O for 5 hours; 3 - removal of H_2O; 4 - sterilization; 5 - rest for 24 hours; 6 - conidia suspension preparation of *F. graminearum*; 7 - 20 mL suspension inoculation in the grains; 8 - Incubation; 9 - colonized grains; 10 - drying of grains; 11 - box with sand; 12 - sandbox with colonized grains; 13 - protection of the grains; 14 - wetting of the grains; 15 - immature perithecia; 16 - mature perithecia. The first nine steps are performed in the laboratory and the other ones outside of the greenhouse.

Keywords: *Fusarium graminearum*; ascospores

CONTRIBUTION OF MOLECULAR BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN URUGUAYAN WHEAT

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Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is one of the most destructive diseases of common wheat (*Triticum aestivum* L.) worldwide and particularly in Uruguay. Resistance to FHB is quantitative, strongly influenced by environment and thus, difficult to evaluate. This makes FHB an ideal trait for the use of molecular marker assisted selection (MAS) to facilitate breeding and cultivar development. Among the many QTL reported, five QTL have been assigned a gene name to date (*Fhb1* - *Fhb5*). *Fhb1* (derived from Sumai 3) is the most widely used and consistent QTL with the largest effect and therefore it is desirable to diversify the resistance sources. FHB outbreaks generally occur every 4-5 years and field inoculated/mist irrigated facilities are available since 1990s, turning Uruguay in an ideal environment for FHB screening. INIA´s wheat breeding program is implementing MAS with the aim of finding and improving sources of resistance different from *Fhb1.* Specifically, we have focused on: i) characterization of breeding material with available molecular markers, mainly for *Fhb1*, ii) double crosses with marker recurrent selection for introgression of FHB resistance in adapted material, iii) developing biparental population for traditional QTL mapping and iv) association mapping. Every year, advanced breeding lines and specific nurseries are evaluated for FHB response in field and greenhouse experiments. This phenotypic information, in combination with pedigree and molecular markers data, is used to design new crosses or select lines that are directly advanced in the breeding program. In the period 1981-2000, the breeding program used germplasm introduced from China, Brazil, and Japan. Since the 2000´s, resistant materials from Brazil, Japan, Austria and Hungry have been used. In a recent screening of advanced breeding material with molecular markers we concluded that the presence of *Fhb1* is not common in our breeding material, but still our lines have high levels of FHB resistance. This would indicate that FHB resistance in INIA´s materials relies on a different basis. Double crosses between resistant material and adapted lines, combining selection with molecular markers are used in order to introduce FHB resistance in adapted material to advance generations. QTL and association mapping with precise phenotyping and high-throughput genotyping are being used to discover the genomic regions associated to the resistance. In a recent GWAS analysis, we found significant QTLs associated with FHB resistance. The in depth study of these new regions and continued work with MAS strategies are essential to achieve superior levels of FHB resistance in our wheat cultivars.

Keywords: MAS breeding; QTL; germplasm; GWAS

CHARACTERISATION OF BRAZILIAN WHEAT CULTIVARS IN RELATION TO THE PRESENCE OF THE GENE *Fhb1* FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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Fusarium Head Blight (FHB) is an important fungal disease caused by *Gibberella zeae*, affecting wheat growing areas in the world. In Brazil, FHB is one of the most important wheat diseases, especially, in the southern states. In addition to the direct damage to yield and reduction in flour quality, the greatest danger attributed to FHB is the contamination of grains with toxic secondary metabolites known as mycotoxins. In Brazil, the most prevalent mycotoxins are deoxynivalenol (DON) and nivalenol (NIV). Genetic resistance to FHB is generally controlled by several genes with moderate or weak effect, defined by “quantitative trait loci” (QTLs). In contrast the resistance of the Chinese wheat cultivar "Sumai 3", is controlled in part by genes of major effect, the most potent being *Fhb 1*. The *Fhb1* has been considered the most effective gene for resistance to FHB. As a result, *Fhb1* is present in many commercial cultivars exhibiting high levels of resistance to FHB around the world. Thus, the characterization of the Brazilian wheat cultivars concerning the presence of this gene is crucial for future attempts to enhance FB resistance in wheat. The "Brazilian collection of wheat cultivars - 2012", consisting of 90 wheat cultivars from different breeding companies was planted at the John Innes Centre in Norwich, Norfolk, England. At the three-four leaf stage, DNA was extracted from leaf tip samples collected from individual cultivars. Then, it was analysed for the presence of *Fhb1*, using the methodology described in RAMIREZ-Gonzalez et al. (2015). Surprisingly, the results showed a complete absence of the *Fhb1* gene in all 90 Brazilian wheat cultivars. Thus, it can be concluded that those 90 wheat cultivars here analyzed, which were present in the recommended list in 2012, and showing different resistance levels to FHB, are carriers of various resistance genes that differ from the *Fhb1* gene. Considering this information, incorporating the *Fhb1* gene in Brazilian wheat cultivars that already have moderate levels of resistance should enhance resistance to FHB. On the other hand, it is also apparent that further studies are needed to clarify the origin of the resistance present in Brazilian germplasm, as well as for the development of new molecular markers to assist in pyramiding these genes present in Brazilian wheat cultivars.

Keywords: fungal disease; breeding; genetic resistance

FUSARIUM HEAD BLIGHT EVALUATION AND GENETIC STABILITY IN SYNTHETIC HEXAPLOID WHEATS

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The Fusarium head blight (FHB), a fungal disease caused by *Gibberella zeae (Fusarium graminearum),* and of great economic importance to the wheat culture, entails quantitative and qualitative losses in world grain production. As there is no effective management of the disease, genetic resistance is desirable. Currently, the breeding programs seek alternative sources of FHB resistance. Synthetic wheats result from the cross between a tetraploid species (genome AABB) and other diploid (DD), leading to a sterile hybrid ABD. To restore fertility, it is made artificial synthesis doubling the chromosomes by using colchicine, yielding a new hexaploid. These wheats are genomically amphidiploid and due to the combination of parental genomes have mainly resistance to fungi and insects. This work aimed to evaluate 20 accessions of synthetic wheats related to FHB, along with six cultivars (controls), previously characterized for resistance to FHB. The experiment was conducted in 2014 and 2015, at Embrapa Wheat, Passo Fundo, RS, in the experimental area with environment simulation favorable to the occurrence of the disease. The design was a randomized block with 26 treatments and the plots consists of a line of 5 m with 60 seeds suitable for linear meter. The spacing between two plots in the row was 1 m. Laterally the spacing between plots was 0.4 m. The spacing between the blocks was 0.8 m to allow the placement of hoses on the ground, aiming to wet the spikes. The phytosanitary control was carried out until the boot stage. In heading stage grains with perithecia and *G. zeae* were spread on the soil surface. Fifty spikes, of uniform size, from each plot were harvested and threshed and FHB was assessed by the incidence of grain symptoms. For the analysis of genetic stability, spikes in the pre-anthesis of five plants per genotype were collected and fixed in Carnoy solution. The cytological slides were made by soaking method and the coloration of cells was done with 1% acetic carmine. Observations were made under an optical microscope, analyzing the presence and absence of micronuclei in tetrad stage. Normal tetrads and micronuclei were counted for calculating meiotic index (MI). The genotypes were considered meiotic stable when the MI was above 90%. The best results for resistance to FHB and genetic stability were obtained with CIGM93.298 and CIGM92.1666 accesses respectively, which showed meiotic index above 90%. These accesses may be indicated to be part of breeding programs as possible FHB resistance sources.

Keywords: *Gibberella zeae*; resistance; micronuclei

USE OF DOUBLED HAPLOID LINES VIA ISOLATED MICROSPORE CULTURE FOR THE INTROGRESSION OF RESISTANCE TO FUSARIUM HEAD BLIGHT INTO WHEAT ELITE CULTIVARS

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Fusarium Head Bligh (FHB) is a devastating disease of wheat and other cereals mainly caused by different species of the fungus *Fusarium*, reducing grain yield and severe affecting quality, mostly due to mycotoxins contamination. The great majority of commercially wheat varieties are highly susceptible to the disease and searching for resistance has been a major focus of many breeding programs. Incorporating resistance genes into improved germplasm is a very attractive and clean approach to control the disease; however, it is a very tedious and long process, due to the innumerous cycles of selfing and lack of homozygosis. Using Doubled Haploid technology is possible to shorten the process of introgression of resistant genes into wheat elite genotypes, enhancing selection efficiency and the development of new varieties. However, many wheat genotypes behave as recalcitrant to haploidization procedures, mostly related to androgenesis. To this end, the first objective of this research was to evaluate the response of a group of wheat genotypes to androgenesis, via isolated microspore culture. Out of a few resistant known genotypes, four were tested to know their ability to respond to microspore culture: a) Frontana; b) Sumai-3; c) BRS 179; and d) BRS Guamirim. Two other genotypes were also tested and used as controls (Fielder and Embrapa 27), showing high and low response to androgenesis respectively. Preliminary analysis of our data revealed that most genotypes responded to microspore culture, producing both embryos and green plants. Of all tested genotypes, Frontana showed the highest response, even higher than the responsive control, producing a massive amount of embryos and plants, followed by Sumai-3 and Fielder. BRS 179 showed moderate response and BRS Guamirim behaved as recalcitrant as Embrapa 27 (few embryos and no green plants were produced). Based on these results, it will be possible to combine different sources and types of resistance and incorporate them into elite wheat genotypes through the aid of microspore culture, since three resistant genotypes were highly responsive to the method. Our findings will be valuable to support wheat breeders on the designing of crosses to develop improved FHB resistant varieties. Further studies are under development, including the formation of a multi-parent mapping population (eight-way cross) aiming for genetic analysis, combining different types and sources of resistance to FHB and the ability to respond to microspore culture.

Keywords: microspore culture; doubled haploids; wheat

PRECISION FIELD-BASED PHENOTYPING FOR FUSARIUM HEAD BLIGHT, WITHIN A NEW MULTIPLE DISEASES PLATFORM IN URUGUAY: 2015 RESULTS

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Breeding for Fusarium head blight (FHB) resistance in wheat is a challenging task since it is quantitatively inherited, thus relying on the accumulation of QTLs involved in resistance. This goal could be achieved by the use of a broad spectrum of resistance sources. Moreover, breeders usually need to test their materials in different abiotic and biotic stress conditions to know their adaptability to diverse environments. In order to improve the quality and speed of wheat breeding, CGIAR-WHEAT Initiative has promoted the establishment of field-based Precision Wheat Phenotyping Platforms (PWPP) accessible to public and private breeding partners. In 2015, a partnership between CGIAR and INIA launched the PWPP-Uruguay to test genotypes for multiple diseases: FHB, Septoria tritici blotch (STB) and Leaf rust (LR). With the aim of adjusting phenotyping protocols, we conducted the first trials in 2015 and results for FHB are presented in this work. A set of 110 genotypes, belonging to three CIMMYT International Nurseries (FHBSN, WYCYT, SATYN) were screened for resistance against FHB at La Estanzuela (Uruguay). Genotypes were planted in 1m-row plots with two replicates. Corn kernels, infected with a mixture of 12 *F. graminearum* isolates of known aggressiveness, chemotypes and representativeness, were applied in the field as spawn inoculum. Misting system worked from one week prior to flowering to milk grain stage, with 5 to 6 sprayings per day. Additionally, plots were spray-inoculated at mid anthesis with conidia inoculum. FHB was assessed at late milky to soft dough stages, with disease incidence and severity estimated to calculate the FHB index. Fusarium diseased kernels (%FDK) and deoxynivalenol content (DON-ppm) were determined after harvest. Plant height, heading date, growth stage at disease scoring dates and agronomic score were also measured. FHB variables were not significantly related ($P>0.05$) to heading date, plant height or growth stage. Linear relationships among FHB variables were significant ($P<0.05$). Frequency distributions of FHB variables were continuous for each of the three International nurseries. 24.4% of the genotypes were selected in the FHB trial. Approximately 2% of the genotypes with high levels of FHB resistance were also resistant to STB or LR, while 1% of the total genotypes were resistant to all diseases. Genotypes with high levels of FHB resistance, low %FDK and DON content could be selected. A small proportion of these genotypes was also resistant to STB and LR. Data from multiple traits obtained in this platform, complemented by molecular selection technologies, would increase the precision and prediction value of phenotype/genotype data for new germplasm emerging from the partners breeding pipelines.

Keywords: resistance; breeding; phenotyping; multiple diseases

P19 *5th ISFHB: Session 1 - Germplasm Development and Breeding for Scab Resistance*

DEVELOPMENT OF DIVERSE FHB RESISTANT WHEAT GERMPLASM

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South Africa (SA) has three main wheat production zones; spring wheat winter rainfall (Western Cape), winter wheat summer rainfall (Free State), and pockets of irrigated spring wheat. Currently, winter wheat production is declining making irrigated spring wheat more important. In SA, Fusarium head blight (FHB) outbreaks have become a serious production concern under the increasing areas of center pivot irrigation and FHB tolerant wheat cultivars are limited. This has initiated the need to improve FHB resistance levels of available cultivars. Additionally, these irrigated wheat production areas require protection against cereal rusts. However, in recent seasons some biotypes of Russian wheat aphid (RWA) were detected on irrigated wheat as well. All of these fungal diseases and insect pests can cause significant yield losses on their own, but in combination can be devastating. This study addresses the need to fast track the transfer of Fusarium head blight resistance with other potential pest/disease resistances into high-potential germplasm. The primary aim of this study was to develop FHB resistant germplasm using diverse FHB resistant wheat germplasm in a marker-assisted backcross pre-breeding programme. High-yielding recurrent parents used contain wheat rust resistance genes (*Lr34* and/or *Sr35)* and/or RWA resistance (SA16491-resistant to *RWASA1, RWASA2* and *RWAS3*). Developed lines contain targeted FHB resistance QTL/genes transferred from nine imported FHB resistant sources which have been validated with a cocktail of South African *Fusarium* isolates. The FHB resistance sources used during line development, namely, Baisanyuehuang (*Fhb1 & 3BSc QTL*), Catbird (*7D-QTL*), Frontana (*3A-QTL*), Heyne (*3A, 4A & 4D-QTL*), Haiyanzhong (*7D-QTL*), Huangfangzhu (*Fhb1 & 7A-QTL*), Ning7840 (*Fhb1 & 5A-QTL*), Sumai &3 (*Fhb1 & 5A-QTL*), Wangshuibai (*Fhb4 & Fhb5)*, with varying levels of success. Multiple delimiting SSR markers targeting the various FHB QTL/genes and certain gene-specific markers for the rust genes were used. A number of promising backcross lines carrying targeted FHB resistance genes/QTL, in combination with different rust genes and potential RWA resistance have been identified. These lines will be backcrossed further and top-crossed to stack the various FHB resistance QTL/genes. The FHB, rust and RWA resistance levels of developed lines will be validated phenotypically in field and glasshouse trials. The unique use of these multiple and diverse FHB resistance sources in combination witth targeted multiple pest/disease resistances in this manner is a significant step forward towards releasing FHB resistant cultivars. This material and research findings should have a significant impact on the South African and international wheat communities. The developed material will be made publicly available in a formal germplasm registration.

Keywords: Fusarium; Fhb1; resistant sources; QTL/gene; SSRs

Fusarium INFECTION AND MYCOTOXINS IN GRAIN OF WILD OATS

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The Russian Federation has the largest oat cultivation area and accounts for nearly 30% of the global oat production (FAOSTAT). The abundance and species composition of the microbiota are the important factors in determining the quality of the grain. In 2014, infection of *Fusarium* fungi was detected in 88.9% analyzed oat grain samples harvested in North-western part of Russia, on the average grain infection ranged 1-64%. T-2/HT-2 toxins were found in 60.7% of samples, with the limits of accumulation 4-186 ppb, deoxynivalenol (DON) was detected in 62.5% of oat samples and its content varied 49-1990 ppb. The N.I. Vavilov Institute of Plant Genetic Resources (VIR) collection contains the global botanical diversity encompassing over 11,000 of cultivated and 2000 of wild oat accessions belonging to 26 species. The genetic potential of *Avena* genus can be serve as useful source of material for breeding new resistant cultivars. Earlier the genotypes of cultivated *Avena* species were evaluated for resistance to *Fusarium* infection and accumulation of mycotoxins in grain, and it was found that genotypes of *A. sativa* and *A. strigosa* are less susceptible to *Fusarium* infection, than *A. byzantina* and *A. abyssinica* (Tekauz et al, 2008; Bjørnstad, Skinnes, 2008; Gagkaeva et al, 2013; Loskutov et al., 2016). In 2015 to study the resistance to *Fusarium* were selected fifty-seven accessions belonged to diploid (16%), tetraploid (32%) and hexaploid (52%) wild *Avena* species from the VIR collection: *A. atlantica*, *A. canariensis*, *A. clauda*, A. *damascena*, *A. hirtula*, *A. longiglumis*, *A. wiestii*, *A. agadiriana*, *A. barbata*, *A. insularis*, *A. magna*, *A. murphyi*, *A. vaviloviana*, *A. fatua*, *A. ludoviciana*, *A. occidentalis*, *A. sterilis*. The genotypes were artificially inoculated with *F. culmorum* at the VIR nursery. All grain samples were examined for DON and zearalenone (ZEN) content by ELISA. DNA was extracted from milled grain samples using CTAB method. Quantification of the trichothecene producing *Fusarium* species DNA (TriDNA) was performed by TaqMan PCR. All analyzed samples of 17 wild oats were infected by *Fusarium*. The amounts of DON ranged from 185 to 2188 ppb. In contrast with DON that which was found in all grain samples, ZEN was detected only in three genotypes of *A. barbata*, *A. ludoviciana*, and *A. wiestii* in the amounts 38-237 ppb. The amount of TriDNA showed substantial variation 0.19-5.19 ng/100 ng of total DNA. The tetraploid oats contained in 3.4-4.3 times more fungal TriDNA, then diploid and hexaploid *Avena* species. The tetraploid oats *A. insularis*, *A. magna* and *A. murphyi*, which are related to the C-genome species, were heavy infected by *Fusarium*. The investigation was supported by the Russian Science Foundation (No. 14-16-00072).

Keywords: wild *Avena*; *Fusarium*; mycotoxins; DNA

TRANSFER OF FHB RESISTANCE FROM *Thinopyrum elongatum* TO SPRING WHEAT

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In an ongoing search for additional sources of FHB resistance it was found that *Thinopyrum elongatum* 2x was a good source. Jan Dvorak of UC Davis had already produced the entire series of chromosome addition and substitution lines in a Chinese Spring (C.S.) background. Chromosome 7E was found to carry the best resistance. (8.0% infected florets compared to C.S. at 57.5%). The 7E (7D) substitution line was crossed to the C.S. *Ph1b* mutant to induce recombination between the two chromosomes. Progeny of plants with increased levels of meiotic chromosome pairing, homozygous for *Ph1b* and showing high levels of FHB resistance were selected for further study. Initially BC_1F_2 progeny were screened with chromosome 7E-specific SSR markers and resistance to FHB. Chromosome 7E-specific markers were obtained from published sequences and 7E-specific expressed markers produced in the lab of T. Ouellet. The objective was to isolate recombinants with minimal segments of chromosome 7E and yet carrying FHB resistance. Marked differences were observed between families for content of 7E chromatin. The number of 7E-specific bands expressed was as high as 23 in some families but reduced to single bands in selected later generations. To date in excess of 500 recombinants have been screened. In later generations, BC_1F_3 and BC_1F_4, large blocks of 7E chromatin were no longer present, but resistance at about the 10 % level persisted. Selected progenies are now being analyzed with SSR markers from chromosome 7D to determine the size and location of the 7E introgressions.

Keywords: 7E introgression; *Thinopyrum elongatum*; FHB-resistance

DEVELOPMENT AND EVALUATION OF N553 CHROMOSOME SEGMENT SUBSTITUTION LINES OF YANGMAI13 BACKGROUND IN BREAD WHEAT

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Narrow genetic base has become a main problem for genetic improvement of wheat scab. N553 is a newly discovered material possessing the same resistant level as Sumai 3, but better agronomic traits with unknown scab resistant genes. In our study, a battery of CSSL lines in Yangmai13 background which could cover 91% genome of N553 were constructed through backcrossing and systematic application of marker-assisted selection to be excavated and utilized new genes/QTLs. The results of field natural incidence of Fusarium head blight in Jianyang, Fujian Province revealed that the percentage of infected spikes (PIS) of N553 was 5.16%, even a little lower than that of Sumai3 (7.04%). Inoculation results of Yangzhou, Jiangsu Province indicate that the number of diseased spikelets of donor parent (NDS) was 11.8 and we could conclude there was no significant difference between N553 and Yangmai13. But, compared to the resistant (Sumai3) and susceptible (Annong8455) control, the difference among them both reached a very significant level. Development of the CSSLs lines: the F_1 was generated by a cross using Yangmai13 as the recipient parent and N553 as the donor parent. The N553× Yangmai13 F_1 progeny were backcrossed with Yangmai13 until BC_2F_1 and self-pollinated to produce BC_2S_1. (Marker-Assisted-Selection)MAS were conducted to identify genotypes of 46 individual BC_2F_1 plants in the Yangmai13 background using 215 polymorphic SSR markers. 27 plants from BC_2F_1 were selected to backcross with recurrent parent to generate BC_3F_1 population according to the results of MAS. A total of 270 BC_3F_1 individuals were also identified by MAS. Thus, a battery of N553 whole genome chromosome substitution Segment lines was developed. The progeny of BC_2F_1 self-pollination were collected for identification the Fhb resistance in continuous of two generations in 2013 and 2014 under field natural incidence. The results showed that the resistance of wheat scab for 8 materials could be significantly increased among the 118 lines both in two places and years. The number of spikes ranged from 2.25 to 2.88 in 2013, 1.50 to 3.33, respectively. For the record, two lines (115 and 268) were expressed stable resistant in two environments. These new sources will be great helpful for wheat scab breeding and lay the foundation for QTL mapping.

Keywords: *Fusarium*; wheat; FHB

MARKER-ASSISTED PYRAMIDING OF GENES CONDITIONING RESISTANCE TO FUSARIUM HEAD BLIGHT AND POWDERY MILDEW IN WHEAT

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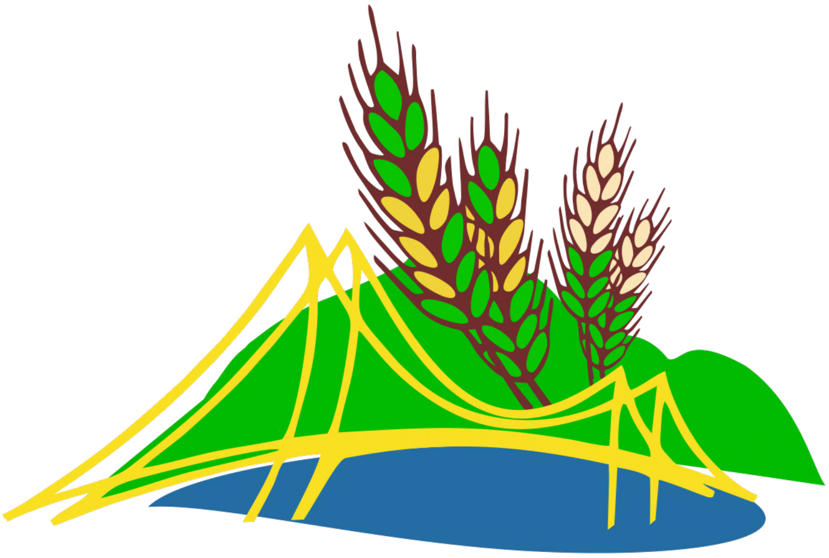
Breeding for FHB resistance in wheat is hindered by complicated genetic mechanisms and poor characterization of resistance sources. In previous studies, our lab identified QTLs for type I and type II resistance QTLs in FHB-resistant Wangshuibai. To evaluate the effects of these QTLs on scab resistance and their usefulness in breeding, these QTL were transferred into elite wheat varieties from different ecological zones in China through molecular marker-assisted selection. Near-isogenic lines (NIL) harboring single or multiple QTLs were developed and evaluated in multiple different geographical environments. These lines showed significantly better resistance than the current parents and lines with multiple QTLs performed even better than those harboring a single QTL. In addition, to breeding lines with multiple resistance, both FHB resistance QTLs and powdery mildew resistance QTLs identified in lab were pyramided, through MAS, into elite wheat varieties from Yangtze River winter wheat area, Huang-huai winter wheat area and Southwestern winter wheat area in china. The preliminary results were very promising. Acknowledgement: This study was partially supported by '973' program (2010CB125900), NSFC programs (30130054, 30025030, 31301308), Fundamental research Funds for the Central Universities (KYZ201202-4), and PAPD project of Jiangsu Higher Education Institutions.

Keywords: wheat; FHB; marker-assisted selection



5th ISFHB

Session 2

Genetics and Genomics of Scab Resistance

HUNTING FOR GENES ASSOCIATED WITH PRIMING-INDUCED RESISTANCE AND WITH SUSCEPTIBILITY TO FHB IN WHEAT

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Lots of attention has been given to the identification of genes for native resistance to FHB in wheat. To complement these efforts, we are focused on two areas of research: identify genes associated with FHB susceptibility and identify genes associated with the priming of the wheat innate immune system. To identify genes associated with susceptibility to FHB, RNA profiling was performed to compare the response of the FHB-susceptible wheat cultivar Roblin when inoculated with a wild type *F. graminearum* (DON+) strain and a related Tri5- knockout (DON-) strain. Several wheat genes that were specifically induced during infection with the DON+ *F. graminearum* strain were identified. Additional experiments showed that many of those genes were induced directly by a treatment with DON. Further analysis of the DON-modulated genes across our wheat head expression profile database showed a correlation between expression level and susceptibility of the genotypes to FHB. Transient silencing of two of those genes, an NFXL1-like regulation factor and an MRP-like ABC transporter in the susceptible Roblin showed reduction in both disease spread and head bleaching. These results support a contribution of DON to FHB-susceptibility in wheat via modulation of gene expression. To identify genes associated with the priming of the wheat innate immune system, we showed that pre-treatment of heads of the susceptible Roblin with non-pathogenic strains of *F. graminearum* can lead to protection of the plant from subsequent challenge with an aggressive strain. RNA profiling was used to characterize the priming phenomenon in the wheat heads. Genes associated with immune surveillance systems and signaling cascades, in addition to selected genes known to directly contribute to defense, were identified. We also observed differences in priming profiles when different non-pathogenic *F. graminearum* strains were used. Our results indicate that this strategy could be used to tease out the complex disease resistance mechanisms in wheat.

Keywords: priming-induced resistance; susceptibility; NFXL1-like; ABC-transporter

FHB RESISTANCE FROM RECEPTORS TO DOWNSTREAM SIGNALLING

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Despite the importance of FHB disease, the signaling mechanisms that underpin the host-pathogen interaction are poorly understood. Pathogen effectors have yet to be characterized, as have the associated receptors. We previously reported the FHB resistance innate to the 'uzu' barley mutant- 'uzu' carries a mutation in the kinase domain of a brassinosteroid receptor. Functional genomics highlighted another receptor up-regulated in 'uzu' in response to FHB and, using gene silencing studies, we showed that this gene has a major influence on FHB resistance. We hypothesize that this protein participates in type I resistance to initial infection. Deoxynivalenol (DON) is a virulence factor that facilitates the spread of FHB disease and in some wheat genotypes resistance to DON is an innate component of type II FHB resistance (resistance to disease spread). Glycosylation of DON reduces its phytotoxicity, but this is not the only means by which plant resist or tolerate DON. We have identified several genes that enhance DON resistance in wheat, but have found no evidence that they degrade or modify the toxin. TaFROG is a novel protein unique to the Pooideae. It is activated in response to DON. Its activation is independent of classical defence pathways and when overexpressed it enhanced both DON and FHB resistance. It interacts with the central stress regulator SnRK1 to modulate its activity. It also interacts with a range of other proteins, including a novel evolutionary divergent transcription factor. We are studying this complex further in order to better understanding the associated signaling network. Other genes that show potential for DON/FHB resistance include an ABC transporter and a cytochrome P450. The latter enhanced FHB resistance when overexpressed in wheat.

Keywords: *Fusarium*; receptors; orphan; P450; ABC

IMPROVED RESOLUTION OF FUSARIUM HEAD BLIGHT RESISTANCE QUANTITATIVE TRAIT LOCI IN TETRAPLOID WHEAT USING A HIGH DENSITY GENETIC MAP

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Fusarium head blight (FHB) is a threat to durum wheat yield and quality worldwide. Breeding for FHB resistance in durum wheat is slow because of the weak and quantitative expression of resistance. Combining genes from the domesticated gene pool with sources from related tetraploid species is one strategy to improve genetic resistance in durum wheat. This can be aided through marker-assisted selection using DNA markers tightly linked to the genes conferring resistance. High density genotyping platforms based single nucleotide polymorphism (SNP) markers have the potential to better identify and resolve QTL. Major stable FHB QTLs have been reported: one from *T. carthlicum* cultivar Blackbird chromosome 1A and two from durum line DT696 on 5A (Singh et al. 2008). In this study, we genotyped with the 90K Infinium iSelect SNP array 90 lines of the A0022 (Strongfield x Blackbird) and 121 lines of the A0132 (DT707 x DT606) doubled haploid mapping populations. Individual component maps were generated for each population using polymorphic SNP markers with simple sequence repeat (SSR) and Diversity Array Technology (DArT) markers used in the original mapping. A consensus map of the two populations was generated by combining the component maps using LPmerge software. This enabled mapping of 14,241 markers spanning 3,047 cM of all 14 chromosomes. The average genetic distance between markers was 0.4 cM. Analysis conducted using the component maps for each population and the multi-location FHB field ratings acquired by Singh et al. (2008) revealed the same QTL but with additional SNP markers within the interval. The 1A QTL intervals calculated from five out of six phenotypic data was from 5.9 to 11.1 cM shorter than those reported by Sing et al. (2008). Two unlinked QTL was detected on 5A chromosome (5A1 and 5A2), confirming the previous results on the presence of two unlinked loci (*gwm156* and *wmc110*) associated with the trait. Intervals obtained for 5A1 using five out of ten A0132 FHB data sets harbored the SSR marker *gwm156*, however in only one instance *wmc110* was within 5A2 intervals. Several SNPs, localized to the centre of the QTL, converted to Kompetitive Allele Specific PCR (KASP) markers were tested on a subset of lines representative of observed haplotypes in the mapping population to assure that the KASP markers reproduce the results obtained from Infinium analysis. About 90% of KASP markers mimic the genotyping output obtained from infinium analysis. We are currently further validating these markers as tools for marker assisted selection in our durum breeding programs.

Keywords: QTL-mapping; SNPs; *T. carthlicum*

CLONING AND CHARACTERIZATION OF A SPECIFIC UDP-GLYCOSYLTRANSFERASE GENE INDUCED BY DON AND *Fusarium graminearum*

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Deoxynivalenol (DON) is one of the most important toxin members produced by *Fusarium species*, which enhances the spread of pathogen in the host. For defense, UDP-glycosyltransferases (*UGT*) family had been deduced to transform DON as the inactive form of D3G, while the functional gene member in wheat is rarely examined. In this study, a DON and *F. graminearum* responsive gene of *TaUGT5*, whoes UTR is specific in resistant cultivars and was cloned with the open reading frame (ORF) of 1542bp encoding 476 amino acids in Sumai3. *TaUGT5* locates on chromosome 2B confirmed by nulli-tetrasomic lines of Chinese Spring (CS) and expresses alone. Overexpression of this gene in *Arabidopsis* conferred the transgenic lines enhanced tolerance when growing on DON containing agar plates. Similarly, the detached leaves and coleoptile of *TaUGT5* overexpressed wheat showed more resistance to *F. graminearum*, slowing down the pathogen destroy and extend in plant tissues. However, the disease expansion in spike did not exhibit significant difference compared with wild type plants. Subcellular localization analysis revealed that *TaUGT5* was localized on plasma membrane of the tobacco and onion epidermal cells. -It is possible that *T*aUGT5 can preservate the cell structure, and then limit the extension of pathogen.

Keywords: UDP-glycosyltransferase; *Fusarium graminearum*; FHB; deoxynivalenol; TaUGT5

ROLES OF SPECIFIC *Fusarium graminearum* SECRETED PROTEINS IN *Fusarium* DISEASE DEVELOPMENT

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Fusarium graminearum (*Fg*) is a major fungal pathogen of wheat crops globally,-causing Fusarium ear blight (FEB) disease. This floral disease results in reduced grain yield and quality and the grain becomes contaminated with various harmful trichothecene mycotoxins. Like many other plant pathogens, *Fg* is predicted to produce *in planta* an array of fungal effector-proteins that modulate plant metabolism to suppress and/ or re-programme plant defences, thereby promoting its own infection. Understanding the molecular functions of *Fg* effectors will help to elucidate the processes underlying wheat spike colonisation and fungal pathogenicity. With the aim of identifying *Fg* effector-proteins that can suppress-host-plant defences-we selected using various criteria a set of small secreted proteins (SSP) to express *in planta* using the-*Barley stripe mosaic virus*-over-expression system (BSMV-VOX).- We then tested whether expression of any of these SSPs enhanced-*Fg*-fungal infection of susceptible wheat spikes. Amongst-the initial set of-*Fg*-SSP tested,-three (FgSSP5, FgSSP6 and FgSSP7) appear to enhance FEB disease when overexpressed in wheat ears prior to infecting with *Fg.* Overexpression another protein, FgSSP8, resulted in necrotic lesion development in *N. benthamiana* leaf tissue, suggesting that FgSSP8 triggers host cell death. FgSSP6 and FgSSP7 belong-to-the cerato-platanin protein (CPP) family. In several other plant pathogenic fungi, CPPs have been implicated in a number of virulence and plant protection mechanisms, including induction of host plant cell death or expansin-like activity. *FgSSP5* encodes a protein that possesses the pfam domain RALF (Rapid alkalinisation factor; PF05498.6). RALF domain-containing proteins are predominately found in plants and play a role in plant development regulating tissue expansion and/or negatively regulating pollen tube elongation. BLAST analyses did not identify RALF domain containing proteins in any fungal pathogen species other than those in Fusaria. FgSSP8 is an annotated protein with a ribonuclease domain. Ribonuclease (RNase) activities contribute to RNA processing or degradation and this activity may play an important role in gene regulation. So far our results suggest that these proteins may be involved in virulence in different ways, either by suppressing plant defence responses (FgSSP6 and FgSSP7), inducing cell death (FgSSP8) or by an as yet unidentified mechanisms (FgSSP5). Once the mechanisms are identified, these genes/proteins could potentially be novel intervention targets either for conventional chemistries or for methods such as host-induced gene silencing to achieve FEB disease and/or mycotoxin control.

Keywords: FEB; effectors; BSMV-VOX; wheat

IDENTIFICATION OF EXPRESSION QTL AND FUNCTIONAL CHARACTERIZATION OF CANDIDATE GENES FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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Fusarium head blight (FHB) is a devastating disease of wheat causing severe yield loss and significantly lowering grain quality. Breeding wheat cultivars possessing genes resistant to FHB is the preferable approach to minimize FHB damage. Resistance to FHB was a complex quantitative trait controlled by multiple genes. Numerous studies have been published on molecular mapping and the phenotypic quantitative trait loci (pQTL) Fhb1 on chromosome 3B was identified as the major QTL for FHB resistance. But till now, no exciting result on cloning FHB resistance gene with known function has been reported. The ability to identify the molecular basis of quantitative traits is being enhanced by genomic methodologies such as transcriptomics, metabolomics, and proteomics. In some cases gene expression levels could be used as a surrogate/biomarker for classical phenotypes, and the gene expression QTL (eQTL) could be an excellent candidate for pQTL. Moreover, the locations of eQTL that regulate gene activity can be correlated with those of pQTL and so provide additional clues as to the genetic basis of quantitative genetic variation. Wheat variety Ning7840, a derivative of Sumai 3, has been used as the promising source in wheat breeding for FHB resistance. We have obtained the positive results on construction of genetic map, identification of pQTL for FHB resistance and the significantly differentially expressed sequence tag (EST) between Ning 7840 and susceptible cultivars after *Fusarium graminearum* infection by microarray analysis, coupled with suppression subtractive hybridization technique. In the present study, 110 ESTs mentioned above were localized on special chromosomes using nulli-tetrasomic lines of Chinese Spring. The expression level of ESTs at 8 hours after *F. graminearum* inoculation in two years was determined. In total 25 eQTLs were revealed in at least one set of gene expression data. Six cis-eQTLs were identified on the chromosome 3BS, indicating existence of candidate gene resistant to FHB in this chromosome region. Two cis-eQTLs, namely Gene273 and Gene395 were further cloned by RACE according to the EST and genome sequence. The analyses of gene expression by qRT-PCR indicated that those two genes were expressed in spikes and leaves, and were in response to the *F. graminearum* at 12 hours after inoculation. Virus-induced gene silencing (VIGS) confirmed that those two genes contributed to the FHB resistance. Silencing of Gene273 and Gene395 by antisense constructs in Sumai3 resulted in 94% and 67% increase in proportion of scabbed spikelets at 14 days after inoculation, respectively. The transgenic lines overexpressing those two candidate genes in susceptible cultivar Yangmai20 will be evaluated their function on disease resistance. Hence, these two FHB-responsive genes warrant further study to determine their potential as FHB resistance breeding targets.

Keywords: FHB; EST; eQTL; VIGS

INTERACTION OF *Qfhs.njau-3B* WITH TWO OTHER LOCI CONFERRED RESISTANCE TO SPREAD OF FUSARIUM HEAD BLIGHT WITHIN SPIKES

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Fusarium head blight (FHB) is a devastating fungal disease of wheat, barley, and other small grain crops worldwide. The complexity of FHB resistance mechanisms and lack of resistance germplasm have greatly hindered the efforts in breeding resistance cultivars. In our previous study, the major QTL conditioning type II resistance, *Qfhs.njau-3B*, was identified on chromosome 3BS of Wangshuibai, an indigenous FHB resistance germplasm, in an interval that might contain the *Fhb1* gene found in Sumai 3. To clone this QTL, a physical map of 497.7 kb was constructed, which contains 40 ORFs. Using two secondary populations derived from near isogenic lines of *Qfhs.njau-3B* in Mianyang 99-323 and PH691 backgrounds, the candidate ORFs were narrowed down to three ORFs within a 23.8 kb interval. The *Qfhs.njau-3B* candidate was finally concluded through comparison of the sequence polymorphism between the resistant and susceptible lines. Virus-induced gene silencing of this gene and haplotype analysis confirmed its effects on FHB resistance. However, not all the wheat germplasms containing *Qfhs.njau-3B* showed resistance to FHB. It turned out that two other loci in the wheat genome interact, epistatically, with *Qfhs.njau-3B*, which led to susceptibility of those lines with *Qfhs.njau-3B*.
Acknowledgments: This study was partially supported by ‘973’ program (2004CB117205, 2010CB125902), NSFC programs (30430440, 30025030, 30671295, 30721140555) and funds for transgenic plants (2008ZX08002-001 and 2009ZX08009-049B).

Keywords: *Fhb1*; cloning; haplotype analysis; interaction

MOLECULAR ANALYSIS OF NOVEL FHB RESISTANCE

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Fusarium head blight (FHB) caused by *Fusarium graminearum* (*Fg*) is one of the most prevalent diseases of wheat (*Triticum aestivum* L.). Due to their poor agronomics and low yield, it has proven difficult to move existing sources of FHB resistance into adapted Canadian varieties. Recently, germplasm developers at Agriculture and Agri-Food Canada (AAFC) generated a novel cultivar, FL62R1, which has high FHB resistance, good agronomics and quality, and resistance to rust and mildew. However, little is known on the molecular and genetic nature of this novel source of FHB resistance and its relationship to existing FHB resistance sources. Understanding the molecular basis of resistance has the potential to accelerate the deployment of novel and native sources of FHB resistance with better agronomics and end-use quality. In this study, multiple approaches were applied to characterize resistance in FL62R1 with the goal of introgressing desirable alleles into Canadian elite wheat varieties. In addition to FL62R1, the well-known FHB resistant line, Sumai3, and two Canadian elite wheat varieties with moderate to low FHB resistance, Stettler and Muchmore, were analyzed. Disease assessment revealed that Sumai3 has the highest type II FHB resistance, with FL62R1 being moderately resistant to *Fg*, and Stettler and Muchmore being more susceptible. Microscopic observations demonstrated that *Fg* growth was limited to the inoculated spikelets in the resistant varieties and did not spread to rachis and uninfected spikelets. Rachilla and rachis of resistant varieties displayed strong cell wall-related defense responses, such as callose deposition and lignification. These results indicate that rachis of the resistant varieties play an important role in the type II FHB resistance. To better understand the molecular basis of type II resistance among the four varieties, *Fg*-infected spikelets and rachis were collected separately for RNA-Sequencing, metabolomics and hormone profiling analysis. Preliminary results suggest that multiple defense-related pathways and responses contribute to type II FHB resistance in Sumai3 and FL62R1.

Keywords: resistance sources; elite varieties; RNA-Sequencing; type II resistance

THE DURABLE RACE NON-SPECIFIC WHEAT RESISTANCE GENE *Lr34* CONFERS INCREASED RESISTANCE TO FUSARIUM HEAD BLIGHT (FHB)

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Lr34 is one of the most widely deployed wheat resistance genes worldwide and confers resistance to the biotrophic fungi *Puccinia triticina* (leaf rust), *Puccinia striiformis* (stripe rust) and *Blumeria graminis* (powdery mildew). However there is a body of evidence that suggests the presence of *Lr34* increases the susceptibility of wheat to pathogens with a hemi-biotrophic / necrotrophic lifestyle including *Zymoseptoria tritici* (Septoria tritici blotch) and *Magnoportha grisea* (wheat blast). FHB is an important disease of wheat due to its potential to cause large yield losses and grain contamination with mycotoxins. It is caused by hemi-biotrophic pathogens, two of the most important of which being *Fusarium graminearum* and *F. culmorum*. In this study on the effect of *Lr34* on FHB we employed a series of pairs of wheat Near Isogenic Lines developed in the varieties Avocet, Jupateco, Thatcher and Lal Bahadur that differ for an effective allele of the resistance gene *Lr34.* These pairs of lines were evaluated for Type 1(initial infection) and Type 2 (spread of the fungus in the ear) resistance to *F. culmorum* under field and glasshouse conditions to ascertain the effect of *Lr34* against this hemi-biotrophic pathogen. Combined Type1 and Type2 resistance was assessed in field and glasshouse trials conducted at the JIC (UK) with spray inoculated field plots under mist irrigation to encourage high disease pressure and on individually sprayed heads under glasshouse conditions with raised humidity. Type 2 resistance was assessed in glasshouse studies employing a single floret point inoculation technique. In both field and glasshouse evaluations contrary to what may have been predicted the presence of *Lr34* was shown to have a positive effect on reducing disease symptoms. The point inoculation assay demonstrated that *Lr34* significantly increased resistance to disease spread (Type 2 resistance).

Keywords: wheat; *Fusarium*; Lr34

EFFECTS OF THE EXOGENOUS APPLICATION OF ABSCISIC ACID, GIBBERELLIC ACID, AUXIN AND ZEATIN ON *Fusarium graminearum* INFECTION IN WHEAT

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The classical roles of phytohormones in plant defense are generally well defined. However, very few of these have been reported with respect to Fusarium head blight (FHB) in wheat. Here, the association between an array of phytohormones and FHB pathogenesis is investigated. Initial experiments in which hormones were co-applied to the wheat head with *F. graminearum* inoculation, showed that while zeatin and auxin had little impact, ABA or GA co-application increased and decreased FHB spread respectively. These phytohormone induced effects may be attributed to alteration of the *F. graminearum* transcriptome, as a high throughput RNASeq analysis of the treated plants showed that ABA promoted expression of early-infection genes including hydrolases and cytoskeletal reorganization genes, while GA suppressed the expression of nitrogen metabolic genes in the pathogen. On the other hand, neither ABA nor GA elicited significant effects on *F. graminearum* fungal growth or sporulation in axenic conditions in shake flask cultures, nor do these phytohormones affect trichothecene gene expression, deoxynivalenol mycotoxin accumulation, or SA / JA biosynthesis in *F. graminearum*-challenged wheat spikes. Overall we conclude that GA and components of its associated metabolic and signaling pathways may serve as effective priming agents, and genetic targets in breeding programs against FHB. However, additional investigations of the impact of the co-application of GA on the wheat transcriptome will be essential, and are ongoing at this time.

Keywords: phytohormones; abscisic acid; giberellic acid

IDENTIFYING GENOMIC REGIONS FOR FUSARIUM HEAD BLIGHT RESISTANCE IN AN INTERNATIONAL DURUM WHEAT PANEL BY ASSOCIATION MAPPING

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Durum wheat (*Triticum turgidum* ssp. *durum*) is an important tetraploid wheat (genome AABB) mainly used for pasta production. Durum is notorious for its high susceptibility to Fusarium head blight (FHB), resistance sources are scarce and on a much lower resistance level than in bread wheat. Our objectives were to identify chromosomal regions affecting FHB resistance in durum wheat, to analyze their covariation with heading date and plant height, respectively, and to unravel the relative magnitude of main effect quantitative trait loci (QTL) using a genome-wide association (GWA) mapping approach. A panel of 184 durum lines, 170 winter and 14 spring types, from 16 countries was phenotyped by inoculation with *Fusarium culmorum* at five environments (location-by-year combination) in Germany and Austria, and genotyped by 30,611 polymorphic GBS (genotyping-by-sequencing) markers, an allele-specific marker for the *Rht-B1* locus and copy number variation for the *Ppd-B1* locus. All traits showed significant ($P<0.001$) genotypic variation with high heritabilities (0.74-0.98), thus providing an ideal basis for association mapping. FHB resistance was negatively correlated with both heading date (-0.61, $P<0.001$) and plant height (-0.37, $P<0.001$). As a consequence, we corrected FHB data for heading date and reduced correlation considerably ($r=0.16$, $P<0.05$). The dwarfing gene *Rht-B1* significantly affected FHB resistance. Additionally, eight QTL on chromosomes 3A, 4A, 5A, 7A, 2B, 3B, 4B, and 5B had significant effects on heading-date corrected FHB resistance explaining 1 to 14% of genotypic variation. In conclusion, inheritance of Fusarium resistance in durum wheat seems to be governed by similar mechanisms like in bread wheat. According to the large number of QTL reported and their small effects, genomic selection approaches should be pursued in future.

Keywords: FHB; winter durum; GBS; Rht-B1

DETOXIFICATION OF MYCOTOXINS AS A SOURCE OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN CEREALS: AN INNOVATIVE TRANSLATIONAL BIOLOGY APPROACH BETWEEN *Brachypodium distachyon* AND BREAD WHEAT

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Fusarium Head Blight (FHB) caused by fungi of the *Fusarium* genus is a widespread disease of wheat (*Triticum aestivum*) and other small grain-cereal crops. The main causal agent of FHB, *Fusarium graminearum*, can produce mycotoxins mainly belonging to type B trichothecenes, such as deoxynivalenol (DON), that can negatively affect humans, animals and plants. Several QTLs for resistance to FHB have been identified some of which have been correlated with efficient DON detoxification, mainly through the ability to conjugate DON into DON-3-*O*-glucose (D3G) *via* UDP-glucosyltransferases (UGTs). Neverthless, only few studies have conducted functional analyses to directly correlate DON glucosylation and resistance *in planta* and none were performed on endogenous detoxification gene(s) of a cereal species. In order to develop efficient strategies of FHB resistance in wheat, there is a need for more detailed functional analyses of the relationship between detoxification of mycotoxins and resistance to FHB. Our team, using the model cereal species *Brachypodium distachyon*, has recently demonstrated that the Bradi5g03300 UGT is able to confer tolerance to DON following glucosylation of DON into DON-3-*O*-glucose and is involved in quantitative resistance to Fusarium Head Blight. Our results have further shown that early and efficient DON glucosylation is essential to establish a good level of resistance. In this present work, we aim at transferring the functional analyses conducted on the model species *Brachypodium distachyo*n to bread wheat. In a first approach the *B. distachyon Bradi5g03300* gene has been introduced through biolistic-mediated transformation in the wheat variety Apogee, susceptible to FHB. Homozygous plants were selected and phenotypic analyses are in progress. In parallel, using a syntenic approach between *Brachypodium distachyon* and bread wheat genomes we identified wheat candidate genes orthologous to the *B. distachyon Bradi5g03300* gene. The expression of the identified wheat genes following fungal infection and DON application is in progress and will be presented. Transformation of one of the most interesting wheat candidate genes into *B. distachyon* is underway to rapidly determine its ability to conjugate DON into D3G *in planta* and its relationship with FHB resistance. Production of the corresponding recombinant protein in *Escherichia coli* will soon achieved to determine its ability to conjugate DON *in vitro.* In conclusion, this project will contribute to increase the knowledge concerning the functional relationship between DON glucosylation and FHB resistance in wheat and provide candidate genes to include in selection processes.

Keywords: mycotoxins; detoxification; translational biology

CHARACTERIZATION OF A QTL ON 3DL CHROMOSOME ASSOCIATED EXCLUSIVELY WITH DON CONTENT IN A BREAD WHEAT MAPPING POPULATION

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Deoxynivalenol (DON) contamination in food and feed is a major concern in case of Fusarium head blight (FHB) infection in wheat. Type III resistance has been proposed for the mechanism of preventing fungal DON production or degrading the produced DON. QTL responsible for this type of resistance usually coincided with those for Type I (for initial infection) and/or Type II (for fungal spread), and so far no QTL exclusively associated with DON content has been validated. This made many researchers think that Type III resistance is a consequence of Type I and Type II resistance and not being independent resistance component. In this study, a FHB susceptible Moroccan cultivar 'NASMA' was hybridized with a FHB resistant CIMMYT breeding line 'IAS20*5/H567.71' to generate 197 recombinant inbred lines. The population was phenotyped for FHB and associated traits in spray inoculated field experiments at CIMMYT-Mexico in 2010, 2013 and 2014. Genotyping was done with the Illumina 15K wheat SNP chip and SSR markers. Plant height (PH), anther extrusion (AE) and days to heading (DH) usually showed moderately negative correlations with FHB index, whereas their negative correlations with Fusarium damaged kernels (FDK) and DON content were much lower. QTL mapping results indicated that the field FHB resistance was mainly controlled by QTL at *Rht-D1*, *Vrn-A1* and the one on 3BL. The latter was also consistently significant for FDK and DON. Interestingly, a major QTL was identified on 3DL responsible exclusively for DON content, accounting for 12-15% of phenotypical variation. Resistance alleles of all the aforementioned QTL were contributed by 'IAS20*5/H567.71'. Additionally, putative QTL with minor effects were detected on chromosomes 1BL, 2AL, 4AL, 4BS (at *Rht-B1*), 5BL and 7A. Seven QTL were mapped for AE and six of them coincided or linked to QTL for FHB, in agreement with previous findings that the two traits are related to each other. More markers are being added to the 3DL QTL region, in the hope to find more closely linked markers to be used in marker-assisted selection. Incorporation of this QTL in breeding program will facilitate the development of cultivars with low DON content even under heavy FHB disease pressure.

Keywords: deoxynivalenol; Type-III; mapping; wheat; *Fusarium*

EVALUATION OF THE EFFECTS OF FIVE SUMAI 3 QTL ON FUSARIUM HEAD BLIGHT RESISTANCE IN CANADIAN SPRING WHEAT

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The cultivar Sumai 3 is a commonly used source of resistance in wheat (*Triticum aestivum* L.) breeding programs. The aim of this study was to evaluate the effect of Sumai 3 QTL to validate the transmission of loci that contribute to Type I and Type II FHB resistance, and Fusarium damaged kernel (FDK) and deoxynivalenol (DON) reduction in breeding populations through Sumai 3 derived parents. The three doubled haploid (DH) populations, Infinity/ND3085, Infinity/ND744 and Alsen/Helios utilizing Sumai 3 derived parents Alsen, ND3085 and ND744 were evaluated. In 2007 and 2008, 80 DH lines of each population and the parental genotypes were grown in FHB screening nurseries near Carman MB, Ottawa, ON and Charlottetown, PEI. The percentage of incidence (Type I), severity (Type II), FDK and DON accumulation were measured and FHB index calculated. DNA markers at five FHB resistance QTL detected in Sumai 3 on chromosomes 2DL, 3BS, 4B, 5AS, and 6BS were evaluated on the populations. For each trait, a *t*-test was applied to means of observations pooled by parental type of each marker to determine which loci contributed to resistance. The alleles at the 3BS and 5AS genomic regions most frequently contributed to Type I and Type II FHB resistance as well as to a reduced FDK and DON in all three populations. Interaction between loci across populations and environments was observed. Over the years and across the environments some loci showed a consistent effect and strong association with resistance, while the effect of some other loci was infrequent and irregular. The results of this study showed that resistance loci from Sumai 3 at the 3BS and 5AS QTL were transmitted to the progeny from the resistant parents to effectively reduced FHB damage.

Keywords: FHB; wheat; validation; FDK; DON

RELATIONSHIP OF TRANSMISSION OF RESISTANCE HAPLOTYPE AT 2BL AND 5A QTL ON FUSARIUM HEAD BLIGHT IN DT696 AND STRONGFIELD DERIVED LINES

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Among the few sources of resistance to fusarium head blight (FHB) previously identified in durum wheat (*Triticum turgidum* L. var. *durum* Desf.) are quantitative trait loci (QTL) on chromosome 5A of the advanced Canadian durum breeding line DT696 and on chromosome arm 2BL of the Canadian cultivar Strongfield. The objective of our research was to evaluate the relationship of resistance haplotypes from Strongfield and DT696 with FHB Type II resistance, FHB-damaged kernels (FDK), and deoxynivalenol (DON) accumulation in durum wheat lines with DT696 and Strongfield in their background. In 2015, fifty-nine lines, all with DT696 and twenty-five with Strongfield in their background, representing field resistant and susceptible phenotypes, were grown with parental checks in a greenhouse at the Swift Current Research and Development Centre, AAFC. Point inoculation was used to evaluate resistance to the spread of *Fusarium graminearum* infection. The percentage of severity (Type II), FDK and DON accumulation were measured. Lines were examined with single nucleotide polymorphism markers (SNPs) mapped near the previously reported FHB resistance QTL microsatellite markers to assess whether or not DT696 and Strongfield haplotype was transmitted. Data from the greenhouse confirmed the resistant phenotype of the majority of the Strongfield derived lines identified as resistant in the field. Genotypic data from the FHB resistance region on chromosomes 2BL revealed that six lines showed a common allelic makeup with Strongfield and that four of these were consistently associated with the resistant phenotype in both the field and greenhouse, expressing an improved Type II resistance and a lower FDK and DON than Strongfield. The results of this study show that Strongfield alleles were transmitted and the SNP markers associated with 2BL may be informative markers for improvement of FHB resistance. A similar haplotype analysis of the 5A resistance region of DT696 was inconclusive.

Keywords: FHB; durum; resistance; SNP; haplotype

THE SIGNALING PATHWAYS-OF WHEAT RESISTANT TO SCAB REVEALED BY BMSV-INDUCED GENE SILENCING

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The resistant mechanism of wheat to scab is still largely unknown. In this study, we carried out the transcriptomic analysis during the time course of pathogen infection using the resistant cultivars of Sumai3 and Sdau1881. The results showed that salicylic acid (SA) may play important roles in the initial biotrophic stage, while the jasmonic acid (JA), Auxin signaling and detoxification process may be involved in the later necrotrophic stage. To convince such hypothesis, we applied the BMSV induced gene silencing (VIGS) system in wheat spike and identified the resistant function of marker genes related with the above pathways, such as *TaICS1* for SA, *TaOPR3* for JA,*TaAMI1* for Auxin and *TaERF1* for Ethylene. When knowing down the expression of *TaICS1* and *TaERF1*, the pathogen expansion in wheat spikes were not apparently affected, while depressing the expression of *TaOPR3* and *TaAMI1* lead to the very fast expansion of the pathogen, revealing the functional roles of JA and Auxin signaling in scap resistance. These results were further confirmed by the phenotypic observation using wheat tilling mutants. Subsequently, we carried out the metabolomic analysis using NIL and found that the tryptophan content, the indirect precursor of Auxin, was higher in the resistant line from 24 to 72 h during the infection time course, indicating the positive roles of Auxin involved in the resistant cultivar.

Keywords: FHB; pathway; VIGS; Fhb7

WHEAT JACALIN-RELATED LECTIN TaJRL2.1 IS RELATED TO FUSARIUM HEAT BIGHT RESISTANCE

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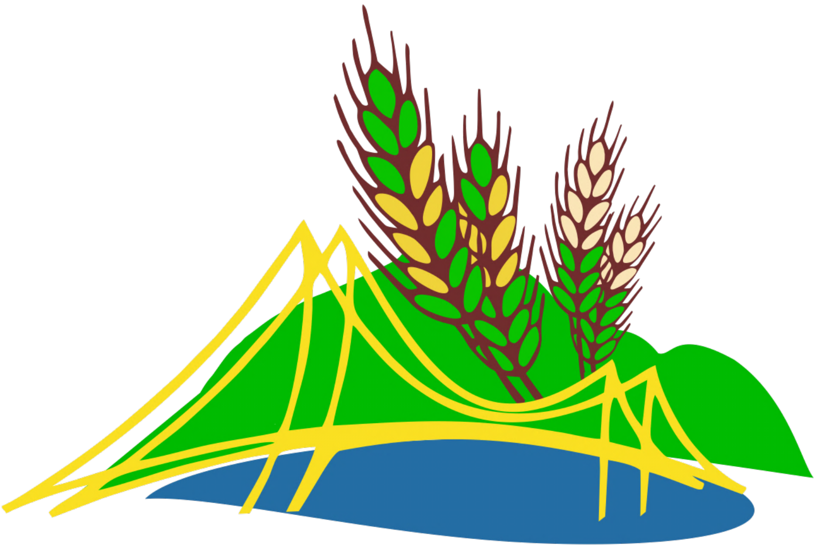
Plant lectins superfamily includes proteins as storage substances or as functional molecules exhibiting biological properties related to protection of plant tissue against pests and pathogens and to tolerance to abiotic stresses. Jacalin-related lectins (JRLs) are lectins have one or more domains with sequences similar to the jacalin protein isolated from jackfruit (*Artocarpus integrifolia*). In transcrptome analysis, we identified a *Triticum*-specific jacalin-related lectin gene *TaJRL2.1* that showed differential expression after *Fusarium graminearum* infection through. *TaJRL2.1* encodes a jacalin-related protein with low homology to other known JRLs. Semiquantitative RT-PCR revealed that *TaJRL2.1* transcript was abundant in young spikes and was almost negligible in other tissues. The expression of *TaJRL2.1* was rapidly induced by *F. graminearum* infection and jasmonic acid treatment and inhibited by BTH application. Attenuating *TaJRL2.1* expression in Fusarium head blight resistant Wangshuibai through virus-induced gene silencing led to susceptibility to *F. graminearum*, whereas over-expression of *TaJRL2.1* in Fusarium head blight susceptible PH691 reduced disease severity. Acknowledgement: this work was partially supported by NSFC program (31201270, 30130054, 30025030), '973' program (2010CB125900), Fundamental research Funds for the Central Universities (KYZ201202-4), and 111 project of Ministry of Education (grant no. B08025).

Keywords: wheat; lectin; FHB; resistance



5th ISFHB

Session 3

Genetics and Genomics of Fusarium Species

USING A BESPOKE 'OMICS' APPROACH TO DEVISE A FLEXIBLE NEW WAY TO CONTROL *Fusarium* IN WHEAT

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Fusarium head blight (FHB) disease has re-emerged as a major problem in most cereal growing regions and now threatens global food security. New ways to control FHB disease are urgently required. Here, we are using a bespoke genome, transcriptome and reverse genetics guided approach to understand and pinpoint the *Fusarium* genes and pathways required to cause disease in wheat heads. Our intention is to identify a new suite of target genes for intervention, enabling the development of flexible new ways to control FHB disease. The genome analyses: The completed *F. graminearum* (*Fg*) genome for the North American reference isolate PH-1 became available in 2015. To predict the core *Fg* proteome prevalent in one of the most severely FHB affected regions of the world, namely Brazil, the genomes of eight well characterised *Fg* isolates from Rio Grande do Sul were sequenced using Illumina and PacBio technologies. The annotated core *Fg* proteome was then inter-compared with the new genomes of two closely related species, *F. culmorum* (a wheat pathogen) and *F. venenatum* (a non-pathogen), to identify to core proteome involved in pathogenicity. The *in planta* transcriptome analyses: During compatible wheat-*Fg* interactions, the advancing *Fg* hyphae colonise the extracellular spaces in wheat tissue without causing macroscopic symptoms. Later, hyphal penetration of wheat cells coincides with the induction of host cell death. By using both RNAseq and *Fg* Affymetrix analyses, we have characterised the early *Fg* transcriptome during symptomless wheat infection. Over 3,000 genes coding for the core *Fg* proteome were found to be either rapidly up-regulated or down-regulated post-infection, revealing candidate gene targets that may be involved in the establishment of infection. Host-induced gene silencing (HIGS): Two recently published independent studies have revealed that transgenic plants expressing RNAi silencing constructs that specifically target *Fg* genes, such as cytochrome P450 lanosterol C-14α-demethylase (CYP51) or chitin synthase, display high levels of resistance to this pathogen. Using the predicting sub-set of genes coding for the core *Fg* proteome that were expressed during symptomless infection, we are now in a position to target additional *Fg* genes that may be essential and/ or required for virulence towards wheat. Transgenic wheat plants harbouring specific RNAi gene combinations have been generated and these will be functionally characterised in the T_1 and T_2 generations, revealing the efficacy of this novel HIGS approach aimed at combatting FHB in Brazil.

Keywords: genome-wide analyses; transcriptomics; *Fusarium venenatum*; *Fusarium culmorum*; host induced gene silencing

1 CHROMOSOME, 1 CONTIG: *Fusarium* IN THE GENOMICS ERA

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Fusarium is a very wide genus with animal and plant pathogenic species as well as saprophytes, that are used in biocontrol as well as for industrial applications. We integrated various disciplines on representatives across the genus. Chromosome numbers (CN) were determined using Germ Tube Burst Method (GTBM) and different sequencing platforms were applied to generate high quality assemblies. In several species remarkable genome plasticity is observed, including variable CNs and the presence of supernumerary chromosomes that differ markedly from the core chromosomes. It appears that several fusion events between core chromosomes happened during speciation. In *F. poae*, the supernumerary chromosomes (~8 Mb) show clear differences to the core chromosomes: 25% of transposable elements (TEs) *vs*. 2% on the core genome. The TEs in the core genome show clear signs of repeat-induced point mutation (RIP), while no RIP was found on the supernumerary genome. In addition, no gene duplications are present on the core, but many are found on the supernumerary genome. Exchange of genetic material occurs between the core and supernumerary genomes. Intact TEs from the supernumerary genome integrate into the core chromosomes, where they are subjected to RIP. In addition, large blocks of supernumerary sequence (>200kb) have recently been translocated to the core genome. *Vice versa*, genes from the core chromosomes are duplicated to the supernumerary genome, where they may show an increase in copy number. This "living apart together" crosstalk bestows significant opportunities for adaptation and evolution on the organism. This is reminiscent to the compartmentalization of genetic material in *F. graminearum*, where non-conserved regions are found at various places on the four core chromosomes (Zhao *et al.,* BMC Genomics 15:191, 2014).

Keywords: *Fusarium poae*; *Fusarium graminearum*; genomics

COMPARATIVE GENOMICS OF CLOSELY RELATED FUSARIUM HEAD BLIGHT FUNGI: *Fusarium graminearum*, *F. meridionale* and *F. asiaticum*

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The *Fusarium graminearum* species complex is composed of several distinct fungal species that cause several diseases in economically important crops, including the disease Fusarium Head Blight of wheat. Despite being closely related, these species and individuals within the species have distinct phenotypic differences in toxin production and pathogenicity, with some isolates previously reported as non-pathogenic on certain hosts. In this report, we compare genomes of six *F. gramineraum* strains and gene contents with two isolates of *F. asiaticum* and two isolates of *F. meridionale* strains. Total DNA was isolated from *Fusarium* isolates using the E.Z.N.A. Fungal DNA Mini Kit (Omega). DNA was sequenced according to manufacturer instructions using 74bp reads from paired-end libraries by Illumina GAII sequencing. The sequencing reactions were performed by the Center for the Analysis of Genome Evolution and Function (CAGEF) at the University of Toronto, Canada. Sequencing reads were imported, processed, and *de novo* assembled by 'CLC Genomics Workbench', and contigs ordered according to the PH-1 reference (MIPS database v3.2). Comparisons of genome structure and gene content revealed a 93 - 99% overlap across all ten genomes. We discovered more than 700kb of SNPs within common regions of the genome indicating genetic diversity within species. We also constructed a non-redundant 'pan' gene list containing 15,297 genes from the ten genomes. Among them, 1,827 genes or 12% were not conserved in at least one isolate. A majority of the poorly conserved genes were localized in telomeric regions and select regions within chromosomes with corresponding increase in SNPs and Indels. Many of these poorly conserved genes between isolates were either involved in secondary metabolism or had other functions associated with disease. In conclusion, the genomic resources we have established will be a valuable resource for the identification of genes that contribute to the phenotypic variation and niche specialization that have been reported for the *F. graminearum* species complex.

Keywords: *Fusarium*; comparative genomics; pangenome

USING THE PHENOTYPIC INFORMATION IN THE PATHOGEN-HOST INTERACTIONS DATABASE (PHI-BASE) TO EXPLORE *Fusarium* AND *Magnaporthe* GENOMES, TRANSCRIPTOMES AND PROTEOMES

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The PHI-base database (www.phi-base.org) stores expertly curated molecular and biological information on numerous virulence and effectors genes for which the effect on pathogen-host interactions has been tested experimentally. Fungal, protist and bacterial pathogens and nematodes which infect plant, animal, fish, insect and/or fungal hosts are included to allow comparative analyses. Information is also given on the target sites of some anti-infective chemistries. The PHI-base phenotypic information is provided to Ensembl and mapped to pathogen genomes (www.ensemblgenomes.org and www.phytopathdb.org). The latest release of PHI-base available provides information > 3,500 pathogen genes described in >5000 pathogen-host interactions (80% plant hosts), involving 230 pathogenic species. Each entry in PHI-base is checked by curators and is supported by strong experimental evidence (e.g. gene disruption/deletion, complementation experiments) and literature references. Several case studies will be described to show how the phenotypic data in PHI-base can be used in data mining approaches in *Fusarium* and *Magnaporthe* species to enrich *in silico* predictive, comparative genomic and network analyses and thereby enhance understanding of the pathogenic process. PHI-base phenotypes can be used to annotate outcomes of transcriptomic, proteomic and protein-protein interaction analyses, by linking genes of unknown function with those of known function. The PHI-base entries can also be used to fast track the interpretation of results from forward and reverse genetic experimentation. Since 2016 we provide an improved data display, a single and multiple sequence BLAST search functionality and an online curation tool (beta version) to capture phenotypes, genetic and molecular information from your paper directly into PHI-base. PHI-base receives BBSRC support as a National Capability (grant BB/J/004383/1) and from the PhytoPath BBR project (grant K021201/1) done in collaboration with the EBI Cambridge, UK.

Keywords: virulence; phenomics; genomics; ENSEMBL; database

MOLECULAR AND PHENOTYPIC STUDIES ON THE QUANTITATIVE INHERITANCE OF- AGGRESSIVENESS AND DEOXYNIVALENOL PRODUCTION IN *Fusarium graminearum* POPULATIONS CAUSING HEAD BLIGHT OF WHEAT

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Fusarium graminearum (Fg) is the most important pathogen causing Fusarium head blight (FHB) in cereals with deoxynivalenol (DON) as the most observed mycotoxin worldwide. Fg is known to exist in genetically highly variable populations. Isolates from individual, naturally infected wheat fields shared about 60% of the genetic diversity from an international collection as shown by DNA markers. Phenotypically, Fg isolates display in bread wheat a large variation for quantitative pathogenicity (=aggressiveness) and DON production in the grains. No specific reactions of individual isolates to wheat cultivars of different resistance level were found. Quantitative inheritance of both traits with a moderate to high heritability was found in genetic studies of biparental populations with Fg isolates from different origin. Because of this complex inheritance, quantitative-genetic methods must be established to analyze the genetic variation of Fg populations in more detail. Our objectives were to: (1) study the genetic variation of 150 Fg isolates for aggressiveness and DON content across four natural field environments, (2) to assess nucleotide diversity in candidate genes by SNP analyses, and (3) to associate phenotypic results with polymorphic SNPs to detect genes that contribute to the quantitative variation of aggressiveness and DON production. Results should offer new insights in the factors governing quantitative traits in Fg and the potential durability of host resistances. They will be discussed according to a review with the results obtained previously. All Fg isolates were analyzed by spray inoculation on a moderately susceptible cultivar of bread wheat in a chess-board like design with two replicates across each of two locations and two years. DON concentrations were analyzed by a commercially available immunotest. Candidate genes reported in literature were Sanger sequenced in all phenotypically analysed isolates and the associations were computed by established biometrical procedures. As a result, individual SNPs in several genes (e.g. *Gmpk1, MetAP1, Mgv1, Erf2, TRI1, TRI6*) contributed significantly to aggressiveness and DON production of Fg in natural field environments. Their individual effects were rather small, ranging from 3 to 26% of genetic variation as expected from quantitative-genetic theory. In conclusion, quantitative variation of aggressiveness and DON production in Fg is caused by a complex network of genes with contribute to the traits, each with small effects. This exactly corresponds to the quantitative nature of host resistance and lets expect, on a theoretical basis, no rapid changes of Fg populations due to the growing of resistant hosts.

Keywords: aggressiveness; association mapping; candidate genes

Fusarium graminearum TRICHOTHECENE GENOTYPES IN EUROPE

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The 3ADON genotype of *F. graminearum* predominates in northern Europe, where oats and barley have been the most important cereals, while the 15ADON genotype is predominant in Central and southern Europe including southern Russia, where wheat and maize are the most important cereals. The 15ADON genotype is spreading to the north in western Europe, while the 3ADON genotype has spread to north-western Russia, probably from Finland. A collection of 190 *F. graminearum* isolates was sampled from three regional locations as defined in Yli-Mattila et al. (2009): Finland and northwestern Russia (F+NWR; N = 40), southwestern Russia including 10 isolates from central Russia (SWR; N = 54), and Siberia and the Russian Far East (S+RFE; N = 96). In addition, 106 *F. graminearum* isolates were included from a previously published (Aamot et al. 2015) VNTR analysis of population structure in Norway (NOR; N = 106). Data from 8 previously published VNTR markers were collected as described by Kelly et al. (2015) and used to assess population structure and diversity. Results indicate that the *F. graminearum* population in Finland and northwestern Russia is closely related to the Norway population. Low levels of differentiation were also observed between the SWR and S+RFE populations. However, significant differentiation was observed between the populations in northern Europe (NOR and F+NWR) and those in southern and eastern Russia (SWR and S+RFE). Northern European populations had substantially less genetic diversity than was observed in the other regions. As previously reported, trichothecene genotype composition was significantly different across the sampling locations. 3ADON was predominant in F+NWR and Norway, 15ADON was predominant in the other regions sampled in SWR, and S+RFE had a balanced composition of these two trichothecene genotypes. The NIV genotype was not observed. Simulations with five populations (K = 5) provided the highest likelihood value in STRUCTURE analyses. However, simulations with two populations (K = 2) provided the greatest change in likelihood values and appear to capture the major structure in the data. According to the VNTR results the southern and northern European populations are strongly differentiated at least in Russia. 3ADON isolates in northern Europe produce more DON than 15ADON isolates from southern Russia and they are more pathogenic against seedlings of winter wheat seedlings. The northern European 3ADON population may be more specialized to oats, which is supported by the fact that only the 3ADON genotype has been found in oats in Europe (Pasquali et al., 2016, submitted), while the 15ADON population is shown to be more aggressive to wheat in Norway. The highest DON levels in Finland, Norway and Sweden have been found in oats and the DNA levels of *F. graminearum* are in agreement with DON levels.

Keywords: *Gibberella zea*; population; structure; deoxinivalenol; genetic diversity

Fusarium culmorum BASELINE SENSITIVITY TOWARDS TRIAZOLE FUNGICIDES AND NEW INSIGHT ON RESISTANCE MECHANISMS

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Fusarium culmorum is an important pathogenic and saprophytic fungal species occurring worldwide on various crops and weeds. During the past decades in Europe, *F. culmorum* was progressively replaced in the Fusarium head blight species complex by *F. graminearum.* Nevertheless, in 2011 *F. culmorum* was detected as the predominant species on wheat in Belgium and in Luxembourg. This brief takeover was accompanied by a significant increase in nivalenol concentration in mature grains. In an assay to characterise this species sensitivity towards triazoles, significant differences were found between strains from a mostly European collection, with up to 10-fold differences in EC_{50}. Interestingly, the resistant strains do not seem to have gain a phytopathological advantage as no increase of the number of resistant strains could be noticed over the years. Nevertheless, *F. culmorum* strain UK99 cultivated in liquid medium with increasing tebuconazole concentrations yielded strains more resistant than the parental strain while sometimes maintaining its pathogenic features. Moreover, in a greenhouse assay, curative treatment with tebuconazole applied on inoculated wheat ears showed less effect on one of the adapted strains as compared to the parental strain. This adapted strain also developed cross-resistance towards other demethylation inhibitors (e.g. epoxiconazole and prochloraz) but not towards tested fungicide from other families (e.g. benzimidazole and morpholine). In order to unravel the biochemical mechanisms behind this new phenotype the transcriptomes of both the adapted and the parental strains submitted to tebuconazole were sequenced by Illumina RNA-Seq. Gene expression analysis was performed to identify differentially expressed transcripts that could be involved in azole tolerance. Candidate genes will be validated by comparison with naturally sensitive and resistant *F. culmorum* strains.

Keywords: wheat; survey; DMI; EC50; transcriptome

GENETIC VARIATION BETWEEN *Fusarium anguioides*, *F. avenaceum* and *F. arthrosporioides* ISOLATES

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American Plant Pathologist Constantine Sherbakoff (1878-1965), who was originally from Russia, identified, described, and named 19 new species and 3 new sections of the genus *Fusarium* (1915). Among them were section *Arthrosporiella* Sherb. and such species as *F. sporotrichioides* Sherb., *F. anguioides* Sherb. and *F. arthrosporioides* Sherb. According to his opinion in section *Arthrosporiella* microconidia are typically present, usually spindle-shaped, 0-3-septate. Sporodochial macroconidia when present are sickle-shaped, mostly 5-septate, of Roseum type, from slightly curved to straight and anguiform; true chlamydospores are absent. The section is a connecting link between sections Roseum and Sporotrichiella (through *F. arthrosporioides*). But in section Roseum microconidia are typically absent, conidia are broad ellipsoid, typically of the same diameter for their length, comparatively narrow, and true chlamydospores are always absent. *Fusarium* a*nguioides* is a representative of section *Arthrosporiella*, having conidia of diverse type, ranging from arthrosporial (short spindle shaped, with more or less rounded ends) to typically slightly curved or nearly straight and anguiform, 1- to 15-septate. No conspicuous sporodochia can be found. Color of conidia in pseudopionnotal layer is ranging from light pinkish cinnamon to cinnamon; arthrosporial conidia are common on aerial mycelium. In our view *F. anguioides* is a common species on different plants, including cereals, but it is often identified as closely related *F. avenaceum* (Corda: Fr.) Sacc. or *F. arthrosporioides.* The aim of research was to identify genetic differences between these closely related *Fusarium* species and make sure that *F. anguioides* belongs to a separate species. Beta-tubulin DNA sequences and ISSR fingerprinting patterns of 13 *F. anguioides* isolates were compared to eight *F. avenaceum* and two *F. arthrosporioides* isolates. All isolates are single spore isolates and they were originally morphologically identified. According to beta-tubulin sequences there are three groups: one *F. anguioides* isolate (Vladivostok, *Rudbeckia* sp.), which has some similarities with *F. tricinctum,* six *F. avenaceum* isolates and one *F. anguioides* isolate, which have similarities with *F. avenaceum* isolates of main group I, and the rest of *F. anguioides* isolates, which have similarities with *F. avenaceum* isolates of main group II. According to the preliminary ISSR results four *F. anguioides* isolates, which have identical beta-tubulin sequences, are closely related to each other and form their own phylogenetic group. Two other slightly supported groups also consists of *F. anguioides* isolates, while three slightly supported groups consists of *F. avenaceum* isolates. The last slightly supported group includes two *F. arthrosporioides*, one *F. avenaceum* and one *F. anguioides* isolate. We will further analyze the ISSR patterns and compare the beta tubulin sequences of the 23 isolates to known sequences to find out, if *F. anguioides* isolates form their own phylogenetic group and species.

Keywords: Genetic diversity; beta-tubulin; *Fusarium* species

A REGIONAL STUDY ON TRICHOTHECENE-B TYPE COMPOSITION OF THE *Fusarium graminearum* SPECIES COMPLEX FROM WHEAT AND BARLEY IN SOUTHERN PARANÁ STATE, BRAZIL

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Fusarium head blight (FHB) is a disease of primary concern to wheat and barley in southern Brazil. Recent published studies have shown that the *Fusarium graminearum* species complex (FGSC) is prevalent in that region, with predominance of *F. graminearum s.str*. of the 15-acetyldeoxynivalenol (15-ADON) genotype, followed by *F. meridionale* of the nivalenol (NIV) genotype; while other FGSC species such as *F. cortaderiae* and *F. austroamericanum*, either the NIV or the 3-ADON genotype, and *F. asiaticum* with NIV genotype occurs occur with low frequency. Aiming to get more information on the composition of trichothecene genotypes at regional level, this study characterized FGSC strains isolated from wheat and barley ears with symptoms of FHB from Southern Paraná, for the presence of *TRI* cluster gene that determine the type-B trichothecene genotypes: 3-ADON, -15-ADON, or NIV. The survey included a total of 860 single spore isolates from wheat and 336 isolates from barley, collected in commercial fields of wheat (n=35-38) and barley (n=32-36) during four years (2011, 2012, 2013, and 2014). The greatest distance between the sampled fields was approximately 365 km. These isolates were examined for polymerase chain-reaction-based (PCR) trichothecene genotype based on the amplification of portions of *Tri3* and *Tri12*. On wheat, 15-ADON was the dominant trichothecene genotype (68.8-67.8%; average 65.0%), followed by NIV (27.0-31.1%; average 29.5%), and 3-ADON (3.2-6.7%; average 5.5%). Also on barley 15-ADON was the dominant trichothecene genotype (41.9-55.5%; average 50.2%), followed by NIV (30.7-45.2%; average 37.3%) and 3-ADON (8.5-14.9%; average 12.5%). Such knowledge is important for developing mycotoxins management strategies, including the selection of wheat and barley germplasm more resistant to FHB and with less mycotoxin accumulation in grains.

Keywords: *Hordeum vulgare*; *Triticum aestivum*; scab

INVOLVEMENT OF THREONINE DEAMINASE FGILV1 IN ISOLEUCINE BIOSYNTHESIS AND FULL VIRULENCE IN *Fusarium graminearum*

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In this study we characterized FgIlv1, a homologue of the *Saccharomyces cerevisiae* threonine dehydratase (TD) from the important Fusarium head blight (FHB) fungus *Fusarium graminearum*. TD catalyzes the first step in the biosynthesis pathway of isoleucine (Ile) for conversion of threonine (Thr) to 2-ketobutyrate (2-KB). The *FgILV1* deletion mutant ΔFgIlv1-3 was unable to grow on minimal medium or fructose gelatin agar which lacked Ile. Exogenous supplementation of Ile or 2-KB but not Thr rescued the mycelial growth defect of ΔFgIlv1-3, indicating the involvement of FgIlv1 in the conversion of Thr to 2-KB in Ile biosynthesis. Additionally, exogenous supplementation of Methionine (Met) could also rescue the mycelial growth defect of ΔFgIlv1-3, indicating a crosstalk between Ile biosynthesis and Met catabolism in *F. graminearum*. Deletion of *FgILV1* also caused defects in conidial formation and germination. In addition, ΔFgIlv1-3 displayed decreased virulence on wheat heads and a low level of deoxynivalenol (DON) production in wheat kernels. Taken together, results of this study indicate that FgIlv1 is an essential component in Ile biosynthesis and is required for various cellular processes including mycelial and conidial morphogenesis, DON biosynthesis, and full virulence in *F. graminearum*. Our data indicate the potential of targeting Ile biosynthesis for anti-FHB management.

Keywords: Threonine dehydratase; *Fusarium graminearum*

CROPS ARE A MAIN DRIVER FOR SPECIES DIVERSITY AND THE TOXIGENIC POTENTIAL OF *Fusarium* ISOLATES IN MAIZE EARS IN CHINA

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In recent years increasing demands and the relatively low-care cultivation of the crop have resulted in an enormous expansion of the acreage of maize in China. However, particularly in China, Fusarium ear rot (FER) forms an important constraint to maize production. In this study, we showed that members of both the *Fusarium fujikuroi* species complex (FFSC) and the *Fusarium graminearum* species complex (FGSC) are the causal agents of Fusarium ear rot in the main maize producing areas in China. Fumonisin producing *F. verticillioides* was the most prevalent species, followed by fumonisin producing *F. proliferatum* and 15-acetyldeoxynivalenol (15ADON) producing *F. graminearum*. Both *F. temperatum* and *F. boothii* were identified for the first time in the colder regions in China, extending their known habitats to colder environments. Mating type analysis of the different heterothallic FFSC species, showed that both two types co-occur in each sampling site suggestive of the possibility of sexual recombination. Virulence tests with *F. boothii* (from maize) and *F. graminearum* from maize or wheat showed adaptation to the host. In addition, *F. graminearum* seems to outcompete *F. boothii* in wheat-maize rotations. Based on our findings and previous studies, we conclude that wheat/maize rotation selects for *F. graminearum*, while a wheat/rice rotation selects for *F. asiaticum*. In contrast, *F. boothii* is selected when maize is cultivated without rotation. A higher occurrence of *F. temperatum* is observed on maize in colder climatological regions in China, while *F. meridionale* seems restricted to mountain areas. Each of these species has their characteristic mycotoxin profile and deoxynivalenol (DON) and fumonisin are the potential threats to maize production in Northern China.

Keywords: FGSC; fumonisin; trichothecene; maize; wheat

LARGE GENETIC DIVERSITY AND CHEMOTYPE INSTABILITY IN A PROMINENT *Fusarium* SPECIES

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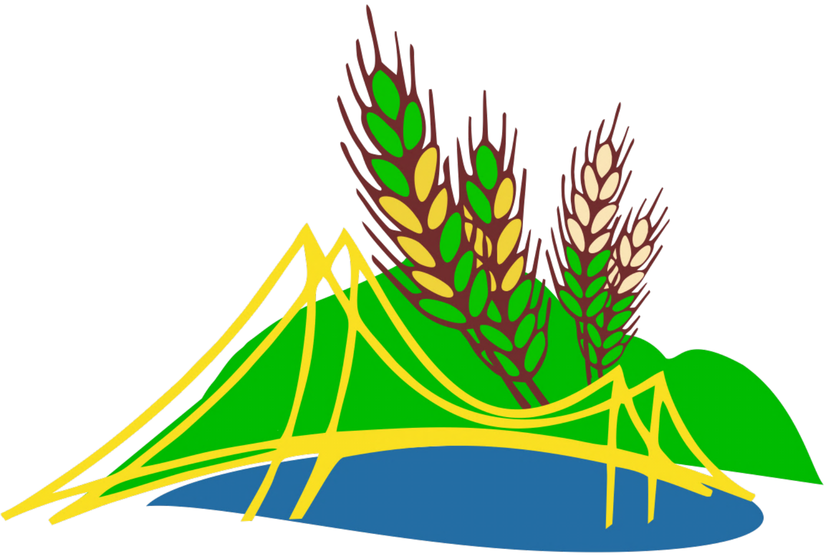
Fusarium Head Blight is a devastating disease of small grain cereals, and ears may be infected by a number of *Fusarium* species simultaneously. *F. poae* has been described as a prominent member of this complex of species. In this study, we took the recently released genome of *F. poae* and employed it in a polyphasic approach to provide clues for the genetic profile, the chemotype and lifestyle of this species. To this end, a representative collection of isolates was set up, including four isolates for which genome sequence is available. We demonstrated for the first time that *F. poae* isolates combine both sexual and asexual reproduction and possess an active transposable element (TE) arsenal. Consequently, this species meets two important FRAC criteria of highly challenging organisms for durable disease management. The high inter-isolate genetic variability present within this species was confirmed, unlinked to host, geography and speciation, and we show that TE proliferation and the species' active genome dynamics in general may confound the clonal nature of some isolates. The trichothecene chemotype, made up of a strictly sequential type A + type B pattern, was unravelled and consisted of diacetoxyscirpenol (DAS), neosolaniol (NEO), fusarenon-x (FUS-X) and nivalenol (NIV) in the listed order. Opposed to the situation in the *F. graminearum* species complex (FGSC), this chemotype was not fixed in vitro, and genetic chemotyping (i.e. determining the 'genotype') may be uninformative for a universally NIV-producing *F. poae* population. Genomic determinants such as high variability at the Tri1 region and, a TE-invaded main trichothecene cluster do not explain the unstable chemotype. This study offers valuable insights into the lifestyle of an enigmatic *Fusarium* pathogen that stands as one of the most frequently detected members of the FHB complex in many countries.

Keywords: *F. graminearum*; *F. poae*; trichothecene-chemotype



5th ISFHB

Session 4

Epidemiology and Management

TECHNOLOGIES FOR PATHOGEN AND DISEASE DETECTION

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Many pathogens are dispersed by airborne spores, which can vary in space and seasonal timing. We can use air sampling integrated with suitable diagnostic methods to give a rapid warning of inoculum presence to improve the timing of control options, such as fungicides. Air sampling can also be used to monitor changes in genetic traits of pathogen populations such as the race structure or frequency of fungicide resistance. Although some image-analysis methods are possible to identify spores, in many cases, species-specific identification can only be achieved by DNA-based methods such as qPCR and LAMP and in some cases by antibody-based methods (lateral flow devices) and biomarker-based methods ('electronic noses' and electro-chemical biosensors). Many of these methods also offer the prospect of rapid on-site detection to direct disease control decisions. Thresholds of spore concentrations that correspond to a disease risk depend on the sampler (spore-trap) location (whether just above the crop canopy, on a UAV or drone, or on a tall building) and also need to be considered with weather-based infection models. Where disease control by spore detection is not possible, some diseases can be detected at early stages using optical sensing methods, especially chlorophyll fluorescence. In the case of *Fusarium* infections on wheat, it is possible to map locations of severe infections to segregate harvesting of severely affected areas of fields to avoid toxins entering the food chain.

Keywords: spore-trap; qPCR; LAMP; optical sensing

THE ROLE OF CULTURAL PRACTICES IN THE CONTROL OF FUSARIUM HEAD BLIGHT OF WHEAT AND OTHER CEREALS

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Fusarium head blight (FHB) has become an intractable problem in many in wheat production regions of the world. Following a global upsurge of FHB, starting in the 1990's, resources directed to supporting research aimed at controlling FHB in wheat and other cereals has been greater than at any other time. As a result of the concerted effort of many researchers significant progress has been made in combatting FHB over the past two decades. Sources of genetic resistance to FHB have been identified in wheat germplasm and the loci conferring resistance introgressed into adapted wheat germplasm. The development of genetic and genomic tools has increased the ability of pant breeding programs to utilize multiple loci with small genetic effects to confer resistance. Fungicide efficacy and application technologies have also been studied providing a much better understanding of the benefits and limits of the use of chemical control. The development of models, aimed at predicting FHB epidemics and/or mycotoxin accumulation, have enabled the most efficient and judicious use of the available fungicides and/or to inform markets and ensure a supply of safe wheat for consumers. However, despite the impressive achievements in deploying genetic resistance and fungicides, neither strategy alone, or indeed in combination, has provided adequate protection to prevent FHB from remaining a significant risk to wheat production, especially in environments where conditions are conducive to disease development. The historical evidence supports the argument that changes in production practices, particularly the widespread adoption of reduced tillage systems and the expansion of corn production, have been drivers in the global increase of this residue-borne disease. Factors influencing the observed changes in the pathogen population, including shifts among the species inciting FHB as well as within species variation, are less well understood. Cultural practices that reduce the survival of the fungal species that incite FHB and the production of inoculum from host residues in subsequent cropping seasons provide an additional strategy to control FHB. Research in multiple environments has demonstrated that residue reductions strategies and rotation to non-host crops can reduce the risk of FHB. While the body of evidence suggests that cultural control strategies directed to reducing primary inoculum are likely to be effective against FHB, the challenge lies in identifying cultural practices that are compatible with commercial wheat production practices such that they can be implemented on a sufficiently large proportion of the wheat production area that they will contribute significantly to disease control.

Keywords: *Gibberella zeae*; rotation; residues; survival

O15 *5th ISFHB: Session 4 - Epidemiology and Management*

MORE THAN A DECADE OF COORDINATED RESEARCH TO DEVELOP INTEGRATED MANAGEMENT PROGRAMS FOR FUSARIUM HEAD BLIGHT OF WHEAT

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For more than a decade, field experiments were conducted through the US Wheat and Barley Scab Initiative to evaluate the effects of fungicide and host resistance on Fusarium head blight (FHB) and deoxynivalenol (DON) at multiple locations across major US wheat-producing states. Several aspects of these strategies were quantified through coordinated projects that involved four key phases. FHB index (field severity: mean proportion of diseased spikelets per spike) and DON data were collected from *Fusarium graminearum*-inoculated wheat research plots and meta-analyzed to quantify the relative efficacy of different demethylation inhibitor fungicides (phase I); the efficacy, additivity, and stability of integrating host resistance and fungicide treatment (phase II); the efficacy and economics of integrating in-field and grain harvesting strategies (phase III); and the relative efficacy of fungicide applications made before, at, and after anthesis to cultivars with different levels of FHB resistance (phase IV). **Phase I**: The efficacy of propiconazole (Tilt), prothioconazole (Proline), tebuconazole (Folicur), metconazole (Caramba), and prothioconazole+tebuconazole (Prosaro) against FHB and DON was determined thorough meta-analyses, with results showing that Prosaro, Caramba, and Proline were the most efficacious fungicides, reducing index by 48 to 52% and DON by 42 to 45%. Relative to Folicur, the industry standard at the time, Proline, Caramba, and Prosaro further reduced index by 14 to 20% and DON by 25 to 29%. **Phase II**: Results from meta-analyses showed that all five FHB resistance class x Prosaro treatment combinations evaluated (susceptible treated [S_TR]; moderately susceptible treated [MS_TR] and untreated [MS_UT]; moderately resistant treated [MR_TR] and untreated [MR_UT]) significantly reduced index and DON ($P < 0.001$) relative to the untreated susceptible check (S_UT). MR_TR was the most efficacious combination, reducing index by 76% and DON by 71%, followed by MS_TR (71 and 58%, respectively), MR_UT (54 and 51%, respectively), S_TR (53 and 39%, respectively), and MS_UT (43 and 30%, respectively). Results from nonparametric analyses showed that MR_TR was also the most stable management combination across environments, and based on the principle of treatment independence, the effect of combining an anthesis Prosaro treatment with moderate resistance was additive in terms of percent control for index and DON. **Phase III**: Relative to S_UT, FHB management programs that included combinations of an anthesis application of Prosaro, a moderately resistant cultivar, and grain harvested with a combine adjusted to remove lightweight kernels resulted in 30 to 51% reduction in estimated price discount, \$127 to 312 ha^{-1} increase in gross cash income, and economic benefit ranging from \$31 to 272 ha^{-1}. **Phase IV**: The highest overall percent control of index and DON relative to S_UT was observed when Prosaro or Caramba was applied to a moderately resistant cultivar at anthesis or at 2 to 6 days after anthesis.

Keywords: Integrated management; meta-analysis; percent control

USING A BAYESIAN MODEL FOR ESTIMATING AIR BORNE INFECTION RISKS: FUSARIUM HEAD BLIGHT

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Among the most significant diseases affecting wheat worldwide, Fusarium head blight (FHB) has increased in concern to growers in many production regions. Apart from losses in- yield and reductions in baking and seed quality, the major impact associates with- FHB epidemics is the contamination of grain with mycotoxins. FHB has a worldwide distribution although the severity of the outbreaks is highly variable and influenced by local weather conditions. Disease development is enhanced when wetness conditions occur during the flowering and grain-filling stages of wheat. In Brazil, the main causal agent of the disease is *Gibberella zeae* (Schwein.) Petch., the teleomorphic phase member of the *Fusarium graminearum* species complex. The fungus infects others hosts besides wheat and can survive on crop residues. Under favorable weather conditions, ascospores and macroconidia are produced on infested residues and transported to wheat spikes by rain splash and turbulent air currents. Forecasting airborne inoculum density can be useful for disease management and new data, and statistical approaches can improve the accuracy of predictions. Currently, most methods for risk assessment rely upon deterministic weather-driven models based on regression techniques. In contrast, this work describes the use of a probabilistic approach to estimate wheat spikes exposure risk to FHB airborne inoculum. The data we utilized consist of daily counts of *Gibberella zeae* colony forming units in a semi-selective agar media and corresponding meteorological variables collected at hourly intervals. Models were fit to the data using a hierarchical auto-regressive Bayesian approach. Models were fit using MC-Stan software based on HMC U-Nuts, called from R via the RStan package.- Our program generated posterior predictions which allow us to assess prediction uncertainty in the density of *G. zeae* airborne inoculum. Model parametrization varied according to El Niño Southern Oscillation (ENSO) phases. Finally, a framework was developed for combining new information with existing models, in which prior probabilistic models are auto-updated based on new observations.

Keywords: *Triticum*; ENSO; forecasting; posterior estimation

RAIN FORECAST TO TIME FUNGICIDE FOR FHB CONTROL IN WHEAT

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Fusarium head blight (FHB) is a floral infecting disease whose infection is dependent on the heads long wetting period (rain). In field experiments, with Ametista wheat cultivar, the rain forecast was used to time fungicides application for FHB control. The fungicides were applied 24 - 48 hours before the expected occurrence of the rain. Fungicides mixtures of strobilurins containing metconazole (Opera Ultra) and prothioconazole (Fox) with the greatest potential for disease control were tested with one and two sprayings. A boom with adjustable twin nozzles launching a forward jet at 30° and other back to 70° was used. Applications were made after the beginning of flowering and before the rain onset forecasted by CPTEC/INPE (http://avaliacaomodelos.cptec.inpe.br/obs/qpf/index.php). It was considered a 15 days heads protection by the fungicides. Thus, in the treatments with two applications, the second was performed after 15 days, but before the occurrence of new forecasted rain. Disease incidence in heads and in spikelets and grain yield were assessed. The best control was reached by the two application of metconazole + pyraclostrobin with 67% control. Infection in check treatment was 24.7%. The control is still considered low and can be enhanced by improving technology for fungicide deposition on the heads side. Springs rains are forecasted with 95% precision. Thus rain occurrence can be used to time fungicide application in FHB control in wheat.

Keywords: control; *Fusarium graminearum*; giberela; scab

TRICHOTHECENE CHEMOTYPES AND FITNESS OF THE *Fusarium graminearum* SPECIES COMPLEX IN THE MAJOR WINTER WHEAT PRODUCING AREAS OF CHINA

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In China, epidemics of Fusarium head blight (FHB) occur frequently in the Yangtze River Basin. With changes in climate and tillage practices, FHB has gradually spread north from the Yangtze River Basin. FHB in China is mainly caused by *Fusarium graminearum* species complex (FGSC), which was divided into 3 chemotypes: 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON) and nivalenol (NIV). Previous works revealed that *F. asiaticum* was the predominant phylogenetic species in the Yangtze River Basin and *F. graminearum* dominant in the north of China. The 3-ADON and 15-ADON chemotypes were predominant in the Yangtze River Basin and areas northe of the Yangtze River Basin respectively, and it is suggested that geographic distribution is associated with differences in temperature as well as crop rotation systems. In order to predict the spreading trend of 3 chemotypes in China, we randomly selected 20 isolates for every chemotype and studied their biological characteristics. The results showed that the optimum temperature for growth of 3-ADON, 15-ADON and NIV isolates was 25 °C. The growth rate of 3 chemotype populations at 15 °C , 20 °C and 25 °C was similar. The growth rate of NIV isolates was the highest at 30 °C . The isolates of 15-ADON chemotype formed more perithecia and earlier at 4 different temperatures, and the ascospore release of 15-ADON isolates was also the highest, and there was no significant difference between 3-ADON and NIV populations. The virulence test on wheat seedlings suggested that there was no significant difference between 3-ADON and 15-ADON populations, but virulence of NIV population was significant lower than that of 3-ADON and 15-ADON population. The virulence tests on wheat ears showed same results. DON content in grains after the inoculation was assayed and the results showed that 3-ADON population produced more DON than 15-ADON and NIV populations. The toxin production in the rice culture of 60 isolates was also evaluated. The mean DON content that 15-ADON chemotype isolates produced was the highest. The fitness and spreading trends of three chemotype population were discussed.

Keywords: *Fusarium*; chemotype; fitness; mycotoxin

APPLICATIONS OF BIO-MOLECULAR DIAGNOSTICS AND HIGH-THROUGHPUT TECHNOLOGY FOR SURVEILLANCE OF FUSARIUM HEAD BLIGHT PATHOGENS IN CANADA

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Fusarium head blight (FHB) is an important disease of cereal grains that affects several segments of the grain industry, from production to processing and marketing. Losses from reduced yields are compounded by mycotoxin contamination and reduction in grain quality. Until the end of the last century, mainly three *Fusarium* species (*F. graminearum*, *F. avenaceum* and *F. culmorum*) were associated with FHB infected wheat in western Canada. But over the last 20 years, *F. graminearum* has become the predominant species, which is known to produce a number of toxic trichothecenes including deoxynivalenol (DON), nivalenol (NIV) and calonectrin derivatives (NX). These important phenotypic traits are also reflected by different genotypes of the pathogens. Agronomic practices and environmental conditions impose selective pressure on indigenous pathogen populations that are continuously adapting to changes of external factors. Shifts in the population structure of FHB pathogens cannot be detected or monitored by traditional agar plating and morphological observations. For this task, quantitative PCR (qPCR) methods have proven to be a powerful technology for the detection, identification and characterization of plant pathogens. Diagnostic tools utilizing species and/or trait specific qPCR assays allow high resolution strain typing of multiple target organisms simultaneously. However, sample preparation and limitations of proprietary platforms often pose bottle necks in the workflow, interfering with a more rapid characterization of *Fusarium* and other pathogen species. For the surveillance and monitoring of FHB and other grain pathogens in Canada, we developed a high-throughput workflow including single kernel DNA extraction, bio-molecular assays and employing an open design qPCR system. The system consists of a quantitative real-time PCR unit and a multi-sample dispenser to rapidly change configuration of the nano-reaction chips allowing for more flexibility in the choice of diagnostic assays and for higher throughput.-The newly developed workflow significantly increased our testing capacity, which comprises the analysis of more than two thousand harvest samples of cereal grains annually. Each crop year since 2014, 15,000 - 5,000 individual seeds of wheat and oats were tested for common and quarantine plant pathogens as well as phenotypic traits such as trichothecene chemotype. Statistics and distribution maps showing the annual occurrence and frequency of *Fusarium* species and their chemotypes in spring wheat were generated for western Canada. For grain producers and government authorities alike, results of the national surveillance program on FHB and other fungal pathogens provide essential support to an integrated approach to on-farm risk management. In addition, open access to monitoring data and statistics allow the agrochemical industry to tailor more customized solutions to control FHB outbreaks and mycotoxin contamination in cereal crops. Our single-kernel workflow has also potential for assisting wheat and other cereal breeding programs with the selection of new lines for disease resistance.

Keywords: *Fusarium graminearum*; trichothecenes; genotyping; qPCR

AGGRESSIVENESS, TOXIGENIC POTENTIAL AND SAPROPHYTIC GROWTH OF FIVE *Fusarium graminearum* SPECIES COMPLEX MEMBERS

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The most common *Fusarium graminearum* species complex (FGSC) members causing Fusarium head blight (FHB) of wheat in southern Brazil, in order of prevalence, are *Fusarium graminearum* (Fgra), a typical deoxynivalenol (DON)-producer, and *F. meridionale* (Fmer), a typical nivalenol (NIV)-producer. However, other members, such as *F. cortaderiae* (Fcor), *F. asiaticum* (Fasi) and *F. austroamericanum* (Faus), which are able to produce NIV or 3-acetyl-deoxynivalenol (3-ADON), have also been found at low frequency in wheat heads. In this study, two isolates representative of these five phylogenetic species were compared with regards to saprophytic and pathogenic fitness and trichothecene production. In the pathogenicity assays, aggressiveness was evaluated using a central-spikelet inoculation in two wheat cultivars (susceptible and moderately resistant). Trichothecene production - NIV, DON and two acetylates of DON - was evaluated in vitro (rice grain substrate) and *in vivo* (moderately resistant wheat cultivar). Saprophytic fitness was assessed based on measures of mycelial growth rate (MGR, mm/day) at 15 and 30 °C, sporulation (SPO, number), macroconidia germination rate (GER, %) and a sexual fertility index (FER, %) calculated based on frequency and percent coverage of perithecia formed on rice, corn and wheat grain substrate. The pathogenicity assay showed that Fgra was more aggressive than Fmer in both cultivars. The *in vivo* trichothecene production patterns were coherent with their toxigenic potential. Fgra consistently produced 15-ADON (in vitro =1.700 µg/kg and in vivo = 666 µg/kg) and Fasi and Fmer produced nivalenol (mean in vivo = 243 µg/kg and 205 µg/kg, respectively). Faus and Fcor produced only 3-ADON (mean in vivo = 944,5 µg/kg and 894,5 µg/kg, respectively). Fasi grew faster at both temperatures and Fmer showed higher SPO and GER. Fgra showed the highest fertility index in all three substrates: rice (61,5%), corn (49,5%) and wheat (32,3%), compared to the other species. Our data suggest that the enhanced sexual fertility, toxigenic potential and aggressiveness of Fgra isolates compared to the other species may help to explain its dominance in wheat agroecosystems.

Keywords: *Triticum aestivum*; trichothecenes; aggressiveness; FGSC

MONITORING *Fusarium* spp. ON *Triticum* L. AND *Hordeum* L. IN CENTRAL REGION OF RUSSIA

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The distribution of facultative phytopathogenic fungi of the genus *Fusarium* in cereals creates threat of increase an infectious background in the soil and on seed material. The agents causing Root Rot, Snow Mold and Fusarium Head Blight belong to a parasitic species with low specialization and these diseases have been found in all regions of cereals cultivation. The study of the influence of environmental factors on mycocenosis above-ground and underground parts of spring wheat have shown the annual dominance of *Fusarium* spp. along with the genera *Bipolaris*, *Alternaria*, *Penicillium*, *Cladosporium*, *Aspergillus* et al. The purpose of the work was the collation of data on species structure, frequency of occurrence, pathogenic and phytotoxic properties of fungi of the genus *Fusarium* with the aim of selection of strains for keeping in the collection and using in breeding studies as infectious material. The agents of Root Rot, Snow Mold and Fusarium head blight were isolated from plants of spring and winter wheat and spring barley collected in 1995-2014 in the Central regions of the Russian Federation. Mycological analysis of seeds and roots samples of cereals was carried out on potato-dextrose agar according to standard methods. Pathogenicity and toxicity of *Fusarium* spp. isolates were studied with using of the biological test method on seeds. In the Central region of Russia there were found out fifteen *Fusarium* species: *F. culmorum, F. heterosporum (Gibberella gordonii), F. sporotrichioides, F. oxysporum, F. nivale (Monographella nivalis), F. graminearum (G. zeae), F. avenaceum (G. avenacea), F. gibbosum (G. intricans), F. sambucinum (G. pulicaris), F. moniliforme (G. moniliformis), F. semitectum (F. incarnatum), F. poae, F. lateritium (G. baccata), F. solani (Nectria haematococca), F. redolens*. The frequency of occurrence of *Fusarium spp*. depended on weather conditions of the year, the localization of the pathogen on plants, previous crop, etc. The incidence of *F. culmorum, F. heterosporum, F. sporotrichioides, F. oxysporum* together accounted for more than 50% of all identified isolates of fungi. Species such as *F. lateritium, F. semitectum, F. poae, F. solani, F. redolens* met seldom. Levels of pathogenicity and toxicity of the strains of the different *Fusarium* species have been varied. The prevalence of strains with high pathogenic and toxic activity was revealed for *F. sporotrichiodes, F. culmorum, F. sambucinum*. *F. oxysporum* and *F. heterosporum* strains were mainly non-pathogenic weakly pathogenic but they showed high toxicity. Isolates of *F. gibbosum* and *F. avenaceum* were characterized by weak and moderate pathogenicity and relatively low toxicity. Most strains of *F. graminearum* showed weak and moderate pathogenicity and high toxicity. Prevalence of *Fusarium* spp. is caused by plasticity and viability in the soil, on weed roots and on the remains of many crops, and also high competitive ability in a mikobiota of agricultural plants.

Keywords: *Fusarium*; cereals; pathogenicity; toxicity

EFFECT OF AN ADDITIONAL SPRAY AT WHEAT TILLERING STAGE IN A MANAGEMENT STRATEGY TARGETING FUSARIUM HEAD BLIGHT CONTROL

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Fusarium Head Blight (FHB) has great economic importance in wheat (*Triticum aestivum* L.), causing grain yield losses. This disease is caused by several species of *Fusarium* and, in Brazil, *Gibberela zeae* (anamorphic form *Fusarium graminearum*) is the main pathogen. Besides yield reduction, the disease affects grain quality and threatens human and animal health due to the mycotoxins produced. Fungicides and resistant cultivars are commonly used to reduce the damage caused by FHB in wheat. The objective of this study was to evaluate the efficacy of different active ingredients and an additional spray at tillering stage for FHB control in wheat in Erechim, RS, Brazil. The experiment was conducted in the experimental area of the Universidade Federal da Fronteira Sul, Campus Erechim. The experimental design used was completely randomized with five treatments and four replications. Each experimental unit consisted of a plot with 12 m^2. The treatments were: Azoxystrobin + Tebuconazole three (T1) and four (T2) applications; trifloxystrobin + Prothioconazole three (T3) and four (T4) applications; and a control treatment (T5) without fungicide application. In the treatments with three applications, fungicides were applied at phenological stages of: elongation, booting and flowering. Treatments with four applications were made at the phenological stages of: tillering (preventive), elongation, booting and flowering. Harvest was done in a portion area of 3 m^2 where it was rated the hectoliter weight (pH) and total productivity (kg ha^{-1}). To evaluate FHB incidence, grains with symptoms were visually separated of healthy grains and then weighed. From the total sample weight and the weight of grains with symptoms, it was determined the percentage of affected grains. Data were submitted to analysis of variance by test F at 5% probability, and the averages compared by Tukey test also with 5% probability. In the pH evaluation, fungicide treatments were statistically different compared to the control. For yield, T2, T3 and T4 presented the best performance. As to FHB incidence, the treatments T2 (1.7% incidence) and T4 (1.6% incidence) showed better results compared to the control (2.1% incidence). In addition, treatments T1 and T3 did not differ statistically from the other treatments in this evaluation. In conclusion, the results showed that treatments with four applications were more effective in FHB control indicating that the maintenance of healthy plant since the beginning can provide higher resistance to late season disease, thereby ensuring production without losses in grain yield and quality. However, futher experiments should be performed, preferably under higher disease pressure aiming an effective control of FHB.

Keywords: *Fusarium graminearum*; disease assessment; fungicide

SENSITIVITY TO TEBUCONAZOLE AND METCONAZOLE FOR A *Fusarium graminearum* SPECIES COMPLEX POPULATION FROM BRAZILIAN BARLEY

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Fusarium head blight (FHB) is among the most important diseases of barley in Brazil. A few members of the *Fusarium graminearum* species complex (FGSC) have been reported in previous studies, with the potential to produce a range of trichothecene-B mycotoxins such as nivalenol (NIV) and DON and its two acetylated derivatives (15-ADON and 3-ADON). Barley growers rely on sequential fungicide sprays at flowering to manage the disease and reduce mycotoxin levels. Information on the sensitivity of the pathogen population to most commonly used fungicides is limited and is unknown whether the sensitivity levels differ among FGSC members. In this study, a collection of 62 FGSC strains were isolated from barley kernels originated from commercial fields located at sixteen municipalities in the main barley-growing regions of Rio Grande do Sul state, Brazil, which were harvested in the 2011 year. All isolates were simultaneously assigned to FGSC member and trichothecene genotype using a Luminex-based multilocus genotype assay. The sensitivity to tebuconazole (Folicur 200 EC, BAYER S.A.) and metconazole (Caramba 90, BASF S.A.) was determined based on the effective concentration that reduced 50% of the mycelial growth (EC_{50}) on agar-based media amended with increasing fungicide dosages (0, 0.1, 0.5, 1.0 and 5.0 mg/L). Three FGSC members were found. *Fusarium graminearum* (Fgra) of the 15-ADON genotype the most dominant (46 isolates), followed by *F. meridionale* (Fmer) (15 isolates) of the NIV genotype. Only one *F. austroamericanum* isolates, of the 3-ADON genotype, was found. Irrespectively of the species, the isolates were around 4 × less sensitive to tebuconazole compared to metconazole: EC_{50} for tebuconazole and metconazole ranged from 0.005 to 4.346 mg/L (mean = 0.773 mg/L; median = 0.492 mg/L) and from 0.000 to 1.318 mg/L (mean = 0.136 mg/L; median = 0.046 mg/L), respectively. A non-parametric test suggested that the sensitivity to tebuconazole of the Fgra and Fmer isolates were sampled from populations with different distributions ($P = 0.0006$), with Fgra isolates being less sensitive. Such difference was not observed for metconazole isolates ($P = 0.47$). Thirteen isolates (eleven Fgra and two Fmer) showed EC_{50} values > 1.0 mg/L to tebuconazole, and only one isolate (Fgra) showed EC_{50} value > 1.0 mg/L to metconazole. Among thirty five isolates with EC_{50} for tebuconazole >0.50 mg/L, three (one Fgra and two Fmer) were able to exceed 50% of mycelial growth at a discriminatory dose of of 2.0 mg/L. Three isolates were considered less sensitive to tebuconazole. Cross-resistance was suggested based the correlation coefficients between EC_{50} values for the two fungicides (Pearson's $r = 0.44$; $P = 0.0003$). Further studies are needed to investigate whether the reduced sensitivity to tebucunazole found in a few isolates is due to resistance associated with increasing use of triazole fungicides in barley crops.

Keywords: Fusarium head blight; *Gibberella zeae*; fungicide resistance; *Hordeum vulgare*

SPRAY NOZZLES AND FREQUENCY OF APPLICATIONS TO THE CONTROL FUSARIUM HEAD BLIGHT IN WHEAT

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The Fusarium head hlight is considered a floral disease infection. For the full development are necessary temperatures above 20°C and wet periods of the largest spikes than 72 h during anthesis of wheat (*Triticum aestivum* L.). This disease presents great difficulties of control, due to factors such as: sporadic of its occurrence, the appropriate time to carry out the control, difficulty of the fungicide in reaching the sites of infection, and reduced control efficiency of available fungicides. The objectives of this study were to determine the incidence and severity, the control of Fusarium head blight, and the yield of wheat in response to two fungicide applications with different spray tips, on the wheat, cultivar Pioneiro. The experiment was conducted in a randomized block design with factorial arrangement (9 x 2) and three repetitions. Two fungicide applications were made, the first in the beginning of anthesis, with the combination of azoxystrobin + cyproconazole (0.33 L.ha^{-1}) + propiconazole (0.3 L.ha^{-1}) + Nimbus® mineral oil (0.5 L.ha^{-1}). At fourteen days after the first application (half anthesis), the second application was made with a combination of pyraclostrobin + epoxiconazole (0.6 L.ha^{-1}) + propiconazole (0.3 L.ha^{-1}) + Assist ® (0.25 L.ha^{-1}) + Tensor Plus® adjuvant (0.15 L.ha^{-1}). In both applications, only the control not received fungicide. The following treatments (spray nozzles) were used: T1. XR 11001, T2. Cone JA-2, T3. DB 110 1.5, T4. DGTJ60 110015, T5. TJ60 11002, T6. AD 11002 T, T7. Teejet Duo TT 11001, T8. AI 3070 110015, T9. Control. These being combined with different pressure values (2.5 to 3.0 bar) application rate (150 to 200 L.ha^{-1}) and speed (3.2 to 4.5 km.h^{-1}). The use of AD 11002 T and Teejet TT Duo 11001 nozzles provide greater Fusarium Head Blight control because they reduce the number of infected spikes and spikelets by scab when compared to control and other treatments, as they help in reducing the incidence and severity of the disease, providing a greater yield.

Keywords: chemical control; fungicides; *Fusarium graminearum*

EFFICACY OF FUNGICIDES FOR FHB CONTROL AND REDUCTION OF DEOXYNIVALENOL IN WHEAT

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Fusarium head blight (FHB) is caused by *Gibberella zeae* (anamorph *Fusarium graminearum*) and occurs in the main wheat producing areas. The disease affects ears, causing reduction in grain weight and contamination with mycotoxins. One of the main mycotoxins is deoxynivalenol (DON), a trichothecen. DON is popularly called "vomitoxin", for inducing vomiting and rejection of food in several species. Due to the harmful effects to humans and animals, maximum limits were established in food, by national and international agencies. In this scenario, it is essential that researches identify products to better control the disease in the field, but mainly, reduce the levels of mycotoxins in grains. To this end, it was evaluated the effectiveness of different active ingredients, applied at flowering, at an experiment in a randomized complete block design with five repetitions, in the winter of 2014, at the experimental area of CCGL Technology in Cruz Alta, RS. Until flowering diseases were controlled uniformly in all treatments. It were evaluated the control of FHB and the fungicides effects on reducing the levels of DON in wheat cultivars TEC 10 and TEC 01/14, respectively moderately susceptible (MS) and moderately resistant (MR) to FHB. For both, seven treatments were tested, beginning from 25% to 50% flowering, varying between one and two applications (performed 10 days after the first application). They are: 1) untreated control during flowering; 2) Metconazole + Pyraclostrobin (1x); 3) Metconazole + Pyraclostrobin (2x); 4) Fluxapyroxad + Pyraclostrobin (1x); 5) Fluxapyroxad + Pyraclostrobin (2x); 6) Epoxiconazole and Pyraclostrobin + carbendazim (1x) and 7) Epoxiconazole and Pyraclostrobin + Tebuconazole (1x). The amount of rainfall in the flowering period favored the occurrence of high levels of DON, since we found 10.8 ppm in the untreated control of MS wheat material and 5.7 ppm in MR. However, treatments with Metconazole + Pyraclostrobin and Epoxiconazole + Pyraclostrobin + Carbendazim, reduced the levels of DON between 48 and 68%, when compared to the untreated control, in both materials. Treatments with Fluxapyroxad + Pyraclostrobin were not effective in reducing DON, reinforcing the hypothesis of the importance of triazole to control the disease. In this study, the best FHB controls provided higher grain yields. In this sense, the MR wheat material had higher yield efficiency and lower levels of DON, in relation to MS wheat. From these results, we inferred that in years with high disease pressure, besides the choice of the most effective active ingredients, the use of varieties with increased resistance are fundamental decisions to control of FHB and reduced levels of DON.

Keywords: chemical control; deoxynivalenol; FHB

P45 *5th ISFHB: Session 4 – Epidemiology and Management*

ASSESSMENT OF FUNGICIDE PERFORMANCE TO CONTROL FUSARIUM HEAD BLIGHT IN WHEAT AND TO REDUCE THE PRODUCTION OF DEOXYNIVALENOL

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Environmental conditions in southern Brazil favor the occurrence of diseases in winter cereals. One of the main is Fusarium head blight (FHB), caused by the fungus *Gibberella zeae*. Research results indicate that two fungicide applications, the first at 25 to 50% flowering, and the second 7 to 10 days after the first, would be ideal to control FHB, added to the earlier applications to control foliar diseases. However, due to high production costs and wheat low prices, most farmers realize a maximum of three applications throughout the crop cycle. In order to test whether this management is sufficient to ensure the FHB control and to lower the DON levels, it was conducted a comparative experiment of fungicides, in 2015 winter season, at the experimental area of CCGL Technology, in Cruz Alta, RS. In the occasion, it were evaluated 13 treatments in wheat cultivar TBIO Sintonia, moderately susceptible to FHB, in a total of three applications, the first in elongation, the second in the boot stage and the third at flowering. The treatments were: 1) Pyraclostrobin + Epoxiconazole; 2) Trifloxystrobin + Prothioconazole; 3) Tebuconazole + Carbendazim + Kresoxim methyl; 4) Azoxystrobin + Cyproconazole; 5) Picoxystrobin + Cyproconazole; 6) Tebuconazole + Azoxystrobin; 7) Tebuconazole + Kresoxim methyl + Carbendazim> Azoxystrobin + Cyproconazole> Prothioconazole + Trifloxystrobin; 8) Azoxystrobin + Cyproconazole > Carbendazim + Tebuconazole + Kresoxim methyl > Trifloxystrobin + Prothioconazole; 9) Azoxystrobin + Cyproconazole> Carbendazim + Tebuconazole + Kresoxim methyl > Metconazole + Pyraclostrobin; 10) Azoxystrobin + Propiconazole + Cyproconazole; 11) Propiconazole > Carbendazim + Tebuconazole + Kresoxim methyl > Metconazole + Pyraclostrobin; 12) Azoxystrobin + Cyproconazole + Mancozeb and 13) Untreated control. In the untreated control, the grain yield was 1856 kg/ha bags/ha. All treatments overcome the yield of treatment control in at least 1835 kg/ha, not differing from each other. In relation to the FHB index, treatments 2, 4 and 6 did not differ from untreated control. The DON levels in the control were 5.6 ppm, while at these treatments, the levels were 4.5 ppm, 5.9 and 4.4, respectively. The most effective treatment in reducing DON levels and that ensure the accordance with levels allowed by ANVISA, with regard to wheat grains for further processing, were those that contemplated Metconazole + Pyraclostrobin and Cyproconazole + Azoxystrobin with Propiconazole reinforcement in flowering, resulting in reduced DON levels more than 50%, compared to the untreated control. The results suggests that the choice of active ingredients to be used in flowering plays an important role in FHB control and in reducing DON levels, even in favorable years to the occurrence of the disease and with only one fungicide application during flowering.

Keywords: FHB control; fungicides

EFFICACY OF FUNGICIDES FOR THE CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT: COOPERATIVE TRIALS RESULTS - 2014 CROP SEASON

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Wheat scab, caused by the fungus *Gibberella zeae*, is one of the most important diseases of this cereal. In favorable weather conditions, the fungus can cause large reductions in yield. Furthermore, there may be mycotoxin accumulation in grain being Deoxynivalenol (DON) the most common and extremely toxic to humans and animals. To control the disease is recommended integrated management, including the use of less susceptible cultivars, crop rotation, choice of appropriate time and place, and fungicide application. To identify the most effective fungicides for control of this disease, a network of experiments with standardized protocol was started in 2011 in different regions of Rio Grande do Sul and Parana. In 2014, experiments were carried out in Cruz Alta-RS, Passo Fundo-RS, Giruá-RS, Agua Santa-RS, Londrina-PR, Guarapuava-PR and Ponta Grossa-PR.-We evaluated the efficacy of fungicides in reducing disease, mycotoxin content and reducing the loss in grain yield. The experimental design was randomized blocks, with eight treatments with different fungicides (T1 to T8) and 4 repetitions. Analyzing all the experiments, the best values for grain yield were obtained by T3 - trifloxystrobin + prothioconazole (two locations), T5 - propiconazole (three locations) and T8 - pyraclostrobin + metconazole (1 site). The lowest values in incidence/disease-severity were obtained in T3 (3 places), T5 (3 locations), T8 (3 locations). The lowest values in accumulation of DON were obtained in T6 – propiconazole + carbendazim (3 places) and T8 (2 locations). Other product reviews did not get highlights. We conclude that, although the results have been varied according to the location of the experiment, the active ingredients trifloxystrobin + prothioconazole, pyraclostrobin + metconazole, propiconazole and propiconazole + carbendazim, were most often highlighted in control of FHB, in reducing accumulation of DON and reducing loss caused by the disease in grain yield in wheat.

Keywords: wheat; scab; fungicides; control

FUNGICIDE EFFICACY FOR MANAGING FUSARIUM HEAD BLIGHT OF WHEAT IN BRAZIL: SYSTEMATIC REVIEW AND META-ANALYSIS

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Fungicides are commonly applied for managing Fusarium head blight (FHB) with the aim of protecting yield and improving grain quality, but especially targeting mycotoxin reduction. Several active ingredients are available in Brazil and the most common belong to the demethylation-inhibitor (DMI) and benzimidazoles (MBC) groups, with the former commonly used in mixture with fungicides of the quinone-outside inhibitor (QoI) group. Given the increasing importance of FHB epidemics in Brazil, the use of fungicides is increasing considerably and usually two sprays are recommended. However, results on fungicide efficacy have been inconsistent and variable for a same fungicide. Quantitative summary of fungicide data available in the country is lacking and could be useful to point to the most efficacious products and scenarios where efficacy can be improved. The objectives of this work were to a) systematically review the peer- and non-peer reviewed literature on chemical control studies for FHB management conducted in Brazil during the last 15 years and b) conduct a meta-analysis of the control efficacy and yield return of selected fungicides. A search in the literature identified 19 studies that reported FHB severity (same as FHB index) for least one fungicide treatment compared to a non-treated check, and also a measure of sampling variance in the trial. Only three and two studies reported mean values of Fusarium-damaged kernels (FDK) and deoxyninvalenol (DON), respectively. Studies were scrutinized and eight fungicide treatments with at least 10 entries were selected for the meta-analysis study. They included DMI (tebuconazole, metconazole and propiconazole) and MBC (carbendazim) fungicide treatments formulated solo and a pre-mix of DMI+QoI (azoxystrobin + tebuconazole, pyraclostrobin + metconazole, trifloxystrobin + prothioconazole and trifloxystrobin + tebuconazole). For most of these fungicides, two applications were tested, the first at full flowering and the second 10 days later. Hence, entries with one application were excluded, and a total of 171 entries, including the check, were used in the meta-analysis. A multivariate meta-analytic model was fitted to the log of the means for each fungicide and check treatment of the trial. The difference in the mean estimates of the log of severity between treatment and the check were used to calculate the log of the response ratio, and then the mean percentage control efficacy ($\bar{C}$) and percentage yield return ($\bar{Y}$). Mean C and Y ranged from 48.9 to 70.2% and from 6.8 to 14.9%, respectively. Metconazole performed best to reduce disease severity ($\bar{C}$ = 70.2%) and increase yield $\bar{Y}$= 14.9%). Propiconazole was the least efficacious among the DMIs ($\bar{C}$ = 48.9%), followed by carbendazim ($\bar{C}$ = 53.8%). In general, the most efficacious fungicides resulted in higher yield return. When fungicide groups were compared, the DMIs and the mixtures performed best in general, especially in trials conducted under high disease pressure (>7% in the check treatment). Results showed that mean control efficacy in Brazilian studies when using two sprays are higher than one spray, as reported in other meta-analysis study in North America. If different fungicides are to be applied sequentialy, the most efficacious should be chosen at full flowering, avoiding propiconazole and carbendazim. Future studies should focus on the effect on DON levels, especially for the mixtures including QoIs, which are strategically used for the control of foliar diseases.

Keywords: *Gibberella zeae*; chemical control; fungicide

SENSITIVITY TO TEBUCONAZOLE AND CARBENDAZIM FOR A A *Fusarium graminearum* SPECIES COMPLEX POPULATION CAUSING FUSARIUM HEAD BLIGHT OF WHEAT IN SOUTHERN PARANÁ, BRAZIL

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Fungicides are the main tool available for Brazilian farmers to reduce the risk of damage and mycotoxin contamination in wheat by Fusarium head blight (FHB) epidemics. Experimental trials where fungicides have been applied for FHB control were monitored in 16 municipalities at the Guarapuava region, Southern Paraná, during four years (2011 to 2014). A total of 227 isolates resembling those of the *Fusarium graminearum* species complex were obtained from wheat heads with FHB symptoms. Of those, 173 isolates were assigned to trichothecene genotypes based on PCR assays. A random sample of 35 isolates from 2011 to 2013 was selected to determine the concentration of tebuconazole and carbendazim fungicides that reduces 50% of the mycelial growth (EC_{50}). The following doses were used: 0, 0.5, 1.0, 2.0 and 4.0 µg/ml. In the PCR assays, most isolates (67%) were of the 15-(A)cetyl-deoxynivalenol (DON), 29% of the nivalenol (NIV) and 4% of the 3-ADON genotype. The estimated means of the EC_{50}, irrespectively of the genotype, were 0.39 µg/ml (0.0004 to 3.0) and 1.25 µg/ml (0.91 to 2.65) for tebuconazole and carbendazim, respectively. The mean EC_{50} were 1.32 µg/ml and 1.21 µg/ml for carbendazim and 0.58 µg/ml and 0.05 µg/ml for tebuconazole, for isolates of the 15-ADON and NIV + 3-ADON isolates, respectively. A Kolmogorov-Smirnov non-parametric test suggested that the sensitivity to tebuconazole of the 15-ADON and NIV+3-ADON isolates were sampled from populations with different distributions ($P = 0.002$). Discriminatory doses of the 1.4 µg/ml and 2.0 µg/ml were used to screen for less-sensitive isolates in the whole sample. Based on those doses, two tebuconazole-less sensitive and six carbendazim-less sensitive isolates were found, all were originated from Guarapuava municipality across three growing seasons and possessed the 15-ADON genotype. There was as significant correlation between the EC_{50} values of tebuconazole and carbendazim ($r = 0.45$; $P = 0,007$). One isolate exhibited the highest EC_{50} values for both fungicides and showed a mutation (R31K) in the *cyp51A* gene. The presence of less sensitive and a resistant isolate reported for the first time in Brazil suggest that selection may drive changes in the populations with impact in disease management. Future studies should focus on the attributes of fitness, competitiveness and *in vivo* assays to check whether fungicide efficacy is hindered in the presence of resistant isolates.

Keywords: *Triticum aestivum*; *Gibberella zeae*; fungicide

INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT IN URUGUAY

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Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, represents one of the main constraints for wheat production in Uruguay. With the aim of optimizing FHB control measures for Uruguayan cropping systems, field experiments evaluating cultivar resistance, fungicide strategies and their interaction were conducted during 2012-2015. Commercial cultivars and advanced lines were characterized under intermediate to high disease pressure in field trials and inoculated nurseries. Although a few commonly grown cultivars had acceptable levels of resistance, the area planted to most of these shifted from 10% in 2012 to more than 20% in 2015. Metconazole + epoxiconazole, prothioconazole + tebuconazole and an experimental product were the most effective fungicides in controlling FHB, by reducing FHB index (FHBI), *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) content. Fungicide efficacy in reducing FHB and DON and in increasing grain yield and test weight was greater if used in moderately resistant cultivars (INIA Genesis 6.87 or INIA G2375) than in a susceptible cultivar (INIA Don Alberto). Results from this study highlight the importance of managing FHB by the integration of cultivar resistance and timely applications of recommended fungicides.

Keywords: resistant cultivars; fungicides; integrated management

METHODOLOGY TO DETERMINE THE NUMBER OF INFECTION POINTS OF *Gibberella zeae* IN WHEAT SPIKES

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Fusarium head blight (FHB) caused by *Gibberella zeae* (*Fusarium graminearum*) affects wheat spikes and grains. The evaluation of FHB in spikes is generally performed by incidence and severity. The objective of this work is to describe a methodology to determine the number of infection points of *G. zeae* in wheat spikes in experiments at field conditions. To access the number of infection points in each spike it has to be considered that wheat spikelets are arranged opposite ways and alternately to each other, on the rachis, being the odd ones at one side of the spikes and the even ones at the other side. After identifying the spike with one affected spikelet, the number of spikelets is counted, starting from the base of the spike to find the numeric position of affected spikelets. This should be repeated each two or three days, until soft dough grain stage, repeating the FHB assessment in the spike to identify new spikelets with symptoms, observing the numerical position location. The first point of pathogen infection is determined by the lowest number, which identifies a spikelet with symptoms. The second one is considered when the next FHB spikelet occurred interleaved at least one soundly spikelet in one side and the other points and so on. This methodology was applied in 90 wheat spikes and was identified up to three distinct points of infection.

Keywords: Fusarium head blight; evaluation; scab

SUSCEPTIBILITY STAGES OF BARLEY TO FUSARIUM HEAD BLIGHT

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Fusarium head blight, caused by *Gibberella zeae* (*Fusarium graminearum*) is an economical damaging disease in barley spikes. The objective of this study was to determine the susceptibility stages to infection by the pathogen. The experiment was conducted in greenhouse, with BRS Marciana cultivar, in a complete randomized design with eight treatments and three replications of ten spikes each. The treatments were: 1- spikes completely enclosed by the flag leaf sheath (SC); 2- five days after SC; 3- ten days after SC; 4- 14 days after SC; 5- 20 days after SC; 6- 24 days after SC; 7- 27 days after SC; and 8- 31 days after SC. The inoculation was done with a suspension of *F. graminearum* at $5x10^4$ conidia mL^{-1}. Plants were then kept in mist for 72 hours at 20 ± 4 ° C. Spikes were harvested 15 days after inoculation, dried and hand threshed. Grain infection by the pathogen was done in a PDA culture medium, after sterilization, in a complete randomized design with three replications of 100 seeds each. Scoring was performed five days after the incubation period. The incidence of FHB on the spike was 100% in all, but treatment 1, where the disease did not evolved. Symptoms in the flag sheath started in treatment 2 when the spikes had not emerged completely. The incidence (%) *of F. graminearum* in the grains of treatment 1 (2.67) differed from the others at 1%, by Tukey test. The treatments 2 (59.33) and 3 (66.33) were statistically equal to each other. The treatments 4, 5, 6, 7 and 8 were statistically equal, with higher incidence of infection in grains. Under favorable environment FHB affects barley from the exposure of the spikes and the sheath colonization can occur even when the spike has not emerged completely.

Keywords: scab; infection; *Fusarium graminearum*

INFLUENCE OF SOWING TIME ON THE FUSARIUM HEAD BLIGHT IN TRITICALE

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Fusarium head blight (FHB), caused by *Gibberella zeae* (*Fusarium graminearum*), affects triticale spikes, reducing the production and the quality of grains and by-products. The objective of this study was to evaluate the influence of sowing dates on the intensity of the FHB. The experiment was conducted in the field at Wheat Embrapa, in Passo Fundo, in 2013, with four genotypes (BRS 203, BRS Harmonia, BRS Minotauro and PFT 1212) in four sowing dates (11th June, 03rd, 11th and 17th July). The plots were constituted of three rows of 3.00 m spaced by 0.20 m. The disease control was carried out until the booting stage. Thirty spikes of each cultivar in pre-flowering stage were inoculated in the central spikelet by injection of 0.025 mL of *F. graminearum* suspension at concentration of $5x10^4$ conidia mL^{-1} with syringe DosysTM Classic 173. The real severity was assessed every two or three days by counting the number of affected spikelet, until the stage of grain soft dough. Means were compared by Tukey test at 1% probability with the statistical program Bioestat 5.0. In the first sowing, BRS 203, BRS Minotauro and PFT 1212 were statistically equal showing the highest severity values. In the second sowing there was no statistical difference between the genotypes while the third, BRS Minotauro was statistically different from the others, with the greatest severity. In the fourth sowing, BRS 203 and PFT 1212 were statistically equal with the lowest severity and BRS Harmony and BRS Minotauro did not differ statistically, with the greatest severity of FHB. The interaction of intensity was not obtained just in the second sowing. The maximum values of average severity obtained, for each genotype, in this work support to consider them as susceptible.

Keywords: *Gibberella zeae*; *Fusarium graminearum*; management

PROGRESS OF FUSARIUM HEAD BLIGHT IN THE WHEAT CULTIVARS BRS GUAMIRIM AND FRONTANA

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Fusarium Head Blight (FHB), caused by *Gibberella zeae* (*Fusarium graminearum*), affects wheat spikes, reduces yield, quality and compromises human and animal health due to mycotoxins and the control is a world challenge. FHB resistance was divided into five types: (i) resistance to initial infection; (ii) resistance to colonization; (iii) resistance expressed in the grain; (iv) resistance of mycotoxins; (v) tolerance. The objective of this study was to evaluate the disease progress, occurring naturally in field conditions of wheat cultivars with different types of resistances. The experiment was conducted on the base of a randomized complete block design with 10 wheat cultivars and three replications, cultivated in a 1.4 m x 6 space, with seven rows and 0.20 space between them. All cultivars were sown on 16 July 2008 and the progress of FHB was measured for two cultivars with different types of resistance: BRS Guamirim (type II) and Frontana (type I). The diseases control occurred until booting stage. Wheat grains with mature perithecia of *Gibberella zeae* was spread among replicates, on the soil surface, at the beginning of silking. Fifteen spikes with only one spikelet with FHB symptoms were identified, on the same day, totaling 45 spikes per cultivar and the number of spikelets, per spike, was quantified. Six assessment of the number of affected spikelets occurred periodically until the stage of grain soft dough. The weather data was obtained from Embrapa Trigo meteorological station (Lat: 28° 15' 46" S, Lon: 52° 24' 24" W), the average rainfall and the daily precipitation was used to determine the number of favorable periods for FHB. Regarding FHB spike progress, the adjusted mixed model showed that the intercept coefficient for the initial stages was lower in Frontana (0.096) than in BRS Guamirim (0.257). The inclination coefficient was lower in BRS Guamirim (1.80) and higher in Frontana (2.77) what means that the progress is higher in Fontana than in BRS Guamirim and increased over time. The average severity was 31.76% in BRS Guamirim and 81.87% in Frontana. The environment of rainfall during FHB progression assessment from October to November was favorable to FHB, in 2008. In October, there were 16 days with precipitation > 0.5 mm and the total rainfall was 351.7 mm, exceeding the normal average that is 167.1 mm. Different types of genetic resistance among cultivars were, probably, the main responsible factor for the double severity in Frontana.

Keywords: *Gibberella zeae*; scab; resistance

EFFECTS OF SOWING DATES AND WHEAT GENETIC RESISTANCE IN FHB CONTROL

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Fusarium head blight (FHB) caused by *Gibberella zeae* (*Fusarium graminearum*) affects wheat cultivation in southern Brazil. The objective of this work was to evaluate the effects of sowing dates and the degree of genetic resistance in controling FHB. The experiment was conducted at the experimental field of Embrapa Trigo, in Passo Fundo, Rio Grande do Sul state in 2008. Ten wheat cultivars with different degrees of genetic resistance to FHB were sown at three different seasons (17th June, 2nd and 16th July). The experiment was performed in randomized blocks, in split-plot design with three replications with sowing dates (3) in main plots and cultivars (10) in subplots that measured 1.4 m x 6.0 m, with seven rows and 0.20 m space between them. The disease control occurred until booting stage. Wheat grains with mature perithecia of *G. zeae* were spread among replicates, on the soil surface, at the beginning of silking. One hundred spikes were sampled in soft dough grain stage and the severity was assessed using a visual scale (Stack & McMullen, 1995). FHB index (ID) was calculated by ID=(I*S)/100. The grain crop was done mechanically closing the air entry. The percentage of troublesome grains (TG) was obtained visually in 1,000 grains. The dates were analyzed by Scott Knott test. For ID in the first sowing, BRS Camboim (8,36), BRS Guamirim (5,89) and BRS Umbu (5,00) were grouped with lower values. In the second sowing, BRS 177 (14.17), BRS Camboim (14.39), BRS Guamirim (9.90) and BRS Umbu (12.77) belonged to the smaller ID group. Only BRS Guamirim (4.61) and BRS Tarumã; (2.89) in the third sowing, were part of group with the lower values of ID. For TG in the first sowing, BRS Umbu (3.60%) was statistically different from the others, with the lowest percentage followed by BRS Guamirim (6.70%). In the second sowing, BRS 177 (4.63%) and BRS Umbu (6.37%) belonged to the group of smaller TG. In the third seeding, only two groups were separate, statistically, and BRS Camboim (7.00%), BRS Guabiju (7.57%), BRS Louro (7.87%) and BRS Timbaúva (9.07%) were in the highest TG. Intensity of FHB varied with the sowing date and resistance of cultivar.

Keywords: scab; managment

FUSARIUM HEAD BLIGHT IN ARGENTINA: AN EPIDEMIOLOGICAL STUDY

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Fusarium Head Blight (FHB) is one of the most important diseases of wheat in Argentina. In the country, the disease is caused principally by *Fusarium graminearum* (teleomorph *Gibberella zeae*). The management of FHB is hindered by the capacity of *G. zeae* to survive saprophitically; by the prevalence of the inoculum and its dispersal to long distances; by the ample range of alternative hosts; and by the weather conditions that induce disease. As FHB is a monocyclic disease, the quantity of primary inoculum available at the time of anthesis is related to the occurrence of epidemics. This pathology affects yield and quality of the grain due to losses in the protein content and contamination with mycotoxins produced by the pathogen during the pathogenic process. The most frequent mycotoxins found on FHB-affected grains are deoxynivalenol (DON), nivalenol (NIV) and zearalenone, and they affect both human and animal health. Usually *F. graminearum* produces one of three chemotypes (15-ADON, 3-ADON or NIV chemotype). Genetic variation and variability in aggressiveness were observed in populations of *F. graminearum* and trichothecenes have been proposed to act as aggressiveness factors. To study the impact of different sources of inoculum on FHB, *F. graminearum* isolates were recovered from weeds and debris of several crops and identified by conventional mycology and PCR. Aggressiveness of a group of these isolates on wheat was evaluated by point inoculation under field conditions. *In vivo* capacity to produce mycotoxins (evaluated by ELISA); and genetic variability (using RFLP markers) were also analyzed. Additionally, pathogen dynamic on cereal and soybean residues naturally infected was evaluated through conventional methodologies and real-time PCR, by pre-optimizing a protocol for DNA extraction from crop residues. Fifty four weed species were identified as alternative hosts of the pathogen for the first time. Furthermore, inoculum presence along to the year and its viability were confirmed and quantified in these species. *Fusarium graminearum* inoculum was present in cereals and soybean residues by at least 18 and 14 postharvest months, respectively. All isolates induced FHB symptoms on inoculated spikes in pathogenicity tests. Even though differences in aggressiveness were found, no significant effect of the source of the isolate was identified. Genetic variability on *F. graminearum* DNA fragments of 2427 bp, showed that there were scarce genetic differences between isolates. Furthermore, no evident relationship between the RFLP profile and the source and/or aggressiveness was found. A positive correlation between symptom severity and *in vivo* DON production was observed. The information provided in this work could prove valuable to the understanding of the epidemiology of *F. graminearum* and for the development of practices aimed at reducing the FHB inoculum.

Keywords: alternative hosts; crop debris; trichothecenes

SILICON REDUCES THE SEVERITY OF FUSARIUM HEAD BLIGHT

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Fusarium head blight (FHB) (*Fusarium graminearum* Schwabe) is one of the major diseases of wheat (*Triticum aestivum* L.) due to reduction in grains yield and its contamination with mycotoxins, such as deoxynivalenol. The objective of this study was to evaluate the calcium silicate, a source of soluble Si, applied in the soil associated with fungicide sprays to the wheat ears in the management of the FHB. The experiments were carried out in 2013 and 2014 under field condition. A 2×2×2 factorial experiment, consisting of cultivars (Fundacep Horizonte and Quartzo), Si supply (without (-Si) or with calcium silicate (+Si)) and fungicide spray [without or with trifloxystrobin (75 g/ha) and prothioconazol (87.5 g/ha)], with four replicates (10 m^2). The incidence and severity of FHB, in percentage, were evaluated on 100 ears per replicate at soft dough stage. For incidence, fungicide spray and cultivars were significant. The FHB incidence was lower up to 21.5% in the cultivar Fundacep Horizonte compared to cultivar Quartzo. The fungicide treatment reduced the FHB incidence up to 29 and 16.3% in 2013 and 2014, respectively. For FHB severity, the factors Si, fungicide and cultivar were significant. Considering the factors Si and cultivar, Si reduced the FHB severity on the cultivar Quartzo up to 55.5 and 30% in 2013 and 2014, respectively. The FHB severity on the cultivar Fundacep Horizonte was lower than that on the cultivar Quartzo up to 50 and 17% for plants -Si and +Si, respectively. Considering the factors Si and fungicide, in plants not treated with fungicide, Si reduced the FHB severity up to 45 and 33.7% in 2013 and 2014, respectively. Fungicide treatment reduced FHB severity up to 49 and 36% for plants -Si and +Si respectively. Considering the factors cultivar and fungicide, the FHB severity was lower in 41.7 and 31.8%, in 2013 and 2014, respectively in the cultivar Fundacep Horizonte, compared to the cultivar Quartzo, for plants not treated with fungicide. Fungicide treatment reduced the FHB severity up to 25.3 and 51.4%, for the cultivar Fundacep Horizonte and Quartzo, respectively. Results of this study show the Si supply to wheat plants reduces FHB severity. Although this is an important result indicating that Si may be included in the integrated management of FHB, more studies at biochemical and histological level are necessary to understand the mechanisms by which the Si affect the disease, as well as the effect of Si treatment on the concentration of mycotoxins produced by species of *Fusarium* in wheat grains.

Keywords: *Fusarium graminearum*; *Gibberella zeae*

Bacillus velezensis RC 218 AS A BIOCONTROL AGENT TO REDUCE FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL ACCUMULATION: GENOME SEQUENCING AND SECONDARY METABOLITE CLUSTER PROFILES

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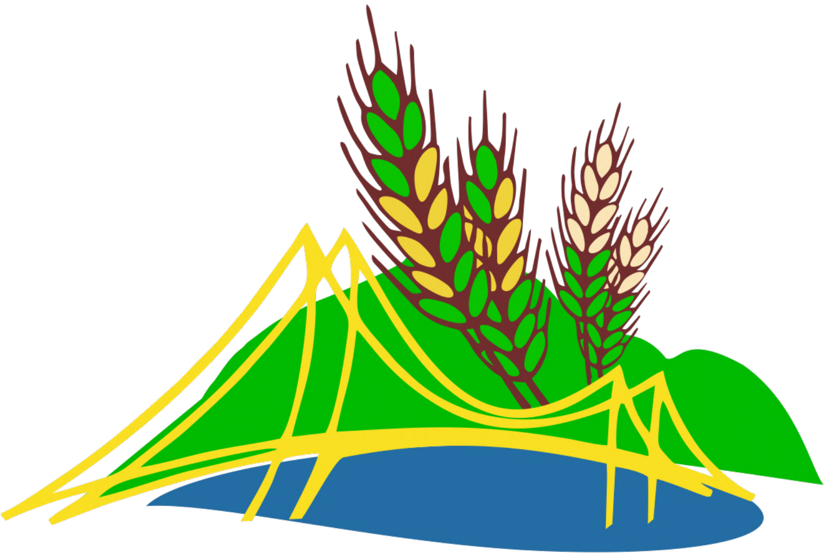
Bacillus subtilis RC 218 was originally isolated from wheat anthers as a potential antagonist of *Fusarium graminearum* sensu stricto, the causal agent of Fusarium head blight (FHB) in Argentina. In previous studies we demonstrated the ability of *B. subtilis* RC 218 to reduce disease severity and DON accumulation *in vitro*, under greenhouse and field trials. The current study extends characterizing *B. Subtilis* RC 218 under a field trial, genome sequencing and secondary metabolite production. The field trial was conducted in Córdoba province, Argentina, during the 2014 harvest season. The wheat cultivar BioInta 1005 (susceptible *to F. graminearum*) was used. Application of the biocontrol agent was done at the anthesis stage with the inoculation of *B. subtilis* RC 218 first and then a mixture of *F. graminearum* strains. FHB disease incidence and severity were evaluated 21 days after inoculation. The genome of *B. subtilis* RC 218 was sequenced using MiSeq DNA sequencer. Genome comparisons and alignments for phylogenetic trees were made using BIGSdb software. Secondary metabolite clusters were identified with antiSMASH3.0 or direct blasting. Culture supernatants containing secondary metabolites were analyzed by LC-MS. The field study demonstrated that *B. velezensis* RC 218 could reduce FHB severity by 26 % and the associated mycotoxin (deoxynivalenol) production to undetectable levels. The genome sequencing allowed us to accurately determine the taxonomy of the strain using a phylogenomic approach, which places it in the *B. velezensis* clade. The genome mining allowed us to identify 9 active secondary metabolites conserved by all *B. velezensis* strains and one additional secondary metabolite, the lantibiotic ericin, which is unique to this strain. This represents the first confirmed production of ericin by a *B. velezensis* strain. The genome also allowed us to do a comparative genomics with its closest relatives and compare the secondary metabolite production of the publically available *B. velezensis* genomes. The biocontrol activity can be related to the ability of the strain to produce several lipopeptides, from the surfactin, fengycin and iturin families since the gene cluster for these compounds were detected. The results showed that the diversity in secondary metabolites of strains in the *B. velezensis* clade is driven by strains making different antibacterials.

Keywords: *Bacillus*; biocontrol; wheat; cluster profiles



5th ISFHB

Session 5

Toxicology and Food Safety

WHEAT INITIATIVE EXPERT WORKING GROUP ON IMPROVING WHEAT QUALITY AND SAFETY

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Wheat demand is expected to increase around 60 % by 2050. To achieve this demand more research is needed with significant investment and collaboration between public and private partners. The wheat Initiative was created in 2011 following endorsement from the G20 Agriculture Ministries. The Wheat Initiative provides a framework to establish strategic research and organisation priorities for wheat research at the international level in both developed and developing countries. The strategic Research Agenda established the key challenges and priorities for wheat research which are organized around four thematic core themes: (1) Increase wheat yield potential, (2) Protect yield potential, (3) Protect the environment and increase the sustainability of wheat production systems, (4) Ensure the supply of high quality, safe wheat and two cross-cutting themes, (5) Enabling technologies and shared resources, (6) Knowledge exchange and education. Expert Working Groups (EWGs) were established with the aims to bring together experts with a focus on a topic of relevance to the Wheat Initiative's aims and objectives. Among these EWGs, one of them is devoted to Wheat Quality and Safety. The expert group will focus on wheat quality and safety in the broad sense including seed proteins, allergens, carbohydrates, nutrition including micronutrients, processing, food safety, genetic resources and gene nomenclature. Mycotoxins in human and animal food supplies have been a recognized safety issue for many years. Wheat (*T. aestivum*/*T. durum*) are susceptible to fungal contamination at different stages of the food and feed chains, pre- and post-harvest. *Fusarium* species within the *Fusarium graminearum* complex have been associated with FHB outbreaks worldwide. The production of trichothecenes and other mycotoxins in wheat are of concern due to the toxic effects of these secondary metabolites to human and animal populations. Advances have been done in the last few years to reduce the impact of the mycotoxins, but more research is still needed to provide the consumers with wheat based products of good quality and safety. The scenario of climate change, changes in cultural practices (non tillage), resistance of pathogens to chemical treatment focus to evaluate new strategies to reduce the impact of mycotoxins in the wheat food chain. The main aspects that the food safety subgroup considered will be discussed.

Keywords: wheat Initiative; food safety

K12 *5th ISFHB: Session 5 – Toxicology and Food safety*

FUSARIUM HEAD BLIGHT IN MALTING AND BREWING: SUCCESSES AND FUTURE CHALLENGES

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Beer, the most widely consumed alcoholic beverage in the world, is generally considered a safe product in terms of the growth of pathogenic microorganisms, However, the presence of mycotoxins has presented maltsters and brewers with challenges, as several can be transferred from the malt or adjunct grain to the final beer. Toxins that are not solubilized during brewing may remain on the spent grains, which are commonly used for livestock feed. The most common and best studied of these mycotoxins has been deoxynivalenol (DON). Severe epidemics of FHB in the main barley producing regions of North America during the 1990's raised alarm within the industry. Early research showed the potential of *Fusarium* species to grow during the malting process and produce additional DON, that DON was quantitatively transferred from malt to beer, and that additional fungal metabolites on barley could cause gushing in packaged beer. The industry responded by self-imposing DON limits on barley and malt. These limits can be viewed as being largely successful, but did contribute to major shifts in malting barley production regions. A 2013 survey analyzed the levels of DON in 374 beer samples from 38 countries. DON was found in 77% of all samples, which initially might seem alarming. As the as mean level in beer was 8.4 ppb, mass balance calculations actually suggest brewers are not accepting malt at levels above either USA guidelines or EU limits for DON for in finished grain products destined for food. However, the North American brewing industry has seen large changes over the past 20 years, with the growth of craft brewing perhaps being foremost. Today there are over 3,500 brewers in the USA, and a recent phenomenon has been the growth of craft malting, who by definition must use locally produced grain. A large number of these craft maltsters are concentrated in regions that are prone to FHB, and there is thus renewed need for education and research. Craft maltsters do not have the diversity of grain supply available to larger operations, and there will thus be more pressure to utilize infected grain in some crops years, In addition, craft maltsters also utilize significant portions of wheat and rye, which are prone to FHB. The USA craft beer, malt and local grain phenomenon is now being mirrored in a number of countries around the world.

Keywords: beer; malt; deoxynivalenol

BREAKDOWN OF MYCOTOXINS IN GRAIN: IS IT A GOOD FORTUNE OR REALLY A MISFORTUNE?

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Masked mycotoxins, plant metabolites of the parent mycotoxins, are an emerging food safety concern. Deoxynivalenol-3-glucosides (D3G), which has been discovered relatively recently is one of the most common forms of masked deoxynivalenol (DON). These mycotoxins are often not detected at grain elevators nor regulated by proper legislation. Moreover, these forms can be hydrolyzed to their precursors in the digestive tracts of animals and can exert toxic effects comparable to their initial mycotoxins. The objective of this study was to analyze the DON and D3G content in Canadian spring wheat cultivars inoculated with different chemotypes of *Fusarium graminearum*. Ten spring wheat cultivars were grown at two locations in Manitoba, Canada and spray inoculated with a mixture of 3-acetyldeoxynivalenol (3ADON) producing isolates and a mixture of 15-acetyldeoxynivalenol (15ADON) producing isolates. Fusarium head blight (FHB) disease incidence and severity of each row was rated 21 days post inoculation. At maturity rows were hand harvested and threshed. Fusarium damaged kernel (FDK) percentage was counted from a 10 g subsample from each row. The same subsample taken for FDK assessment was ground, and used for DON and D3G testing using LC-MS. Analysis of variance demonstrated that total DON and D3G content was significantly different among the ten spring wheat cultivars used. The total DON content was significantly different between the two chemotypes of *F. graminearum* but not the D3G content. These results indicated that the amount of D3G content in the infected wheat kernels was maintained by the resistance mechanisms within the wheat cultivars, not by the chemotypic origin of the *F. graminearum* isolates. Correlation analysis showed a strong positive correlation between DON and D3G among wheat cultivars. The highest DON/D3G ratio was observed in moderately resistant cultivars such as Carberry and CDC Kernen, compared to susceptible cultivars. Taken together, this study suggests that tolerance of *Fusarium* infection in a wheat cultivar is based on the ability of that cultivar to convert DON into D3G during the detoxification process. This is the first study done in Canada to determine the amount of D3G in Canadian spring wheat cultivars after inoculating with different chemotypes of *F. graminearum*. The findings from this study help to understand the occurrence of D3G in commonly grown spring wheat cultivars in Canada. So far, D3G content is not assessed in routine food and feed safety protocols in many countries. Therefore, this study shows the importance of including D3G in food and feed safety assessments in Canada and elsewhere as these masked mycotoxins might be converted back to the parental toxins inside animal and/or human body.

Keywords: trichothecenes; masked mycotoxins; wheat scab; chemotypes

MYCOTOXINS PROFILE IN BRAZILIAN WHEAT

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Deoxynivalenol (DON) and zearalenone (ZEA) are the only mycotoxins regulated by the Brazilian legislation specifically for wheat grain, wheat bran, whole wheat grain flour (WWGF) and refined wheat flour. However, besides *Fusarium graminearum* species complex (FGSC), other fungi may also be present in the environment, which may produce health hazardous metabolites. Therefore, the aim of this study was to evaluate the content of DON and its acetylated forms, 3-acetyldeoxynivalenol plus 15-acetyldeoxynivalenol (ADON), ZEA, as well as the type A trichotecenes [HT-2, T-2 and diacetoxyscirpenol (DAS)], ochratoxin A (OTA), aflatoxins B1, B2, G1 and G2 (AFLA B1, B2, G1 and G2) and fumonisins B1 and B2 (FUMO B1 and B2) in wheat genotypes developed for the humid subtropical conditions in southern Brazil. The experiments were conducted in field plots. The experimental design was factorial, and the factors studied were genotypes (n=19) and growing season (2013 and 2014). The genotypes were artificially inoculated by spraying a conidial suspension of FGSC at mid-anthesis and the plots were appropriately irrigated. Grains were milled into WWGF with a laboratory mill. WWGF mycotoxin content was determined with UPLC-MS/MS. DON, ADON and ZEA contents were above the level of detection, whereas HT-2, T-2, DAS, OTA, AFLA B1, AFLA B2, AFLA G1, AFLA G2, FUMO B1 and FUMO B2 contents were below the level of detection in both years of assessment. Significant differences in *Fusarium* head blight (FHB) incidence and severity, *Fusarium*-damaged kernels, DON, ADON and ZEA contents were observed among wheat genotypes. These results not only increase the knowledge of the genetic resistance of wheat genotypes to FHB and accumulation of DON, ADON and ZEA, but also show that other important mycotoxins were not present.

Keywords: food contamination; mycotoxin survey

022 *5th ISFHB: Session 5 – Toxicology and Food safety*

CONTAMINATION RISK OF DON BY CONSUMPTION OF CRACKER BISCUITS

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The *Fusarium* fungi are pathogenic to crops worldwide, especially wheat, resulting in economic damage to the crop and a threat to the health to consumers due to the possibility of the complex toxigenic potential expression. Trichothecenes are among the toxic compounds produced by *Fusarium* when there are under biotic and abiotic propitious conditions. Among them, deoxynivalenol (DON) is the most studied and regulated for maximum levels in the legislation of many countries. The chemical structure of DON has an epoxide ring quite stable, which provides acute or chronic toxicity and the consolidation of strategies for food safety. In this work was carried out the distribution of DON present in cracker biscuit during the digestive process to better assess the risk of chronic intake. Samples of ground crackers biscuits (-1 (average value surveys). The determination of DON was performed in two main stages of the digestive process, after hydrolysis promoted by pepsin at 37°C (3 h) and pancreatin (24-h). DON was extracted of the solid residue using acetonitrile: water (96:4, v/v) under horizontal stirring being the extract identified and quantified by HPLC-DAD with Gemini C18 analytical column (250 x 4.60 mm ID, 5 µm, 110 Å), isocratic elution with acetonitrile and ultra-pure water, acidified with formic acid to pH 3.5 (70:30 v / v), flow 0.5 mL min^{-1}, detection at 217.9 nm, and run time of 15 min. At the end of hydrolysis with pepsin, 76% of DON (1961 µg kg^{-1}) remained in the bolus (precipitate) and therefore it can estimate that 24% (619 µg-kg^{-1}) of DON (supernatants) present in the biscuits, could be available for absorption in this stage. After the action of pancreatin only 14% (274 µg kg^{-1}) of the remaining DON stayed in the precipitate, estimating thus that 86% (619 µg kg^{-1}) could be absorbed by the body. The lipophilic nature of this mycotoxin suggests that when it is in the digested liquid portion it can be absorbed and trigger its chronic toxicity to the consumer. Therefore, the control of acceptable limits from the knowledge of metabolic processes of mycotoxins is essential.

Keywords: *Fusarium*; toxin; cereal products; digestibility

CURRENT RISK OF *Fusarium* TOXINS OF WHEAT IN CHINA

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In recent years, Fusarium head blight of wheat has been epidemic much more-frequently than before, especially in the middle-lower Yangtse valley， Huai valley and the southern Huan-Huai valley. The disease caused the great loss of yield, and even more the harvest grains which contain *Fusarium* toxins harmed to livestock and human health. *Fusarium* toxins, including Deoxynivalenol (DON, also called vomitoxin), zearalenone (ZEN), nivalenol (NIV) and fumonisin (FB), can cause animal digestive disorder, nervous and immune inhibition, reproductive system abnormalities, even cancer. In this paper, we present the chemotype and classification of mycotoxin-genic *Fusarium graminearum* species complex*,* current situation of the occurrence of *Fusarium* toxins and their influencing factors, mycotoxin contamination risk of wheat in China, which hopefully benefit to the understanding of *Fusarium* toxins and to protect the development of wheat industry in China.

Keywords: *Fusarium* toxins; risk; wheat scab

O24 *5th ISFHB: Session 5 – Toxicology and Food safety*

GENOMIC ANALYSES OF *Fusarium incarnatum-equiseti* SPECIES COMPLEX FROM CEREALS REVEAL GENETIC DIVERSITY OF SECONDARY METABOLISM GENE CLUSTERS

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Toxigenic filamentous fungi constitute a health risk to humans and animals all over the world. Especially, the genus *Fusarium* comprises the most destructive plant-pathogenic species worldwide, involved in many plant diseases that lead to large agricultural and economic damage. Among toxigenic *Fusarium* species, members of *Fusarium incarnatum-equiseti* species complex (FIESC) have been commonly reported as contaminants of cereals, less often associated with major disease epidemics, although regularly identified with other pathogens in field surveys. Moreover, members within FIESC are described as moderately aggressive, even though they are reported in literature as producers of apicidin, beauvericin, butenolide, equisetin, enniatins, both type and type B trichothecenes, and zearalenone. Previous DNA-based phylogenetic analyses showed a high level of cryptic speciation within FIESC, and revealed 31 phylogenetic species belonging to the complex, resolved into two major clades: the Equiseti and the Incarnatum clades. In this study, twelve strains, identified as members of FIESC, were selected for whole genome sequencing and comparative analysis. We have performed the first comprehensive study aimed to illustrate in depth the evolutionary relationships of the phylogenetic species within the FIESC, and identify "in silico" the genetic set potentially involved in the biosynthesis of some selected mycotoxins. Phylogenomic analysis of twelve FIESC strains, based on both the combination of 26 housekeeping genes and the whole genome, inferred a phylogeny that was consistent with but more highly resolved than previously phylogenic analyses inferred from four genes. Comparative analysis revealed a different distribution of secondary metabolism gene clusters among FIESC phylogenic species, which appear unrelated with the evolutionary relationships of species. The observed discontinuous distribution could be explained by genome reorganizations and individual losses, but also by horizontal gene transfer events. However, further investigations are needed to confirm such hypothesis. The result of this work not only gave insight into the evolutionary history of FIESC, but also identified species-specific toxicological profile, providing the basis for implementation of further research to verify the function of secondary metabolite gene clusters, as well as to define prevention programs against mycotoxin contamination of cereals.

Keywords: FIESC; wheat; mycotoxins

DISTRIBUTION OF *Fusarium* MYCOTOXINS IN WHEAT MILLING PROCESS

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Fusarium head blight (FHB) is a fungal disease that affects cereals and is capable of producing mycotoxins of increasing health concerns. In Southern Brazil, FHB of wheat is caused by *Fusarium graminearum* species complex, which produces mainly deoxynivalenol (DON) and zearalenone (ZON) mycotoxins. There is a need for research-based information on how different contamination levels affect these mycotoxins' distribution in the milling process. The objective of this study was to analyze the Fusarium mycotoxin distribution within each milled fraction, extracted from wheat lots artificially contaminated with a crescent gradient of deoxynivalenol (3000 µg kg^{-1}). Wheat samples produced in 2013 season in Southern Brazil region were obtained from plots of Embrapa breeding program. The wheat samples were artificially contaminated with residues of cleaning and pre-cleaning process, including light and shriveled grains. Pilot-scale milled wheat fractions were collected, comprising finished flour and bran. The Fusarium mycotoxin content was determined by chromatography (UHPLC-MS/MS). Ergosterol, a fungal marker was analyzed, to elucidate the relationship between toxin accumulation and fungal dispersion in wheat layers. The distribution of ergosterol in the wheat milled fractions was similar to DON, it presents higher concentration in bran than in finished flour. The mycotoxin concentration in the inner grain layers may be associated with fungal growth within the grain rather than the toxin diffusion. The results obtained show that *DON* increased exponentially relative to the initial levels of mycotoxin in wheat milled fractions (finished flour and bran). DON concentration was significantly higher in bran, when compared with milled wheat and finished flour, with DON levels lower than 1,000 µg kg^{-1}. Therefore, the milling process cannot be solely used as an effective tool for DON reduction in the finished flour, especially in high-contaminated wheat lots.

Keywords: deoxynivalenol; ergosterol; wheat; milling process

EFFECTS OF BAKING PROCESS ON MYCOTOXIN CONTENT IN WHOLE AND WHITE BREADS

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The wheat is the main source of nutrients to the world population and most of its production is converted into flour for human consumption. In Southern Brazil, where 90% of the national wheat is produced, *Fusarium* head blight (FHB), a fungal disease, is a major concern. Apart from yield loss and mycotoxin contamination, *Fusarium graminearum* may reduce wheat milling and baking performance. The objective of this work was to evaluate the effect of baking on the mycotoxin content on white and whole bread, made with artificially contaminated wheat flour, in order to obtain information about wheat by-products' safety. Wheat samples were artificially contaminated with addition of *Fusarium* damaged kernels and a *gradient* was obtained *with* four different levels of deoxynivalenol (DON), ranging from 5000 μg kg^{-1}. The wheat was milled in a Laboratory Mill 3100 Perten, for producing whole bread. A pilot-scale mill Brabender Quadrumat Senior was used to obtain wheat flour for baking white bread. Bread loaves were baked on the straight-dough breadmaking, using a formulation without fat. After fermentation, the dough was baked in an electric oven for 35 min at 180 °C. After cooling, the bread loaves were weighed and the specific volume (SV) of each loaf was measured. *Fusarium's* toxin levels (DON and zearalenone - ZON) were determined by chromatography (UHPLC-MS/MS). The statistical analysis used paired t-test, and the data from four contamination levels were individually considered. After the breadmaking process, a reduction in DON levels was observed, in both whole (55%) and white (65%) breads, when compared with milled wheat and wheat flour. DON levels in whole bread were lower than in milled wheat, from the second level (500 μg kg^{-1}) to the higher level of contamination. Similarly, in white bread, DON content decreased significantly in the two higher levels of contamination, from 2000 to 5000 μg kg^{-1}. ZON levels presented slight increase in final breads when compared with initial levels in flour, but it was not statistically significant. ZON was detected only in the two higher levels in the whole wheat flour (mean 33.1 μg kg^{-1}) and in the two derived whole breads (mean 42.4 μg kg^{-1}). The mycotoxin content affected the specific volume (SV) of bread, increasing in the gradient 2000-5000 μg kg^{-1}, although decreasing in the higher level (>5000 μg kg^{-1}). Both trends were statistically significant. DON levels decreased during the bread processing. So, baking process can be a complementary strategy to reduce the mycotoxin content in wheat by-products, especially for wheat lots with high DON levels (>2000 μg kg^{-1}).

Keywords: Artificial inoculation; deoxynivalenol; breadmaking

ASSESSMENT OF DEOXYNIVALENOL LEVELS IN WHEAT FLOUR BY QuEChERS-HPLC/UV

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The climatic conditions in the wheat-producing regions of Brazil favor proliferation of diseases that significantly affect this crop, such as those caused by *Fusarium* (primarily *Fusarium graminearum*). Besides the direct damage to the crops, infected *Triticum* grains are contaminated with the *Fusarium* mycotoxin deoxynivalenol (DON), which is toxic to both humans and animals. A study was carried out in order to assess deoxynivalenol leves in Brazilian wheat flour samples by applying the QuEChERS method with some modifications (Sospedra et al. 2010), and subsequent extract analysis by using high-performance liquid chromatography with UV detection. The method was validated. The study also assessed the samples compliance with the Brazilian regulations for DON in cereals and cereal-based products. Fifty samples from wheat produced in 2012 in the states of Rio Grande do Sul, Paraná, and Minas Gerais were provided for analysis by the Brazilian Agricultural Research Corporation (EMBRAPA). For DON extraction, 5 g of the sample and 10 mL of a methanol: acetonitrile solution (85:15) were added to centrifuge tube containing 2.3 g of anhydrous MgSO4 and 0.8 g of NaCl, followed by agitation in a vortex for 2.5 min and centrifugation at 4000 rpm for 9 min. For clean-up extract, 3 mL of the supernatant obtained in the extraction phase, 300 mg of anhydrous MgSO4, and 100 mg of PSA were all added to a centrifugation tube, and agitated in a vortex for 1 min, and centrifuged at 4000 rpm for 9 min. Once cleaned, the extract was dried with N2, resuspended in the mobile phase, and filtered through a 0.22 µm membrane before being injected into the HPLC/UV. Six samples from Rio Grande do Sul were contaminated with 143 (3,689 µg·kg^{-1} of DON. Three of these were contaminated with 1,700(3,689 µg kg^{-1} of DON, with an average of 2,753 µg kg^{-1}, thus exceeding the limits stated in the Brazilian (1,750 µg kg^{-1}) regulations. Only one sample from Paraná was contaminated with deoxynivalenol, with a concentration of 877 µg kg^{-1}, thus complying with the Brazilian regulations. None of the 20 samples from Minas Gerais showed any detectable level of DON contamination. Of all the samples, 14% were contaminated with DON between (range: 147(3,688µg kg^{-1}), and 6% showed an average contamination of 2,753 µg kg^{-1}, thus exceeding the limits stated by the Brazilian regulations. As DON is a natural contaminant of wheat, it has a significant impact on human and animal health; therefore, the contamination by this mycotoxin demonstrates the importance of constant monitoring, for minimizing the associated health risks.

Keywords: deoxynivalenol; wheat; quenchers; HPLC/UV; regulations

P61 *5th ISFHB: Session 5 – Toxicology and Food safety*

EMERGING RISK OF T-2 AND HT-2 CONTAMINATION IN DURUM WHEAT COLLECTED IN SOUTHERN-ITALY AT THE HARVEST

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Fusarium Head Blight represents one of the most economically worldwide devastating disease of durum wheat, caused by a complex of species belonging mostly to *Fusarium* genus. Many of these species can produce a wide range of mycotoxins that can be accumulated in wheat kernels at maturity, among which the trichothecenes are the most common. One-hundred-fifteen samples of durum wheat, collected in Italy in 2013, 2014, and 2015 were analysed for the occurrence of *Fusarium* species and the related mycotoxins, trichothecenes and zearalenone. The *Fusarium* strains isolated from the wheat kernels were morphologically and molecularly identified. The analysis of mycotoxins revealed a higher level of contamination in 2014 compared to 2013. Deoxynivalenol (DON) was detected at relevant levels only in the samples collected in Central and Northern Italy, while T-2 and HT-2 toxins and zearalenone occurred at higher levels in samples collected in Southern Italy. Thirty-six out of 44 wheat samples from Southern Italy in 2013 and 2014 (range, 100-335 and 155-486 ppb, respectively) were over the recommended limits suggested by the European Union for the sum of T-2 and HT-2 toxins in the wheat kernels. The most occurring species were *Fusarium graminearum sensu stricto* in samples in which DON occurred at high levels, and *F. langsethiae* when T-2 and HT-2 toxins were detected. Mycotoxin contamination occurring in the kernels was reflected in the spectrum of *Fusarium* species isolated and identified. The results of this study show that a real mycotoxin risk related to *Fusarium* mycotoxins does exist along the whole Italy.

Keywords: *Fusarium langsethiae*; trichothecenes

VOLATILE ORGANIC COMPOUNDS PRODUCTION BY TRICHOTHECENE NON-PRODUCING *Fusarium graminearum* MUTANTS

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Fusarium graminearum Schwabe, the main fungal pathogen associated with Fusarium head blight (FHB), contaminates agricultural crops and commodities with mycotoxins, mostly the trichothecenes deoxynivalenol (DON), nivalenol (NIV), and their acetyl-derivatives. It has been demonstrated that DON plays a role in pathogenesis. The first cyclic intermediate in the biosynthetic pathway of trichothecenes is the trichodiene, which synthesis is catalyzed by the enzyme trichodiene synthase, codified by the *TRI5* gene. During trichothecene metabolism several volatile organic compounds (VOC) are produced, which can be detected using the solid phase microextraction (SPME) technique coupled to capillary gas cromatography (CGC) mass selective detection (MS). With the objective of comparing the VOC production profiles of trichothecene producing and non-producing strains of *F. graminearum*, trichothecene non-producing mutants were obtained using a split marker recombination approach and VOC were measured and identified using SPME-CGC-MS. In a DON producing *F. graminearum* strain, *TRI5* was replaced with a marker gene (*hph*, which confers resistance to hygromicin B) by protoplast transformation with two constructs each containing a portion of *hph* and of a ~500 bp region flanking either the upstream or downstream side of the target gene. Replacement of the gene was confirmed by PCR and Real-time qRT-PCR. Furthermore, decreased aggressiveness of the knock-out mutants was verified in a field test on point inoculated wheat spikes which were later checked for DON content using a commercial enzyme-linked immunosorbent assay (ELISA) kit. For VOC analysis, mutants were cultivated on 50 gr of 80% humidity (w/w) rice in 500-ml flasks. VOCs were extracted from the head space of fungal cultures 7 days after inoculation using a polidimethylsiloxane/divinylbenzene (PDMS/DVB) fiber for 30min. CGC-MS analysis was performed using a Hewlett Packard 6890 gas chromatograph coupled to an HP 5975C VL Agilent mass selective detector employing a non-polar HP-5 capillary column. Seventeen *F. graminearum* transformants were obtained of which 76% were confirmed to be *(TRI5* mutants by PCR, qRT-PCR, aggressiveness tests (F = 23.28) and DON production (no detectable amounts of DON were observed). The remaining 24% of the transformants were ectopic mutants that behaved as the wild type strain in all tests. Both the wild type and ectopic strains showed the presence of several VOC in the elution zone of sesquiterpenes. A major peak eluting at ~ 15 min was identified as trichodiene by interpretation of its mass spectral fragmentation. On *(TRI5* mutants, on the other hand, neither trichodiene nor other sesquiterpene compounds were detected. The results obtained showed that *TRI5* was correctly and efficiently replaced by the marker gene in the *F. graminearum* strain tested and that this replacement hindered the production of TRI and other sesquiterpenes by the mutants obtained.

Keywords: gene knock-out; sesquiterpenes; trichodiene; SPME-CGC-MS

KINETIC EVALUATION OF THE ALCOHOLIC FERMENTATION USING *Saccharomyces cerevisiae* IN THE PRESENCE OF NIVALENOL

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Barley is the primary raw material for beer production with frequent occurrence of *Fusarium* toxins. This mycotoxin can cause toxic effects in humans, animals and microorganisms, generating substantial economic losses. During the fermentation process, the presence of mycotoxins can be indicated by the accumulation of molecules produced by the yeast *Saccharomyces cerevisiae*, such as glutathione (GSH) which are involved in the biochemical pathways of detoxification. The objective was to monitor the kinetics of submerged cultures of *S. cerevisiae* in the presence of nivalenol (NIV), and evaluate the production of the molecule GSH during alcoholic fermentation. The cultivation was performed using *S. cerevisiae* inoculum with 6,6% (v.v^{-1}), cultured in Yeast Peptone Dextrose (YPD) in the presence of NIV (0,1 μg.mL^{-1}) (Treatment) at 26°C for 96 h, compared to the group (Control), in the absence of NIV. The parameters kinetics was determined by evaluating cell growth, reducing sugar concentration, GSH concentration, final concentration of NIV, conversion factors $Y_{P/S}$ (substrate to product), $Y_{X/S}$ (substrate to cell), $Y_{P/X}$ (cell to product) and $\mu_{máx}$ (maximum specific velocity of growth). The GSH concentration was also assessed on 20 commercial samples of beer, together with the quantification of NIV when the profile was changed. Higher cell concentration was obtained in 48 h of cultivation (2,4 mg.mL^{-1}) followed by the consumption of 79 and 76% of reducing sugar in the first 24 h, without statistical difference between Treatment and Control. GSH concentration in 96 h indicated a profile change in the presence of NIV, with 60% reduction in concentration, possibly due to the oxidation of this molecule. This data is confirmed by the conversion factor $Y_{P/S}$ and $Y_{P/X}$, where the Control showed 6,87 mg$_{GSH}$.g glucose^{-1} and 139,98 mg$_{GSH}$.g cells^{-1} and the Treatment 2,53 mg$_{GSH}$.g glucose^{-1} and 65,54 mg$_{GSH}$.g cells^{-1}. For $Y_{X/S}$ both experiments showed 0,04 mg$_{cells}$.mg glucose^{-1}. Furthermore, it was observed that $\mu_{máx}$ of the Treatment occurred between 2 and 8 h of the cultive, with 0,2 h^{-1}, different of the Control period (4 and 12 h) and 0,18 h^{-1}. Thus, a presence of the NIV (0,1 μg.mL^{-1}) altered the kinetics of alcoholic fermentation and the system related to cellular stress and contamination of fermented final product. Only one sample of commercial beer showed profile change in GSH (0,003 μg.mL^{-1}) in which NIV was detected at 980 μg.L^{-1}. Therefore, GSH can be an effective indicator of the presence of the *Fusarium* toxin and GSH detection tests may result in low cost and easy execution of chemical analysis.

Keywords: alcoholic fermentation; glutathione; kinetics

ENZYMATIC REDUCTION OF *Fusarium* TOXINS IN MODEL SOLUTION

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Even though chemical and physical processes get good results in *Fusarium* toxins reduction, their application present economic and environmental disadvantages. In order to fill this gap, the use of enzymes in bioreduction has stood out in the food field. Its application aims the change at chemical structure through its stereospecific action, which may lead to the mycotoxins detoxification. In the case of *Fusarium* toxins, Deoxynivalenol (DON), 3-Acetyldeoxynivalenol (3-ADON) and T-2 Toxin, the detoxification occurs when epoxide ring is breaked (between C-12 and C-13) or by the action in hydroxyl groups. Enzymes Peroxidases (PO) catalyze redox reactions of several substrates, mainly those that serve as electron donors having hydrogen peroxide as an oxygen donor. Thus, the objective of this study was to evaluate the *Fusarium* toxins reduction by PO application in model solution. The assays of DON, 3-ADON and T-2 toxin reduction by applying the PO were carried out in optimal conditions previously determined by PO manufacturer (phosphate buffer 0.1 mol L^{-1} pH 5.0 and hidrogen peroxide), the concentrations were fixed at 1 µg mL^{-1} and the PO at 10 µg mL^{-1}. The reaction occurred during 180 min under orbital shaken at 150 rpm, where aliquots (1 mL) of the system were used to *Fusarium* toxins quantification by salting-out assisted liquid-liquid extraction. In the aliquot were added 2.3 mL of acetonitrile (ACN) (extraction solvent) followed by vortexing for 30 s and 3 min in an ultrasonic bath. Then sodium chlorate was added until the medium was saturated. This extraction was repeated twice. After extraction, the ACN phase was concentrated. The quantification was carried in HPLC-UV and the confirmation was carried in HPLC-MS/MS. The methods performance is within the criteria adopted by the European Regulatory Committee for acceptance of analytical methods used for the determination of trichothecenes (Commission Regulation, EC No. 401/2006). The reduction for DON was 44.5% (1.9), 3-ADON was 24.5% (2.6) and T-2 Toxin was 0% (1.0). These results can be explained by PO application, because the oxidative action can break in the epoxide ring, a reduction reaction that leads the formation of a compound with the presence of a double bond in C-13. There are others two possibilities, hydroxyl groups can be reduced to carbonyl groups and hydrogen bond can promove an adsorption process. In both possibilities are associated with the number of hydroxyl groups in each structure (DON has 3 groups, 3-ADON has 2 groups and T-2 Toxin has one). Though the mechanism of PO action in this *Fusarium* toxins is still in evaluation and confirmation process, these results emphasize the PO action in these toxins reduction.

Keywords: peroxidase; reduction; *Fusarium*; model solution

P65 *5th ISFHB: Session 5 – Toxicology and Food safety*

ANTIFUNGAL ACTIVITY OF MICROALGAE EXTRACTS AGAINST 14C1 STRAINS OF *Fusarium graminearum* COMPLEX

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The fungal colony halo measure is a way to track the fungal growth, however determination of chemically structural compounds provides better insight for understanding the mechanism of action of the extract. The ergosterol, a cell membrane component, is a good fungal growth indicator because it can provide correlation with metabolically active fungal biomass. In this experiment it was tested the ability of microalgae phenolic extracts (*Spirulina* sp. and *Nannochloropsis oculata*) in inhibiting the growth of *Fusarium* strains, isolated from barley culture, using ergosterol as an indication efficiency. The phenolic compounds of microalgae were extracted with methanol, clarified and after evaporation the extracts were suspended in water. According to the phenolic acid profile, synthetic mixtures were prepared, containing 50-µg-mL^{-1}, such as the natural extracts. Natural and synthetic extracts, together with fungal mycelium disks (diameter 1 cm) were inoculated in the center of petri plates with potato dextrose agar at 25 °C with a 12 h of photoperiod for 8 days. In the control, was used only the agar. The potential inhibition was determined by measuring the halo of fungal growth and the ergosterol reduction, determined by high performance liquid chromatography with ultraviolet detector. The highest reduction in ergosterol content was observed at the treatment with the extracts from *Spirulina* (88% reduction) and his synthetic extract inhibited 34%. The natural extract of *N. oculata* reduced 71% of ergosterol production, while the synthetic extract reduced 25%. Studies using the antioxidant potential of phenolic compounds showed that they do not act alone, but in synergy, being reinforced by other antioxidants present in the matrix itself. In this way the antioxidant activity of phenolic mixtures is not well represented by their synthetic mixtures or compounds isolated, due to their synergistic action. The same can be applied to antifungal activity of natural extracts in this study, where the antifungal capacity was higher than that one found for the synthetic mixtures, indicating that this synergy is also required for efficient fungal inhibition.

Keywords: nannochloropsis oculata; phenolic compounds; ergosterol

DEVELOPMENT OF AN IMMUNOCHROMATOGRAPHIC STRIP TEST FOR THE RAPID DETECTION OF ZEARALENONE IN WHEAT AND MAIZE

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A colloidal gold immunochromatographic strip (ICS) test was developed for rapid detection of zearalenone (ZEN) in wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) samples. The mAb against ZEN was prepared in our laboratory and labelled with colloidal gold as a probe for the ICS test. The antigen ZEN-OVA and goat anti-mouse IgG were coated onto a (NC) nitrocellulose membrane as test and control lines, respectively. The conditions were optimized and 30 nm colloidal gold nanoparticles were chosen for optimal performance, in terms of both stability and detection limit. Millipore 135 was chosen as the NC membrane for its level of sensitivity. The optimum amount of coated antigen ZEN-OVA was 0.5 mg/mL and the amount of anti-ZEN mAb was 8 μg for 1 ml colloidal gold. The ICS test, which has a detection limit of 15 ng/mL for ZEN, could be completed in 5 min. Analysis of ZEN in wheat and maize samples revealed that data obtained from the ICS test were in a good agreement with liquid chromatography with tandem mass spectrometry (LC-MS/MS) data. This result demonstrated that the ICS test could be used as a qualitative tool to screen on-site for ZEN.

Keywords: zearalenone; ICST; detection

TEMPORAL DYNAMICS, POPULATION CHARACTERIZATION AND MYCOTOXINS ACCUMULATION OF *Fusarium graminearum* IN EASTERN CHINA

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Members of the *Fusarium graminearum* species complex (FGSC) cause Fusarium head blight in small cereal grains all over the world. Trichothecene genotype composition, mycotoxins production, genetic diversity and population structure were analyzed with 190 *Fusarium* strains collected from wheat in Jiangsu province of five years (1976, 1983, 1998, 2006 and 2014). The results showed that 3ADON was consistently the predominant type in this region over 40 years and NIV type emerged since 1998. Long term rotation of wheat and rice, rather than fungicide application, fitness or weather conditions, may be the main cause of this phenomenon. The genetic diversity results from two toxins synthetic gene, *Pks4* and *Tri10*, and variable number of tandem repeat (VNTR) markers revealed the largest variance in the population from 1998 which was also found to be with the greatest production of mycotoxins. Population differentiation analysis indicated that major temporal population comparisons from the same area were not significantly differentiated. Our results showed dominant species could maintain genetic stability for a long time and *Pks4* would be of great help in genetic and population study.

Keywords: temporal dynamics; population structure; mycotoxin; *Fusarium graminearum*

EFFECT OF ENVIRONMENTAL FACTORS ON *Fusarium* POPULATION AND ASSOCIATED TRICHOTHECENES IN WHEAT GRAIN GROWN IN JIANGSU PROVINCE, CHINA

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The present study was performed to identify the prevailing *Fusarium* species and the environmental factors affecting their frequencies and the contamination of grain with the main mycotoxins in Jiangsu province. The precipitation were 184.19mm, 156.44mm, and 245.80mm from 2013 to 2015, and the temperature fluctuated averaging at 10.62±7.15°C in 2013, 10.93±7.19°C in 2014, and 10.55±6.26°C in 2015. The average concentrations of DON were 879.32±1127.79, 627.84±640.50, and 1628.61±2168.03μg/kg from 2013 to 2015, respectively. The average concentrations of 3ADON were 43.50±58.98, 71.16±102.52, and 33.52±111.92μg/kg during 2013-2015. We found that the average concentration of DON in wheat was positively correlated to precipitation ($r=0.998$, $p<0.01$) and 3ADON was negatively correlated to precipitation ($r=-0.887$, $p<0.05$). However, there was no correlation between precipitation and 15ADON. The differences for temperatures were not as great as the differences in rainfall amount for a short time period. Therefore, there were no correlations between temperature and the concentration of trichothecenes except 3ADON ($r=0.996$, $p<0.01$). Besides, our data indicated that *F. asiaticum* was the primary pathogenic fungus prevalent in the FHB disease nursery. The trichothecene chemotype composition was shown to be different between *F. graminearum* s. str. and *F. asiaticum* isolates. 3ADON chemotype was found only among the strains of *F. asiaticum*. NIV chemotype was not observed among the strains of *F. graminearum,* while the 15ADON chemotype represented 100% of the *F. graminearum* strains collected. It was apparent from this study that there were no correlations between environmental conditions and the species and genetic chemotype composition of the pathogens causing FHB. These results suggested that a pathogen population from a small, isolated disease nursery may keep stable for some period of time in Jiangsu province.

Keywords: environmental; *Fusarium*; trichothecenes; wheat

TRICHOTHECENE GENOTYPE AND CHEMOTYPE AMONG *Fusarium graminearum* SPECIES COMPLEX ISOLATED IN ARGENTINA DURING TWO WHEAT HARVEST SEASONS

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Members of the *Fusarium graminearum* species complex (FGSC) are the causal agents of Fusarium Head Blight (FHB) in wheat worldwide. The disease causes reductions in grain yield, quality and safety due to the contamination with trichothecenes, mainly deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV); and zearalenone (ZEA). Determination of chemotype is relevant in the analysis of *Fusarium* species populations that causes FHB. Shifts in trichothecene chemotypes have been observed in Europe, China and Canada, this aspect is of concern to strains aggressiveness and toxin production. In Argentina, 17 FHB outbreaks had occurred in the last 50 years with loses ranging from 30% to 70%. The aim of the present study was to characterize trichothecene genotype and chemotype of two populations of *F. graminearum* collected from wheat grains in Argentina during an epidemic and a non-epidemic year. A total of 229 *F. graminearum* isolates were collected from the wheat growing Zone II North during 2012 and 2014 harvest season. PCR assays were carried out to predict DON, 15-ADON, 3-ADON and NIV genotype. Chemotypes were determined by LC-MS/MS. From the total isolates evaluated in 2012 harvest season, 110 isolates belong to 15-ADON genotype and 3 isolates were NIV genotype. Among the isolates from 2014 harvest season, 103 belong to 15-ADON genotype and 4 were NIV genotype. The isolates collected from 2012 harvest season were all DON producers (mean 8,63 mg/kg), 84 were 15-ADON producers (mean 2,17 mg/kg), 22 were 3-ADON producers (mean 0,47 mg/kg), 1 isolates was NIV producer (0,1 mg/kg) and 116 isolates were ZEA producers (mean of 2,11 mg/kg). All the isolates collected from 2014 harvest season were DON producers (mean 9,77 mg/kg), 65 were 15-ADON producers (mean 0,85 mg/kg), 47 were 3-ADON producers (mean 2,83 mg/kg) and 2 were NIV producers (mean 0,10 mg/kg) and 114 isolates were ZEA producers (mean 15,3 mg/kg. The chemotype profiles observed in this study; showed the potential of the species within the FGSC to produce a wide range of Type B trichothecenes and high levels of zearalenone. It is important to indicate the difference in chemotype between the isolates from the two harvest seasons evaluated. In the actual condition of climate change it is relevant the evaluation of the toxin ability of the fungal populations for the implication on food safety.

Keywords: *Fusarium graminearum*; wheat; chemotype; genotype

Fusarium FUNGI AND MYCOTOXINS OF CULTIVATED FORAGE CROPS

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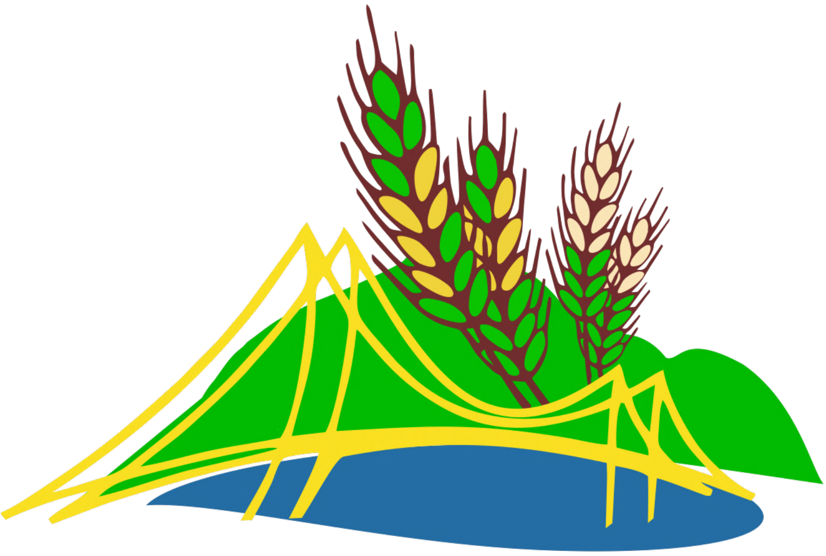
The forage crops constitute the major feed component in the diet of domestic animals, making them the basis for the agricultural and rural economies. The growth of commonly occurring filamentous fungi in forages may result in the production of mycotoxins, which can cause a variety of negative implications on animal health and welfare. The present study aimed at a combined study of the *Fusarium* occurrence and mycotoxin content in the types of grass- and legume-based forages. The plant material (44 samples) was collected from the fields of stock-farms located in five districts of north-western part of Russia. Collections included three forage types: grasses (type I), mixture of clover and grasses (type II), and alfalfa mixed with timothy (type III). The method of dilution plating was applied to count the number of *Fusarium* fungi in the samples of forage. The occurrence of deoxynivalenol (DON), diacetoxyscirpenol (DAS), T-2 toxin (T-2), zearalenone (ZEN), and fumonisins (FUM) was performed by indirect ELISA. Mixtures of clover and grasses found to be the most intensively contaminated by fungi (34000-552000 CFU/g), including *Fusarium* 1.7-4.6% of the total amounts. The intensity of *Fusarium* contamination was less in grasses (1-2% from 43000-80000 of CFU/g) and in alfalfa-timothy mixtures (2-3% from 17000-37000 of CFU/g) than in clover-grass mixtures. The greatest diversity of *Fusarium* species composition was also established in forages type II (*F. avenaceum*, *F. anguioides*, *F. sporotrichioides, F. tricinctum, F. semitectum, F. culmorum*, *F. equiseti*, *F. proliferatum* and *F. poae*). The proportion of samples contaminated with *Fusarium* mycotoxins, in forages type I was the lowest, whereas in legumes mixtures with types II and III. The most of the positive samples in legumes mixtures had combined contamination, consisting of two, three or four mycotoxins together, whereas as single components found only T-2 or FUM. The maximum levels of mycotoxins in all types of forages reached for T-2 (36 μg/kg), DAS (490 μg/kg), DON (160 μg/kg), ZEN (50 μg/kg) and FUM (165 μg/kg). The changes of content and number of microorganisms in infested plants during the growing season, including toxigenic fungi, certainly has an impact on the range and amount of accumulated mycotoxins. A better understanding of the environmental and cropping factors and the interaction between the representatives of plant mycobiota could contribute towards reducing the potential risk of the contaminated feed to the animal health. The investigation was supported by the Russian Science Foundation (No. 14-16-00114).

Keywords: forages; *Fusarium* fungi; mycotoxins



2nd International workshop on wheat blast

Keynotes and Poster Presentations

Session 1 - - Wheat blast biology, genetics and genomics of resistance

Session 2 - Screening of germplasm for blast resistance

Session 3 – Pathogen Population Biology, Host Specialization and Origins

Session 4 – Ecology, epidemiology and management

K13 *2nd IWWB: Session 1 - Wheat blast biology, genetics and genomics of resistance*

WHEAT BLAST: BIOLOGY, GENETICS AND GENOMICS

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Wheat blast, caused by the *Triticum* pathotype (MoT) of *Magnaporthe oryzae* (synonym to *Pyricularia oryzae*) has become a serious constraint to wheat production in Brazil, Bolivia and Paraguay since its identification in 1985. Whole genome analysis confirms that MoT strains are related to, but distinct from strains of *M. oryzae* population causing rice blast disease (the *Oryza* pathotype, MoO). Key fundamental infection mechanisms, such as host surface penetration using a highly pressurized appressorium, appear conserved in blast diseases on rice and wheat, but some differences occur in the field. Unlike rice blast, symptoms on wheat occur mainly on wheat heads with fewer symptoms on leaves. In contrast to the complex race structure in the rice blast fungus, few MoT races have been identified so far, and only a few blast resistance genes have been identified in wheat. We have shown that the 2NS/2AS translocation from *Aegilops ventricosa* confers wheat blast resistance in some genetic backgrounds (Cruz et al, 2016, Crop Science). This wild wheat chromosome segment has already been widely deployed because it carries the Lr37, Yr17 and Sr38 rust resistance genes as well as a nematode resistance gene. Molecular markers are available for its use. Additional wheat resistance genes identified for rust and mildew resistance are being tested for efficacy toward wheat blast. Extensive knowledge gained for rice blast, as well as cloned rice resistance genes, is being leveraged to facilitate wheat blast control. We have shown that some MoT strains contain a functional allele of the *AvrPizt* avirulence gene that is required for triggering *Piz-t* mediated resistance in rice. We are currently testing if the rice *Piz-t* gene confers blast resistance after transformation and expression in wheat.

Keywords: *Pyricularia*; *Triticum*; MoT; host resistance;

OCCURRENCE OF WHEAT BLAST IN BANGLADESH

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Wheat blast caused by *Pyricularia* sp. (teleomorph: *Magnaporthe* sp.) has been recorded in Bangladesh for the first time in 2016. This report is new to Asia and should be taken with utmost attention knowing the significant crop losses sporadically observed in South America characterized with warm and humid conditions. The disease symptoms suddenly appeared in several southwestern and southern districts of the country in late February to early March. The areas affected covered about 15% of Bangladesh's total wheat area in 2015-16 and present a serious threat to the country's wheat production. Initial estimates showed as much as 90% losses in susceptible varieties while potentially resistant varieties had 5-10% losses. After the 2009 outstanding outbreak in Latin America, scientists have underscored a concern about the potential spread of the blast to other wheat producing areas in Bangladesh, South Asia and beyond. Conspicuous symptoms consisted in partial or complete whitening of spikes starting from an apparent dark colored point of infection at the level of the rachis or the base of the infected spikes. A light gray mycelium developing at the point of infection with abundant production of *Pyricularia* sp. conidia was clearly observed in independent observations of samples collected in field surveys in affected districts by the scientists from Wheat Research Centre, Bangladesh Agricultural Research Institute (WRC-BARI) and the International Maize and Wheat Improvement Center (CIMMYT). The fungus attacks the base or other parts of the rachis, causing spikelet sterility above the point of infection, similar to symptoms described in South America. The portion above the infection point does not produce grains whereas the lower part of the spike remains apparently unaffected. Initial field infection occurred in patches and spread rapidly to the whole plot whereas the canopy remains green. With this new epidemic it is necessary to further and fully characterize the fungal isolates to determine the type of *Pyricularia* involved, and to identify the origin of the inoculum. Infected samples have been sent to USA for molecular identification. Spike infection is the most conspicuous symptom of wheat blast in South America with complete or partially bleached spikes occurring within few days. Rice blast caused by *P. oryzae* is a common disease on rice nearly everywhere rice is grown including Bangladesh and neck blast is known to occur. However, the origin of the fungus observed in wheat is not known and pathogenicity test need to be conducted to clarify the host range. Wheat blast is known to be seed transmitted and it is considered that several grasses may serve as alternate hosts. However, the epidemiology and life cycle of the wheat blast pathogen is still largely unknown. In the current cropping season, late-planted wheat was largely affected due to rain occurring during heading stage. Farmers in affected areas have never observed the symptoms and commented on rains followed by warm temperature that coincided with heading and flowering. After initial symptoms, the disease rapidly worsened within two weeks. Disease severity varied with sowing times and cultivar. This outbreak poses a serious threat to wheat production in Bangladesh. It underlines the urgent need for collaborative research, policy support and awareness among farmers and seed producing agencies in the public and private sectors to mitigate the threat of wheat blast.

Keywords: *Pyricularia*; wheat blast; Bangladesh

K15 2nd IWWB: Session 2 - *Screening of germplasm for blast resistance*

RESISTANCE TO HEAD BLAST IN U.S. WHEATS

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The head phase of wheat blast, caused by *Magnaporthe oryzae* (Triticum pathotype) (MoT) can be extremely severe in South America. Although MoT is not known to occur in the U.S., it could arise from native strains of *M. oryzae* or be introduced from South America. Because of that, it is important to prepare for the possible appearance of wheat blast in the U.S. One major knowledge requirement is to quantify the reaction of U.S. wheat cultivars to the head phase. Inoculation protocols were developed and carried out in the U.S. under biosafety-level-3 conditions at Ft. Detrick, Maryland and Kansas State University, Manhattan, Kansas. These protocols were used to phenotype over 500 U.S. wheat cultivars for reaction to head blast. For both spring and winter wheats, there is a continuum of reaction from highly susceptible (>90% spikelet mortality) to highly resistant (<10% spikelet mortality). For winter wheats, at least two physiological races of MoT have been identified. An example of a race differential cultivar is the hard white winter wheat RonL; it is resistant to race 1 but susceptible to race 2. Furthermore, the 2NS translocation from *Aegilops ventricosa* to wheat has been shown to confer high levels of resistance to head blast (races 1 and 2) under artificial inoculation. The translocation also showed 85% reduction in blast in three field experiments over two years in South America. However, the degree of resistance due to 2NS is different depending upon the genetic background that contains the translocation. Therefore, there may be other genes that modify the effect of the translocation. In related experiments, there is preliminary evidence that accessions of *Aegilops tauschii* may contain resistance to MoT. Similarly, efforts are underway to identify non-2NS resistance in other potential sources such as the Nested Association Mapping population, emmer wheats, and synthetic wheats. All of these data are necessary to help prepare for the possible appearance of MoT strains in the U.S. If this occurs, informed recommendations can be made to wheat producers about which cultivars may show useful levels of resistance to this important disease threat.

Keywords: resistance; head blast; U.S. wheats

EFFICIENT BREEDING STRATEGY FOR WHEAT BLAST DISEASE (*Magnaporthe oryzae* Triticum) RESISTANCE IN BOLIVIA - USE OF THE EXPERIENCE ACQUIRED ON RICE BLAST

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Blast is the main wheat disease in Bolivia and to tackle it we are applying a breeding strategy, which has proven its efficacy for obtaining rice varieties with a high level of durable partial resistance to blast. Incomplete information is available on wheat blast resistance, so, firstly we base our strategy on the worst hypotheses. Next, the possible invalidation of every hypothesis can allow simplifying the breeding schema and its implementation. These successive or complementary hypotheses are the following: $H_0$1: The obtaining of a sufficient resistance is impossible; $H_0$2: Only a partial resistance with a low level is available; $H_0$3: The partial resistance is polygenic; $H_0$4: The partial resistance is little heritable; $H_0$5: The partial resistance is partly specific; $H_0$6 and $H_0$7: A complete resistance is obtained and is specific; $H_0$8: The disease resistance penalizes the yield. For every hypothesis, the consequences of its consideration, the study of its validity and the consequences of its possible invalidation are explained. Scientific arguments, references, and materials and methods details are provided. Briefly, these hypotheses lead 1) to the obtaining and to the cheapest multiplication of genetically pure blast-free seeds for blast-free cropping areas, 2) to the search of new resistance genes or new genetic backgrounds for the expression of such genes in core collections and in recurrent populations with a very broad genetic base, and 3) to the implementation of the participatory recurrent selection in populations with a narrow genetic base with the extraction of lines for providing the participatory creation of varieties. Every sub-program, i.e., for obtaining new varieties for a specific set of agro-ecological, socio-economical, industrial and commercial conditions and purposes, needs less than 1.000 m2. This own strategy is focused on the obtaining of adequate resistant varieties as fast as possible in the respect for the Bolivian scientific sovereignty, by a complementary approach of those of the other teams and by an application of the subsidiarity principle.

Keywords: wheat blast; partial resistance; recurrent selection; participatory breeding

THE RECENT EMERGENCE AND EVOLUTION OF THE WHEAT BLAST SPECIES COMPLEX IN BRAZIL

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Wheat blast is caused by *Pyricularia* species closely related to the rice blast fungus *Pyricularia oryzae*. It was first detected in the 1980s in Paraná State, Brazil and has since spread across wheat growing areas in Brazil, Bolivia, Argentina and Paraguay. We conducted a series of studies to determine the phylogenetics and the population genetic structure of the wheat blast pathogen and elucidate its origins. We collected hierarchical samples of the fungus from wheat and other grass species invasive to wheat fields across the known geographical range in Brazil, generating a sample of 553 isolates. These isolates were characterized for sequences of 10 housekeeping genes, 11 SSR loci, mating types, two genes associated with fungicide resistance (*cit b* and *cyp51*), and an avirulence gene (*AvrCO39*). A subset of strains was phenotyped for virulence on a set of wheat differentials and resistance to QoI and azole fungicides. By comparing sequences of 10 housekeeping genes in over 120 isolates, we found that the wheat blast and the rice blast pathogens are closely related, but phylogenetically distinct. We also concluded that blast disease on wheat and other invasive Poaceae hosts from the Brazilian agroecosystem is caused mainly by two *Pyricularia* species, occurring in similar proportions: the newly described *Pyricularia graminis-tritici* sp. nov. (Pgt), and the known *P. oryzae* pathotype *Triticum* (Pot)*.* The distribution of the wheat blast pathogens varied according to a temperature cline with *Pyricularia graminis-tritici* prevalent in warmer Central Brazil and *P. oryzae* pathotype *Triticum* predominant in Southern Brazil. Since its emergence the wheat blast pathogens have evolved rapidly to become resistant to QoI and azole fungicides and specialized to include at least 14 pathotypes. The population genetic structure is consistent with a mixed reproductive system that includes regular recombination and significant gene and genotype flow over spatial scales of 1,000s of km. Ascocarps were abundantly formed in senescent wheat tissues inoculated with both fungal mating types. Isolates of *Pyricularia* from several poaceous hosts such as *Avena sativa*, *Cenchrus echinatus*, *Cynodon* spp, *Digitaria sanguinalis*, *Elionurus candidus*, *Echinochloa crusgalli*, *Eleusine indica*, *Rhynchelytrum repens*, and *Urochloa* spp. were indistinguishable from *P. graminis-tritici* isolated from wheat and populations from the two host groups had the same genetic structure. Because *Urochloa* is a widely grown pasture grass occupying more than 90 million ha in Brazil, we propose that *Urochloa* provides a major source of wheat blast inoculum and may be the preferred host for pathogen recombination.

Keywords: phylogenetics; population structure; gene-flow; reproductive mode

K18 *2nd IWWB: Session 3 – Pathogen Population Biology, Host Specialization and Origins*

WHEAT BLAST AND GRAY LEAF SPOT: DISEASES CAUSED BY A SINGLE, GENETICALLY DIVERSE PATHOGEN SUPER-POPULATION?

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Disease scouting in a University of Kentucky Research plot in late May of 2011 identified a single wheat plant with bleaching symptoms that were different to those of surrounding scab infected plants. Classical and PCR-based diagnostic methods positively identified the disease agent as *Magnaporthe oryzae*. Fortunately, further scouting after the diagnosis failed to identify any additional blast infected plants. The PCR-based markers provided equivocal information about the origin of the suspect strain because, while one marker indicated it was possibly an exotic wheat blast strain (*M. oryzae Triticum* - MoT), another suggested it was most likely an endemic *Lolium*- infecting pathogen (MoL). Therefore, to gain a better insight into the suspect strain's origin we determined genome sequences for a representative single spore culture (WBKY15) and compared this sequence to the genomes of various *M. oryzae* isolates from wheat, perennial ryegrass (*Lolium perenne*) and annual ryegrass (*L. multiflorum*). Analysis of genome-wide, single nucleotide polymorphisms revealed that WBKY15 is most closely related to *M. oryzae* strain PL3-1, which was isolated from annual ryegrass growing in Kentucky in 2002. In contrast, it was quite distantly related to the wheat blast strains. Thus, we conclude that the Kentucky wheat blast incidence was probably caused by an endemic MoL strain that had jumped hosts. Subsequent analysis of genome sequences from additional wheat blast strains and a range of *M. oryzae* host-specific forms revealed that some wheat blast strains group within the MoL clade. This, in turn, suggests that MoT and MoL may belong to a single pathogen superpopulation. Additionally, there was unusually high genetic diversity within the MoT and MoL populations which belies the relatively recent emergence of the wheat blast and gray leaf spot diseases. Possible reasons for this discrepancy will be discussed.

Keywords: *Triticum aestivum*; *Lolium* sp.; genetic diversity

K19 *2nd IWWB: Session 4 – Ecology, epidemiology and management*

WHEAT BLAST: PROGRESS IN ELUCIDATING ITS ECOLOGY AND DEVELOPING STRATEGIES FOR CONTROL

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Wheat blast, caused by the *Triticum* pathotype of *Magnaporthe oryzae* (MoT), is an emerging disease considered to be a limiting factor to wheat production and regarded as very difficult to control. Wheat producers in blast endemic regions of Bolivia, Brazil, and Paraguay are managing the disease with limited information on MoT ecology and epidemiology. Availability of sources of resistance and information on fungicide efficacy for this disease are also limited. Given the status of wheat blast as a high consequence plant disease, studies are being performed to understand the ecological factors associated with blast outbreaks and to develop effective management strategies. The evidence presented in this seminar is derived from experiments conducted under Biosafety Level-3 conditions in the U.S., as well as field conditions in Bolivia and Brazil. Experiments in the U.S. and Bolivia were conducted to elucidate the importance of basal leaves in disease etiology. Inoculations of wheat seedlings showed that certain cultivar and isolate combinations caused more disease on old wheat leaves than young expanding leaves. Under optimum conditions, MoT had the potential to produce tens to hundreds of thousands of conidia on small amounts of wheat basal leaves. A mean of 1,7 million conidia were produced on 1 g dry basal leaves of a highly susceptible cultivar under optimum conditions for sporulation. Conidia production on leaves coincided with spike emergence under both greenhouse and field conditions. Although additional studies are needed to understand the significance of auto-infection in the wheat blast pathosystem, preliminary studies in the field in Bolivia have shown that, in the absence of foliar fungicides, blast might be able to progress from older to youngest leaves as the season progresses. In Bolivia, a prominent breeding program has included phenotyping efforts to select for wheat blast resistance at both the leaf and head stages. According to our results from field research, several foliar fungicides had activity against wheat head blast, but Picoxystrobin + Cyproconazole in Bolivia and Thiophanate-methyl + Mancozeb and Mancozeb in Brazil were most effective at reducing head blast severity and increasing yields. The data reported in these studies should contribute to better management of the wheat blast disease in the future.

Keywords: wheat blast; brusone; ecology; management

SILICON PAVES THE WAY IN SUPPRESSING BLAST DEVELOPMENT ON WHEAT

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Blast, caused by the fungus *Pyricularia oryzae*, is a major disease affecting wheat crop in Brazil. We summarize here the research carried out in the Laboratory of Host-Parasite Interaction at the Department of Plant Pathology of Viçosa Federal University over the past ten years using silicon (Si) as an alternative to control blast. We demonstrated that the incubation period of blast was significantly increased while the area under blast progress curve and the number of lesions per cm^2 of leaf significantly decreased for plants supplied with Si (+Si plants). The concentration of lignin and the activities of chitinases, peroxidases and polyphenoloxidases were higher for +Si plants. The supply of Si contributed to improve gas exchange performance of plants with less dysfunction at the photochemical level. For the inoculated +Si plants, net CO_2 assimilation rate, stomatal conductance to water vapor and transpiration rate were significantly higher in contrast to the inoculated plants not supplied with Si (-Si plants). The maximum quantum quenching as well as total chlorophylls concentration and the chlorophyll *a*/*b* ratio significantly decreased for the -Si plants in comparison to the +Si plants. Significant differences between -Si and +Si inoculated plants occurred for photochemical and non-photochemical quenching coefficients and the electron transport rate. Although the expression of the defense-related genes *Pathogenesis-related 1* (*PR-1*), *β-1,3-glucanases*, *chitinases*, *peroxidases*, *phenylalanine ammonia-lyases* and *lipoxygenases* significantly increased in response to *P. oryzae* infection, such increases were 2- to 3-fold higher for the +Si plants relative to their -Si counterparts. Microscopically, epidermal and collenchyma tissue of rachis of -Si plants was extensively colonized by fungal hyphae. For +Si plants, fungal hyphae colonized the epidermis and the collenchyma cells to a lesser extent and the accumulation of phenolics in the parenchyma constrained fungal colonization. On the leaves of -Si plants, hyphae of *P. oryzae* grew successfully and formed an extensive branched mycelium in the first-invaded epidermal cell and invaded several neighboring cells, while on the +Si plants fungal hyphae was restricted to the first-invaded epidermal cell. Strong fluorescence, which was an indication of the presence of flavonoids, was detected in the leaf cells of +Si plants suggesting that the phenylpropanoid pathway was potentiated contributing, therefore, to an increase on wheat resistance to blast. Even though several aspects of wheat resistance to blast potentiated by Si have been elucidated, more research is needed to better understand the wheat-*P. oryzae* interaction at both physiological and molecular levels.

Keywords: photosynthesis; host resistance

FINE PHENOTYPING UNLOCKS WHEAT MECHANISMS OF REACTION TO *Magnaporthe oryzae*

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Wheat blast is the most recent economically important disease in Brazil. Although the development of symptoms in all aboveground parts of the wheat plant is possible, spike infection causes the greatest losses of grain production. Infected spikes show characteristic bleaching. Despite the intense search for sources of wheat blast resistance in South America, little is known about the genetic of resistance. Since 2009, a Brazilian research initiative has been undertaken to identify and molecularly characterize wheat genotypes with contrasting blast resistance phenotypes. Dozens of resistant genotypes were identified in nurseries of wheat blast in Brazil. We selected twelve of these genotypes for a detailed phenotyping under controlled conditions. The wheat cultivar BRS 209 was used as susceptible control. It is the maternal parent of a doubled haploid population created at Embrapa Wheat for genetic studies of wheat blast resistance. Wheat plants were cultivated in pots until the adult plant stage. Spikes of adult wheat plants were inoculated at the heading stage with the isolate Py0925 at 2×10^5 conidia/mL. Control spikes were misted with solution without conidia (mock inoculation). Wheat spikes were inspected daily. The total number of spikelets per spike was counted. Disease severity (in percentage) was estimated by the ratio of number of spikelets with lesions, or bleached spikelets, in relation to the total number of spikelets over time until the plants attained physiological maturity. For each inoculated spike, the lesion type was also evaluated. After harvest, wheat rachises were searched for the infection point on the rachis (ipr), which corresponds to the penetration site of the pathogen. Previous results of our group identified variability in the number of ipr of wheat varieties. The data were analyzed using analysis of variance (ANOVA) and the genotypes were grouped according to Scott-Knott test at 5% significance. The Pearson's correlation coefficient was applied to evaluate the associations between the variables. There was no significant difference among the genotypes for blast severity, considering the percentage of spikelets with lesions, at five days post-inoculation (dpi). When we analysed bleaching severity at seven dpi, four groups of wheat genotypes were identified. Cultivars BRS 209 (susceptible control) and Shanghai showed the highest bleaching severities at seven dpi and the less total number of ipr. Under our experiment conditions, some genotypes had more than 30 ipr. A significant negative correlation (-0.58) occurred between bleaching severity at seven dpi and the total number of ipr. Scientific literature reports that susceptible genotypes commonly presented many ipr. We intend to conclude grain production evaluation of inoculated and mock-inoculated spikes to analyze the possible association between resistance and tolerance mechanisms of wheat faced with the blast pathogen. Acknowledgements: Thanks to CAPES-Embrapa and CNPq-PIBIC for fellowships supporting graduate and undergraduate research studies of the first and fifth author, respectively.

Keywords: host resistance; tolerance; *Pyricularia oryzae*

P72 *2nd IWWB: Session 2 - Screening of germplasm for blast resistance*

PRECISION FIELD-BASED WHEAT PHENOTYPING FOR THE IDENTIFICATION OF NOVEL SOURCES OF RESISTANCE TO WHEAT BLAST

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The CGIAR (Consultative Group for International Agricultural Research) Research Program on WHEAT envisions a network of Precision field-based Wheat Phenotyping Platforms (PWPP) developed with co-investing National Agricultural Research Systems (NARS). The PWPP for Wheat Blast will act as a hub for generating and sharing data and knowledge in this particular wheat disease. Diverse wheat materials will be phenotyped in South America under defined field screening protocols in partnership with NARS collaborators. The platform will generate high quality phenotyping data, complementing the potential of new molecular selection technologies. Therefore, it is expected an increase in the precision and prediction value of data, contributing to the development of new germplasm from WHEAT and partner breeding pipelines. The resistant germplasm, diagnostic tools, and information on epidemiology and pathogenic virulence from this project will assist plant breeders and pathologists in developing resistant varieties and strategies to manage wheat blast. Training of technical and scientific personnel in different aspects of wheat blast screening will also form an important component of this project. This will enhance the expertise necessary to secure the adequate and reliable screening and phenotyping for this emerging disease. The information generated will ultimately contribute to a faster development of broad genetic based resistant, high yielding wheat varieties.

Keywords: phenotyping; novel resistance; wheat blast

CHLOROPHYLL FLUORESCENCE IMAGING AS A TOOL TO DETERMINE THE EFFECT OF *Pyricularia oryzae* INFECTION ON THE PHOTOSYNTHETIC PERFORMANCE OF WHEAT PLANTS

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Blast, caused by *Pyricularia oryzae*, is considered the most important disease affecting wheat grown in different regions in South America. It is known that the infection process of *P. oryzae* on wheat leaves decrease the net carbon assimilation rate (A) due to biochemical limitations likely related to the reduced activity of RuBisCo coupled to reductions on both stomatal conductance to water vapor (g_s) and transpiration rate (E). This study aimed to determine the photosynthetic performance of wheat plants from a susceptible (Guamirim) and a partially resistant cultivar (BR-18) to *P. oryzae* infection based on the electron transfer rate (ETR) as well as on other chlorophyll fluorescence parameters to photosynthetic photon flux density (PPFD). The fluorescence imaging parameters were determined at 48, 72, 96 and 120 hours after inoculation (hai) using the Imaging-PAM M-Series chlorophyll fluorometer and the software Imaging WIN version 2.32. From 72 to 120 hai, blast severity increased from 7 to 22% for plants from cv. BR 18 and from 10 to 45% for plants from cv. Guamirim. There were drastic reductions on the F_v/F_m values for the infected plants from cv. BR-18 (14%) and cv. Guamirim (22%) in comparison to the not infected plants. The observed patterns in the light-response curves (LCs) indicated that the photochemical dissipation [Y(II)] decreased on the infected leaves of plants from both cultivars, especially at 120 hai. Increases in the values of non-photochemical dissipation [Y(NPQ)] and ETR depended on the light intensity. A the blast severity increased, there were drastic reductions in the response of Y(NPQ) and ETR, especially for plants from cv. Guamirim. Values for the non-regulated dissipation [Y(NO)] were higher for the infected plants in comparison to the not infected ones. For the infected plants from cv. Guamirim, the values for Y(NO) were higher in contrast to the infected plants from cv. BR-18. Impaired photosynthetic capacity of the wheat plants infected with *P. oryzae* was due to the occurrence of photoinhibition and photooxidation processes at the level of the reaction centers. Indeed, the photochemical phase necessary for ATP and NADPH generation to be used for CO_2 fixation was limited. The greater basal level of wheat cultivar resistance to blast resulted in a better photosynthetic response that minimized the negative impact on the processes of light energy dispassion.

Keywords: wheat blast; photosynthesis; photoinhibition; photooxidation; resistance;

WHEAT BLAST EXPRESSION BY NITROGEN FERTILIZATION

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Blast, caused by *Pyricularia grisea*, is one the of most important wheat diseases. Nitrogen (N) is a nutrient furnished to the plants in large amounts. Conditions such as the source of the N and the application timing can influence the increment or decrease of diseases. The objective of this work was to evaluate the effect of nitrogen fertilization in the expression of the wheat blast in the field during the 2015 cropping season. The expression of the wheat blast was compared in two wheat cultivars, BRS Gralha-Azul (susceptible) and BRS Gaivota (moderately resistant). The cultivars received 60 kg/ha of N applied as topdressing utilizing three N sources, [calcium nitrate ($N\text{-}NO_3$), ammonium sulphate ($N\text{-}NH_4$), and ammonium nitrate ($N\text{-}NO_3NH_4$)] in three stages of application (booting, flowering and in both stages). The experiment was carried on in block design, with-three replications, in the factorial 2x3x3 scheme. Blast intensity was evaluated by the destructive method, collecting spikes (n>60) from a linear meter selected at random inside each plot. Statistical analysis ($p<0.05$) of the incidence and severity of the disease was performed by utilizing the Student`s least significant difference (LSD) test. Significant difference was observed only between the wheat cultivars. Lower severity (14%) of blast was observed in the BRS Gaivota and higher severity (24%) occurred in the wheat cultivar Gralha-Azul. The blast incidence (64%) was similar in both cultivars. There was no observed significant difference of the N source and in the application timing, which showed severity and incidence levels lower than 19% and 64% respectively. Possible explanations could be the fact of the blast expression to nitrogen fertilization is mediated by higher levels of N as well by the interaction with other nutrients, like potassium. Therefore, this work needs to be replicated again in order to confirm the obtained results. The nitrogen fertilization effect in the wheat blast expression could have implications in the breeding process, increasing the intensity differences between resistant and susceptible lines.

Keywords: *Pyricularia grisea*; mineral nutrition; *Magnaporthe oryzae*

WHEAT BLAST EXPRESSION BY POTASSIUM FERTILIZATION

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Wheat blast, caused by the fungus *Pyricularia grisea*, is undoubtedly a limiting disease in the production of wheat in South America (URASHIMA ET AL. 2010). Mineral nutrients like potassium (K) can influence the increase or the reduction of diseases, expressing, many times, the resistance or susceptibility of the host. The objective of the work was to evaluate the effect of potassium fertilization in expression of the wheat blast in the field, in 2015. The expression of the wheat blast was compared in two wheat cultivars, BRS Gralha-Azul (susceptible) and BRS Gaivota (moderately resistant). The cultivars received 30 kg/ha of K applied as topdressing utilizing three K sources, [potassium nitrate (KNO_3), potassium sulphate (KSO_4) and potassium chloride (KCl)] in three stages of application (booting, flowering and in both stages). The experiment was carried on in block design, with four replications, in the factorial 2x3x3 scheme. Blast intensity was evaluated by the destructive method, collecting spikes (n>60) from a linear meter selected at random inside each plot. Statistical analysis ($p<0.05$) of the incidence and severity of the disease was performed by utilizing the Student`s least significant difference (LSD) test. Significant difference was observed only between the wheat cultivars. Lower severity (8%) of blast was observed in the BRS Gaivota and higher severity (12%) occurred in the wheat cultivar Gralha-Azul. The blast incidence (48%) was similar in both cultivars. There was no observed significant difference of the K source and in the application timing, which showed severity and incidence levels lower than 10% and 50%, respectively. Some possible explanations of the non blast expression and the potassium fertilization would be due to the effect mediated by higher levels of K and by the interaction with other nutrients like nitrogen or due to higher responses in deficient plants.

Keywords: *Pyricularia grisea*; mineral nutrition; *Magnaporthe oryzae*

BREAD AND HARD WHEAT TOLERANT TO *Pyricularia* IN QUIRUSILLAS, SANTA CRUZ, BOLIVIA

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With the purpose to identify tolerant cultivars against wheat blast, a germplasm was introduced form CIMMYT (1th Wheat Blast South America Nursery). The nursery was established in Quirusillas valley in Santa Cruz during the 2012-2013 crop season. The germplasm was screened for sources of tolerance to wheat blast. Eleven variables were evaluated in field. Statistical package R was used to operationalize parametric univariate and multivariate responses. Seventy one entries were identified with some degree of tolerance to wheat blast. Furthermore, some of those entries were also identified as tolerant to helmintosporiosis (entries: 857, 545, 578, 675, 66, 64, 611, 80, 571 and 55). Of the 290 entries of durum wheat, none of them were significantly tolerant to wheat blast, only 47 showed moderate responses to wheat blast.

Keywords: wheat; blast

GENETIC DISSIMILARITY AMONG AVENA CULTIVARS IN RESPONSE TO THE BLAST REACTION

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The oat blast caused by *Pyricularia oryzae* Cavara (teleomorfa *Magnaporthe oryzae* B. Couch), has increased the incidence and has attracted the attention of plant scientists worldwide. The disease causes significant reduction in oat grain yield. The search for blast resistance sources amongst oat germplasm is as a priority in Brazilian oat breeding programs. Genetic dissimilarity may help in better understanding of the different genotypes within and between groups. This study aimed to evaluate the genetic dissimilarity between cultivars of *Avena strigosa* (Schreb) e *A. brevis* (Roth) in response to blast reaction. An experiment was carried out at Embrapa Trigo, in 2015. We used four cultivars of *A. strigosa* (Embrapa 29 Garoa; Embrapa 139 Neblina; Agro Planalto; Agro Zebu) and two cultivars of *A. brevis* (BRS Centauro; BRS Madrugada). The experimental design was a randomized block with four replications. A mixture of four isolates of *P. oryzae* at a concentration of 2 x 10^5 conidia/mL was sprayed at tillering stage of oats development. The lesions number, lesion size (length x width) and severity was recorded, at three days-interval, during 27 days. The data were subjected to multivariate analysis, clustering groups average using Euclidean distance and UPGMA methods from the statistical program Gene. The six cultivars were separated in three groups. The group I, clustered the cultivars Embrapa 139 Neblina, Agro Zebu and BRS Centauro. This group represents cultivars with large lesions. The cultivars Embrapa 29 Garoa and Agro Planalto formed the group II characterized by small lesions. The group III, represented solely by BRS Madrugada showed a low number of lesions and low severity. According to the Euclidean average matrix, the maximum distance (84%) was between cultivars Embrapa 29 Garoa and BRS Centauro and the minimum distance (18%) between the cultivars Embrapa 129 Neblina and Agro Zebu. As to the relative contribution of characters for genetic divergence, the severity was the most important variable (91.86%), followed by the number of lesions (4.77%), lesion length (3.24%) and width (0.13%). There was dissimilarity between cultivars of *A. strigosa* and *A. brevis* in response to the reaction to oat blast.

Keywords: oat; *Pyricularia oryzae*; euclidean distance

REACTION OF BRAZILIAN WHEAT CULTIVARS TO WHEAT BLAST IN THE CERRADO REGION

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Wheat blast, caused by *Magnaporthe oryzae*, is the main disease of the wheat crop in the Cerrado Region in Brazil. Nowadays, this disease is the main factor that limits the expansion of the wheat crop in that region. Due to the limited efficiency of the fungicides in years of severe occurrence of wheat blast, association of genetic resistance and chemical control has been the best strategy to control the disease and improve the cost-effectiveness for the farmers. In order to evaluate the preliminary reaction of Brazilian wheat cultivars to wheat blast under field conditions, a collection with 69 wheat cultivars was evaluated according to the occurrence of wheat blast on the heads in a field experiment conducted in Santa Juliana, Minas Gerais State, located about 1,000 m of altitude. The experiment was sowed on March 16, 2015, and the evaluation was done on May 27 (64 days after emergence) on the heads that already were reached the heading stage until May 17. Only 49 cultivars were under this condition at that moment. Later cultivars were not evaluated because the weather conditions on their heads were not appropriate for wheat blast development. A scale (1 to 5) of visual notes was used, considering 1= no heads with symptoms of wheat blast, and 5= almost 100% of the heads with severe symptoms, was used. Intermediate values (e.g.: 3/4= 3.3; 4/3= 3.6) also were noted, to try better discriminating the disease reaction. The cultivars were classified in R: Note 1.0 to 1.6; MR: 2.0 and 2.3; MS: 2.6 to 3.3; S: 3.6 to 4.3; AS: 4.6 and 5.0. Groups to heading were used. The reaction of the cultivars were: **Group 1** (34-40 days to heading) **S**: BRS 210 and BRS 394; **AS**: BRS 254, BRS 264, Embrapa 22 and Embrapa 42; **Group 2** (41-45) **MR**: BR 18, BRS 404 and CD 111; **MS**: CD 1550; **S**: BRS 248, BRS Guamirim, CD 123 and Valente; **Group 3** (46-50) **R**: BRS 229 and Fundacep Cristalino; **MR**: BRS 207, BRS 220, BRS Louro, CD 117, CD 118, CD 151, Quartzo, TBIO Itaipu and TBIO Seleto; **MS**: BRS 179, CD 1252, IAC 24, IPR Catuara, Mirante and Topázio; **S**: Ametista, CD 105, CD 108, FPS Nitron and Fundacep Horizonte; **Group 4** (51-55) **R**: CD 116 and IPR 128; **MR**: Fundacep Raízes, IPR 85, IPR 130, IPR 136 and TBIO Sinuelo; **MS**: BRS 208, BRS Gralha Azul, MGS Brilhante and Supera; **S**: BRS 327 and CD 150. The most resistant cultivars were BRS 229, BR 18, BRS 404 and CD 111. The high resistance of CD 116, IPR 128 and Fundacep Cristalino is questioned. Because of the weather conditions after heading (low rainfall), caution is necessary to consider the reaction for other cultivars of the groups 3 and 4.

Keywords: *Magnaporthe oryzae*; genetic resistance; BRS 229

WHEAT BLAST IS CAUSED BY MULTIPLE *Pyricularia* SPECIES, INCLUDING *Pyricularia graminis-tritici* sp. nov.

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Pyricularia oryzae is a species complex that causes blast disease on more than 50 species of poaceous plants. The *P. oryzae* pathotype *Oryza* is considered the widely distributed rice blast pathogen, whereas *P. oryzae* pathotype *Triticum* is the South American wheat blast pathogen. Our first objective was to determine if the *Oryza* and the *Triticum* pathotypes of *P. oryzae* are distinct species that should be given different names. Our second objective was to describe a new *Pyricularia* species causing blast on wheat and several other poaceous hosts in Brazil. We conducted phylogenetic analysis on ten housekeeping genes using sympatric populations of *Pyricularia* adapted to rice (N = 24), wheat (N = 79) and other poaceous hosts (N = 23) from Brazil. The Bayesian phylogenetic analysis grouped the isolates into two major monophyletic clusters (Clusters I and II) with strong Bayesian posterior probabilities (P = 0.99). Cluster I contained isolates originating from wheat as well as from another Poaceae (P = 0.98). Cluster II was divided in three host-associated clades with high posterior support (Clades 1, 2 and 3; P > 0.75). Clade 1 contained isolates obtained from wheat and one isolate obtained from signal grass plants invading a wheat field in Paraná state. Clade 2 contained exclusively wheat-derived isolates, and Clade 3 comprised isolates associated only with rice. Our interpretation was that Cluster I and Cluster II corresponded to two distinct species: *Pyricularia graminis-tritici* sp. nov. (Pgt), newly described in this study, and *Pyricularia oryzae* (Po). The host-associated clades found in *P. oryzae* corresponded to *P. oryzae* pathotype *Triticum* (Po-T, Clades 1 and 2), and *P. oryzae* pathotype *Oryza* (Po-O, Clade 3). Our analyses did not clearly separate *P. oryzae* pathotypes *Triticum* and *Oryza* into two distinct *Pyricularia* species. No morphological or cultural differences were recorded among species. However, distinctive pathogenicity spectrum was observed. Pgt and Po-T were pathogenic and highly aggressive on wheat, barley, signal grass, and oats. Po-O was highly virulent on the original host (rice), and also on wheat, barley, and oats, but not on signal grass. Our study demonstrated that blast on wheat and other Poaceae in Brazil is a complex disease caused by more than one species of *Pyricularia*. Given this finding, recognizing and properly naming the causal agents of wheat blast will not only increase our understanding of the biology and epidemiology of the disease, but will also enable the establishment of proper quarantine regulations to limit the spread of these pathogens into disease-free areas that grow susceptible wheat cultivars, such as Europe, Asia and North America.

Keywords: cryptic species; host adaptation; phylogenetics; *Triticum aestivum*

CULTURE MEDIA INFLUENCE ON VEGETATIVE GROWTH AND IN VITRO CONIDIA PRODUCTION OF ARGENTINIAN WHEAT ISOLATES OF *Pyricularia oryzae*

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Culture media influence on vegetative growth and in vitro conidia production of wheat isolates of *Pyricularia oryzae* from different geographical origin was studied. Four monoconidial isolates from wheat blast tissue samples collected from Argentina, Bolivia, Brazil and Paraguay were tested on solid media, V8 agar, Rice polish agar (RA) and Oat meal agar (OMA) under two different light regimes (12h light-darkness and 24h darkness). Agar blocks (5 mm diameter) were cut from actively growing margins of each colony and centrally inoculated on plates. The cultures were incubated 25 ± 2°C. Growth of the isolates was measured from the circumference of the agar plug to the leading edge of mycelial growth at 2-day intervals. Four replications were maintained for each isolate tested. The morphocultural characteristics to study variability of isolates were: cultural texture, colour, conidia shape and sizes, sporulation and growth rate. Mean diameter and rate of colony growth in cm/24 hr were also determined. Size of conidia (length and width) was measured using a micrometer in microscope. Results revealed that isolates showed variation among them in mycelial growth, pigmentation or conidial production in different culture media and light regime tested. Significantly variation in growth rate among isolates was seen in all media. PyPaG and PyBr isolates shown the highest growth rate on OMA ranging from 0.71 to 0.79cm/day while isolate PyBol had the slower growth rate on V8 (0.47 cm/day). The growth rate of the isolates on OMA was faster than V8 or RA. Thus, OMA resulted a favorable media for the fungus growth with a significant highest growth rate of all the isolates under both light conditions. In all isolates, the shape of the conidia was typically pyriform and 2-septate with basal appendages. Differences in spore size were seen among the isolates tested. The isolate PyBol had the longest (24.00µ (48.00µ) and width conidia (9.60µ -19.20µ). The lowest conidial length was for isolate PyArg22 (12.00µ (19.20µ). Maximum variation in the length:wide (L:W) relationship ranged from 1.7:1 to 3.8:1 on RA media under 12h light-12h darkness for PyArg22 and PyBol isolates respectively, and 1.7:1 to 2.9:1 on V8 and darkness incubation for PyArg22 and PyBra respectively. Interestingly under continuous darkness conidia were shorter according the L:W relationship and the visual appearance. The L:W relationship resulted variable under the 12 h light-12 h darkness condition. Under darkness, some isolates not shown sporulation. Isolate PyArg22 produced the highest conidial production in all media tested with a maximum shown in OMA. These results could help to understand the in vitro requirements for development of the pathogen, from which no previous information is available in Argentina. Moreover, to contribute with the knowledge about the behavior of the fungus and its handling to produce mass inoculum for future experiments.

Keywords: *Magnaporthe oryzae*; phenotypic traits

SEED BORNE INFECTION OF WHEAT SEEDS BY *Pyricularia oryzae* AND ITS TRANSMISSION TO SEEDLINGS

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The effect of *Pyricularia oryzae* on wheat seed infection and germination is little known in Argentina. Thus, the aim of this study was to examine the seed borne infection and transmission of *P. oryzae*, in different wheat cultivars currently grown in Argentina. Seeds of BioInta 3004, Baguette 18 and Klein Proteo were inoculated (1,5x105 spores/ml) with isolates PyArg22, PyBol, PyBra and PyPG1 collected from wheat plants, and isolate PyAZ1 collected from rice plants. The effect of the pathogen was studied in laboratory conditions by standard blotter test method. Seeds were kept in three layers of moistened white blotting paper, in plastic trays. Two hundred seeds per each isolate-cultivar combination were analyzed in four replicated experiments. Evaluation of seed germination and infection symptoms was registered at the seventh day, through visual emergence of germ tube. After 7 and 14 days, each seed was examined microscopically to detect sporulation of *P. oryzae* and a total number of infected seeds was counted. Data analyzed by Kruskal-Wallis non-parametrical test shown differences among wheat cultivars and isolates tested. Klein Proteo germination was not affected by any of the isolates of the fungus tested; in Baguette 18, isolates PyBra and PyBol caused a significant reduction of germination of 15%. All the four isolates tested affected significantly the germination of BioInta 3004 causing a reduction between 50-66%. Symptoms observed were total or partial rot of the seeds, and necrotic symptoms with brown dots and brown or grey spot discoloration. Presence of fungal mycelium was observed and recovered under microscope. Incidence of symptoms indicated that cultivar BioInta 3004 was the most severely infected (64.16% of seeds with presence of the fungus). Seedlings emerged from BioInta 3004 infected with isolate PyArg22 shown symptoms of the disease (necrotic spots on coleoptile and first leaf emerged). A preliminary screening of seed transmission was evaluated on superficially disinfected and then liophyllized BioInta 3004 seeds by PCR technique. These results showed a positive result confirming the presence of *P. oryzae* in wheat seeds. Given the importance of the seed as a major source of diseases transmission and origin of primary inoculum of infection in wheat seeds, this fungal disease could be considered and evaluated on young seedlings. In this way, the use of pathogen-free seeds will be an important component of integrated wheat blast management in Argentina. Moreover, the reduction in germination and symptoms of rot and discolored seed are considered as indicator for negative characters of seedlings regarding the high cost of their production.

Keywords: *Magnaporthe oryzae*; seed pathology; wheat blast

UPDATE ON RECENT WHEAT BLAST RESEARCH PROGRESS IN ARGENTINA

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The *Magnaporthe oryzae* Triticum pathotype (MoT) is a new fungal problem that requires knowledge in our country. The disease, known as "Brusone" or Wheat Blast (WB) is recognized as a serious threat to wheat worldwide. Although currently confined to South America, MoT is considered a high consequence pathogen given the yield failures and the significant economic losses in neighboring countries of Argentina (Bolivia, Paraguay and Brazil). Since the first detection of the disease in Chaco Province, Argentina, in 2007, the progress on research has been as follows: In 2012, wheat plants were found with symptoms compatible with WB in a routine monitoring of fungal diseases conducted in Lavallol, Province of Buenos Aires. In 2015, the first international report of the research period 2012-2015 at CIDEFI was published. Results to date show the susceptibility of the germplasm currently used in Argentina against the infection of the fungus at both the leaf and heading stages. Moreover, the ability of the fungus to infect grain and its transmission to seedling has been studied. The main actions being undertaken involve the characterization of the fungus; in this sense, a collaboration with the Institute for Biodiversity Research and Biotechnology (INBIOTEC-CONICET), and Applied Biological Research Foundation (FIBA), Mar del Plata, Argentina has been established for the implementation of molecular techniques for identification and characterization of MoT, and other *Magnaporthe* strains from poaceae and weeds. Between 2013-2015, the virulence of different strains of the fungus was evaluated and the MoT isolates collection was enriched. In addition, the incidence and severity on leaves and spikes of several commercial wheat cultivars was evaluated under greenhouse conditions. The effect of MoT infection on grain quality (weight reduction and protein content) was analyzed. During 2014, association mapping studies were initiated in order to localize resistance genes to WB and genes that determine morpho-physiological characters. This work was performed using a germplasm collection/population of 110 genotypes from 32 countries and 5 continents provided by the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. We also focused the role of the different components that participate in the cross talk amongst hormone-induced signaling pathways triggered in wheat plants by abiotic and biotic stress caused by MoT. In this sense, a platform of collaboration between National University of La Plata, Buenos Aires, and Universitat Illes Balears, Spain, was initiated. No reports of new detection of the disease exist to date in Argentina. However, the potential for this to occur can be high. Moreover, the disease may be masked or confused with other diseases of similar symptoms in both leaf and spike, or go unnoticed due to the lack of a correct diagnosis. All these factors require immediate attention by the main actors involved in wheat crop protection to prevent outbreak in Argentina.

Keywords: wheat blast; host resitance

AGGRESSIVENESS OF *Pyricularia oryzae* ISOLATES ON SEEDLINGS AND ADULT PLANTS OF ARGENTINIAN VARIETIES AND THEIR EFFECT ON 1,000-GRAIN WEIGHT

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Wheat blast caused by *Pyricularia oryzae* is a new disease of *Triticum aestivum* in Argentina and a threat to wheat cultivation in the region of Buenos Aires Province where the fungus was recently observed. During the spring of 2015, eight isolates of P. oryzae recovered from diseased rice and wheat plants in four countries (Argentina, Bolivia, Brasil and Paraguay) were evaluated for pathogenicity on commercially wheat varieties ACA 303, Baguette 18, Buck Meteoro and Klein Proteo under greenhouse assays. Results indicated that isolates were able to successfully infect both seedling and adult plants. A differential reaction was shown at vegetative (seedling) and reproductive stage (head). Interestingly, this study confirms that P. oryzae isolates from rice were pathogenic on wheat cultivars tested at both stages. Resistance or incompatible reaction after leaf inoculations was observed on Baguette 18 in combination with isolates PyAz1, PyAz2, PyAz4, and ACA 303 with isolates PyAz4 and PyBr27. Additionally, isolates PyAz3, PyBol8, PyArg22 and PyPaG1 caused compatibility reactions in seedling of all wheat varieties tested. On spikes, first signs of the disease were developed after a week on Buck Meteoro and Baguette 18 cultivars as small necrotic spots in the basal part of the spike. The progress of the disease showed variation in susceptibility according the genotype and isolate inoculated. Disease incidence ranged between 12.50-100%. Low disease severity was shown in ACA 303 in combination with isolates PyAz3, PyAz4, PyBr27 and PBol8 with mean values of 2.18 % -11.50 %. Similarly, Buck Meteoro with PyAz2, PyAz44 and PyBol8 reached mean values of disease severity among 9.09 and 14.43 %. By other hand, Klein Proteo with isolates PyArg22 and PyAz1 was highly susceptible (97.38 % and 99.20 % of severity respectively). Similar behavior was shown by Baguette 18 inoculated with isolates PyAz1 (95.20%), PyAz3 (97.57%) and PyArg22 (97.77). Phenotypic variation in pathogenicity and aggressiveness of the isolates showed significant differences. Greater aggresiveness was caused by the Argentinian isolate PyArg22 compared with isolates from other regions both in young and adult plant growth stages in all varieties tested. Baguette 18 showed high resistance level when tested at young plant growth stage. Contrary, ACA 303 showed less head area affected by blast in adult plants. The relative 1,000-grain weight (TGW) was negatively affected by the infection of P. oryzae with reductions ranging from 18.53 % to 74.94%. Severely infected seeds weighted significantly lower than controls, with a TGW as low as 9.32g in the most affected genotype, ACA303 infected with PyArg22. According to these observed results, further studies to screen wheat blast resistance in current wheat germoplasm used in Argentina needs top priority.

Keywords: wheat blast; host resistance

BLAST SEVERITY OF WHEAT LEAVES IN ARGENTINA CAUSED BY *Pyricularia oryzae*: ASSESSMENT OF VARIETIES AND ISOLATES OF DIFFERENT ORIGINS

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Wheat blast caused by *Pyricularia oryzae* causes significant losses in several South American countries. As a new disease in Argentina, no previous reports about the behavior of wheat cultivars are available. During the spring of 2015, eight isolates of *P. oryzae* recovered from diseased rice and wheat plants in four countries (Argentina, Bolivia, Brazil and Paraguay) were evaluated for pathogenicity on four commercially wheat varieties (ACA 303, Baguette 18, Buck Meteoro and Klein Proteo) under greenhouse assays. Wheat plants were inoculated at tillering (Z2.1) stage with $1x10^5$ spores/mL of each isolate until run off. Then, covered with plastic bags during two days and evaluated 7 and 14 days after inoculation for severity and lesion type. First symptoms appeared 96 h post inoculation in cultivars Klein Proteo and Buck Meteoro- with all the isolates tested. Cultivars Baguette 18 and ACA 303 did not shown any symptoms at that time. Seven days after inoculation most combinations cultivar x strain showed any of typical phenotypic reactions already described according to Urashima et al. (1993). Severity values ranged between 0-32.67 % for ACA 303; 0-8.83 % for Baguette 18; 5.17-17.08% for Buck Meteoro and 1.71-10.42 % for Kein Proteo. Baguette 18 infected with isolate PyAz2 and ACA 303 with isolates PyAz1, PyAz2 and PyAz44, did not show any symptoms of the disease. However in the second evaluation date (14 days) all the combinations variety x isolate shown any type of symptom like minute pinhead-sized spots, small brown to dark lesions with or without grey centers or the typical blast lesions elliptical with gray centers. The highest severity values were reached 21 days after inoculation (60.91%, 37.5% and 26.91%) by the isolate PyAz3 in combination with varieties ACA 303, Buck Meteoro and Klein Proteo respectively. Regarding the phenotypic reactions, ACA 303, Baguette 18, Buck Meteoro and Klein Proteo with isolates PyAz3, PyArg22 and PyPaG1 reached lesions type 4. These lesions showed abundant sign of the disease under 24 hours of humidity chamber at Lab conditions. This is an important information from an epidemiological point of view and indicative that even with low severity values, the spots could have the potential to produce mycelium and spores to spread the disease under favorable field conditions. According to the observed lesions and type of reaction, the cultivars tested displayed a different pattern of reaction across leaves depending of the inoculated strain. Particularly, Buck Meteoro showed a susceptible reaction with 7 of the 8 isolates tested. The results also indicates variation in virulence and/or aggressiveness among *P. oryzae* isolates evaluated. Further studies monitoring new strains and wheat cultivars are needed to be tested before a wheat blast outbreak occurs in the main Argentinian growing area.

Keywords: wheat blast; epidemiology

WHEAT BLAST CAUSED BY *Magnaporthe oryzae* (*Pyricularia oryzae*): VARIABILITY AND DIVERSITY OF VIRULENCE IN WHEAT GENOTYPES

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Wheat blast caused by *Magnaporthe oryzae* (*Pyricularia oryzae*) Triticum pathotype (MoT) constitutes a sanitary adversity which is taking place at many productive locations in South Cone of South America with losses up to 100% under certain weather conditions. Detections of the disease in the North East of Argentina and Buenos Aires region have been recently reported. *P. oryzae* shows mechanisms of genetic variability that provides great adaptability to different environments and hosts. Given these large diversity and virulence of the fungus, the genetic resistance of wheat has been hard to identify. Taking into consideration the presence of the disease in the neighboring countries (Brazil, Paraguay, Bolivia), the conducive weather conditions in many of the agroecological area currently under crop production and the recent detection of the fungus infecting wheat plants in Argentina, the threat of a future outbreak of Wheat Blast is hight. This study was started with the aim to determine the virulence's diversity of *P. oryzae* in wheat using two strains from Argentina (ArW22) and Paraguay (PyG1), tested on a set of 110 wheat genotypes selected from the germplasm repository provided by the Genbank of IPK, Gatersleben, Germany under greenhouse conditions for two years (2014-2015). Inoculations were made at a concentration of $2x10^5$ conidia/mL. Preliminary results about the variability of virulence showed on wheat genotypes are currently been analyzing. Results of both years shows the high agressivity of the ArW22 isolate on TRI4056 and TRI5984 genotypes, and PyG1 on TRI10625 affecting the 100% of the spikes with the disease. Interestingly, the 23% of all genotypes tested were infected with high severity values (60 to 80%) after inoculating with ArW22 isolate, particularly TRI3931, TRI6094, TRI3664, TRI10311 and Baguette18 genotypes. Similarly, isolate PyG1 infected the 11,5% of the all genotypes tested with 60% to 80% of disease severity in combination with TRI3477, TRI10311, TRI4081 and TRI4130 genotypes. By other hand, low values of disease (under 20%) were found in the 21% and 29,5% of the affected genotypes inoculated with ArW22 and PyG1 isolates, respectively. The results indicate diverse patterns of virulence on 110 wheat genotypes tested under greenhouse conditions. There is a differential behavior depending on growth stage (seedling / adult plant), genotype and tested organ (leaf / head). It is intended to determine the location of resistance through mapping by association and determine the location of genes that determine morphophysiological characters (flowering time, plant height, peduncle length, number of grains / spike weight grains, earing date, length and weight of the number of spikelets / spike, number of spikelets / spike sterile, disease resistance, protein content and sedimentation value). Partial results are under evaluation.

Keywords: wheat blast; epidemiology

P86 *2nd IWWB - Session 4 – Ecology, epidemiology and management*

SAPROTROPHIC SURVIVAL OF *Magnaporthe oryzae* IN INFESTED WHEAT RESIDUES

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Wheat blast caused by *Magnaporthe oryzae* is a relative new disease that has caused considerable damage on wheat fields of several South American countries such as Brazil, Bolivia and Paraguay. In temperate regions, inoculum overwinter is limited by low temperatures. However, the effects of not limiting low temperature conditions on the survival of *M. oryzae* in infested wheat residue had not been fully explored. Therefore, a time course study was designed to assess sporulation of infested wheat residue exposed to ambient and predetermined treatments. The main objective of this study was to monitor the saprotrophic development of *M. oryzae* on wheat debris and to explore the relative importance of crop residues as a source of inoculum. The wheat cultivars BRS 229 and Anahuac 75, respectively, a moderate and high susceptible were grown under greenhouse. At heading stage the cultivars were inoculated with a spore suspension of 100,000 conidia ml^{-1} using a more virulent (Py 12.1.209) and a less virulent (Py 12.1.132) isolate. The plants were incubated in a moist chamber for seven consecutive days. Immediately after, the infested plants were transferred to a greenhouse. At maturity, a portion of leaves, stems and spikes were detached from plants. A group of ten lesions were randomly selected and marked in each plant organ. The air-dried plant organs were placed separately inside bags and exposed outside. Every 14 days, the marked lesions were examined under a dissection microscope for the presence of *M. oryzae* conidiophores bearing conidia. The results showed that the number of sporulating lesions on leaves, spikes and stems diminished with time. Sporulation was no longer observed after 140 days of exposure. Estimation and group comparison of survival curves were obtained by a survival analysis. A longer survival curve was observed in the cultivar BRS 229 compared to the cultivar Anahuac 75. A shorter survival curve was observed in the spikes compared to leaves and stems. The survival curves for the isolates were similar. Although preliminar, the results presented here, make very unlikely that infested wheat residues serve as a source of wheat blast inoculum for the next wheat crop. The management of crop residues is not a key point to control the development of wheat blast. A strong emphasis should be placed on the presence of other hosts.

Keywords: wheat blast; crop residues; disease cycle

IN VIVO SENSITIVITY OF WHEAT *Magnaporthe oryzae* TO FUNGICIDES

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Wheat blast, caused by the fungus *Magnaporthe oryzae*, represents a threat to wheat production in Brazil and other South American countries. The pathogen was first reported in Brazil infecting naturally wheat plants during the mid-1980s. In some years and locations, control of wheat blast has been difficult due to highly favorable weather conditions and the lack of adequate genetic resistance to the disease. Currently, application of foliar fungicides is one of the main measures of the integrated control of wheat blast. The objective of this study was to verify the *in vivo* sensitivity of wheat *M. oryzae* isolates to fungicides. The wheat cultivars BRS 229 and BRS 220 (moderately resistant and susceptible to wheat blast, respectively) and *M. oryzae* isolates, selected previously according their *in vitro* sensitivity to fungicides (Py 12.1.132, high sensitivity; Py 12.1.209, low sensitivity), were used in the study. Trifloxystrobin + prothioconazole and prothioconazole were sprayed on wheat plants at three-four leaf stage. After 24 h, the plants were inoculated with a spore suspension of 100,000 conidia ml^{-1}. Immediately after inoculation, the plants were transferred to moist chamber kept at the temperature of 25 C. After 96 h, the plants were transferred to a greenhouse and the number of lesions on individual leaves recorded. The data was fitted to a generalized linear model with a Poisson distribution. The results revealed a significant interaction for all factors. The cultivar BRS 229, in combination with the fungicide trifloxystrobin + prothioconazole, and the isolate Py 12.1.209 showed the lower number of lesions per leaf. The differences between the two *M. oryzae* isolates used in this study in relation to fungicide sensitivity were not as clear as those observed in the experiments conducted previously in culture medium (*in vitro*). Nevertheless, despite the effect of interaction with the cultivar and fungicide factors, the lower number of lesions observed on the leaves infected by the isolate Py 12.1.209 is an important indication that wheat *M. oryzae* in Brazil differ for sensitivity to fungicides when is infecting the plants. This situation may be one of the main factors that contribute to the inefficiency of the chemical control of wheat blast in the fields, observed mainly in the spraying of strobilurin fungicides.

Keywords: chemical control; wheat; wheat blast

WIDESPREAD DISTRIBUTION OF RESISTANCE TO TRIAZOLES IN POPULATIONS OF *Pyricularia* ASSOCIATED TO WHEAT BLAST IN BRAZIL AND EVOLUTION OF *cyp51A* GENE

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Pyricularia oryzae-has worldwide distribution as rice pathogen, but in the last century emerged as an important pathogen of wheat and has become one of the most important diseases of the aerial part of wheat in Brazil. Strobilurin and-azole-fungicides-have-been intensively used for controlling wheat diseases in the last three decades.-Resistance to strobilurin in populations of the wheat blast pathogen has already been reported. The inefficacy showed by azole-fungicides in managing wheat diseases, might indicates a similar scenario. Triazoles are the main representatives of the sterols demethylation inhibitor (DMI) fungicides group. These fungicides interfere in the biosynthesis of ergosterol, a major component of cell membranes of fungi by inhibiting the 14α-demethylase enzyme that is encoded by the *cyp51* gene. In this study, we determined the frequency distributions of EC_{50} values for tebuconazol and epoxiconal of 180 isolates of *P. oryzae*-associated to wheat sampled from south-central Brazil. In addition, we described the presence of mutations in the *cyp51A* gene and built their reticulate phylogeny to identify the evolutionary relationship among haplotypes. All populations sampled-were-insensitive to azoles when compared to the field dose recommended for the management of disease. Based on the sequence of *cyp51A* gene, eight distinct haplotypes were distinguished and seven non-synonymous mutations could be correlated with resistance to DMI's. We alert for the importance of correct fungicides management, as our results indicate a slow but steady change towards reducing the sensitivity of the wheat pathogen to triazole fungicides in Brazil.

Keywords: fungus; sensitivity; fungicides; mutations; *cyp51*

EFFICACY OF FUNGICIDES FOR THE CONTROL OF WHEAT BLAST: COLLABORATIVE TESTS RESULTS - 2014 SEASON

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The blast wheat caused by the fungus *Pyricularia oryzae*, has great importance in the culture of cereal, limiting their production in many states of Brazil, due to disruption of the transport of water and nutrients to the grain formation. Their control can be accomplished through the use of less susceptible cultivars and healthy seeds, choose the time of sowing and the use of chemicals, although most fungicides is ineffective in controlling rice blast. Seeking to identify best chemical control levels of this disease, a network testing using standardized protocol was established in 2010. The first trials were conducted in 2011 in regions of Paraná, Mato Grosso do Sul, Mato Grosso, São Paulo, Minas Gerais and the Federal District. In 2014 and 2015 they were tested six fungicides against the witness (7 treatments) with four replications in an experimental design of randomized blocks. Some fungicides and specific adjuvants were included only in certain regions totaling 11 treatments in all regions. The efficacy of the fungicides was evaluated by ratings of incidence, severity, and index of disease, as well as the total yield of the grains. By analyzing all the experiments, it is seen that the lower values of incidence / severity / index of the disease were obtained by active ingredients Azoxystrobin 12% + Tebuconazole 20% (4 places), Trifloxystrobin 10% + Tebuconazole 20% (1 site), Mancozeb 80% (6 locations), Trifloxystrobin 15% Prothioconazole + 17.5% (1 site), Thiophanate methyl 14% + Mancozeb 64% (2 sites), Tebuconazole 20% (site 1) + Mancozeb 80% polyether Corpolímero and silicone (1 site), and tricyclazole (1 site). Already the treatments had better grain yield were Thiophanate methyl 14% + Mancozeb 64% (2 locations), Azostrobina 12.5% + Tebuconazole 24% (1 site), Thiophanate methyl, Azoxystrobin 12% + Tebuconazole 20% (2 sites), Mancozeb 80% (4 sites), Mancozeb + 80% Corpolímero and silicone polyether (one site), Trifloxystrobin 15% Prothioconazole + 17.5% + soybean oil methyl ester (1 site). Thus, it is concluded that, although the results have proven quite variability from one region to another, the products composed of the active ingredients Thiophanate methyl 14% + Mancozeb 64% Mancozeb 80% and Mancozeb 80% + Corpolímero polyether silicone It was the most efficient for *Pyricularia oryzae* control recommendation in the wheat crop, especially to Mancozeb, which is present in all three products mentioned above.

Keywords: blast; wheat; control; fungicide

P90 *2nd IWWB - Session 4 – Ecology, epidemiology and management*

EVALUATION OF FUNGICIDE EFFICACY FOR WHEAT BLAST CONTROL IN PLANALTINA-DF, BRAZIL

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The non-irrigated wheat cultivated in the off-season in the Federal District of Brazil has been successfully used in rotation with soybean, corn and cotton. It has suppressive effect on the weeds that invade fallow areas, breaking the disease cycle and reducing its inoculum in the area, as well as maintaining soil quality over time by forming mulch on the soil. The wheat blast caused by *Pyricularia oryzae* is becoming a serious problem for tropical wheat regions. This disease occurs under high temperature and humidity conditions and has become the main problem for wheat grown in the Federal District region. There are no resistant cultivars when there are favorable climatic conditions to the disease, so it is necessary to use other management strategies to control this disease, as chemical control. The aim of this study was to evaluate the efficiency of nine fungicides used to control wheat blast during five seasons (2011 to 2015), as well as quantifying the wheat blast incidence, severity and crop yield. Significant differences were found in-disease-incidence and severity during the 2012 and 2014 seasons. However significant differences in the yield were observed only in 2011, 2014 and 2015. The incidence and severity ranged from 3.5% to 100% and 1.72% to 97.2%, respectively. These patterns reflect variation in climate across years. In 2014 and 2015, rain-coincided with the spike emergence period, providing high levels of incidence and severity. In June 2012, no disease was found because air humidity was too low, even when irrigation was provided one hour daily. The wheat yields ranged from 15.62 to 2,876 kg/ha on non-irrigated crops, while in the irrigated area ranged from 3,250 to 4,754 kg/ha. The variation was associated with wheat blast incidence. However in 2014 and 2015, no effect of fungicides on leaf diseases was observed. The hectoliter (HL) was negatively associated with blast incidence, as expected, since diseased spikes produce low quality grains. The 2012 experiment, when symptoms were absent, higher HL levels were determined on grains, while 2014 and 2015- HL was below- 50, confirming that the great damage of wheat blast to the grains. Financial support: FAPDF.

Keywords: *Pyricularia oryzae*; phytopathometry; fungicide evaluation

CHANGES IN THE SOURCE-SINK RELATIONSHIP ON WHEAT DURING THE INFECTION PROCESS OF *Pyricularia oryzae*

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Blast, caused by *Pyricularia oryzae*, is one of the most important diseases occurring on wheat and can cause yield losses. On leaves, the symptoms are elliptical or rounded lesions with dark brown margins and gray centers. However, it is on the spikes that the blast symptoms are much more pronounced, which including the bleaching and death of the infected tissues in the rachis. Wheat grain yield is considered to be a dynamic process determined to a great extent by the capacity of the source leaves to export photoassimilates to the sink grains tissues as well as by the capacity of these sinks to convert these photoassimilates into starch. This study aimed to determine the changes associated with photoassimilates production and partitioning in source-sink relationship on flag leaves and spikes of wheat plants infected with *P. oryzae*. Flag leaves and spikes were inoculated at 10 days after anthesis (daa) (Exp. 1) or at 20 daa (Exp. 2) with a conidial suspension of *P. oryzae*. There was an impairment on chlorophyll *a* fluorescence parameters (analyzed as F_m, F_v/F_m YII and Y(NO)) on both the infected flag leaves and spikes coupled with reduced concentrations of chlorophyll *a* + *b* and carotenoids as well as lower capacity for CO_2 fixation by RuBisCO in the infected flag leaves. In the flag leaves and grains (obtained from the infected spikes) there were lower concentration of soluble sugars and an increase on the hexoses-to-sucrose ratio only on flag leaves. In the infected flag leaves, there was a lower sucrose phosphate synthase (SPS) activity and lower expression of sucrose synthesis (*Susy*) gene while there were higher expression and activity of acid invertases. At advanced stages of fungal infection, the concentration of starch was reduced on grains whereas on the infected flag leaves its concentration was kept high. There were reduction on the activity of ADP-glucose pyrophosphorylase and on the expression of *ADP-glucose pyrophosphorylase* gene and down-regulation on the expression of the *β*- and *α-amylase* genes at advanced stages of fungal infection on flag leaves and spikes. In conclusion, the greatest effect of blast on both grains quality and yield can be associated with alterations in the production and partitioning of carbohydrates during the grain filling process.

Keywords: wheat; blast; sucrose, starch; grains

WHEAT BLAST AND ITS EFFECT ON TECHNOLOGICAL QUALITY OF GRAIN FROM TWO SOWING DATES

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The fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*) causes blast disease (or “brusone”, in Portuguese), which is a usual disease on rice. On wheat, the first report was in 1985 in Paraná State, south of Brazil. Wheat blast produces detrimental effects on the wheat production of Brazilian Cerrado areas, as for wrinkled grains and low specific volume. It is supposed that the sowing date has influence in brusone wheat occurrence. So, the aim of this study was to investigate if wheat technological quality is affected by blast disease in grain samples obtained from two sowing dates in 2015 (first and second, with high and low brusone content, respectively), in Patos de Minas, MG, Brazil. Twelve wheat samples were analysed in each sowing date, totalizing 24 samples, in duplicate or triplicate, according to the used method. Wheat samples were characterized for blast incidence, severity, and grain yield (GY) in the field, and for wheat technological quality (WTQ), with their respective parameters, in the laboratory. WTQ was performed by hectoliter weight (HW), thousand kernel weight (TKW), grain hardness index (GHI), grain diameter (GDIAM), grain falling number (GFN), flour yield (FY), grain protein (GP), solvent retention capacity (SRC), gluten content (GI, gluten index; WG, wet gluten; and DG, dry gluten), flour color (L*, a*, and b* values), alveography (W, gluten strength; P, tenacity; L, extensibility; P/L ratio; G, swelling index; and EI, elasticity index), farinography (WA, water absorption and STB, stability), and bread evaluation (SV, specific volume; BS, bread score; and crumb color). The incidence of brusone presented negative correlations with GY, HW, and TKW. Severity showed positive correlations with TKW, GDIAM, and a* value (tendency to red color). Grain yield was positively correlated with HW, TKW, GDIAM, SRC (lactic acid), DG, L, and G, and negatively correlated with P/L ratio. The baking test showed that brusone samples did not affect specific volume and internal characteristics of bread, but affected its external characteristics, as for aroma and taste of bread, although, comparing the two dates in bread evaluation, no differences were observed between the breads. In the first sowing date, with the higher brusone content, some mean values were different from the second sowing date: GY (lower value), TKW (lower value), GFN (higher value), and dough extensibility (lower L); for the other parameters, there was very slight variation in the results. The small variation on the most of WTQ characteristics of samples, with high and low content of wheat blast, may be a consequence of the similar severity degree found between them.

Keywords: *Magnaporte*; *Triticum*; bread; physicochemical analysis; rheological properties

EXPRESSION OF DEFENSE-RELATED GENES IN WHEAT LEAVES INFECTED WITH *Pyricularia oryzae* MEDIATED BY CALCIUM

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Blast, caused by the fungus *Pyricularia oryzae*, emerged in late 1980s as a novel and serious threat to the wheat production in Brazil. Despite the researchers' efforts to obtain strategies for disease management, yield losses are often reported mainly in the Brazilian Cerrado. Mineral nutrition can be easily manipulated in order to increase host resistance, but for the wheat-*P. oryzae* pathosystem, only the effects of silicon and magnesium (Mg) have been investigated. Intriguingly, recent reports demonstrated that Mg increased wheat susceptibility to leaf blast, fact that was ascribed to the reduced foliar calcium (Ca) concentration observed in plants that were grown in nutrient solution containing high Mg concentrations. Here, therefore, we examined whether Ca could increase wheat resistance to leaf blast by increasing the abundance of defense-related transcripts. To address this issue, wheat plants from cultivar BRS Guamirim (susceptible to leaf blast) were grown in nutrient solution containing three Ca concentrations: 0.26, 2.6 and 5 mM. When the plants reached the growth stage 65, they were inoculated with a conidial suspension of *P. oryzae* (10^5 conidia mL^{-1}). Flag leaf samples from wheat plants that were either challenged or not challenged with *P. oryzae* were collected at 24, 48, 72 and 96 hours post inoculation. Quantitative real time PCR analysis was performed to investigate the relative expression of the defense-related genes *Pathogenesis-related 1* (*PR-1*), *Chitinase* (*CHI*), *β-1,3-glucanase* (*GLU*), *Phenylalanine ammonia lyase* (*PAL*), *Peroxidase* (*POX*), *Polyphenol oxidase* (*PPO*), *Tryptophan decarboxylase* (TRI), *Allene oxide cyclase 1* (*AOX*) and *Allene oxide synthase* (*AOS*). Foliar Ca concentration was significantly increased from 1.3 to 4.1 dag/kg, whereas blast severity was significantly decreased from 36.8 to 8.2%, as Ca concentration in nutrient solution increased from 0.26 to 5 mM. The expression of all defense-related genes was significantly increased upon *P. oryzae* infection, but plants grown at the highest Ca concentration displayed increases in *PR-1, CHI, GLU, PAL, POX, PPO* and *AOS* expression that were significantly higher in comparison to plants grown at the lower Ca concentrations. Therefore, it is postulated that Ca, in addition to its well-known importance for cell wall strengthening and membrane functionality, played an active role in wheat resistance to leaf blast. Further studies examining Ca-mediated activation of defense-signaling cascades will provide invaluable information to better understand the effect of this macronutrient in the resistance of plants to diseases.

Keywords: wheat blast; mineral nutrition; *Triticum aestivum*

PRELIMINARY ANALYSIS OF WHEAT BLAST EPIDEMIOLOGY UNDER NATURAL FIELD AND CONTROLLED CONDITIONS

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In order to understand the epidemiology of wheat blast and develop effective management programs, it is important to characterize the pattern of pathogen/disease spread and quantify environmental conditions that are conducive for disease development. For instance, the pattern of blast development within a field provides clues about the origin of the pathogen as well as pathogen characteristics that affect the spatiotemporal dynamics of the disease. In this study, the epidemiology of wheat blast was investigated for disease incited by the *Triticum* pathotype of *Magnaporthe oryzae* (MoT) under natural infection in Bolivian wheat fields, and by the *Lollium* pathotype of *M. oryzae* (MoL) under controlled conditions. Inoculum build-up and temporal and spatial spread from naturally occurring in-field inoculum sources (hotspots) were investigated in six 27 x 27 m^2 plots at three locations in Bolivia during the 2015 growing season. Every three days, the plots were assessed for spike blast incidence and severity as well as leaf blast severity on flag leaves. Assessments were made on a grid, with approximately twenty spikes rated every 2.7 m along transects. Hotspots increased in size over time and were associated with the highest disease incidence and severity ratings. Both spike and leaf blast incidence and severity increased in space and time relative to the hotspots. Initially, plants adjacent to the hotspots had higher disease levels than plants located farther from the hotspots. However, as the disease progressed, the steepness of the disease gradient decreased away from hotspots, and high levels of incidence and severity were observed throughout the plots. This trend was observed across the three locations, although there was higher severity in locations with higher relative humidity. Preliminary spatial analyses suggest that the hotspots are important in-field sources of inoculum. In addition to studying wheat blast epidemiology in the field, we quantified conditions for MoL-incited spike blast development under controlled conditions. Several growth chamber studies were conducted using split plot randomized complete block designs to quantify disease development as influenced by temperature (20, 25, 30° C), duration of high (100 %) relative humidity (0, 3, 6, 12, 24, 48 hours), infection timing (Feekes 10.1-3, 10.5, 10.5.1, 10.5.4), cultivar resistance, and inoculum concentration (2e2, 2e3, 2e4, 2e5 spores/ml). Preliminary results indicated that higher disease incidence and severity levels occurred at higher temperatures, longer durations of high relative humidity, and high spore concentrations. These results suggest that the conducive conditions for MoL may be very similar to those previously reported for MoT. Subsequent experiments and analyses will be conducted to determine whether interactions among the aforementioned factors are significant.

Keywords: disease gradient; hotspot; MoL; epidemiology

USING A BAYESIAN MODEL FOR ESTIMATING AIR BORNE INFECTION RISKS: WHEAT BLAST

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The effect of weather on plant diseases has been widely researched, and the ability to forecast meteorological events is able to offer valuable insights about the magnitude of an epidemic. In addition, better predictions of outbreaks that are more sensitive to fluctuations in weather can help in the establishment- of more suitable control practices. Wheat blast caused by *Magnaporthe oryzae* is a relative new disease that has been responsible for considerable damage on the wheat fields in several South American countries such as Brazil, Bolivia and Paraguay. Wheat blast outbreaks vary from year to year and location to location. The erratic occurrence of the disease can be attested, in some years, by comparing wheat fields varying on sowing dates. The erratic development of the disease is probably environmental in origin. The fungus *M. oryzae* produces light and dry hyaline conidia that may be removed by wind, from sporulating lesions, and transported over long distances. A study was carried out to examine the relationship of conidia density and weather factors. A spore sampler was prepared using microscopes slides coated with petroleum jelly, protected by a rain shelter, exposed to the air at 1.50 m height. The angle of the glass slides to wind was approximately 45º. The glass slides were replaced every 24 hours starting at 00:00 GMT from February 2, 2013 through June 7, 2014 in Passo Fundo, RS, Brazil. After being exposed, the slides were examined under light microscope and the number of conidia-like structures resembling those of *M. oryzae* were recorded. At the monitoring site, the weather variables such as temperature, relative humidity, wind, solar radiation, dew point temperature and rainfall were recorded at every hour. We used a hierarchical auto-regressive Bayesian model adjusted using MC-Stan software based on HMC U-Nuts, called from R via the RStan package. Our program generated posterior predictions which allow us to assess prediction uncertainty in the density of *M. oryzae* airborne inoculum.The hierarchical Bayesian model built here for estimating *M. oryzae* like conidia in the air showed that daily atmospheric temperature range (C), mean relative humidity (%), accumulated solar radiation (MJ/m^2) and accumulated rain (mm) appeared to be the meteorological variables of prime importance. This information may help growers reduce economic and environmental risk through eliminating unnecessary fungicide applications.

Keywords: *Triticum*; Brusone; forecasting; posterior estimation

DEVELOPING A RISK ASSESSMENT FOR PREDICTING WHERE MAJOR OUTBREAKS OF WHEAT BLAST COULD OCCUR IN THE UNITED STATES BASED ON THE PATHOGEN'S FREEZE-THAW TOLERANCE

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Wheat blast (*Magnaporthe oryzae Triticum*) has emerged as a serious problem for wheat production in South America. To prepare for the possible introduction of wheat blast in the United States, it would be helpful to identify areas of the country most at risk for blast epidemics. Therefore, the objective of the research was to quantify the freeze-thaw tolerance of this pathogen on naturally infected wheat rachises from Bolivia. Four treatments, plus a control, comparing moisture and number of freeze-thaw periods were analyzed. Treatment A was moistened and exposed to five freeze-thaw cycles, treatment B was moistened and experienced a five-day freeze with no thaw periods, treatment C was dry and exposed to five freeze-thaw cycles, treatment D was the control with no freeze periods, and treatment E was moistened and exposed to one freeze-thaw cycle. Each treatment and the control had five replicates which consisted of a glass plate, filter paper, five rachises, and an equal number of incubation hours. Several measurements were taken on the replicates including total spore count, total rachis length, total weight, and germination rate. The results indicate that freezing and thawing does have an effect on the fungus's ability to produce spores when the residue is moistened. Exposing dry residue to five freeze-thaw cycles had little effect on the ability of the fungus to produce spores relative to residues that remained unfrozen. When the residue was moistened however, 5 freeze-thaw cycles reduced the number of spores produced by 48% relative to the residue that remained unfrozen and 67% relative to the dry residue exposed to five freeze-thaw cycles. A single five-day freeze event had less of an effect on the sporulation of the fungus and produced 45% more spores than the moistened residue exposed to five freeze-thaw cycles. When moistened residue was exposed to a single freeze-thaw cycle, the sporulation was reduced by 21% relative to the residue that remained unfrozen. In conclusion, freezing and thawing of moistened residue significantly affected the pathogen's ability to produce spores.

Keywords: *Magnaporthe oryzae*; survival; wheat blast

A WEATHER-BASED SIMULATION MODEL FOR WHEAT BLAST INCIDENCE

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Wheat Blast incited by *Magnaporthe oryzae* is a serious disease of wheat causing yield failures and significant economic losses during epidemic years. The disease was first identified in 1985 in Paraná State of Brazil and has since spread to important wheat-producing regions of Brazil, Bolivia, and Paraguay. Despite the sporadic nature of wheat blast outbreaks, it is now considered a major threat to wheat production in this region. Warm temperatures and high humidity favor disease development. The pathogen infects all aboveground parts of the wheat plant, but spike infection is the main concern. The potential damage can account for 10 to 100% crop losses. Disease control is limited by lack of effective fungicides and resistant varieties. This study investigated relationships between climate and occurrence of wheat blast. Recorded incidence of wheat blast was obtained from fields trials carried out in 2009 at Itaberá, SP. The wheat blast susceptible cultivar IAC 370 was sowed on April 17th and May 5th of 2009, respectively. The plots measuring 3 x 10 meters were replicated six times. The plots were assessed for wheat blast incidence at 10, 18, 21, 25 and 32 days after spike emergence. Wheat blast was only observed in plots with late sowing date. A two-compartment model was used to predict wheat blast incidence. In the first compartment, inoculum potential is estimated based on the frequency of days considered favorable for sporulation. In the second, spike infection risk is estimated based on daily maximum temperature (>23 ^{o}C), temperature amplitude (>13 ^{o}C) and relative humidity (>70 %). The parameterized wheat model indeed reproduced the field observations. Finally, the wheat blast model was loosely coupled to the CSM-CROPSIM: WHEAT simulation model. The date of first spike emergence simulated by the wheat model launches the wheat blast model. A user friendly- application tool was created to run the model over multiple locations, years and sowing dates. Risk maps were produced from different years (2002-2014) and locations in the state of Paraná, Brazil. In general, the disease risk maps coincided with historical observations.

Keywords: *Triticum aestivum*; brusone; forecast; mapping

Author index