The Global Fusarium Initiative for International Collaboration

T. Ban, J.M. Lewis, and E.E. Phipps, editors

A Strategic Planning Workshop
held at CIMMYT, El Batan, Mexico,
March 14-17th, 2006,
with support from Government of Japan,
Japan-CIMMYT FHB project

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INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER

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Session 2: Fusarium Consortium

Session 3: International Scab Nursery Consortium

Foreword

Many institutions around the world have devoted substantial resources to combat *Fusarium* diseases on cereal crops, and have met a measure of success. However, the global community is facing the threat of imminent epidemics. Even worse, the mycotoxins produced by the *Fusarium* fungus cause acute food poisoning in people and farm animals that are fed infected grain. The risk to human and animal health and food security is a problem that concern both developed and less developed countries. Unless steps are taken to defeat the disease, this threat will materialize into a much greater problem and as such requires a global response.

Fusarium head blight (FHB) is a grave threat that requires an integrated research approach to overcome. The International Maize and Wheat Improvement Center (CIMMYT) is working to facilitate the development of a global platform for international collaboration on these *Fusarium* diseases including FHB and Fusarium crown rot (FCR). Our vision is that this platform will serve to streamline and aid the exchange of information, development of collaborative projects, germplasm enhancement and germplasm distribution. We recognize the need to enhance international relationships as a part of each national/international project and consortium. CIMMYT is taking a proactive stance to elevate the work of resistance breeding and to raise the profile of this global challenge.

The development of this Global Fusarium Initiative has been supported by the Government of Japan since 2004. The challenges and specific activities are based on the new paradigm which arose from the JIRCAS Workshop held in February 2004 in Tsukuba, Japan. The concept of the Global Fusarium Initiative was proposed and accepted at the 2nd International Symposium on Fusarium Head Blight, incorporating the 8th European Fusarium Seminar, 11-15 December 2004, Florida USA. A new global collaboration for consensus QTL mapping of FHB and FCR resistance in wheat, involving the world's most advanced FHB researchers, will be one of the activities. *Fusarium*, the pathogen that causes FHB, also causes FCR in Australia, Turkey and other places, another constraint on global wheat production. We have integrated research and germplasm enhancement for both FHB and FCR under the Global Fusarium Initiative. This initiative will encourage communication and cooperation among individuals, institutions and governments focusing on this disease.

It is our vision that this workshop will facilitate the development of a Global Fusarium Initiative to provide a platform for international collaboration on *Fusarium* research and facilitate information exchange, germplasm enhancement and the development of breeding methods and materials worldwide.

We will use the untapped potential of this global communication to combat these *Fusarium* diseases and contribute to international efforts in increasing grain availability and safety.

Masa Iwanaga
Director General
CIMMYT

Preface

Fusarium head blight (FHB, scab) and Fusarium crown rot (FCR) are important threats to sustainable wheat and barley production worldwide. The efforts to combat these diseases have been increasing around the world throughout numerous countries and research communities. The plan of this workshop was to join together these various research communities so that we, as a global community, can have greater impact and efficacy in our efforts against these diseases. With such a global platform we can support the exchange of information, collaborative research, development and exchange of germplasm enhancement, the development of breeding methods and other activities. CIMMYT has been conducting a holistic operation to enhance resistance to *Fusarium* diseases in wheat and barley germplasm through systematic and intensive screening in multiple environments and cutting-edge genetic research. Novel genetic variation for wheat is found among CIMMYT's genebank accessions and synthetic wheat derivatives. The FHB research at CIMMYT has been systemized in a simple workflow on four levels: evaluation of resistance in the field (phenotyping); genetic characterization by DNA markers (genotyping); gene discovery; and development of DNA marker assisted selection (MAS) for use in breeding. To raise the profile of and consolidate these efforts, CIMMYT organized an international workshop—the first in a series—to highlight the importance of *Fusarium* diseases, the status of collaborative efforts to address these diseases, and future prospects for international collaboration. The agenda involved discussion, planning, and prioritization for the most critical research needs, opportunities for web-based knowledge sharing, opportunities for international collaboration, and action plans in the following topics:

1. FHB-QTL consortium: Wheat FHB-QTL comparative study for the deployment of resistance genes, including the analysis of the bases of resistance, the development of an effective MAS system and the pursuit of germplasm enhancement.

2. Fusarium fungus consortium: Global compilation/monitoring system of genetic diversity, pathogenicity, and toxigenicity from studies on Fusarium fungi, to control FHB and FCR.

3. International scab nursery consortium: Development of new international interactive Fusarium resistance screening nurseries for germplasm enhancement and global compilation of Genotype x Environment x Management effects on resistance to FHB/FCR.

This workshop was an invaluable opportunity to exchange ideas regarding the current status of FHB/FCR research around the globe.

Hans J. Braun

Director, Global Wheat Program, CIMMYT

Acknowledgments

The editors and workshop beneficiaries would like to thank the Government of Japan for its financial support through the Japan-CIMMYT FHB collaborative research project, and The Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF) which made this strategic planning workshop and attendant proceedings possible. We would like to acknowledge the contribution of Japan International Research Center for Agricultural Sciences (JIRCAS) through the collaborative projects. We wish to give great thanks for the contributions of all of the participants who willingly shared data, the latest status of research, experience and your thoughts on the development of targeted consortium within the Global Fusarium Initiative. We learned much from your excellent presentations and benefited greatly from the many discussions during the workshop. We are happy to have achieved fruitful outcomes and action plans to launch the Global Fusarium Initiative.

Tomohiro Ban and Richard R. Ward,
Workshop Co-organizers, CIMMYT

GLOBAL FUSARIUM INITIATIVE FOR INTERNATIONAL COLLABORATION IN GENETIC STUDIES AND BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT AND BARLEY

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ABSTRACT

Fusarium head blight (FHB) is a grave threat that we will only overcome if we integrate all our wisdom and expertise. Many institutions around the world have devoted substantial resources to combat FHB, and have met with a measure of success. However, the global community is facing the threat of imminent epidemics. Unless steps are taken to strategically combat the disease on a global scale, this threat will materialize into a much greater problem and as such requires a global response. CIMMYT has adopted a holistic approach to enhance novel FHB resistance among genebank accessions and synthetic wheat derivatives and combine their resistance using systematic screening in multiple environments and genetic characterization by DNA markers.

For this reason, CIMMYT is facilitating global communication through the Japan-CIMMYT FHB project supported by the Japanese government since 2004. The challenges and specific activities are based on the new paradigm which arose from the JIRCAS Workshop held in February 2004 in Tsukuba (Ban, 2004). The concept of the Global Fusarium Initiative was proposed and accepted at the 2nd International Symposium on Fusarium head blight, incorporating the 8th European Fusarium Seminar, 11-15 December 2004, Florida USA (Van Ginkel and Ban, 2004). A new global collaboration for consensus QTL mapping of FHB resistance in wheat, involving the world's most advanced FHB researchers, will be one of the activities. *Fusarium* fungi, the pathogen of FHB, also causes Fusarium crown rot (FCR) in Australia, Turkey and other places, and is another constraint to global wheat production. We have integrated research and germplasm enhancement for both FHB and FCR under the Global Fusarium Initiative. This initiative will encourage communication and cooperation among individuals, institutions and governments focusing on this disease.

CIMMYT's role in the Global Fusarium Initiative is to provide a platform for international collaboration on *Fusarium* research, and facilitate information exchange, germplasm enhancement and the development of breeding methods and materials globally. This Global Fusarium Initiative will encourage communication and cooperation among individuals, institutions and governments focusing on this disease. Specific activities will be linked using a web site and on-site forums (http://www.fusarium-net.org). Global Genotype x Environmental meta-data compilation, updated global information, and the development of a global crop information system on FHB data will be features of this web site. The Global Fusarium Initiative provides the platform to fight this grave threat. We are leading a new paradigm for international cooperation and collaborative research to combat the disease, which will contribute to the reduction of poverty and hunger world-wide.

The challenges of this initiative are:

- Identification of new sources of resistant germplasm and pre-breeding
- Delineation of the nature of wheat resistance to FHB and host-pathogen interaction
- Development of effective cropping systems adjusting pathogen cycle and wheat growth

- Germplasm sharing and intellectual property rights (IPRs) management
- Knowledge sharing among the global community

The specific activities of this initiative are:

- Linking with relevant *Fusarium* research activities
- Website and e-News, http://www.fusarium-net.org
- Global compilation of Genotype x Environment x Management meta-data through new international interactive screening nursery system
- Up to date global information on FHB epidemics, toxins and resistant breeding
- Biennial meetings for information sharing and focused discussion
- Global Crop Information System on FHB

We aim to acquire potentially novel sources of resistance from global hotspots through our widespread contacts. Expectations are high that useful resistance genes may be identified during the screening of germplasm accessions and that the effects of Genotype x Environment x Management interactions and the distribution of *Fusarium* isolates will be better understood. In addition, we are working to develop a compilation/monitoring system for *Fusarium* genetic diversity, pathogenicity, and toxigenicity to further our abilities to control FHB.

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MARKER-ASSISTED SELECTION FOR FHB RESISTANCE IN WHEAT

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ABSTRACT

Wheat varieties with a higher level of resistance to Fusarium head blight (FHB) would make a substantial contribution to reducing the losses from this devastating disease. With the recent establishment of the USDA-ARS Small Grains Genotyping Centers in the U.S., we have increased our efforts in marker-assisted selection (MAS) for FHB resistance. During 2005 we screened more than 5,000 F_2 or F_3 individuals for their genotype at *Fhb1*, the major quantitative trait locus (QTL) on chromosome 3BS. This will increase to more than 7,000 individuals in 2006. Because additional loci (e.g. high molecular weight glutenins, leaf rust resistance) are also being subjected to MAS in the same individuals, we are practicing allele enrichment, i.e. selection against the homozygous undesirable types, as a means of maintaining an adequate population size for subsequent selection. In the future, a greater emphasis will be placed on screening BC_1F_1 individuals with markers. Enriched populations will undergo phenotypic selection for FHB resistance, and other yield, disease resistance, and end-use quality testing necessary to produce FHB resistant germplasm and variety candidates. We believe that substantial efforts in phenotypic assessments for FHB resistance will still be necessary, even with an increase in MAS for this trait, because there are likely numerous "minor" effect genes that need to be combined with the major QTLs in order to obtain the desired level of resistance.

There is a need to characterize additional FHB resistance genes and to identify associated diagnostic markers. Our germplasm screening and QTL mapping efforts are focused on materials that do not contain the *Fhb1* QTL. Tightly linked markers at the *Fhb1* locus are being used to identify this germplasm. This increases our chances of finding novel, major QTL, versus finding yet another source of resistance containing *Fhb1*. Although other QTLs for FHB resistance have been located using DNA markers (e.g. loci on 5AS and 6BS), they are not as suitable for widespread use in MAS because of lack of polymorphism of the markers and/or insufficient linkage disequilibrium between the markers(s) and QTLs. A global consortium could assist in the prioritization of germplasm for genetic studies and development of diagnostic markers for new and existing QTLs.

EUREKA CEREQUAL: RESEARCH STRATEGIES TOWARDS IMPROVING WHEAT QUALITY BY RESISTANCE TO FUSARIUM HEAD BLIGHT (FHB)

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OBJECTIVES

This collaborative project between a private plant breeding company and two scientific institutions has the following objectives: (1) Mapping and validation of quantitative trait loci (QTL) for resistance to Fusarium head blight (FHB) in two winter wheat and two spring wheat populations, (2) Enrichment of the QTL regions with AFLP and SSR markers and development of appropriate STS markers, (3) Comparison of phenotypic with marker-based selection for FHB resistance in one spring- and one winter-wheat population, (4) Determination of the correlated response for reduction in deoxynivalenol (DON) content of the grain.

INTRODUCTION

FHB is one of the most destructive diseases in small-grain cereals. In Europe, the damage is mainly caused by the pathogens *Fusarium graminearum* and *F. culmorum*. Infections lead to severe yield losses, poor grain quality, and contamination with mycotoxins in the grain, especially with DON. Resistance to FHB is quantitatively inherited. The overall aim of the project was to improve the breeding progress by using molecular markers that can be applied to practical plant breeding.

MATERIALS AND METHODS

Two populations of spring wheat (CM82036 [resistant, Sumai 3/Thornbird-S] x Remus [susceptible], Frontana [resistant] x Remus) and two populations of winter wheat (G16-92 [resistant] x Hussar, Dream [resistant] x Lynx) have been tested for FHB by artificial inoculation with *Fusarium culmorum* in multiple environments. Genomic constitution was analysed with microsatellite and AFLP markers. In spring wheat, three QTL were located on chromosomes 3A, 3B and 5A contributing between 15 and 32% of the phenotypic variance in the respective mapping populations (Buerstmayr et al. 2003, Steiner et al. 2004).

For establishing a broad-based selection project, a spring- and a winter-wheat double cross with the above mentioned resistance donors, but different susceptible crossing partners (Nandu, Munk for spring wheat, LP-strain and Brando for winter wheat) were used as source materials. In spring and winter wheat, 1,200 and 600 F_1 plants, respectively, were tested in multi-locational infection trials and selected for their FHB resistance in a two- and one-step procedure, respectively. The 20 best lines were slightly less diseased than the resistant parents. Moreover, they were significantly less contaminated by DON than moderately and highly susceptible genotypes (Wilde and Miedaner 2006). They were crossed in a factorial design and selfed. In parallel, both source populations were analysed for each of three donor QTL alleles that have been associated with FHB resistance (6A and 7B from Dream, 2B from G16-92, 3B and 5A from CM82036 and 3A from Frontana) by one to two single-sequence repeat (SSR) marker per QTL. Across selfings, all eight expected donor QTL alleles and allele combinations were found. In 2004, the populations selected by phenotype and markers, respectively, were grown together with the source populations and standard varieties at four locations. Inoculum of two highly aggressive, DON-producing isolates of *Fusarium culmorum* (FC 33, FC 46) was

applied with density of 5 x 10^5 spores ml^{-1}. To consider the variation in flowering date between entries, all genotypes including the parents were inoculated four times. Accordingly, FHB rating was assessed three times by rating the percentage of infected spikelets per plot (0-100). For data analysis, the arithmetic mean of all three ratings was used. In spring wheat, FHB ratings were adjusted to heading date by multiple regression analysis (adjusted FHB rating).

RESULTS AND DISCUSSION

In each of the two winter wheat populations, QTL for combined resistance to initial infection and spread within plant tissue were detected (Schmolke et al. 2005). In the Dream/Lynx population, two main FHB resistance QTL were detected on chromosomes 6AL and 7BS which explain 19% and 21% of the phenotypic variation. QTL on chromosome 6AL overlapped with a QTL for plant height. Additionally, minor QTL were identified on chromosomes 2BL and 1B. In the G16-92/Hussar population, a major QTL was detected on chromosome 2BL explaining 17% of the phenotypic variation. Another QTL was found on chromosome 1A explaining 14% of the phenotypic variation and overlapping with a QTL for plant height.

For validation of the identified resistance QTL, near-isogenic lines were created by backcrossing and selfing combined with marker-based selection. Selected lines of the BC_2S_3- and BC_2S_4- generation were tested for FHB resistance in field trials at four and three environments in 2005, respectively. In the Dream/Lynx population, the main resistance QTL on chromosomes 6AL and 7BS had significant effects on FHB severity, between 7% and 10%. Among the genotypes for the QTL validation are some lines carrying one or both of the resistance alleles, which are of agronomic interest combining short plants with a good to medium FHB resistance. The minor QTL had no significant effects on FHB severity. In the G16-92/Hussar population, the detected QTL could not be validated due to loss of resistance probably during backcrossing of the lines. A new backcross population will be developed and investigated following this project.

Most exotic and adapted donor QTL could be verified in elite wheat background (Table 1). Even in the case where the individual QTL allele showed no effect compared to the class without any donor QTL allele it had a positive additive effect in combination with other QTL. Highest effects were found for each combination with donor QTL 6A or 7B in winter wheat and the combination of donor QTL 3B and 5A in spring wheat. Effects were clearly smaller for the adapted winter wheat QTL than for those originating from CM82036 or Frontana.

Marker-based selection on the donor QTL 3B and 5A in spring wheat resulted in an indirect selection gain on reduced DON content in the grain (Figure 1). Donor QTL 3A from Frontana did not contribute to this effect. Individual bulks within the respective QTL class nevertheless showed a large significant (P=0.05) variation in both traits. This is most likely caused by additional QTL alleles for FHB resistance in the donors or the susceptible elite cultivars (for the class without donor QTL alleles) not followed by molecular markers.

Table 1. Means and effects of eight QTL classes for adjusted FHB rating after inoculation of 12 to 15 $F_{3:5}$ bulks per class by *Fusarium culmorum* across four locations in 2004

Winter wheat			Spring wheat		
QTL class	FHB rating	Effect[1]	QTL class	FHB rating	Effect[1]
	%			%	
2B + 6A + 7B	18.7 a[2]	**10.6**	3B + 5A + 3A	14.2 a	**17.3**
6A + 7B	19.0 a	**10.4**	3B + 5A	16.2 ab	**15.3**
2B + 6A	18.0 a	**11.3**	3B + 3A	20.6 b	**10.9**
2B + 7B	21.1 ab	**8.2**	3A + 5A	19.1 ab	**12.4**
2B	22.4 ab	**6.9**	3B	21.1 bc	**10.4**
6A[3]	23.7 ab	**5.6**	5A	21.4 bc	**10.1**
7B	26.6 bc	**2.7**	3A	26.6 cd	**4.9**
Susceptible	29.3 c	-	Susceptible	31.5 d	-

[1] Difference to the susceptible class.
[2] Different letters mark significant differences (P=0.05).

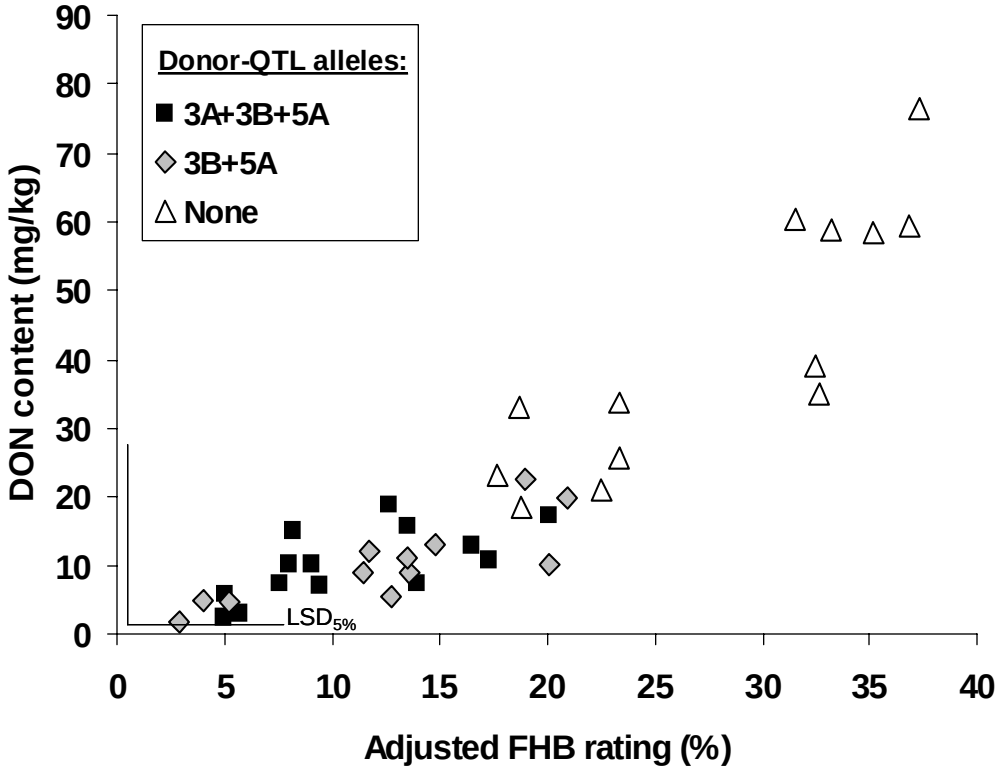

Figure 1. Association between adjusted FHB rating and DON content of $F_{3:5}$ bulks selected for the given donor QTL alleles by SSR markers and tested phenotypically by inoculation with *Fusarium culmorum* across three locations in 2004

Substantial gain from phenotypic and marker-based selection for FHB resistance was found in the spring and winter wheat population. Higher gain from selection could be achieved with exotic donor QTL from spring wheat compared to the adapted QTL from winter wheat. Their introgression also led to an indirect selection gain for DON content in the grain in both selection variants. Application of DNA markers almost doubled the realized selection gain per year in spring wheat, but not in winter wheat. Here, both variants had a similar selection gain per year. Economically, the marker-based selection is cheaper than the phenotypic selection when only the major QTL for FHB resistance are followed. For introgression of exotic donors, however, a background selection for the genome of the elite parent would be highly recommendable. Caused by the high genetic variation within the best marker-based selected variants additional phenotypic selection is useful to achieve the maximum selection gain.

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MOLECULAR MAPPING OF QTLS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN ASIAN WHEAT

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OBJECTIVES

1) To identify the quantitative trait loci (QTLs) for type II FHB resistance in different Asian cultivars.
2) To identify closely linked molecular markers to these QTLs for marker-assisted breeding.
3) To elucidate genetic relationship between QTLs from different sources.

INTRODUCTION

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, is an economically important disease of cereal crops worldwide. FHB can cause dramatically reductions in grain yield and grain quality (Bai and Shaner, 2004). The accumulation of mycotoxins in the infected grain has become a major concern for human health and animal production (Bai and Shaner, 2004).

Using resistant wheat cultivars is the most cost-effective and environmental safe method to reduce losses caused by FHB. Different types of FHB resistance have been described (Bai and Shaner, 2004). Resistance to initial infection (type I) and to FHB symptom spread within an infected spike (type II), and low deoxynivalenol (DON) accumulation in infected grain (type III) have drawn more breeders' attention. Type II is a more stable type of resistance in most of the resistant cultivars identified to date (Bai and Shaner, 2004). Many wheat cultivars or landraces from Asia have been reported to have reasonable type II resistance (Yu et al., 2006). However, some of the QTLs in these cultivars have not been well characterized.

MATERIALS AND METHODS

Plant materials

Four populations of recombinant inbred lines (RILs) were developed by single seed descent from the crosses Wangshuibai/Wheaton (Zhou et al, 2004), Ning 7840/Clark (Bai et al., 1999), Chokwang/Clark (Yang et al., 2005) and CS-SM3-7ADS/Annong 8455 (Ma et al., 2006a). Wangshuibai is a Chinese landrace with a high level of FHB-resistance. CS-SM3-7ADS is a Chinese breeding line highly resistant to FHB that was derived by replacing chromosome 7A of Chinese Spring with the corresponding chromosome from Sumai 3 (Zhou et al., 2002a). Ning 7840 is a Chinese FHB-resistant cultivar derived from Sumai 3, while Chokwang is a moderately FHB-resistant cultivar from Korea (Yang et al., 2005). Wheaton, Clark and Annong 8455 are all highly susceptible to FHB.

Evaluation of FHB and DON content

All RILs were evaluated for FHB resistance in several greenhouse experiments by inoculating with conidiospores of *F. graminearum*, GZ 3639, a field isolate from Kansas. Type II resistance was measured by injecting 1000 conidiospores of isolate GZ 3639 into a central floret of a spike at anthesis with a syringe. The RILs were prepared for inoculation as following: after vernalization at 4ºC in a growth chamber for eight weeks, six seedlings were transplanted into a 5'x 5' tora pot (Hummert Int., St. Louis, MO) containing Metro-mix 360 (Hummert Int., St. Louis, MO), and grown in a greenhouse bench with 12-h daylight period. All plants in each pot were inoculated with a single head per plant, and incubated in a moist chamber for three days to initiate infection. The inoculated plants were then moved to the original

bench positions and grown at 25ºC during the day and 22ºC during the night. The infected and total spikelets in a spike were counted at 21st day after inoculation and the proportion of symptomatic spikelets (PSS) was calculated for the final disease severity. DON content was determined by direct quantification of DON in the harvested kernels of *Fusarium* inoculated spikes using high-pressure liquid chromatography (HPLC) and electrospray ionization mass spectroscopy (ESI-MS) (Mirocha, et al. 1998).

Marker analysis

DNA was isolated from leaf tissue using CTAB method (Saghai-Maroof *et al*. 1984). AFLP and SSR analyses followed Bai *et al*. (2003). For SSR detection, an 18bp M13 tail sequence (5'-ACGACGTTGTAAAACGAC) was added at 5'-end of each forward SSR primer and an additional M13 primer was labeled with IRdye-700 or IRdye-800 (Li-Cor, Inc. Lincoln, NE). To amplify SSRs, a touchdown PCR profile started at 95°C for 5 min, followed by 5 cycles of 45 s at 95°C, 5 min at 68°C, and 1 min at 72°C with the annealing temperature that was lowered by 2°C in each following cycle; for another 5 cycles, the annealing time was 2 min and the temperature was lowered 2°C in each following cycle; in the last 25 additional cycles, the annealing temperature was constant at 50°C with 5 min at 72°C for a final extension. AFLP was analyzed in a Li-Cor 4200 DNA Sequencer (Li-Cor, Inc. Lincoln, NE) and scored by visual inspection. SSR was analyzed in either a Li-Cor 4200 DNA Sequencer or ABI 3100 DNA Analyzer (Applied Biosystems, Foster City, CA).

Data analysis

Linkage maps were constructed with JoinMap 3.0 (van Ooijen & Voorips, 2001) with the Kosambi mapping function (Kosambi, 1944). A minimum logarithm of odds (LOD) threshold of 3 was used for determining linkage groups. Simple interval mapping (SIM) and composite interval mapping (CIM) were performed using average values over each individual experiment and on the overall average across all experiments by using Qgene (Nelson 1997), MapQTL (van Ooijen & Voorips, 2004) or Cartographer 2.0 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). The threshold of the LOD score for declaring the significance of a QTL was determined by a 1,000-permution test. Determination coefficients (R^2) for each QTL were calculated through multiple linear regressions of the QTLs on the phenotype data using SAS REG procedure.

RESULTS AND DISCUSSION

QTLs for type II resistance and low DON content in Ning 7840

Type II FHB resistance of 133 RILs derived from cross Ning 7840/Clark was evaluated in four greenhouse experiments (Bai et al, 1999). One major QTL and several minor QTLs were identified (Bai et al 1999, Zhou et al 2002b). The major QTL on 3BS explained up to 50% phenotypic variation for type II resistance (Bai et al 1999, Zhou et al 2002b) and 25% for low DON (Bai et al, 2000). It is a stable QTL, was detected in all four experiments and physically mapped between breakage points 3BS-3 and 3BS-8 (Zhou et al 2002b). Several AFLP and SSR markers were identified in the QTL region. Markers *Xgwm533*, *Xgwm493* and *Xbarc147* are the closest SSR markers for the QTL. In addition, one closely linked AFLP marker linked to the major QTL was converted into a sequence tagged site (STS) marker (Guo et al, 2003). These SSR and STS markers have been used in marker-assisted selection (MAS) for the major QTL in many breeding programs.

Beside the major QTL on 3BS, additional QTLs with minor effects were also identified on chromosome 2BL and 2AS for type II resistance using SSR markers (Zhou et al, 2002b). More recently, a number of resistance gene analog (RGA) markers were screened and five RGA markers were identified as associated with FHB resistance in the Ning7840/Clark population (Guo et al, 2006). Three of them associated with a QTL on chromosome 1AL that explained 12% phenotypic variation. One of the RGA markers on 1AL was converted into a STS marker. Significant positive interaction was detected between QTLs on 3BS and on 1AL. This STS marker can be used in MAS to pyramid QTL on 1AL with others.

QTLs for type II resistance in Chinese Spring-Sumai 3-7A-disomic substitution line

Chinese Spring is a moderately resistant landrace from China. Its Sumai 3-7A-disomic substitution line (CS-SM3-7ADS) showed the same high resistance as Sumai 3 (Zhou et al, 2002a). A population of 97 RILs was developed from the cross CS-SM3-7ADS/Annong 8455 and evaluated for type II resistance in the greenhouse (Ma et al 2006a). The result indicated that CS-SM3-7ADS carries FHB-resistance alleles at five QTLs on chromosomes 2D, 3B, 4D and 6A. One QTL on 3BS had the largest effect, and explained 30.2% of the phenotypic variance for type II resistance and was located at the same location as that in Sumai 3 and Ning 7840. In addition, several susceptible factors were mapped on chromosomes 1A, 1D, 4A and 4B of CS-SM3-7ADS. No QTL for enhanced FHB resistance was detected on chromosome 7A of CS-SM3-7ADS,

suggesting the increased FHB resistance in CS-SM3-7ADS was not due to any major FHB-resistance QTL on 7A of Sumai 3, but more likely due to removal of susceptible factor(s) on 7A of Chinese Spring. Further evaluation of a set of ditelosomic lines derived from Chinese Spring for FHB resistance indicated that ditelosomic lines DT1AS, DT2AS, DT3AS, DT3BL, DT6BL, DT1DL and DT1DS had a significant greater FHB than Chinese Spring; whereas lines, DT7AL, DT3BS, DT6BS, DT7BL, and DT4DL had a significantly lower FHB than Chinese Spring (Ma et al 2006b). The results suggested that some wheat cultivars may have both FHB-resistance QTLs and susceptible factors. In breeding practice, adding FHB-resistance-enhancing QTLs or removal of susceptible factors may both significantly increase the level of wheat resistance to FHB in a wheat cultivar.

FHB resistance in Wangshuibai

Wangshuibai is a FHB-resistant Chinese landrace unrelated to 'Sumai 3' based on marker data (Bai et al, 2003). A mapping population of 139 RILs was developed from the cross Wangshuibai/Wheaton (Zhou et al 2004). After screening about 1300 simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers, we detected seven QTLs on chromosome 3BS, 1A, 5AS, 5DL, 7AL, and 3DL for type II resistance, and six of them were also associated with lower DON content except the one on 3DL (J-B. Yu et al, unpublished data). These QTLs jointly could explain as much as 63.5% of phenotypic variation for type II resistance and 48.0% for low DON. The QTL on 3BS showed major effect on both type II resistance and low DON and was stable across all four experiments. However, its effect on type II resistance appears to be smaller than that in Ning 7840. This QTL in both Ning 7840 and Wangshuibai was mapped in the same region of 3BS and is most likely the different alleles of the 3BS QTL.

A new QTL in Chokwang

Chokwang is a moderate FHB-resistant cultivar from Korea. A population of 79 RILs were derived from the cross Chokwang/Clark in Purdue University (Yang et al, 2005). The population was screened with both SSR and target-region amplified polymorphism (TRAP) primers. One major QTL, *Qfhb.ksu-5DL1,* was identified on chromosome 5DL. The SSR marker *Xbarc* 239 was mapped in the QTL region, and also physically located to the bin of 5DL1-0.60-0.74 by using Chinese Spring deletion lines. Major QTL was detected on 5DL suggested that Chokwang contains a new QTL for FHB resistance that is different from the one on 3BS of Sumai 3 or Ning 7840. A second QTL *Qfhb.ksu-4BL1* linked to SSR *Xbarc*1096 was tentatively mapped on 4BL. In addition, a minor QTL (*Qfhb.ksu-3BS1)* was detected on 3BS with marginal significance in Wangshuibai and mapped on the same location as that in Ning 7840.

In summary, resistant and moderately resistant cultivars from Asian sources usually carry one major QTL and several minor QTLs for type II resistance. The QTL on 3BS is a consistent QTL for type II resistance in four FHB resistant cultivars, but variation in effects on type II resistance was observed among cultivars: major effects in Wangshuibai, Ning 7840 and Chinese Spring, and minor effect in Chokwang. This QTL is most likely allelic among cultivars studied. The chromosome locations of three QTLs were in common between two cultivars: a QTL on 1A and another near centromere of 3BS presented in both Wangshuibai and Ning 7840, and the QTL on 5DL showed a minor effect in Wangshuibai and a major effect in Chokwang. Minor QTLs on 2A, 2B and 1A are unique in Ning 7840; QTLs on 4D, 6A are unique in Chinese Spring; QTL on 3D, 5A and 7A are unique for Wangshuibai; and QTL on 7B is unique for Chokwang. QTLs for low DON content were usually overlapped with QTLs for FHB resistance. Several QTLs for FHB susceptibility were identified in Chinese Spring, suggesting that removing susceptible QTLs can be a useful strategy for improving FHB resistance in wheat cultivars.

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QTL FOR THE RESISTANCE TO WHEAT FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL ACCUMULATION IN WANGSHUIBAI UNDER FIELD CONDITIONS

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ABSTRACT

Wheat Fusarium head blight (FHB) may cause serious losses in grain yield and quality. Production of deoxynivalenol (DON) by *Fusarium graminearum* in infected grain is detrimental to livestock and is also a safety concern in human foods. Cultivation of cultivars with resistance to FHB and DON accumulation is the most effective strategy for disease control. Wangshuibai is a Chinese landrace with a high level of resistance to FHB and DON accumulation, and an F_7 population of recombinant inbred lines (RILs) derived from the cross between Wangshuibai and susceptible cultivar Annong 8455 was developed for molecular mapping of quantitative trait loci (QTL) for the resistance to FHB and DON accumulation. Proportion of scabbed spikelets (PSS) and DON content were assessed under the field conditions over two years. Three hundred fifty-three SSR and AFLP markers were mapped on 38 linkage groups covering a genetic distance of 1594 cM. Composite interval mapping (CIM) revealed that two and three QTL were significantly associated with low PSS and low DON content, respectively, over two years. QTL on chromosome 3B and 2A explained 17% and 11.5% of the phenotypic variance for low PSS, respectively, whereas QTL on chromosome 5A, 2A and 3B explained 12.4, 8.5 and 6.2% of the phenotypic variance for low DON content, respectively. The 3B QTL appeared to be associated mainly with low PSS, and the 5A QTL primarily with low DON content in Wangshuibai. The 2A QTL had minor effect to both low PSS and DON content. The SSR markers linked to these QTL should be useful for marker-assisted selection (MAS) of QTL for low PSS and low DON content from Wangshuibai.

RESEARCH ON MOLECULAR MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT AT IFA-TULLN, AUSTRIA

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ABSTRACT

At IFA-Tulln we have been working on searching for resistance sources, genetic analysis of promising sources, improvement of phenotypic resistance evaluation methods, and the role of fungal toxins in the plant-pathogen interaction for more than 10 years.

We generated several recombinant mapping populations (DHs or RILs) in order to allow for replicated resistance testing using artificial inoculation. Possibly the most important factor for successful QTL mapping is the accurate phenotyping of the lines. We therefore put much emphasis on this aspect by performing artificial inoculation in replicated (min. 2 seasons) experiments. We usually sow replications within each experiment in staggered time intervals. This allows each genotype to reach anthesis and to be inoculated at slightly different micro-environmental conditions within the same experiment. Under our conditions about 2 weeks sowing difference will result in about 2 days flowering difference for winter wheat and about 7-9 days sowing difference will result in 2 days flowering difference for spring wheat.

In several of our projects we applied different inoculation methods to evaluate for components of resistance, like single-spikelet inoculation to test for resistance to fungal spread and spray inoculation to test for 'field' resistance.

For molecular genetic analysis we used SSRs and AFLPs in the past. SSR markers allowed anchoring of linkage groups relative to published maps (e.g. in the graingenes database, http://wheat.pw.usda.gov). With AFLP markers we could generate of many marker data in relatively short time and at reasonable cost.

The first population we analysed was derived from the cross CM-82036/Remus, the second was Frontana/Remus. CM-82036 (a line selected at CIMMYT from Sumai-3/Thornbird) appeared to carry two major QTL for disease severity under field conditions mapping to chromosomes 3BS (*Qfhs.ndsu-3BS*, *Fhb1*) and 5A (*Qfhs.ifa-5A*) (Buerstmayr et al. 2002, 2003). The QTL on 3BS of CM-82036 confers resistance to fungal spread and is the same locus found in a lot of mapping studies based on Asian resistance sources including Sumai#3, Ning 7840 and W14 (e.g. Anderson et al. 2001, Zhou et al. 2002, Chen et al. 2006). The 5A QTL appears primarily involved in resistance to fungal penetration (Buerstmayr et al. 2003, Chen et al. 2006). In a recent study, Lemmens et al. (2005) found that the FHB resistance QTL at 3BS co-localizes with the ability to detoxify the mycotoxin deoxynivalenol.

Compared to CM-82036, Frontana showed more QTL with smaller individual effects of which those on 3A and 5A appeared to be relatively stable (Steiner et al. 2004). In an independent Frontana derived population (Frontana/Seri82) only the QTL on chromosome 3A was consistent with the Frontana/Remus population (Mardi et al. 2006).

In a population from the cross Wangshuibai/Seri82 two QTL were detected, where the major resistance factor mapped near *Qfhs.ndsu-3BS* (Mardi et al. 2005). Unfortunately, different Wangshuibai derived mapping studies in the literature showed only moderate agreement with our results and with each other, apart from the QTL on 3BS. Reasons for the non-agreement between independent mapping studies may be manifold including the use of different seed stocks of the resistant line, the different susceptible parents used, and different inoculation and testing methods applied.

In an attempt to develop higher resolution maps for *Qfhs.ifa-5A* (Buerstmayr et al. 2003) we found severely suppressed recombination in the 5A QTL

region, making fine mapping very difficult. On the other hand several SSR markers appear tightly linked to the QTL, which is advantageous for marker assisted selection (unpublished results).

More recently, we were also involved in QTL analysis using the European winter wheat cross Dream/Lynx (Schmolke et al. 2005). The results indicate that European winter wheat possesses different QTL for FHB resistance than Asian or South-American sources, opening the possibility of resistance gene pyramiding. In a marker assisted selection study for three QTL (3B, 5A, 3A) using spring wheat crosses, all three QTL showed significant effects in reducing FHB severity and DON content (Miedaner et al. 2006).

Currently ongoing projects in FHB resistance involve the use of more distantly related resistance sources like *Triticum macha* for bread wheat and *T. dicoccoum* or *T. dicoccoides* for durum wheat by using advanced back-cross QTL mapping approaches (Tanksely and Nelson 1996).

In addition we work on a project to identify differentially expressed genes involved in the disease response by applying cDNA-AFLP on near isogenic lines for the FHB resistance QTL on 3BS and 5A.

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EVIDENCE THAT RESISTANCE TO FUSARIUM HEAD BLIGHT AND CROWN ROT ARE CONTROLLED BY DIFFERENT GENES IN WHEAT

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ABSTRACT

To test whether resistance against the two diseases are conditioned by the same genes, we carried out two experiments. Firstly, Fusarium head blight (FHB) and crown rot (CR) were assessed in a common set of hexaploid wheat genotypes using one aggressive isolate each of *F. graminearum* and *F. pseudograminearum*. A clear correlation between CR and FHB severity was not detected for either isolate. In the second experiment we created and analyzed populations segregating for the 3BS allele of Sumai 3, a major FHB resistant locus. As expected, plants with the Sumai 3 allele showed significant reduction in FHB severity. However, the presence of the 3BS locus showed no effect on CR resistance. These results provide evidence that, although FHB and CR are caused by the same pathogens, different host genes may control resistance to these two diseases that infect different tissues.

INTRODUCTION

Fusarium pathogens cause two serious diseases in wheat. Of these, FHB has been intensively studied internationally, while CR, which is a major constraint to wheat production in Australia, has received less attention. Some of the outcomes of the international effort include the screening of many thousands of wheat genotypes and the identification of several lines with high levels of resistance (Gilchrist et al. 1997; Lu et al. 2001). Although *F. graminearum* predominantly causes FHB and *F. pseudograminearum* is the most common CR pathogen, recent studies show that both pathogens can cause FHB and CR. This raises the question whether the same genes confer resistance to the two diseases. The FHB resistant genotypes could potential be invaluable in our effort to breed crown rot resistant cultivars if the same genes are involved in resistance to these two diseases. This paper reports on two experiments to address this: in one, a set of hexaploid wheat genotypes was inoculated separately using an aggressive isolate each of *F. graminearum* and *F. pseudograminearum*; in the second experiment the progeny of two populations segregating for a major FHB resistance locus on the chromosome arm 3BS were analyzed. Results from these two experiments were summarized in this paper.

MATERIALS AND METHODS

Plant material

In the first experiment, 24 genotypes were used. FHB bioassay was carried out in a controlled environment facility at the CSIRO Plant Industry Brisbane Laboratories, with 25/15^{o}C day/night temperature and 65/95% relative humidity, and a 13 -hour photoperiod. A *F. pseudograminearum* (CS3096) and *F. graminearum* isolate (CS3255) from CSIRO collection were used. These two isolates are highly aggressive according to a screening of over 650 isolates collected in field surveys from Queensland and NSW (Akinsanmi et al. 2004). Each replicate consisted of two plants in two separate pots and two replicates were used for each isolate. Eight to ten spikes from each of the two replications were inoculated using a modified "cotton wool" method developed at CIMMYT. At anthesis a 3mm filter paper saturated with inoculum (about 10µl suspension of 10^5 conidia/mL) was placed into the fourth spikelet from the tip of a spike. The inoculated spikes were immediately covered with moistened polythene bags for 48 hours and then with a paper bag until disease assessment at 21 days after inoculation. The FHB severity was measured as (a) the

average number of infected spikelets (NIS) below the point of inoculation and (b) the proportion of infected spikelets (PIS) from a counting of infected and total number of spikelets. As susceptible genotypes often rapidly develop bleaching of spikelets above the point of inoculation, we, as suggested by Buerstmayr et al. (2002), excluded these spikelets from data summary and analysis. The same two isolates were used in a glasshouse bioassay to determine CR resistance of the 24 genotypes. The glasshouse is maintained at 24/15°C day/night temperature with natural illumination. Three replications with five seedlings each were tested for each genotype-isolate combination. Ten-day-old seedlings were inoculated by placing a 10µl droplet of inoculum (10^6 conidia/mL) on the stem, 0.5 to 1cm from the soil surface. The inoculated seedlings were kept in a humidity chamber for 48 hours then transferred back to the glasshouse. CR severity was assessed as the length of discolored stem at 35 days after inoculation. Data were analyzed with t-tests using Microsoft Excel and arithmetic means were compared using Duncan's multiple range test.

In the second experiment, two 4-way F2/3 populations were used:
(A) Baxter/3/Lang//EGA Wylie/Sumai 3
(B) Drysdale/3/EGA Gregory//EGA Wylie/Sumai 3

In this experiment, FHB and CR assays were carried out following conditions described above using the *F. pseudograminearum* isolate CS3096.

Identification of individuals with or without the FHB resistant 3BS allele of Sumai 3 by molecular marker analysis
DNA was isolated from all individuals of the two populations and their parents. Leaf tissue was collected during controlled environment facility and glasshouse trials. For each genotype, a small section of a young leaf was ground with 200 µl DNA extraction buffer (100mM Tris-HCl pH8.5, 100 mM NaCl, 50 mM EDTA pH8.0, and 2% SDS) and incubated at 65°C for 1-2 h. The sample was extracted once with phenol/chloroform and DNA was precipitated with ethanol and dissolved in 100 µL of 1 x TE. Aliquots of initial extractions were diluted in water to a final concentration of ~25 ng/µl prior to PCR amplification.

Two SSR markers, gwm493 and gwm533, were used to identify the presence/absence of the 3BS locus of Sumai 3. These two markers, with a map distance of about 7 cM between them, are known to flank the major FHB resistance of Sumai 3 on the short arm of chromosome 3B (Buerstmayr et al 2003; Liu and Anderson 2003). PCR reactions for SSR amplification were performed in a total volume of 10 µL containing 5 nM of each primer, 0.2mM of each deoxynucleotide, 1.5 mM MgCl2, 0.25 unit Taq polymerase and 50 ng template DNA. After an initial denaturing step for 3 min at 94oC, 40 PCR cycles were performed with 30 s at 94°C, 15 s at 58 °C, 30 s at 72 °C, followed by a final extension step of 7 min at 72 °C. PCR products were separated on 3% agarose gels and visualized by staining with ethidium bromide.

RESULTS AND DISCUSSION

1. Correlation between CR and FHB resistance assessed using different genotypes:
Both isolates used caused CR and FHB. CR severity caused by the *F. graminearum* isolate ranged from 0.0 to 12.1 with an average of 3.9, and that caused by the *F. pseudograminearum* isolate ranged from 0.3 to 14.2, with an average of 4.4. FHB severity caused by the *F. graminearum* isolate ranged from 1 to 8.1 (average 3.3), and 1.8 to 12.0 (average 5.3) for the *F. pseudograminearum* isolate. A strong correlation was not detected between CR and FHB resistance for either of the two isolates (Figure 1).

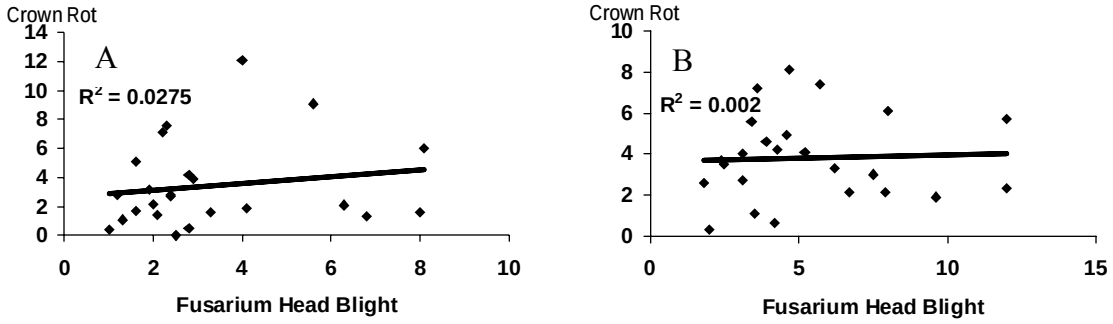

Figure 1. Relationship between FHB and CR resistance in 24 wheat genotypes assessed using *F. graminearum* (A) and *F. pseudograminearum* (B) isolates

2. Identification of individuals with or without the 3BS locus of Sumai 3:

Two SSR markers, gwm493 and gwm533, were used to identify individuals that inherited the 3BS allele of Sumai 3. The marker gwm493 detected a single locus but gwm533 detected two loci, gwm533a and gwm533b (Figure 2). Both of the gwm533 loci were segregating in one (B) of the 4-way F2 populations but only gwm533a was segregating in the other. The single PCR product amplified from either Baxter or Drysdale was located between the two products of gwm533 and was allelic with gwm533a (Figure 2).

To test the associations between the two different loci detected by gwm533 and FHB resistance, FHB severity between individuals with or without the gwm533 allele of Sumai 3 was compared using Population B with 388 inoculated spikes. Individuals with the gwm533a allele of Sumai 3 had significantly lower FHB severity compared with those without this allele, but the difference between individuals with or without the gwm533b allele was not significant (data not shown). This suggests that the gwm533a locus is more closely associated with FHB resistance than gwm533b. Thus, data derived from gwm533a was used here for inferring the presence of the 3BS allele of Sumai 3.

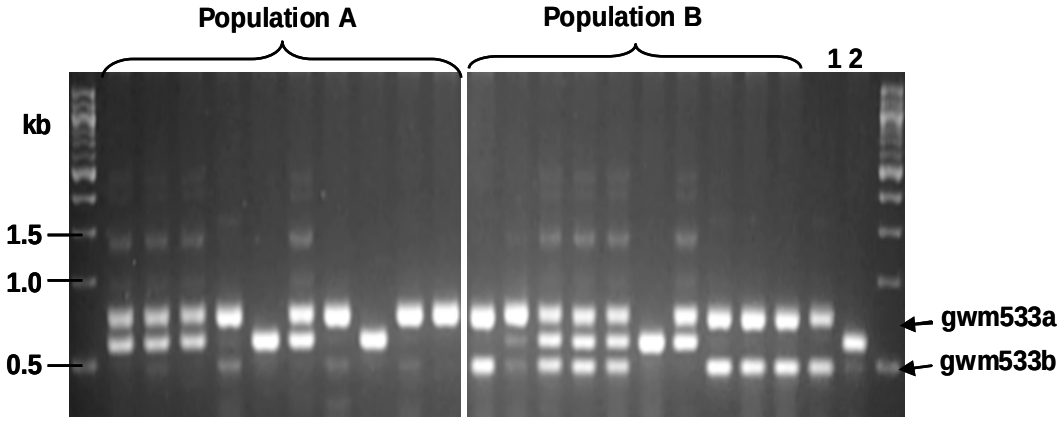

Figure 2. Profiles of SSR marker gwm533 in the two populations, showing that the marker detected two segregating loci in one population (B) but only one in the other (A). Lane 1 is Sumai 3 and lane 2 Baxter.

3. Effect of the 3BS locus of Sumai 3 on FHB resistance:

Significant differences were detected between individuals with or without the 3BS allele of Sumai 3. When only individuals with Sumai 3 alleles of both markers were considered, there was a 27.0% to 42.0% (average 32.0%) reduction in the NIS and a 22.4% to 39.0% (average 29.2%) reduction in PIS among individuals that had the 3BS allele of Sumai 3 (Table 1). Compared with the average severity of the local cultivars used to produce the two 4-way F2 populations (ignoring their different contributions to the progeny), individuals with the 3BS allele of Sumai 3 reduced FHB severity by an average of 42.0% and 47.8%, respectively. These results confirm that the presence/absence of the FHB resistant locus on the short arm of chromosome 3B was effectively selected using the two flanking SSR markers, and that the locus has a significant effect on FHB resistance.

4. Effect of the 3BS locus of Sumai 3 on CR resistance:

The two measurements of CR resistance produced slightly different results. When visual rating was used, the difference between the four families from population A was highly significant. Individuals with the 3BS locus of Sumai 3 showed slightly improved CR resistance (Table 2). When the length of the discolored leaf sheath was used, however, the difference among the four families from this population was not significant. For the four families from population B, highly significant differences were detected using either rating or the length of discolored leaf sheath (Table 2). In contrast to the four population A families, individuals without the 3BS allele of Sumai 3 showed better CR resistance in the four population B families. The combined results of the two populations showed that the presence of the 3BS allele of Sumai 3 had no effect on CR resistance for either measure of CR severity (Table 2).

Table 1. Effect of the 3BS locus of Sumai 3 on FHB severity

Locus	allele	Population A		Population B		Average	
		NIS[b]	PIS[c]	NIS	PIS	NIS	PIS
gwm493	a	7.3	56.9	8.1	53.8	7.5	57.3
	A	5.2	40.2	4.7	32.8	5.2	41.8
	Difference	P<0.01	P=0.01	p<0.01	p<0.01	p<0.01	p<0.01
gwm533	b	7.3	56.9	8.1	54.1	7.6	59
	B	5.2	40.2	5	34.2	5.3	41.5
	Difference	P<0.01	P<0.01	P<0.01	p<0.01	p<0.01	p<0.01
gwm493 and gwm533	ab	7.3	56.9	8.1	53.8	7.5	57.2
	AB	5.2	40.2	4.7	32.8	5.1	40.5
	Difference	P<0.01	P<0.01	P<0.01	p<0.01	p<0.01	p<0.01

a. a and b represent Baxter/Drysdale alleles of gwm493 and gwm533, respectively; A and B represent Sumai 3 alleles of gwm493 and gwm533, respectively

b. NIS = Number of infected spikelets

c. PIS = percentage of infected spikelets

Table 2. Crown rot severity, assessed by either a visual rating or discolored leaf sheath, of individuals with or without the Sumai 3 alleles of gwm493 and gwm533

Locus	Allele[a]	Population A		Population B		Average	
		rating	length	Rating	rating	rating	length
gwm493	a	1.82	1.94	0.67	0.93	1.31	1.49
	A	1.29	1.53	1.09	1.44	1.17	1.47
	difference	P<0.01	P=0.05	P<0.01	P<0.01	p=0.13	p=0.45
gwm533	b	1.95	1.99	0.67	0.93	1.27	1.42
	B	1.39	1.64	1.09	1.44	1.25	1.54
	difference	p=0.08	p=0.18	P<0.01	P<0.01	p=0.44	p=0.21
gwm493 and gwm533	ab	2.04	2.12	0.67	0.93	1.25	1.42
	AB	1.18	1.89	1.09	1.44	1.12	1.55
	difference	P<0.01	p=0.23	P<0.01	P<0.01	p=0.18	p=0.20

a. a and b represent Baxter/Drysdale alleles of gwm493 and gwm533, respectively; A and B represent Sumai 3 alleles of gwm493 and gwm533, respectively

CONCLUSIONS

This paper describes two experiments aimed at examining the relationship between FHB and CR resistance. Results from both experiments suggest that resistance to FHB and CR are controlled by different genes, although these two diseases can be caused by the same *Fusarium* species. This implies that the numerous FHB resistant genotypes identified in the international programs may not be very useful in our CR research and separate screenings for the two different diseases seem to be essential. However, further tests with the use of a much larger number of genotypes would be needed to confirm these results. It would also be highly desirable if genotypes showing high levels of resistance to both FHB and CR could be identified and used to further investigate the genetic bases of resistance to these two diseases.

ACKNOWLEDGEMENTS

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FUSARIUM HEAD BLIGHT RESISTANCE FROM WIDE CROSSES IN BREAD WHEAT AND DURUM

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OBJECTIVES

To identify resistance to Fusarium head blight (FHB) in wild relatives of wheat, to transfer this resistance into bread wheat and durum and to enhance the resistance inherent in those two wheat species.

INTRODUCTION

Fusarium head blight has become a devastating disease of cereals in temperate climate regions of the world. There appears to be sufficient inoculum built up so that the occurrence of rainfall during the flowering period of the crop is certain to cause extensive infection. The single best source of resistance under our conditions has been the variety Sumai 3. However, under intensive infection pressure in our epiphytotic nursery, Sumai 3 will suffer up to 20% floret infection and deoxyivalenol (DON) content as high as 5.0 ppm. We have employed two methods of enhancing the FHB resistance of Sumai 3. The first is by combining the resistance of Sumai 3 with that of unrelated wheats such as Nyu Bay, Frontana and other Brazilian and Chinese wheats. The second method is by screening large numbers of accessions of alien species, selecting lines with resistance and introducing the resistance to bread wheat and durum.

MATERIALS AND METHODS

The *Triticum timopheevii* accessions that were screened for FHB resistance were those previously identified as having multiple pest resistance (Brown-Guidera et al., 1996). Accessions of *T. monococcum* were obtained from Maxime Trottet of INRA Le Rhou, Cedex, France and *Ae. speltoides* accessions from Maria Zahrieva of INRA Centre de Montpellier in France. The *T. miguschovae* (AGD) accessions were provided by Tamara Ternovska of the Mohyla Academy in Kiev, Ukraine and the *Ae. cylindrica* (CD) source of resistance was provided by Alexander Rybalka of Odessa Ukraine.

The accessions of Tritordeum (ABH) that we screened were either produced by ourselves or supplied by A. Martin of Cordoba, Spain. The *T. carthlicum* (AB) accession was obtained from the Vavilov Institute of St. Petersberg, Russia while the ABE amphiploid was supplied by Fangpu Han formerly of the Northeast Normal University at Changchun in China. The screening methods involved growing the plant materials in growth rooms, inoculating (point and spray) spikes at 50% anthesis with a 50,000 spores/ml suspension of *F. graminearum*. Plants with inoculated spikes were "misted" for 48 hours and symptoms scored at 21 days. Inoculation was repeated on accessions showing minimal symptoms. Resistant accessions of *T. monococcum*, *Ae. speltoides* and *T. miguschovae* were crossed onto the cultivar Superb and hybrid embryos were cultured on B5 medium. The *T. monococcum* hybrid was backcrossed to the cultivar Fukuhokomugi; *Ae cylindrica* derivative was crossed to Superb whereas three backcrosses to Superb were required to restore fertility of the hybrid involving *Ae. speltoides*. DT 712 (AC Strongfield) was the recipient parent in the tetraploid manipulations. The ABE amphiploid was crossed with the Capelli *ph* mutant to enhance recombinations whereas repeated backcrosses to AC Strongfield were carried out to produce the addition lines.

The derived lines were seeded as one meter rows in the FHB Nursery, inoculated with corn spawn and irrigated twice a day. Symptoms were scored at 21 days after 50% anthesis. Incidence and severity scores were assigned visually and the percentage of Fusarium-damaged kernels (FDK) was determined on threshed samples. Duplicate five-gram samples of seed were ground. One gram of the ground samples was randomly taken for DON analysis.

RESULTS AND DICUSSION

The first field trial of interspecific derivatives was conducted in the summer of 2004 and the results are shown in Table 1. Since the check varieties performed as expected from previous experiments it was considered that the values for the test lines should be relatively accurate. The DON contents for the seven *Ae. speltoides*-derivatives ranged from 2.9 to 8.5 ppm. Three of the lines approximated DON levels observed in the Sumai 3; the best check variety. The DON levels in the *Ae. speltoides* and *T. timopheevii* derivatives were somewhat higher than in Sumai 3. However, if the resistance is contributed by alleles different from those already present in bread wheat, the new resistance source may be effective in augmenting the resistance of the known genes.

The field screening of the *T. monococcum* and *Ae. speltoides* was repeated in 2005 with 22 and 70 lines respectively. For some reason the DON levels in the checks were very low in 2005, eg. in Sumai 3 it was at 2.9 ppm compared to 5.5 in 2004. Similarly the DON levels in Roblin, the susceptible check, were 35.0 in 2004 but only 9.1 in 2005. The DON levels in the derived lines were very low in 2005. The range for the *T. monococcum* derivatives was 0.1-1.7 and 0.3-2.0 for the *Ae. speltoides* derivative i.e. they overlapped the levels observed in Sumai 3.

Although the DON levels were so drastically different in two years of testing, the values obtained for the derived lines relative to the checks was consistent so we feel that the resistance is real. Although we do not present DON data for TC 67 (*T. timopheevii*-derived) for 2005, we have carried out sufficient study with this line to conclude that its FHB resistance is stable.

Table 1. FHB symptoms and DON content in progenies of interspecific crosses with bread wheat (field data, 2004)

Source of Resistance		Generation	Incidence(%)	Severity(%)	FDK(%)	DON (ppm)
Ae. speltoides						
Line	1	BC_3F_4	30	25	11.7	8.5
	2		15	10	7.0	3.6
	3		10	10	6.3	4.3
	4		15	10	14.7	5.3
	5		15	10	6.7	3.2
	6		15	10	10.0	2.9
	7		10	10	7.0	4.8
T. monococcum						
Line	1	BC_2F_4	10	5	15.3	8.9
T. timopheevi						
TC67		F_9-SSD	19.0	11.3	13.9	5.7
Checks						
Sumai3			10	5	9.0	5.5
Nyu Bay			33	16	13.2	3.4
Fukuhokomugi			15	5	50.0	7.7
Roblin			80	80	90.0	35.0
AC Barrie			45	10	20.3	16.3

Table 2. FHB symptoms and DON content of progenies from interspecific crosses with bread wheat (field data, 2005)

Source of Resistance	Generation	Number of lines	FHB Index	DON Level (ppm)
T. monococcum	BC_2F_6	22	0.3-3.0	0.1-1.7
Ae. speltoides	BC_3F_6	70	0.3-7.5	0.3-2.0
Checks				
Roblin			76.5	9.1
Superb			24.0	9.2
Fukuho			1.5	1.9
Sumai3			1.3	2.9

In our experience, there was a certain amount of linkage drag accompanying the transfer of FHB resistance from exotic sources to bread wheat. Even in transfer of resistance from exotic wheat cultivars of Brazilian, Chinese and Japanese origin it was necessary to grow large populations and select intensively for earliness, lodging resistance and shorter plant stature. The same was true in progeny of interspecific hybrids. The derived lines shown in Tables 1 and 2 all have reasonable agronomics except that they are several days later in flowering and maturity than locally- adapted check cultivars. When tested against local check varieties AC Brio and Hoffman, the derived lines had hectolitre and thousand kernel weights that were equal to the checks. In terms of crude protein content, the *T. monococcum* and *Ae. speltoides* derivatives had levels of 13.9 and 15.3, respectively. We have also been transferring FHB resistance from *Ae. cylindrica* (CD genomes) into bread wheat. For the 2006 season we will have in excess of 100 F_6 lines to be tested in the epiphytotic nursery. These lines were derived from crosses to the cultivar Superb. This source of resistance has also been crossed into the Canadian cultivars Teal, Barrie, Domain, Elsa and HY644. Progenies from the latter are now at the F_4 and F_5 generation.

We are working on the introgression of FHB resistance into durum wheat. We received a sample of tetraploid wheat from the Vavilov Institute labelled as *T. carthlicum*. In our indoor screening facility it showed reasonable Type I resistance to FHB. This line was crossed to the durum cultivar Strongfield and a doubled haploid population of about 150 lines produced in the laboratory of Julian Thomas at Cereal Research Center (CRC) of Winnipeg. The population was phenotyped at the ECORC facility during the winter of 2004. The marker work conducted by Daryl Somers of CRC showed two major QTL on chromosomes 2B and 6B that controlled the resistance. These markers are now being used for MAS in the durum program.

Introgression of resistance into durum wheat is also continuing using Tritordeum (ABH), *T. miguschovae* (AGD) and an amphiploid between *T. turgidum* x *Th. elongatum* 2x (ABE) as resistance donors. The latter has excellent FHB resistance. BC2 F_4 progeny from crosses to Tritordeum were grown in the epiphytotic nursery in 2005. They appeared to have reasonable resistance but were quite late maturing under our conditions. We have produced BC_2 F_4 progeny from crosses with *T. miguschovae* and are now screening progeny indoors before going to field plots.

With amphiploid 8801 we are in the process of advancing the progeny in two different streams. BC_2 progeny have been produced from hybrids with the Capelli *ph* mutant where we hope to isolate recombinants with resistance. In the other stream the cross was made with Strongfield and backcrossed with the idea of producing EE addition lines. We now have 30 chromosomes lines showing resistance. The next step will involve the induction of recombination between the resistant addition lines and the durum chromosomes. Once the resistance from alien sources has been isolated, the next steps will involve identifying the resistance QTL with molecular markers

and using these markers to "pyramid" the various sources of resistance into adapted cultivars. A number of mapping populations have been produced for this purpose and appropriate crosses and intercrosses have already been made.

From our experience in screening wild relatives of wheat for FHB resistance, we find low frequencies of resistant accessions in many alien species. This phenomenon is reflected in several efforts worldwide at introgressing alien resistance into wheat. Major efforts are underway at the Nanjing Agricultural University (Chen et al., 2004), Purdue University (Shen et al, 2006) and North Dakota State University (Oliver et al., 2004). Each of these groups has found high levels of resistance in particular species and is concentrating on those. The species that we are concentrating on are different from the above (Fedak et al., 2005). This appears to be a pragmatic approach as in due time, it should be possible to pyramid resistance genes from numerous unique sources.

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UTILIZATION OF WILD GENETIC RESOURCES FOR THE IMPROVEMENT OF FHB RESISTANCE IN WHEAT BREEDING

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INTRODUCTION

While accumulation of resistance sources from known wheat genetic resources is a major task, we still need to seek additional and more effective sources that we can use in breeding programs. This is especially the case for Type I resistance (against initial infection). A fair number of bread wheat accessions have been reported as Type II resistant (against spread of infection), but none are Type I resistant. The diverse source of genes provided by wheat relatives and alien species has the potential to produce wheats resistant to FHB. Ancestral species would be the first choice to explore because genetic materials can be introduced by recombination. This includes wild types of hexaploid and tetraploid wheat as well as diploid ancestors of A, B, and D genomes. A number of alien species has been reported as resistant, including *Thinopyrum*, *Leymus*, and *Roegneria* (Mujeeb-Kazi 1986; Wan 1997; Fedak 2003). Wan et al. (1997) reported genus *Roegneria* as hyper-resistant for both Type I and Type II resistance, while Sumai 3 was regarded as susceptible in Type I. CIMMYT has been working on D genome synthetics and demonstrating that these are useful sources for FHB resistance in bread wheat. Likewise, synthetic wheat of A and B genomes would be useful for durum breeding. For pursuing further resistances, we are now turning more attention on alien sources. After evaluation of the FHB resistance of amphiploids and addition lines, we started production of translocation lines. However, one problem for this production is that it will require significant investments of time and labor until these become useful in the breeding program. This is a challenge we need to overcome.

MATERIALS AND METHODS

Plant Materials (Table 1)

All synthetic wheats used have been produced in CIMMYT by crossing durum and diploid ancestor species followed embryo rescue and chromosome doubling. There are about 1,100 lines of D genome synthetic (AABBDD), 150 lines of A genome synthetic (AAAABB), and 50 lines of B genome synthetic (AABBBB). Amphiploid and addition lines of *Leymus* and *Elytrigia* were produced in CIMMYT or Kihara Institute (Japan). The mapping populations were obtained after crossing resistance synthetic derivatives and susceptible wheat variety 'Flycatcher' (FCT).

Production of translocation

Amphiploid of *Thinopyrum bessarabicum* was crossed twice with wheat line Chinese Spring (*ph1b*). Translocations between *Th. bessarabicum* and wheat chromosomes were detected by genomic *in situ* hybridization (GISH). The plants with translocations were backcrossed with CIMMYT variety PRINIA several times to produce a translocation line whose chromosome constitution is 42. Addition lines of *Leymus racemosus* were crossed with monosomic lines whose homoeology is supposed to match *L. racemosus* chromosomes in the addition lines. Centromeric translocation was screened in their progenies. Addition lines of *L. racemosus* were also crossed with 2C chromosome addition lines. The 2C chromosome is from *Aegilops cylindrica* and has been known to induce chromosome breakage and translocation in its progeny (Endo 1998). Translocation was screened in the F2 generation. The A and D genome translocation will be induced in the progenies of the crosses between durum line 'Capelli (*ph1c*)' and *Aegilops tauschii* lines.

Evaluation of Type II resistance

The evaluation was conducted in early September in Toluca station in Mexico. Five to ten spikes of each line were point-inoculated with FHB suspension (50,000 spores / ml) and then covered with glassine bags. Spread of infection was measured 30-35 days after the inoculation.

RESULT AND DISCUSSION

Bread Wheat

Synthetic wheat/Aegilops tauschii

After screening of 1,000 synthetic wheats of D genome, we have identified five lines of FHB resistance whose Type II resistance are equal to or higher than Sumai 3. Since all five synthetic wheats were put into different phylogenic groups (data not shown), they may contain different resistance sources. Three lines of synthetic derivatives were employed to propagate nine sets of mapping populations. Quantitative trait locus (QTL) analysis has been conducted on one of the mapping populations, and one strong QTL was detected in chromosome 2DL (Lewis et al. 2004). It is important to investigate whether the other four synthetic wheats have novel sources or not. Resistant synthetic wheats have been crossed with several CIMMYT varieties to transfer resistance. Field testing has been conducted in CIMMYT Toluca station every year with selection of the lines whose infection level is less than 15%. The F8 were propagated in 2005, and these materials can be used as alternative resistance sources in breeding programs.

Leymus racemosus

We used two different *L. racemosus* accessions and obtained two sets of addition lines. The first and the second sets contain ten and six addition lines, respectively. The evaluation of these lines showed one mild resistance in the first set and three lines of resistance in the second set. The difference between the sets may indicate that the choice of parents is important for FHB resistance. To transfer these resistances to bread wheat, we started the production of translocation lines using 2C gametocidal systems. In the last year, we have obtained about 40 translocated chromosomes. Separately from this work, we are now propagating centromeric translocations using monosomic lines. After several backcrossings with CIMMYT varieties, these translocations will be readily transferred into CIMMYT breeding varieties to see the effect of the translocation.

Thinopyrum bessarabicum

It was reported that amphiploids and addition lines between *Thinopyrum* species and wheat would increase FHB resistance, including using species *Th. elongatum, Th. intermedium*, *Th. junceiforme, Th. ponticum* (Jauhar et al. 2001; Fedak et al. 2003; Shen et al. 2004). Since we had a set of addition lines of *Th. bessarabicum*, we focused on this species. Amphiploids between wheat and *Th. bessarabicum* showed FHB resistance higher than Sumai 3 (Table 2), but we observed that only three addition lines possessed mild resistance (~15%; Table 2). Because of complex nature of FHB resistance, the resistance may become diluted in the addition lines, or the resistance may not be expressed in the wheat background. We have already obtained four different translocation lines and also twenty translocated chromosomes. We will evaluate resistance of these lines in the future.

Durum Wheat

Tetraploid and AA and BB genome ancestors

Preliminary screening of wild relatives showed that we would find resistance sources in *Triticum dicoccoides* and *T. dicoccum* (Table 3). The infection level in those species ranged from 6% to 100%, comparatively much lower than the level in durum, which was between 70 and 100%. CIMMYT has about 50 and 200 lines of A genome (AAAABB) and B genome (AABBBB) synthetic wheats, respectively. When we tested the A genome synthetic wheats, there were also some accessions which showed Type II resistance of the same level as Sumai 3 (~10%). The resistance sources in tetraploid relatives and in A or B genome synthetic wheat can be transferred into durum by conventional breeding methodology.

Transfer of resistance from D genome

Resistance from D genome can be also transferred into durum wheat by induction of translocation between A and D genome. From the study of synthetic wheats of D genome, we know which *Ae. tauschii* accessions have FHB resistance. In 2005, we obtained two kinds of amphiploids between *Ae. tauschii* and Capelli (*ph1c*) accessions. We are now screening translocation between A and D genome by GISH (Figures 1 & 2).

One big problem in the use of alien species

We are now heavily involving the production of translocation lines for FHB resistance in both of bread and durum wheat. However, one problem is the time and labor required to induce translocations. Moreover, one translocation seems to be insufficient to achieve the full resistance of alien species (Table 2), even though the resistance of alien species is superior to that of wheat. We may need to combine multiple translocations. The task would be quite a burden if pursued by one institute. Fortunately, several groups

across the world seem to be working in different species, though there may be overlapping to a certain extent. If we can collaborate and divide our task efficiently, we believe we can achieve our goal much more easily.

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Table 1. Plant materials utilized in this study.

Lines		Number of lines	Origin
Synthetic wheat			
	D genome synthetic wheat (AABBDD)	1,100	CIMMYT
	A genome synthetic wheat (AAAABB)	150	CIMMYT
	B genome synthetic wheat (AABBBB)	50	CIMMYT
Amphilpoid			
	Bread wheat - *Thinopyrum bessarabicam*	1	CIMMYT
	Bread wheat - *Thinopyrum elongata*	1	CIMMYT
	Bread wheat - *Elytrigia scythica*	1	CIMMYT
Alien chromsome addition lines			
	Thinopyrum bessarabicum	7	CIMMYT
	Leymus racemosus set#1	10	Japan
	Leymus racemosus set#2	6	CIMMYT
Mapping population (Doubled haploid)			
	TURACO/5/CHIR3/4/SIREN//ALTAR 84/Ae. tauschii (205)/3/3*BUC/6/ **FCT**	128	CIMMYT
	MAYOOR//TK SN1081/Ae. tauschii (222)/3/ **FCT**	171	CIMMYT
	SABUF/3/BCN//CETA/Ae. tauschii (895)/4/ **FCT**	125	CIMMYT

Table 2. Type II resistance in *Thinopyrum bessarabicum* chromosome lines.

Lines	Damage % Type II
Amphilpoid	
Wheat - *Thinopyrum bessarabicum*	8.2
Addition lines	
Thinopyrum bessarabicum 1J	29.1
Thinopyrum bessarabicum 2J	19.8
Thinopyrum bessarabicum 3J	39.4
Thinopyrum bessarabicum 4J	16.6
Thinopyrum bessarabicum 5J	16.0
Thinopyrum bessarabicum 6J	17.2
Thinopyrum bessarabicum 7J	

Table 3. Type II resistance in the field at Toluca, Mexico 2004.

Species	Genome	Number of lines	Damage % Type II Min	Max	Average
Triticum durum	AABB	16	69.4	100	84.4
Triticum dicoccum	AABB	35	6.9	70.3	32.8
Triticum dicoccoides	AABB	46	5.8	100	25.3
Triticum monococcum	AA	41	9.4	45.7	26.2
*A genome synthetic wheat	AAAABB	194	9.5	100	41.8

The analysis was conducted by Dr. Maarten Van Ginkel. *The test for synthetic wheat was conducted Dr. Mujeeb Kazi in 2001.

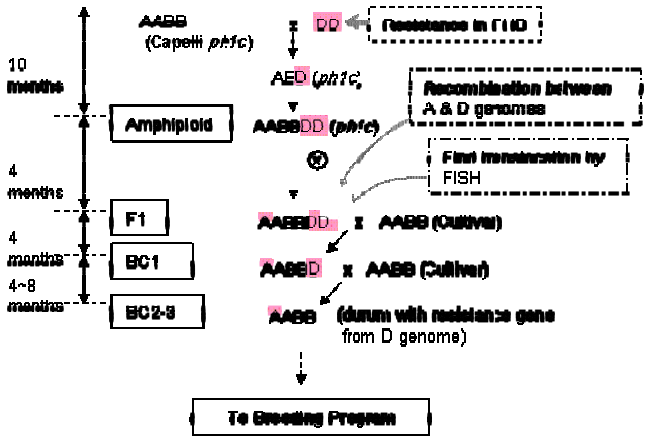

Figure 1. Methodology for transfer of B and D genomes into A genome

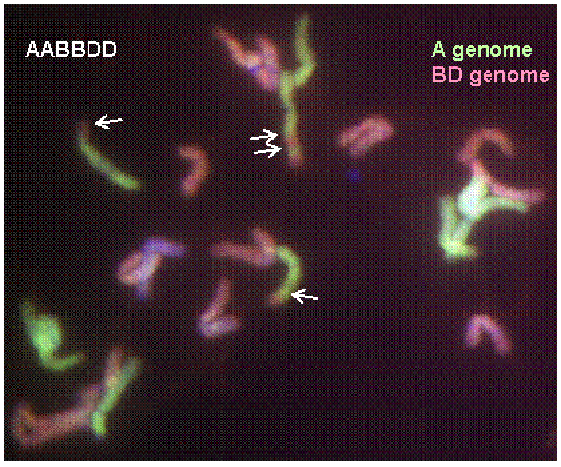

Figure 2. Translocation between B/D genomes and A genome. The arrows indicate transfer of B and D genomes into A genome chromosomes.

NOBEOKA BOZU, AN UNUSED RESISTANCE SOURCE AND ITS UTILIZATION IN IMPROVING RESISTANCE TO FHB

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ABSTRACT

I received Nobeoka Bozu (NB), a spring type FHB resistance source from Japan, 20 years ago from Dr. M. Kohli of CIMMYT. The first tests showed a very high level of resistance in FHB symptoms, kernel infection, yield reduction and deoxynivalenol (DON) accumulation. Several crosses were made with high yielding adapted materials such as Zugoly (Zu), Ringo Star (RSt), Kincso (Ko), Réka (RÉ) (abbreviations in Figure 1) to observe its segregating populations. NB is an agronomically poor, ill-adapted landrace. The heads and grains are small and 1000 kernel weight is about 25-27 g. The straw is thin and the color is light green, though the segregating populations also contained plants with light yellow heads. NB has comparable FHB resistance to Sumai 3 (Sum 3) (Figure 1). Of the progenies derived from the crosses with NB, several highly resistant lines were identified. All are winter type, and therefore they have special value for winter wheat programs. In Figure 1, the genotype first from the left is a highly susceptible genotype: it seemingly did not inherit resistance from NB. Some of these materials have also been tested in Japan, with encouraging results (Ban, unpublished). The agronomic traits of these lines are significantly better than those of NB, as are baking quality, yield potential, and resistance to other diseases, whilst the resistance seen in NB has been successfully retained.

NB has highly effective QTL(s) on 3BS type (Liu et al. 2005, Jian-Bin et al. 2006.), but it does not seem to be identical with the 3BS QTL allele found in Sumai 3 (unpublished), even though it gives strong Type II resistance (Jian-Bin et al. 2006). Therefore it is highly important to identify the genetic background of the NB QTL(s). We now have three mapping populations from NB. Their study began in 2006 in the field in collaboration with the Agricultural University As, Norway. We hope that QTLs may be identified that will have similar significance to the Sumai 3 QTL(s) and that the identity or diversity problem can be answered.

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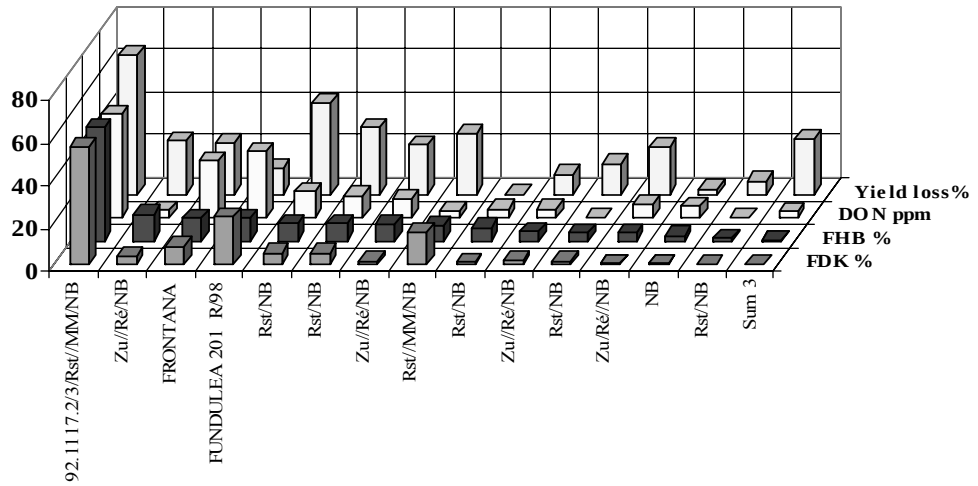
Figure 1. FHB resistance of lines derived from Nobeoka Bozu, 2004



DNA MARKER ANALYSIS FOR FHB-RESISTANCE PYRAMIDING FROM DIFFERENT GERMPLASMS

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ABSTRACT

Use of resistant cultivars is an effective way to control Fusarium head blight (FHB), an epidemic wheat disease around the world. Several resistant germplasms, such as Sumai 3, Wangshuibai, and Nobeokabouzu-komugi, have been screened and widely used in wheat breeding programs. A number of quantitative trait locus (QTL) analyses indicated that the resistance genes are not identical among resistant germplasms. Pyramiding of different resistance genes into one wheat cultivar would be useful for breeders to introgress different resistance genes into their locally-adapted cultivars. By crossing Sumai 3, Wangshuibai, and Nobeokabouzu and following a high FHB pressure selection for six generations, a line with pyramided resistance, WSY, has been developed. In the present study, we analyzed the genetic flow between the three parental cultivars and the pyramided resistance line WSY using DNA markers with a purpose to clarify how many and which resistance genes were accumulated in the pyramided line. Two-hundred and eighty three SSR markers and three STS markers from the 21 wheat chromosomes were analyzed. Of these markers, 115 are from ten FHB resistance QTL regions. The results disclosed that the pyramided line WSY included different chromosome regions that harbored putative FHB QTLs from the three parental germplasms. Haplotypes of DNA markers on these QTL regions demonstrated that the 3BS QTL (the major FHB resistance QTL) was from Nobeokabouzu; QTLs on 1BL 5A and 2BL were from Sumai 3; QTLs on 2AS, 3AS and 6BS were from Wangshuibai; and the 3BSc QTL was from Sumai 3 and Wangshuibai. This study showed that different resistance genes from the different resistant germplasms were indeed accumulated in the pyramided resistance line WSY. The developed pyramided resistance line might be used as a potential resource for FHB resistance in wheat breeding.

GLOBAL BIODIVERSITY IN *FUSARIUM GRAMINEARUM* (*GIBBERELLA ZEAE*) AND *F. CULMORUM* POPULATIONS AND IMPLICATIONS FOR BREEDING RESISTANCE TO FUSARIUM HEAD BLIGHT

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OBJECTIVES

Genetic diversity of a population is the result of all evolutionary processes that have affected a population. Recombination, gene flow and mutation increase genetic variation, selection and genetic drift decrease it. Knowing the amount of genotypic diversity and its spatial and temporal distribution within populations, the level of population subdivision, and its importance for phenotypic traits like aggressiveness and deoxynivalenol (DON) production, will allow us to predict the evolutionary potential of the respective pathogens and generate important implications for deployment of resistance. This paper will briefly review the present knowledge on population-genetic parameters in individual field populations of *F. graminearum* and *F. culmorum*.

INTRODUCTION

Fusarium graminearum (teleomorph *Gibberella zeae*) is the most destructive pathogen causing Fusarium head blight (FHB) worldwide. In Europe other *Fusarium* species may play an important role in this disease, *e.g. F. culmorum , F. avenaceum*, *F. poae*. *F. graminearum* and *F. culmorum* both produce similar mycotoxins: DON, nivalenol (NIV), and zearalenone (ZEA) are among the most important. *F. graminearum* naturally infects wheat during flowering by splash-dispersed ascospores from soil or infected wheat or maize stubble. Secondary infections by conidiospores can additionally occur. Humid weather during flowering is an important prerequisite for epidemics. Kernels are totally destroyed when susceptible varieties are infected early or become discoloured and shrivelled. Seed-borne inoculum of late-infected kernels that look healthy might play a role in long-distance transport of the fungus. Ascospores have been found to be transported at higher altitudes by air streams (Fernando et al. 2000). *G. zeae* is a haploid, homothallic fungus caused by the presence of two mating type idiomorphs (*MAT1-1* and *MAT1-2*) in each isolate. Some outcrossing has been observed in the laboratory (Bowden and Leslie 1999). Crossings between isolates can easily be done, and recombinants have to be identified by use of nitrate-nonutilizing mutants (Bowden and Leslie 1999) or by use of female strains with a manipulated MAT locus to achieve obligatory heterothallism (Lee et al. 2003). Segregation occurs in a haploid fungus in the F_1 generation and dominant molecular markers have the same information content as codominant markers. For *F. culmorum* no sexual stage is known, but two separate MAT idiomorphs were found (Toth et al. 2004). The importance of *F. graminearum* for wheat crown rot and FHB in Australia is reviewed in another chapter of this volume (Chakraborty et al., p. 42).

LEVELS OF BIODIVERSITY

Four levels of biodiversity are obvious: Variation among isolates (1) from different parts of the world, (2) within geographically defined regions, (3) within and between individual field populations, (4) within crossing populations.

Large variation between isolates from different countries and continents is common with each isolate displaying a unique haplotype (Miedaner et al. 2001). O'Donnell et al. (2000) were the first to divide *F. graminearum* into seven phylogenetic lineages of different geographic distribution. Recently, the lineages were extended to nine and given species rank while the postulated geographical barriers dwindle (O'Donnell et al. 2004). Bowden et al. (2006) could achieve fertile crossings between lineage 7, the most common clade in the Northern hemisphere, and all other lineages and between selected other lineages as

well. Genetic variation within one lineage due to traits other than sequenced structural genes analysed by O'Donnell still needs to be evaluated.

More important for assessing the evolutionary potential of *F. graminearum* with respect to resistance breeding is the third and fourth level of biodiversity. Several studies revealed a high level of genetic diversity by RAPD, AFLP and SSR markers within individual field populations or populations sampled across a small spatial scale (Table 1).

In *F. graminearum* populations there is unequivocally a high percentage of unique haplotypes and conseqently a large genotypic and genetic diversity that have been reported. Populations that were sampled 100 to 200 km apart (Naef 2006, Gale et al. 2002) as well as populations from about 2.500 km apart (Zeller et al. 2004) showed no or only a low amount of subdivision. According to these studies, more than 97% of allelic variation is shared among populations. This might be most likely caused by a high gene flow among populations that was estimated with 7 to 74 migrants.

The evolutionary forces behind this genetic diversity might be most likely driven by the large population size of asexually propagated conidia supporting variation by mutation. Additionally, balancing selection between the parasitic and saprophytic subpopulations might contribute to genetic variation. A new study compares genetic diversity within field populations of saprophytic isolates from maize stubbles (Table 1, Switzerland) with a re-analysis of a wheat pathogenic population (Miedaner et al. 2001) by SSR markers (Naef 2006). Genotypic diversity was significantly higher on maize stubble, allelic richness and gene diversity, however, were similar in both populations. A high extent of asexual dispersal was found and the populations shared six multilocus haplotypes (MLHs) across a geographical distance of about 100 km and a temporal difference between both collections of 11 years. If the selection forces in both subpopulations act in at least partially different directions, a balancing selection could maintain large genetic variation. High gene flow together with the occurrence of environmentally stable MLHs could be explained by long-distance transport of ascospores emerged by selfing. Similar results from eight parasitic wheat populations were previously reported by Zeller et al. (2004). Little linkage disequilibrium was detected either in the population as a whole or in any of the individual eight subpopulations sampled from seven US states in three years (Zeller et al. 2004). Similarly, estimates from the Chinese *F. graminearum* populations showed gametic equilibrium in 25 out of 36 locus pairs (Gale et al. 2002). These results are in accord with the hypothesis of a large, randomly mating population. The role of sexual recombination and especially that of outcrossing in natural populations of *G. zeae* is, however, still under debate (Gale et al. 2002). Population analyses of Schilling (1996) and Naef (2006) revealed no gametic equilibrium. Population-genetic theory, however, shows that even rare outcrossings may contribute significantly to genetic diversity.

Table 1. Important quantitative-genetic parameters of individual *Fusarium* populations

Country (No. of popul.)	Sum of isolates	No. of poly-morphic loci	% unique haplo-types	Genotypic diversity G_0/N	Nei's genetic diversity H	Population subdivision R_{ST}	Gene flow N_m
F. graminearum:							
Germany (1)[1]	70	37	76	0.64	0.69	–	–
China (4)[2]	225	9	64		0.31-0.37	0.01-0.07	7-30
USA (8)[3]	523	26-30	91-100	0.95-1.00		0.00-0.07	7-74
Switzerland (5)[4]	395	8	76-98	0.59-0.96	0.67-0.70	≤ 0.02	
F. culmorum:							
Collection[5]	24	20	54	High	–	–	–
Russia (1)[6]	41	28	56	0.50	–	–	–
Hungary (1)[5]	13	20	0	0	–	–	–
Switzerland (1)[4]	29	8	83	0.74	0.34	–	–

[1] Schilling 1996, [2]Gale et al. 2002, [3]Zeller et al. 2004, [4]Naef 2006, [5]Toth et al. 2004, [6]Miedaner et al. 2001.

The high genetic variation found in two *F. culmorum* populations (Table 1, Miedaner et al. 2001, Naef 2006) illustrates that in this species, forces on a population level exist that promote diversity without sexual recombination. In contrast, de Nijs et al. (1997) found in the Netherlands by RAPD analyses only three very closely related haplotypes among 18 *F. culmorum* isolates as expected from a clonally propagating species. Thirteen isolates from Hungary also were of clonal origin whereas the analysis of a collection of different origins with the same markers revealed large genotypic diversity (Toth et al. 2004). These differing results can only be clarified by analysing larger populations from different countries or even continents.

ANALYSES OF SEGREGATING POPULATIONS FROM CROSSINGS AND IMPLICATIONS FOR RESISTANCE BREEDING

Phenotypic traits, especially aggressiveness measured by symptom development and host colonization after inoculation, but also mycotoxin production largely vary on a quantitative scale among isolates. All isolates of *F. graminearum* or *F. culmorum* produce either DON or NIV, their respective precursors, and ZEA (Gang et al. 1998, Miedaner et al. 2000, Toth et al. 2004), *i.e.* each infection in the field will result in toxin-contaminated grain.

If the amount of genetic variation within populations reflects the evolutionary potential of a pathogen, this potential should be rated high for *G. zeae*. In natural infections, often multiple infections occur with different MLHs on the same wheat head (Miedaner et al. 2001, Naef 2006). Additionally, different isolates may have a differing competitiveness in the natural habitat that is not necessarily related to aggressiveness or the amount of toxin production (Miedaner et al. 2004). It is, therefore, of high interest to study the inheritance of these traits.

Significant ($P<0.01$) genetic variation for aggressiveness, host colonization, and DON content was found among 155 progeny of a cross between two medium aggressive isolates of European origin, isolate x environment interactions also was important (Cumagun et al. 2004). Several transgressive segregants towards higher aggressiveness and higher DON production occurred in this population illustrating that both parental isolates had different alleles for these traits that recombined in the progeny. This illustrates the potential of the pathogen to increase its level of aggressiveness by intermating of isolates of the same geographic region.

In the inter-lineage cross between a Japanese barley isolate (R-5470) and a US wheat isolate (Z-3639), a major gene for toxin content (*TOX1*), female fertility (*PERI1*) and colony pigmentation (PIG1) was mapped for the first time (Jurgenson et al. 2002). In close vicinity of these loci, a gene for pathogenicity (*PATH1*) was mapped for the first time (Cumagun et al. 2004) and at least one major QTL for aggressiveness located on a different linkage group but near the trichothecene cluster that contains the DON *vs*. NIV switch. Progeny producing DON were, on average, twice as aggressive as those producing NIV. This has been previously found also for isolate collections (Miedaner et al. 2000).

Resistance to both *Fusarium* species should not be endangered by selection within populations in the near future, because no specific host genotype x fungal isolate interaction occurs in this pathosystem. If, however, the same resistance genes with high effects are used globally and genotypes harbouring them are grown on large acreages, an unspecific increase in aggressiveness and mycotoxin production might occur in natural populations of *F. graminearum* on the long run. To minimize this risk, several genetically unrelated resistance sources should be introgressed in national breeding programmes. Besides the well-known Chinese and South American resistance sources, additively inherited resistance genes exist in other gene pools, *e.g.* among the Middle and East European winter wheats. By recurrent selection a rather high resistance level can be achieved. Mapping studies in these materials detected a high variation for QTLs on nearly all wheat chromosomes.

FUTURE OUTLINES

For a profound understanding of the structure of *F. graminearum* and *F. culmorum* populations more data are necessary on the global diversity. For this, a common array of selection-neutral markers, *e.g.* SSR markers, should be used. This would be especially important for populations of *F. culmorum*, because here substantial data are lacking. Populations from the center of wheat diversity should be included to understand phylogeny of both species. Another feature is monitoring of gene flow between parasitic and saprophytic populations of the same *Fusarium* species in the same fields related to the crop rotation. More populations of *F. graminearum* that have been obtained from crosses within the same lineage should be mapped for phenotypic traits to monitor the amount of segregation variance that might be available in future field populations.

An important issue for the deployment of resistances is the adaptation of the pathogen to resistant varieties. This could be tested in various ways, *e.g.* by growing resistant lines in hot-spot areas of epidemics or by monitoring pathogen populations on hosts differing in resistance.

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DIVERSITY OF FUNGAL POPULATIONS ASSOCIATED WITH FUSARIUM HEAD BLIGHT IN URUGUAY

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OBJECTIVES

(1) To identify and quantify the most prevalent *Fusarium* species in wheat and barley grains.
(2) To characterize the diversity within the population of *Fusarium graminearum* collected in Uruguay

INTRODUCTION

Fusarium head blight (FHB) is a destructive disease of wheat and barley in the Southern Cone of South America. In particular, FHB represents one of the main constraints for wheat and barley production in Uruguay where moderate to severe outbreaks have occurred in one of every two years over the past decade (Díaz de Ackermann and Kohli, 1997; Perea and Díaz, 1980; Pereyra and Díaz de Ackerman, 2003). Epidemics have caused extensive damage through direct losses in grain yield and quality, particularly because of the presence of mycotoxins, such as deoxynivalenol (DON), in harvested grain.

In Uruguay, FHB is primarily caused by *Fusarium graminearum* (Schw.) [teleomorph *Gibberella zeae* (Schwein.) Petch] (Boasso, 1961; Boeger, 1928; Pereyra and Stewart, 2001; Pritsch, 1995). Other species may also incite FHB such as *F. poae* and *F. culmorum* in wheat (Stagno, 1980) and *F. poae* in barley (Pereyra and Stewart, 2001). However, there has not been a systematic survey of *Fusarium* species present in wheat and barley grains harvested from different cultivars, locations, and years.

Previous studies have indicated a differential response in aggressiveness among 14 *F. graminearum* isolates collected during 1991 to 1993 in western-southwestern Uruguay (Díaz de Ackermann and Kohli, 1997). These isolates were tested in the greenhouse against three wheat sister lines (Catbird) with different degrees of reaction (moderately resistant, moderately resistant to moderately susceptible, and susceptible) to FHB, both in the field and greenhouse. No polymorphism was detected among isolates through RFLPs (Pritsch, 1995).

From a phylogenetic approach, lineage 7 (*F. graminearum sensu* O´Donnell) is dominant in Brazil and Uruguay, although other lineages like 1, 2, 6, and 8 are present in minor frequencies (Leslie and Bowden, 2005; O´Donnell *et al*., 2004; Zeller *et al*., 2002; Zeller *et al*., 2003).

Knowledge of *Fusarium* population diversity in Uruguay is essential for effective disease management strategies in the region. This information would be useful for ecological and epidemiological studies, to develop resistant cultivars through improved screening procedures, and to optimize chemical and biological control.

MATERIALS AND METHODS

Fusarium species present in Uruguayan wheat and barley grains

Wheat and barley grain samples (0.2 kg) were collected from epidemic years 2001 and 2002. Five wheat cultivars and five barley cultivars that together comprise the bulk of the commercial hectarage in Uruguay were tested each year at La Estanzuela, Young, and Paysandú and planted at different dates. One hundred arbitrarily selected kernels per cultivar, planting date and location, were examined each year. Surface-disinfected grain samples were plated onto pentachlonitrobenzene (PCNB) agar medium in Petri plates. Twenty grains were placed per Petri plate with five replicates and incubated at 20-22°C with 12-hr light and dark cycles for seven days. Colonies growing with salmon to pink-white color were recorded as *Fusarium* species. The proportion of *G. zeae* colonies was determined by transferring 10 arbitrarily selected *Fusarium* spp. single conidial colonies to carnation-leaf agar (CLA) medium and potato-dextrose agar

(PDA). Cultures were incubated at 20-22°C with 12-hr light and dark cycles for 15 days. The formation of bluish to black perithecia in CLA cultures indicated the presence of *G. zeae*. *Fusarium* colonies not forming perithecia were identified to species, based on procedures and descriptions outlines by Nelson *et al*. (1983) and Burgess *et al*. (1994).

Pathogenicity tests were conducted with single conidial isolates from different species and environments (locations/planting dates) in the greenhouse with susceptible wheat line LE 2294 and susceptible barley cultivar Estanzuela Quebracho. One to two spikes per pot (five pots per isolate per plant species) were inoculated with a concentration of 2 x 10^4 conidia per ml at mid-anthesis in wheat and at heading in barley using an airbrush (model VL3, Paasche Air Brush). Inoculated plants were incubated in a dew chamber at 20-22°C with 12-hr photoperiod and 100% relative humidity for 72 h. Disease severity (percentage of symptomatic spikelets per spike) was evaluated 21 days after inoculation.

***Fusarium graminearum* diversity**

Sixty-four *F. graminearum* isolates were obtained from wheat grains collected in commercial fields in western Uruguay in 2001, 2002 and 2003. Isolates were identified from morphological characters following the procedures of Nelson *et al*. (1983) and confirmed by *F. graminearum*-specific PCR using primers Fg11F/Fg11R (Nicholson *et al.,* 1998). Furthermore, isolates were sent to Dr. K. O´Donnell lab (MGBR, NCAUR, USDA, Preoria, IL) to determine by molecular methods, which of the nine lineages or species within *F. graminearum* clade, they belonged to.

All isolates were tested by a combination of phenotypic and molecular criteria, including chemotype, DON production, fungicide resistance, aggressiveness under greenhouse conditions, and perithecial production under standardized techniques.

Chemotype characterization: PCR assays to determine the presence of trichothecene genes Tri 5, Tri 7 and Tri 13 were performed. Primers for analysis of polymorphisms in Tri 7 and Tri 13 genes were as described by Lee *et al.* (2001) and Kim *et al*. (2003), respectively. The primers for Tri 7 analysis were GzTri7F and GzTri7R. Isolates producing DON have 11 base pairs insertions rendering it non-functional. Primers for Tri 13 analysis were GzTri13F and GzTri13R. Isolates producing DON have deletions of 181 base pairs, rendering it non-functional.

DON production: Each isolate was separately cultivated on 10 g of rice grains moistened with 5 ml of sterile water in a 100-ml Erlenmeyer flask, at 28°C for 30 days. Rice cultures were then removed and the content of each flask was blended with 50 ml methanol for one minute. Extracts were filtered through filter paper. DON was detected by Thin Layer Chromatography according to AOAC and Elisa test (Veratox® DON 5/5 Quantitative DON Test Neogen®) was used in order to quantify DON concentration.

Assessment of fungicide sensitivity: Minimal inhibitory concentration (MIC) was determined for thiabendazole- Tecto 500 SC ® (Syngenta), tebuconazole- Folicur 450 ® (Bayer Crop Science) and metconazole – Caramba® (BASF). The last two fungicides are widely used to control FHB in Uruguay. PDA (200 µl) amended with different fungicide concentrations were dispensed into the wells of sterile disposable microtitre plates. After 72 h of incubation at 25°C in the darkness, fungal growth was determined visually. MIC was defined as the lowest concentration that inhibited fungal growth. Two repetitions per treatment were performed. Fungicide concentrations assayed were 0, 2, 4, 8, 16, 32, 64, and 128 ppm. Experiments were repeated at least twice.

Aggressiveness: Wheat genotypes with different reactions to FHB in the greenhouse and field were planted in the greenhouse: Frontana (resistant), Sumai#3 (resistant), Ringo Sztar-Mini Mano/Nobeoka Bozu, Catbird 1073 (moderately resistant), Onix (moderately resistant to moderately susceptible), INIA Churrinche (moderately resistant to moderately susceptible), INIA Mirlo (susceptible), INIA Boyero (susceptible), and Buck Guaraní (susceptible). The nine *F. graminearum* isolates with the highest DON production were tested in a randomized complete design with 10 plants per cultivar per isolate. One to two spikes per pot (five pots per isolate per plant species) were inoculated with a concentration of 2 x 10^4 macroconidia/ml at mid-anthesis using an airbrush (model VL3, Paasche Air Brush Company, Harwood Heights, IL) at 12 psi to deliver ca. 0.2 ml of inoculum per spike. Controls were mock-inoculated with sterile deionized water. Inoculated plants were incubated in a dew chamber at 20 to 22ºC with a 12-h photoperiod and 100% relative humidity for 72 h. After incubation, plants were returned to the greenhouse and grown under the same light and temperature conditions used prior to inoculation. Disease severity was evaluated 7, 14 and 21 days after inoculation and expressed as the percentage of symptomatic spikelets per spike. A repetition of this experiment is underway; therefore, only preliminary results will be presented.

Perithecia production: Residue of the wheat cultivar INIA Churrinche, collected from a commercial field and without *G. zeae* perithecia, was used to determine perithecia production of 14 *F. graminearum* isolates. These isolates were selected for the highest DON production among the 64 isolates. Residue (1.5-cm long stem pieces including one node) was autoclaved at 120°C for 25 minutes. Three grams of residue pieces were inoculated by submerging for two minutes into an aqueous macroconidia suspension (1×10^5 spores/ml) of each *F. graminearum* isolate. Following inoculation, residue was placed on sterile sand moistened with sterile water in Petri plates (one gram of residue per plate per isolate, three replicates per isolate). To facilitate the development of mature perithecia, residue pieces were incubated at 20-22°C under 12-hr light and dark cycles for 21 days. Plates were covered with a plastic lid for three days and then maintained uncovered for the rest of the experimental period. Gross box weight was checked each four to five days and sterile water was added as necessary to maintain the original weight. Following the 21-day incubation, perithecia were counted in a 7 mm diameter field using a dissecting scope at 30x magnification on each residue piece.

RESULTS AND DISCUSSION

Fusarium species present in Uruguayan wheat and barley grains

Fusarium graminearum was the primary species associated with FHB. It comprised 76% and 60% of all *Fusarium* species isolated from wheat grains in 2001 and 2002, respectively (Figure 1). *Fusarium graminearum* represented 65% and 56% of all *Fusarium* species isolated from barley grains in 2001 and 2002, respectively. The results from isolation and identification of *Fusarium* species in wheat and barley grains clearly confirmed that the main species associated with FHB in Uruguay is *F. graminearum* as suggested by previous studies (Boerger, 1928; Boasso, 1961; Pritsch, 1995; Pereyra and Stewart, 2001). Similar results are reported for North America (Clear and Patrick, 2000; McMullen *et al*., 1997; Salas *et al*., 1999), some parts of Europe (Parry *et al*., 1995) and other countries in South America (Lori *et al*., 2003; Reis, 1988). *Fusarium graminearum* is widespread in the southern cone of South America and has been isolated from a wide range of hosts (Fernández, 1991; Pereyra *et al*., 2004; Reis, 1988).

The frequencies with which *Fusarium* species other than *F. graminearum* were recovered varied depending on both environment and host cultivar. In general, *F. avenaceum*, *F. culmorum* and *F. poae* were the following most common species isolated from wheat grains. Other species included *F. equiseti, F. acuminatum,* and *F. trincictum. Fusarium poae* and *F. equiseti* were the most common species after *F. graminearum* isolated from barley grains. Other *Fusarium* species recovered in barley grains included *F. avenaceum*, *F. sambucinum, F. trincictum*, *F. semitectum*, and *F. chlamydosporum* (Figure 1). *Fusarium avenaceum* was the second most common species found in wheat grains while either *F. culmorum* or *F. poae* was the third most common species, depending on the year. These three species are most often found in cooler production areas (Backhouse *et al*., 2001; Bottalico and Perrone, 2002) and this might explain the higher incidence of these species at the early planting dates in the southern region of Uruguay (La Estanzuela) where cooler conditions than the other sites occur at heading/flowering. *Fusarium poae* was the second most prevalent species in barley grains. Under Uruguayan conditions, infection by *F. poae* usually occurs at boot stage, infecting the spike through the flag leaf sheath in late-August or early-September when lower temperatures are generally more favorable for disease development. The virulence and increasingly wide distribution of *F. poae* in barley should not be overlooked by barley breeding programs when screening lines for resistance to FHB.

All species were pathogenic on wheat and barley in inoculation tests in the greenhouse, except *F. semitectum* on wheat. Greater FHB severity and FHB incidence on wheat and barley spikes were obtained with the *F. graminearum* isolates, followed by *F. avenaceum* and *F. poae*. Cultivars previously characterized as moderately resistant to moderately susceptible showed the lowest FHB incidences, severities, percentages of *Fusarium*-infested grains, and grains infested with *F. graminearum*.

The results from this study have shown that several potentially important toxin-producing *Fusarium* species are common under natural conditions in wheat and barley grains in Uruguay.

Fusarium graminearum diversity

Phylogenetic lineage 7 (*F. graminearum sensu* O´Donnell) was the most common lineage isolated from wheat grains in Uruguay during 2001, 2002, and 2003 (Figure 2). Sixty-two of the 64 isolates corresponded to this lineage, one isolate to lineage 1 (*F. austroamericanum*) and one to lineage 8 (*F. cortaderiae*). Presence of these lineages have already been cited in the Southern Cone of South America by several authors (Leslie and Bowden, 2005; O´Donnell *et al*., 2004; Zeller *et al*., 2002; Zeller *et al*., 2003).

Based on the presence of genes *Tri7* and *Tri13*, all isolates belonged to DON chemotype. Several amplification sizes were obtained from the different *F. graminearum* isolates for *Tri7* gene, indicating considerable diversity among these isolates (Figure 3).

Deoxynivalenol (DON) was produced by all 64 isolates in rice culture. Isolates were classified into three groups according to levels of DON production (Figure 4). Lineage 8- isolate produced less than 10 ppm of DON, while lineage 1- isolate produced DON in the range of 100 to 1000 ppm. All isolates were 15-AcDON producers, except isolate of lineage 1, which corresponded to the 3-AcDON type.

All isolates had high levels of sensitivity against thiabendazole and metconazole. However, different levels of sensitivity were observed against tebuconazole.

Preliminary results showed differences among isolates with high DON production for aggressiveness and perithecia production. All isolates tested caused visible symptoms of FHB, however, some isolates did not cause detectable disease on some resistant wheat genotypes. The effect of the isolate on FHB severity was significant (*P=0.0001*). The average range of FHB severities on inoculated spikes 21 days after inoculation was from 18% to 40%. These results are currently being checked in a repetition of the experiment.

All isolates produced perithecia under the temperature, moisture and light conditions of this study. However, there were significant differences among isolates for perithecia production (*P=0.005*). Average perithecia per gram of residue ranged from 59 to 521.

Results from this study reveal that the population of *F. graminearum* in Uruguay is diverse for several characteristics. Further work is ongoing to understand the genetic and pathogenic diversity of the populations of *F. graminearum* in wheat and barley producing areas. Furthermore, information from these studies will be added to analyses of diversity of *Fusarium* populations in the Southern Cone of South America.

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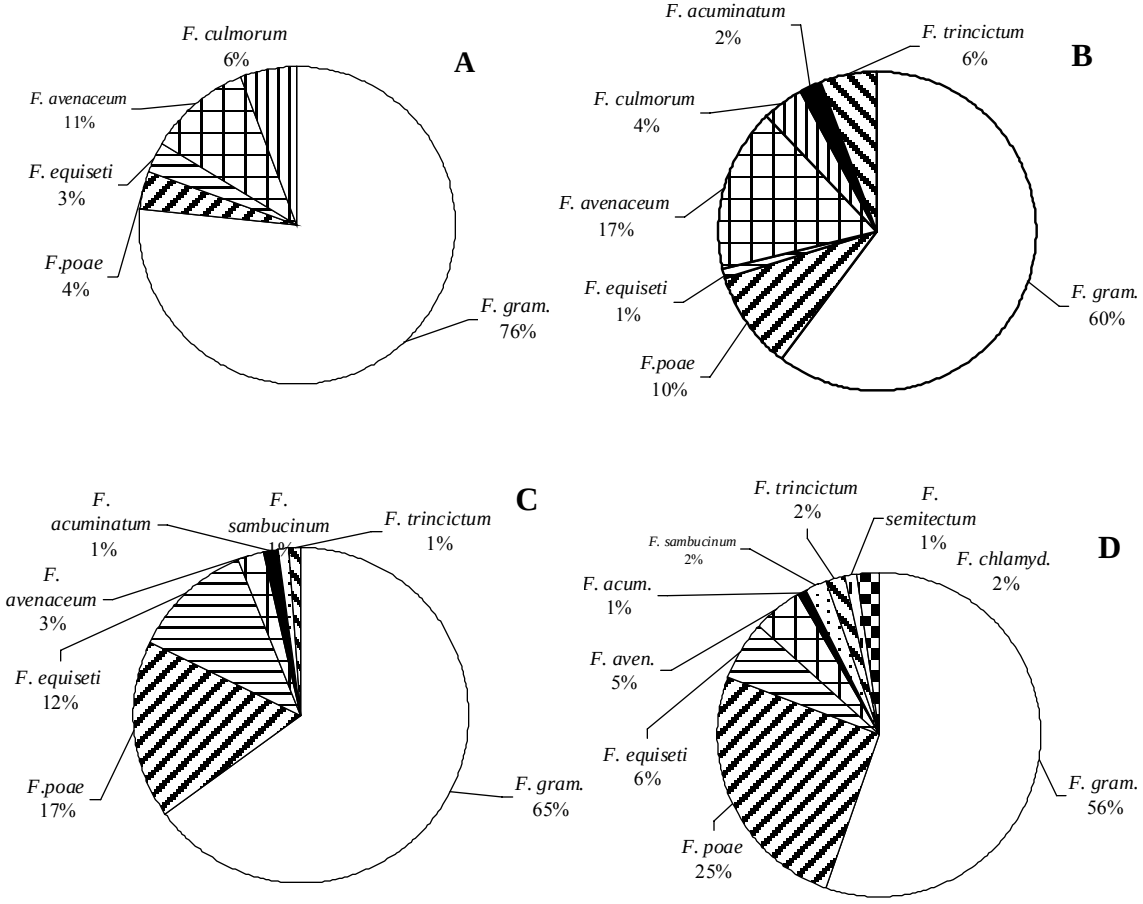

Figure 1. Frequency of *Fusarium* species isolated from wheat (A,B) and barley (C,D) grain sampled from regional field trials in 2001 (A,C) and 2002 (B,D)

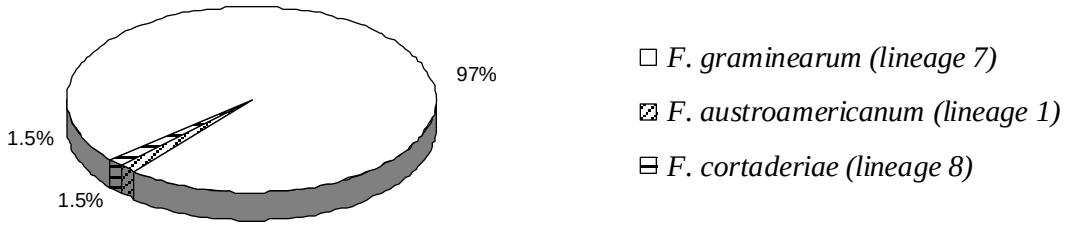

Figure 2. Frequency of *Fusarium graminearum* phylogenetic lineages and corresponding species *sensu* O'Donnell *et al*. (2004) isolated from wheat grains in 2001, 2002 and 2003.

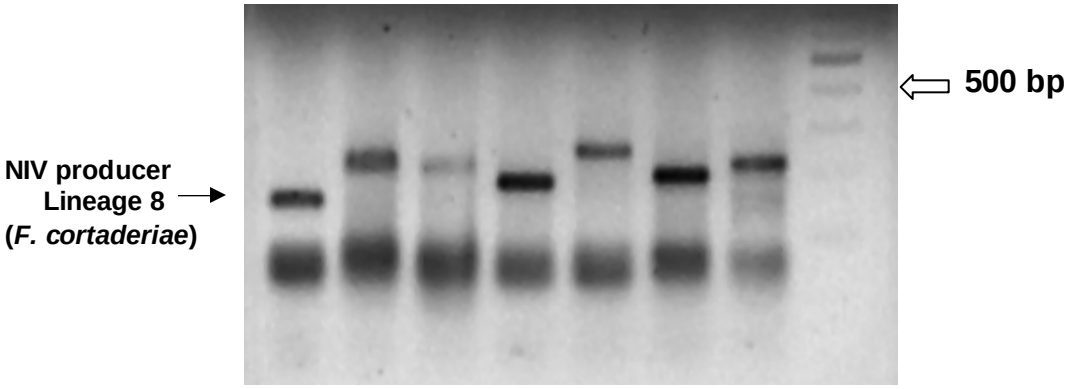

Figure 3. PCR products obtained for *Tri7* gene amplification of different *F. graminearum* isolates. Different sizes correspond to different number of 11bp insertions.

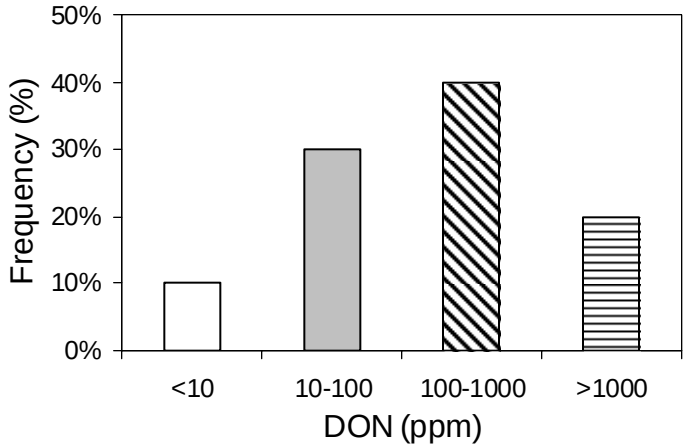

Figure 4. Frequency of *Fusarium graminearum* isolates producing different deoxynivalenol (DON) concentrations in rice grain cultures.

FUSARIUM PATHOGENS OF WHEAT IN AUSTRALIA

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ABSTRACT

This paper deals with a comparative study of Fusarium head blight (FHB) pathogens including *Fusarium graminearum* (FG) and *F. pseudograminearum* (FP) from Australia and the USA using aggressiveness, mycotoxin production and genotypic diversity based on amplified fragment length polymorphism. In addition, it examines phylogenetic relationships within FP using genealogical analysis of four nuclear genes. Both Australian FG and FP are genetically diverse, although perithecia are rarely seen in nature in the heterothallic teleomorph of FP. Both species produced mycotoxins in grain cultures and caused FHB in infection assays. Although toxin levels and FHB severity varied with the isolate, there was no association between these two traits. A multi-locus sequence analysis of FP isolates from Australia, Canada, New Zealand, Turkey and the USA differentiated this species into four well-supported clades, but these were not linked to their geographic origin. The paper stresses the importance of international collaboration in sharing information, tools, and resources in making research outputs applicable to wide geographical regions.

INTRODUCTION

In Australia, crown rot (CR), predominantly caused by *Fusarium pseudograminearum* (FP), and head blight (FHB), predominantly caused by *F. graminearum* (FG), are two important diseases of wheat (Burgess *et al*., 2001). CR imposes a recurring cost of >$56 million per year over vast areas. The impact of FHB is sporadic in Australia and limited to an area in northern New South Wales (NSW) and southern Queensland (QLD) and as a result, there has been limited research. The global significance of FHB has prompted coordinated research efforts in the USA and Europe, among others, to generate a wealth of knowledge (Goswami and Kistler, 2004). In contrast, the majority of CR research originates from Australia despite its presence in most cereal-growing regions of the world. Ongoing Australian research over the past 50 years, reviewed by Burgess *et al*. (2001), has been boosted by recent industry initiatives. New research to improve host resistance to CR and FHB by applying essential knowledge of pathogen biology, genetics and epidemiology started in 2001 with the build up of a culture collection of well characterized isolates of *Fusarium* spp. from Australia and overseas. All 17 *Fusarium* species collected from Australian field surveys caused FHB and all 10 of these 17 tested caused CR in plant infection assays where 20% of FP and FG isolates were aggressive to highly aggressive for both diseases (Akinsanmi *et al.,* 2004). New knowledge of pathogen aggressiveness has led to the development of a new high throughput seedling assay for CR (Mitter *et al.,* 2006) to accelerate the search for host plant resistance.

FHB and CR have linked aetiology and epidemiology but the relationship among *Fusarium* species and strains that cause FHB and CR are far from clear, except that infected crop residue is the primary inoculum source for both diseases. The narrow window of FHB infection and the subsequent removal of infected heads at harvest raise the possibility that the bulk of FHB inoculum may originate from stem and/or crown infection. How CR infected crops contribute to FHB inoculum has never been explored despite increased prominence of CR in the USA in recent years (Smiley *et al*., 2005). Similarly, the wealth of knowledge from global FHB research has not been applied to CR. To do this, the information gained on one location/disease must be relevant to another location/disease. In the USA ascospores are the main FHB inoculum but these are less aggressive than macroconidia in CR infection assays. In Australia macroconidia are the dominant FHB inoculum and both spore types are equally aggressive for CR (Mitter *et al*., unpublished data). In this paper we continue the

comparative study of FG and FP from Australia and the USA using FHB aggressiveness, toxin production and genotypic diversity. Additionally, phylogenetic relationships within FP were examined using genealogical analysis of four nuclear genes.

MATERIALS AND METHODS

Comparison of F. graminearum from Australia and the USA as FHB pathogen

A total of 13 *F. avenaceum*, 6 *F. crookwellense*, 69 FG and 75 FP isolates from Australia and 6 *F. avenaceum*, 5 *F. culmorum*, 130 FG, 5 *F. poae,* 5 FP and 7 *F. sporotrichoides* isolates from the USA were studied using the same methodology. FHB aggressiveness of isolates from the USA was studied at the University of Minnesota on cultivars Wheaton (susceptible), Pioneer 2375 (moderately susceptible) and Alsen (Sumai3-derived moderately resistant) by point inoculation of a central spikelet in each of 10 spikes with 10µl suspension of 10^5 macroconidia/ml at anthesis. The Australian isolates were similarly inoculated on to Wheaton (susceptible), Pioneer 2375 and Sumai3 (moderately resistant) at CSIRO facilities in Brisbane. Each isolate was grown on autoclaved wheat grains, ground and toxins extracted and analyzed following the procedures of Mirocha *et al*. (1998). Genotypic diversity was analyzed using amplified fragment length polymorphism (AFLP) following methods outlined previously (Akinsanmi *et al*. 2006).

Phylogenetics of F. pseudograminearum

DNA from the translation elongation factor-1α, phosphate permease 1, phosphate permease 2 and the intergenic region between these two genes, a reductase gene and the β-tubulin gene were amplified and sequenced for 54 FP isolates from Australia, Canada, Turkey, New Zealand and the USA. Data were analyzed separately and as a multi-locus sequence using both maximum parsimony and Bayesian inference methods.

RESULTS AND DISCUSSION

Comparison of F. graminearum from Australia and the USA as FHB pathogen

Results on the Australian isolates are mainly discussed here. On sterilized wheat grain the Australian FG isolates produced between 0 and 113 ppm deoxynivalenol (DON) (mean 10.3, median 1.9) compared with 0 to 1587 ppm (mean 21.5, median 2.2) for isolates from the USA. Australian FP isolates produced 0 - 45 ppm (mean 1.3) DON compared with 0.1-101 ppm (mean 22.6) for isolates from the USA. In most isolates 3ADON and/or 15ADON were co-produced in smaller quantities but none produced Nivalenol to any significant extent. FG and FP isolates differed in their aggressiveness on the three wheat varieties; Sumai 3 was the most resistant, 2375 was intermediate in resistance and Wheaton the most susceptible. Toxin production in grain culture was not associated with aggressiveness on any variety, indicating that mycotoxins are only one component of aggressiveness. Data on mean FHB severity on three wheat varieties and DON are shown for FG and FP (Figure 1).

The genotypic diversity was high; over 90% of FP and FG isolates had distinct haplotypes. The polyphyletic Australian FG was grouped into five AFLP clusters (Figure 2) with reference isolates of lineages 2, 3, 4 and 6 were distributed within two of the Australian clusters, whereas isolates of lineages 1, 5 and 7 were contained in a single cluster with other Australian isolates. Although this suggests FG lineages 1, 2, 3, 4, 5, 6 and 7 (O'Donnell *et al.*, 2004) in Australia this must be confirmed by gene sequencing since AFLP clustering does not accurately correlate with lineages. So far, sequencing has confirmed lineage 7 (FG) and 8 (*F. cortideriae*) in Australia. Genotypic diversity and FHB aggressiveness in FG were not significantly linked. The 5 clusters for the Australian FP isolates were not as clearly delineated (Figure 3) and unlike FG, AFLP clusters were significantly association with FHB aggressiveness. The population structure of the heterothallic *Gibberella coronicola* (teleomorph of FP) and the homothallic *G. zeae* (teleomorph of FG) was not that different and neither species was panmictic or strictly clonal.

Phylogenetics of F. pseudograminearum

Evolutionary divergence varied between genes and there was phylogenetic incongruence between the reductase gene and the translation elongation factor-1α, phosphate permease and β-tubulin genes. A multi-locus sequence analysis differentiated FP isolates into three well-supported clades while a single Turkish isolate formed a fourth clade. The majority of Australian, all New Zealand, two Canadian and one isolate from the USA grouped in one clade; five of seven Canadian isolates formed another; and four isolates from the USA and two Australian isolates formed the third clade. These clades were not consistent across genes to indicate that FP is a single phylogenetic species as opposed to FG (O'Donnell *et al*. 2004). Evolutionary structure and geographic origin of isolates were not linked, indicating FP to be a recombining species. The high level of genetic variation and sexual recombination in the heterothallic *G. coronicola* is consistent with a recent finding that high levels of genetic variation is generated by single recombination events and that the *MAT1-1* and

MAT1-2 idiomorphs of the mating type locus occurs in 1:1 ratio in most field populations within Australia (Bentley *et al*. 2005).

In Australia the geographical distribution of FG is far more restricted than the widespread FP. This is counter-intuitive, since the long distance dispersal of airborne *G. zeae* ascospores should have made this species more widespread than *G. coronicola* where perithecia are not frequent. The level of genotypic diversity and population differentiation or the range of aggressiveness for FHB as determined from the current work do not indicate a superior pathogenic or saprophytic fitness of FP over FG either. While climate (Backhouse and Burgess 2002) can influence the distribution of some *Fusarium* species, the temperature difference between northern NSW and southern QLD is not large enough to explain the differential distribution of these two species. There is a history of research on epidemiology and management of CR in Australia. New information on the structure, genetics and evolution of the pathogen population is only just beginning to emerge. The vast wealth of FHB knowledge is starting to be examined from a CR perspective by sharing information, tools, and resources through international collaboration. This must continue, evolve and expand to encompass other research groups to make research and development more cost effective and targeted.

ACKNOWLEDGEMENTS

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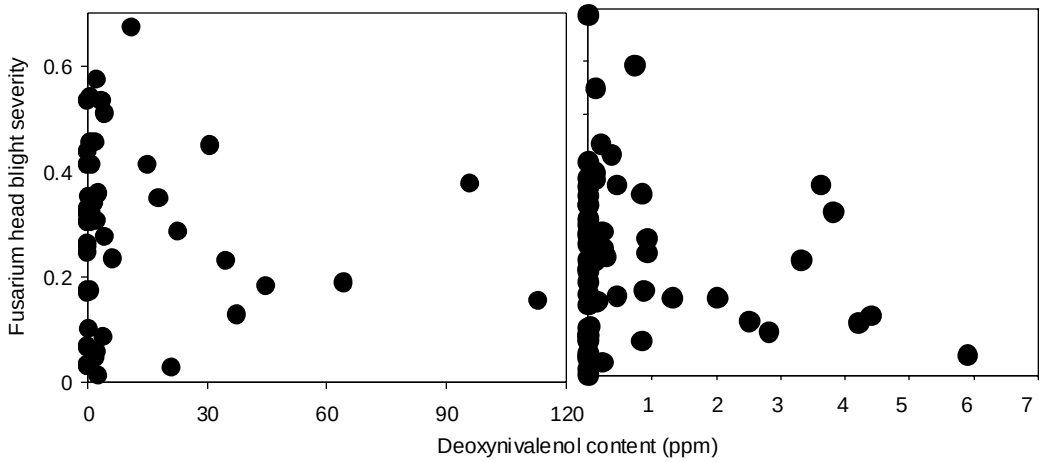

Figure 1. Mean Fusarium head blight severity (proportion of spikelet infected) on three wheat varieties and Deoxynivalenol production on grain culture by isolates of Australian *Fusarium graminearum* (left) and *F. pseudograminearum* (right)

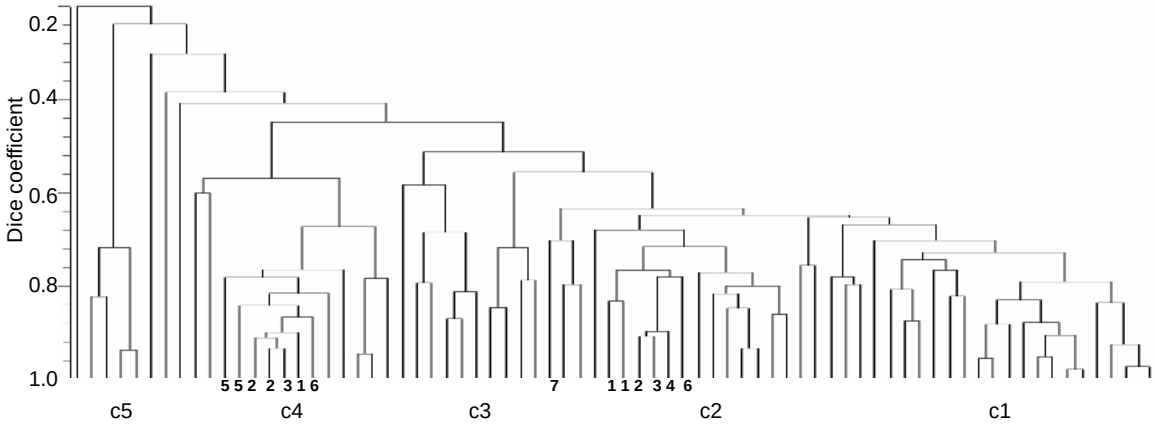

Figure 2. Five genotypic clusters (c1-c5) of Australian *Fusarium graminearum* isolates based on amplified fragment length polymorphism with lineages (small numerals) of reference isolates

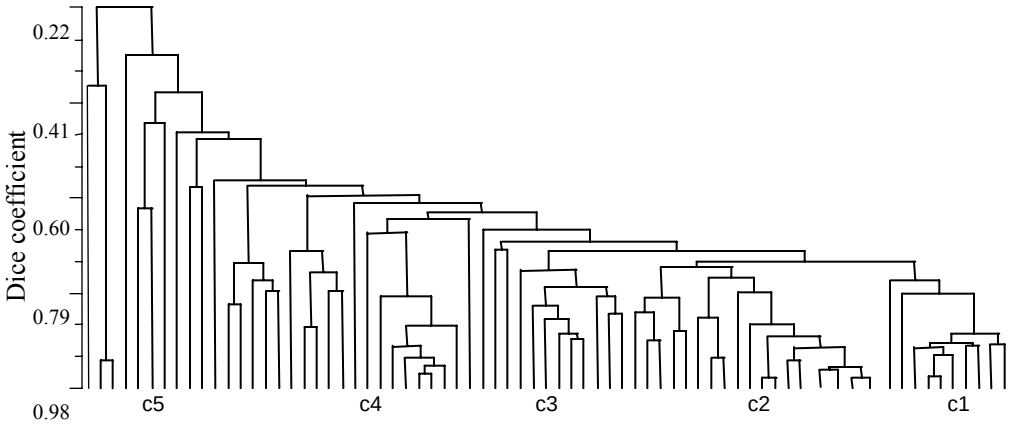

Figure 3. Genotypic clusters (c1-c5) of Australian *Fusarium pseudograminearum* isolates based on amplified fragment length polymorphism

TURKISH *FUSARIUM* ISOLATES FROM WHEAT CROWN AND HEAD CAN CAUSE SEVERE CROWN ROT

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ABSTRACT

This paper deals with the severity of crown rot caused by *Fusarium acuminatum, F. avenaceum, F. chlamydosporum, F. crookwellense, F. culmorum, F. equiseti, F. graminearum, F. oxysporum, F. pseudograminearum, F. semitectum, F. solani, F. subglutinans* and *F. verticilloides* isolated from wheat fields in Turkey surveyed for Fusarium head blight in 2003 and crown rot in 2005. In a seedling assay *Fusarium* species and isolates within a species significantly influenced the level of crown discoloration and biomass of the winter wheat cultivar 'Pehlivan'. There was no significant difference among the 3 most aggressive species, *F. culmorum, F. graminearum* and *F. pseudograminearum*. Where isolates of the same *Fusarium* species were obtained from head and crown tissue, these did not differ greatly in their aggressiveness on the crown tissue.

INTRODUCTION

The genus *Fusarium* is one of the most widespread and economically important groups of fungi with more than one hundred species attacking most plant species. On wheat and barley *Fusarium* species cause two diseases: a basal stem and root rot often known as crown rot (CR) and spike infection commonly known as Fusarium head blight (FHB). Several *Fusarium* and other species including *F. pseudograminearum* (teleomorph *Gibberella coronicola*), *F. crookwellense, F. avenaceum* (teleomorph. *G. avenacea*), *F. culmorum, F. acuminatum* and *Microdochium nivale* (teleomorph *Monographella nivalis*) can cause CR (Cook, 1981, Specht and Rush, 1988). A similar range of species including *F. graminearum, F. culmorum, F. avenaceum, F. sambucinum* var. *coeruleum, F. crookwellense, F. sporotrichoides* and *M. nivale* can cause FHB (Arsenuik *et al.* 1991., Miedaner *et al*., 1993).

Turkey is among the 10 largest wheat producers worldwide with 16-21 million tones from 9.35 Mha at an average yield of 2t/ha (Braun *et al*., 2001). Over half of the area is located in Central Turkey where wheat is grown under rainfed or supplementary irrigation conditions in cereal fallow rotation. In the Central Anatolia Plateau cereal root rots contribute to significant yield losses of between 24-36% in the commonly cultivated winter wheats (Aktaş *et al*. 1999; Hekimhan *et al*., 2004).

The distribution of *Fusarium* species associated with CR in Turkey varies with the geographical region: *F. pseudograminearum* is more widespread than *F. culmorum* in the Northeast Marmara coastal region (Aktaş *et al*., 1996), while in the Central Anatolia Plateau *F. culmorum* was the most commonly isolated species from the crown and sub-crown tissue in three surveys spanning 1994 to 2004 (Aktaş *et al*., 1999; Tunali *et al*., 2006) While most tests show *F. pseudograminearum* and *F. culmorum* as the most pathogenic for CR in Turkey, some reports indicate *F. avenaceum* to be equally or more pathogenic than the other species (Arsenuik *et al*., 1993; Jenkins and Parry, 1994).

In Turkey FHB epidemics are restricted to years of high humidity during anthesis and *F. graminearum* was the primary pathogen in the Northwest Anatolia in 2001/02 (Tunali *et al*., 2006). Although *F. culmorum* is more aggressive on wheat spikes in pathogenicity tests, it is considered a secondary pathogen following *F. graminearum* infection.

In Australia both *F. graminearum* and *F. pseudograminearum* can cause CR and FHB; but the former predominantly occurs on flag leaf node and head tissue while the later is common on crown and stubble (Akinsanmi *et al.*, 2004). Overall, isolates from stem base and crown are more aggressive for CR and

isolates from flag leaf node and head tissue are more aggressive for FHB. Although several species including *F. culmorum, F. graminearum* and *F. pseudograminearum* are commonly associated with CR and FHB in Turkey, their relative aggressiveness for CR has not been studied in details. This paper compares the CR aggressiveness of isolates from several *Fusarium* species originating from crown and head tissue of wheat.

MATERIALS AND METHODS

All isolates of *Fusarium* species used in this work came from two surveys around plant maturity (Zodoks growth stage 92) of wheat growing areas in central and northern Anatolia: survey for FHB in 2003 and for CR in 2005 (Figure1). Details are given for the 2005 CR survey where diseased crown and sub crown tissue was collected from five plants at each of the 32 sites. Small pieces of infected tissue were washed in tap water, surface sterilized with 1% NaOCl solution for 3 minutes and plated on ¼ strength potato dextrose agar (PDA, 9g PDA, 10g Bacto agar, 1L distilled water) amended with streptomycin sulfate (100 mg/L) and Oxytetracyline (60 mg/L). Plates were incubated for 7 days at 25 ºC, 15 h photoperiod under cool white fluorescent light and colonies were transferred to Carnation leaf agar (CLA). To obtain monoconidial isolates water agar plates were seeded with a conidial suspension, incubated (Burgess *et al*., 1994) and a single germinated macroconidium on a small square of agar was transferred to a fresh CLA plate using a sterile needle. *Fusarium* species were identified using morphological and cultural characteristics (Booth, 1977; Gerlach and Nirenberg 1982; Tousson and Nelson, 1985).

The pathogenicity of 51 isolates from 13 *Fusarium* species was tested in a glasshouse assay. Plants were grown in plastic pots (8 cm diameter, 16cm high) with a mixture of autoclaved commercial potting soil and local loam soil. Seeds of the winter wheat cultivar 'Pehlivan' were surface sterilized for 3 minutes in a 1% aqueous solution of NaOCl, rinsed twice in sterile distilled water and coated separately with macroconidia and mycelia of each of the 51 monoconidial isolates by shaking on a CLA plate of a 7 day old culture. Additional inoculum was added by placing a 1 cm diameter agar plug with mycelium and spores from the periphery of CLA cultures of the respective isolate at the bottom of each of the three 3-cm deep holes in each pot (Fernandez and Chen, 2005). One Pehlivan seed coated with the same isolate was placed on top of the agar plug so that the growing colony was in contact with the seed and the holes were covered with 3 cm of potting soil. Sterile agar plugs were used as a control. Three pots were used for each fungal isolate and treatments were arranged in a completely randomized design with each pot treated as one replicate. Plants were watered as necessary and grown at 25±5°C in a greenhouse under a 16 h photoperiod using fluorescent lights.

Plant emergence was counted 15 days after planting and symptoms were assessed at harvest on day 70. Each plant was rated for discoloration of crown and the sub coronal internodes using a 0-3 scale, where, 0= no discoloration, 1= trace to 25% discoloration, 2= 25 to 50%, and 3= 50>%. Plants were dried between 2 layers of paper towels following disease assessment and the fresh weight was recorded as an estimate of biomass.

Data on percentage discoloration were analyzed using a nested model in an analysis of variance where isolates were nested within species using the SAS software.

RESULTS

Eleven isolates comprising *F. culmorum* (2), *F. graminearum* (4) and *F. pseudograminearum* (5), from the 2003 FHB survey were used (Table 1). The remaining 40 isolates of *F. acuminatum* (4), *F. avenaceum* (2), *F. chlamydosporum* (2), *F. crookwellense* (1), *F. culmorum* (13), *F. equiseti* (2), *F. oxysporum* (2), *F. pseudograminearum* (1), *F. semitectum* (2), *F. solani* (2), *F. subglutinans* (4) and *F. verticilloides* (5) originated from crowns of 160 plants sampled from 32 fields in 2005. All species and isolates caused discoloration of crown and the sub-coronal internode. The level of aggressiveness differed significantly both within (Figure 1) and among *Fusarium* species but there was no significant difference among the 3 most aggressive species, *F. culmorum, F. graminearum* and *F. pseudograminearum* (Table 1). The aggressive species also greatly reduced plant emergence and survival (data not shown). The pathogenicity of each species and isolate was confirmed by following Koch postulates where infected tissue from the crown and sub crown internodes were plated onto CLA medium and the identity of the *Fusarium* species was re-confirmed.

The impact of crown rot was evident from a significant reduction of plant biomass with the three most aggressive species, *F. culmorum, F. pseudograminearum* and *F. graminearum*, reducing growth by up to 85% of the uninoculated control (Table 1). As expected, crown rot severity and plant biomass were inversely correlated (Figure 2). All except *F. crookwellense* had more than 1 isolate per

species, but an overwhelming majority of isolates originated from the crown tissue and only *F. culmorum* and *F. pseudograminearum* isolates originated from both spike and crown tissues. Overall, the crown rot severity of isolates originating from the crown tissue was not different to that of the isolates originating from the spike, but the very limited number of isolates from the spike makes this comparison tentative.

DISCUSSION

We have shown that *Fusarium* species and isolates within a species significantly influence crown rot severity and biomass of winter wheat Pehlivan in a seedling assay and *F. culmorum, F. graminearum* and *F. pseudograminearum* are the 3 most aggressive species. Where isolates of the same *Fusarium* species were available from head and crown tissue, these did not differ greatly in their aggressiveness on the crown tissue. The three highly aggressive species also affected seedling emergence and growth. This is similar to Australian findings where *F. graminearum* from wheat spikes and *F. pseudograminearum* mostly from crown tissue were equally aggressive for crown rot under greenhouse tests (Akinsanmi *et al*., 2004). Other findings (Fernandez and Chen, 2005) also show that *Fusarium* species derived from infected wheat heads or sub-crown internode/crown have similar relative pathogenicity on wheat heads. These data indicate that the entire wheat plant may play an important role in the survival of the pathogen and act as inoculum source for both FHB & CR.

Previous work has failed to separate *F. graminearum* and *F. pseudograminearum* based on their crown rot aggressiveness in seedling bioassays where the same 20% of isolates from both species were aggressive or highly aggressive for both CR and FHB (Akinsanmi *et al*., 2004). Our current study showing *F. pseudograminearum* and *F. graminearum* among two of the three most highly aggressive species causing CR in the seedling test, confirms this earlier finding. Extensive studies in the USA (Smiley and Patterson, 1996) with more than 1200 *Fusarium* isolates representing 19 species confirmed *F. graminearum, F. culmorum* and *B. sorokiniana* to be the major species associated with root and crown rot of wheat. Recent work has further highlighted the importance of *F. pseudograminearum* (Smiley *et al.,* 2005). Although many other *Fusarium* species have been isolated from the crown tissue in this and previous research (Uoti, 1976; Arsenuik *et al*., 1993 their importance as a CR pathogen appears to be limited.

Recent extensive surveys of a major cereal producing region in Turkey have clearly identified *F. culmorum* as the main *Fusarium* species associated with the crown tissue (Tunali *et al*. 2006), while *F. pseudograminearum* is the dominant pathogen in some other areas (Aktaş *et al*., 1996). Both are among the most aggressive *Fusarium* species for CR. *Fusarium graminearum*, the other most aggressive CR pathogen is also the dominant FHB pathogen in Turkey (Tunali *et al*., 2006). CR is a chronic biotic constraint in Turkey on dryland wheat while FHB is restricted to years of high humidity during anthesis. Given the global efforts in identifying FHB resistance, future research need to explore whether some of the sources of FHB resistance to also offer resistance to CR. This must be combined with field-based research on the inter-relationships between FHB and CR epidemiology.

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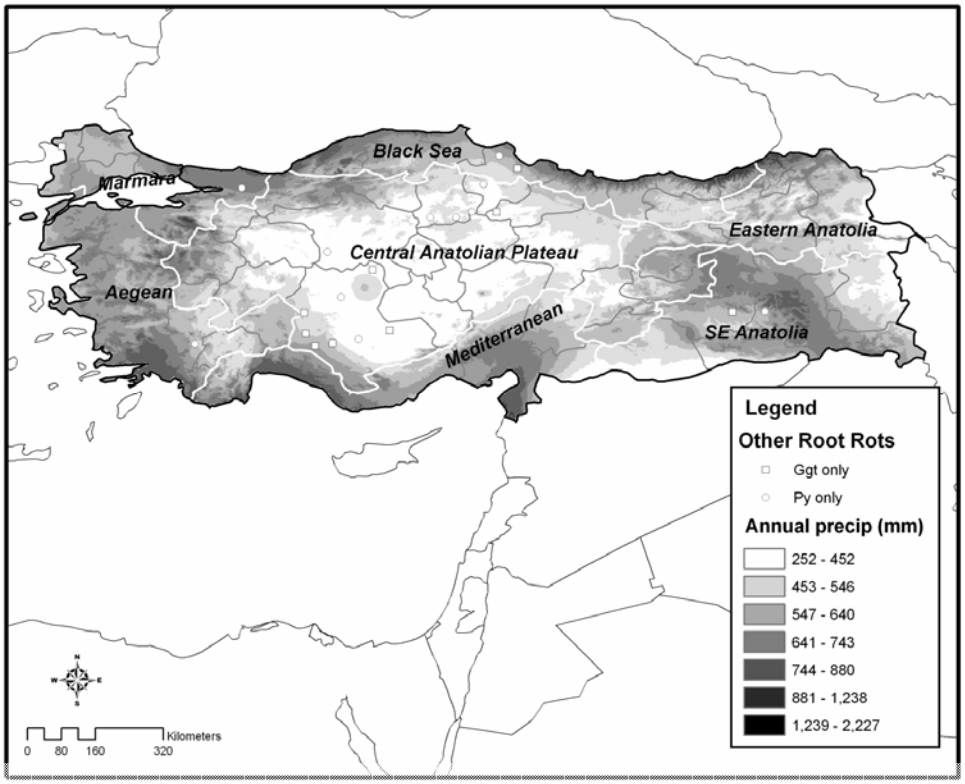

Figure 1. Field sites in Central and Northern part of Anatolia, Turkey sampled for *Fusarium* species associated with crown rot (2005) and Fusarium head blight (2003) of

Table 1. Crown rot severity and fresh weight of winter wheat 'Pehlivan' inoculated in the greenhouse with isolates from 13 *Fusarium* species obtained from different parts of wheat plants growing in farmers' fields in Turkey.

Fusarium species	Isolates from crown	Isolates from spike	Total	Mean severity	Mean fresh weight
F. pseudograminearum	1	5	6	91.98 A	0.28 D
F. culmorum	13	2	15	91.57 A	0.35 D
F. graminearum	0	4	4	88.61 A	0.43 D
F. avenaceum	2	0	2	59.28 B	1.57 ABC
F. crookwellense	1	0	1	55.17 BC	0.89 CD
F. semitectum	2	0	2	48.13 BCD	1.23 BC
F. oxysporum	2	0	2	46.27 BCD	1.01 CD
F. solani	2	0	2	44.43 BCD	1.41 ABC
F. verticilloides	5	0	5	37.01 CDE	1.60 ABC
F. acuminatum	4	0	4	36.97 CDE	2.03 AB
F. equiseti	2	0	2	31.45 DE	1.82 AB
F. chlamydosporum	2	0	2	31.45 DE	1.65 ABC
F. subglutinans	4	0	4	22.21 E	2.07 A
Total isolates	40	11	51		

Within columns, means followed by a different letter are significantly different (P <0.05) according to Ryan-Einot-Gabriel-Welsch Multiple Range Test.

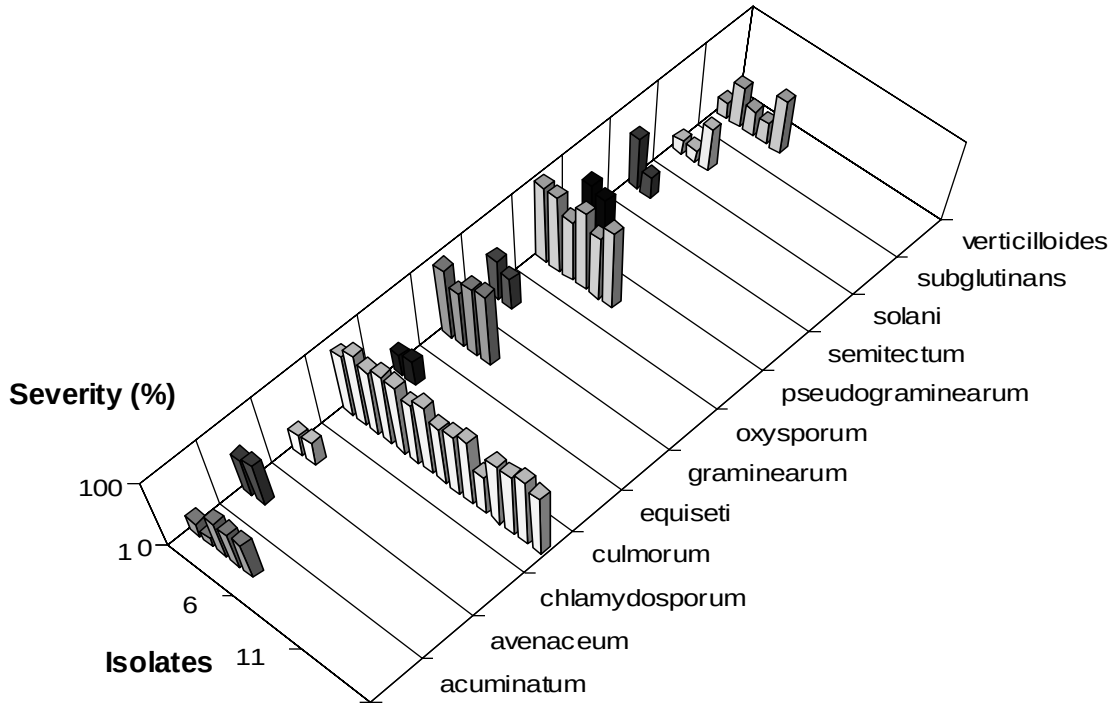

Figure 2. Crown rot aggressiveness (severity) of isolates from 13 different *Fusarium* spp. on winter wheat 'Pehlivan'.

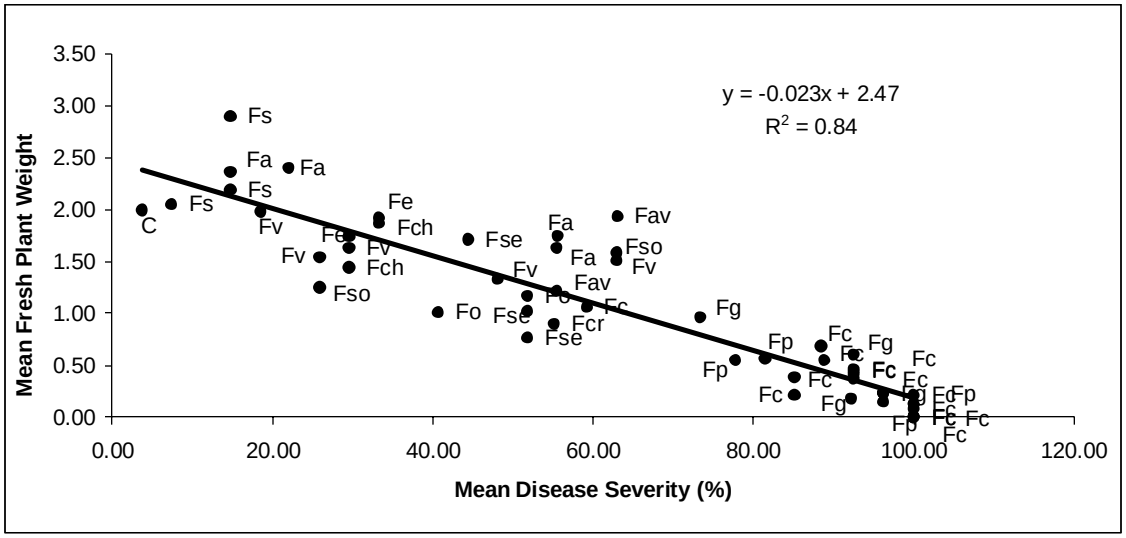

Figure 3. Relationship between fresh plant weight and crown rot severity caused by isolates of 13 different *Fusarium* spp. on winter wheat 'Pehlivan' screened under greenhouse conditions.
F.a: *F. acuminatum*, F.av: *F.avenaceum*, F.c: *F. culmorum*, F.ch: *F. chlamydosporum,* F.cr: *F. crookwellence*, F.e: *F. equiseti*, F.g: *F. graminearum*, F.o: *F. oxysporum*, F.p: *F. pseudograminearum*, F.s: *F. solani*, F.se: *F. semitectum*, F.s: *F. subglutinans*, F.v: *F. verticilloides.*

VEGETATIVE COMPATIBILITY ANALYSIS (VCG) AND SEQUENCE RELATED AMPLIFIED POLYMORPHISMS (SRAP) IN UNDERSTANDING GENETIC DIVERSITY OF *GIBBERELLA ZEAE* ISOLATES FROM TWO MANITOBA FIELDS

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ABSTRACT

Gibberella zeae causes Fusarium head blight of wheat. It is one of the most important diseases of cereals in the Canadian prairies especially the Red River Valley for the last decade. In 2002, 60 isolates of *G. zeae* were collected and single spored from naturally infected spikes of wheat from Carman and Winnipeg in Manitoba. These isolates were compared using vegetative compatibility analysis and PCR-based sequence related amplified polymorphisms (SRAP). Sixteen vegetative compatibility groups (VCG) were found among the 50 isolates tested. Eight SRAP primer pairs identified 59 distinct haplotypes. Principal component analysis and UPGMA separated the dataset into two main groups, each with isolates from both locations. The analysis of molecular variance also revealed that 75% and 20% of the variance were associated to differences among individual isolates and varieties sampled respectively. Geographic location was not a significant source of variation at P=0.05 and accounted for only 4% of total variance. A low correlation between VCG and SRAP marker data was detected. This study showed that though the genetic diversity is high among *G. zeae* isolates, Carman and Winnipeg collections have a similar genetic makeup and are likely part of the same population. A large-scale study has been initiated collecting *Fusarium* isolates from 15 different farmers' fields located throughout Manitoba. The crop history, environmental conditions, cultivars, and soil type are different in these fields. The new study will investigate the genotype and chemo-type differences of isolates from between and within fields.

PRESENT STATUS OF THE *FUSARIUM GRAMINEARUM* CLADE IN EUROPE AND POSSIBLE DEVELOPMENT STRATEGIES

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ABSTRACT

Fusarium head blight caused mainly by *Fusarium graminearum* and *F. culmorum* is the most important disease of wheat in Central Europe. Previous studies clarified that *F. graminearum* is an assemblage of at least nine geographically separated species (O'Donnell et al. 2004).

We examined the mycotoxin producing ability, molecular variability and aggressiveness of *Fusarium graminearum* isolates originated from Central Europe, and representatives of 8 species identified in the *F. graminearum* clade. Mycotoxin producing abilities of the isolates were tested by GC-MS and HPLC analyses. The mycotoxins tested included type B trichothecenes (deoxynivalenol, 3- and 15-acetyl-deoxynivalenol, nivalenol, 4-acetyl-nivalenol (fusarenone X)) and zearalenone. All but one of the isolates produced zearalenone. The Central-European isolates were found to belong to chemotype I (producing deoxynivalenol). Most of them produced more 15-acetyl-deoxynivalenol than 3-acetyl-deoxynivalenol, indicating that these isolates possibly belong to chemotype 1b.

Phylogenetic analysis of random amplified polymorphic DNA (RAPD) profiles of the isolates let us cluster the Central-European isolates into 10 haplotypes. The three Austrian isolates formed a distinct clade on the tree. We also examined the variability of the intergenic spacer region (IGS) of the ribosomal RNA gene cluster using IGS-RFLP analysis. The isolates belonged into 9 haplotypes on the tree based on IGS-RFLP data. Representatives of species of the *F. graminearum* clade exhibited unique IGS-RFLP and RAPD profiles. When RAPD and IGS-RFLP data were combined, almost every single Central-European *F. graminearum* isolate could be differentiated from each other (27/30 haplotypes). Such a lack of strict correlation between trees based on different data sets indicates that recombination took place in the examined population due to frequent outcrossing. Based on RAPD, IGS-RFLP and sequence data, the majority of the Central-European isolates belong to the *F. graminearum sensu stricto* species characteristic to the Northern hemisphere, with the exception of a Hungarian isolate, which was closely related to *F. asiaticum* based on RAPD and IGS-RFLP data. Further sequence analysis revealed that this isolate belongs to a new species, which occurs in Hungary and Japan (*F. vorosii,* species description is in progress; Tóth et al. 2005). The taxonomic assignment of two other Hungarian isolates previously suggested to belong to *F. boothii* based on mitochondrial DNA RFLP data were supported by sequence analysis.

Isolates belonging to the *F. graminearum* species complex exhibited high levels of strain-specific variability in their aggressiveness and their ability to produce trichothecenes on susceptible cultivars (Goswami et al. 2005). We tested twenty wheat genotypes in 2003–2004 under field conditions by spraying inocula of isolates of eight species of the *F. graminearum* species complex representing geographically isolated populations. The various wheat genotypes exhibited similar reactions against the different *Fusarium* isolates, indicating that resistance to *F. graminearum sensu lato* was similar to that for the other *Fusarium* species examined (Mesterházy et al. 2005). This is an important message to breeders as the resistance relates not only to any particular isolate of *F. graminearum sensu stricto*, but similarly to other isolates of the *Fusarium graminearum* species complex as well. Based on our results, the new members of the *F. graminearum* species complex do not seem to have any importance for breeding. Therefore resistant genotypes can be grown successfully in areas where different members of the species complex dominate or occur mixed.

CROSS FERTILITY OF LINEAGES IN *FUSARIUM GRAMINEARUM* (*GIBBERELLA ZEAE*)

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INTRODUCTION

Fusarium graminearum Schwabe is the main causal agent of Fusarium head blight of wheat and barley around the world. The name *Fusarium graminearum* Schwabe is used to designate the anamorph (asexual conidial stage) of the fungus. The name *Gibberella zeae* (Schwein.) Petch is used to designate the teleomorph (sexual ascospore stage) of the fungus. Both names are commonly used in the literature to denote this fungus. *G. zeae* is homothallic, but it can also outcross in the laboratory (Bowden and Leslie, 1999) and there are several lines of evidence that it outcrosses in the field (Schmale et al. 2006; Zeller et al., 2004).

O'Donnell et al. (2000) divided *Fusarium graminearum* into seven phylogenetic lineages based on the partial sequences of six nuclear genes. Phylogenetic analyses of the sequences for each gene gave similar patterns, a phenomenon termed genealogical concordance. The genealogical concordance indicates that members of the phylogenetic lineages in *F. graminearum* have exchanged little or no genetic material with each other in recent evolutionary history. O'Donnell et al. (2000) noted a strong global pattern in the distribution of the lineages, which may help explain the lack of genetic exchange. The number of lineages was later extended to eight and a genealogical discordance involving genes in the trichothecene cluster was reported (Ward et al., 2002). The existence of phylogenetic lineages in this fungus has been independently confirmed and is generally well accepted (Jeon et al, 2003; Vargas et al, 2001).

Recently, O'Donnell et al. (2004) extended the number of lineages to nine and gave them species rank (Table 1). The diagnoses for each of the nine species were based on uniquely fixed single nucleotide polymorphisms (SNPs). The phylogenetic analyses of eleven nuclear genes indicated that the nine species together form a closely related monophyletic group or clade. Two additional phylogenetic species of the

Table 1. Correspondence of lineage and species designations within the *F. graminearum* clade.

Lineage Designation	Species Designation	References
1	*F. austroamericanum*	O'Donnell et al. (2000, 2004)
2	*F. meridionale*	O'Donnell et al. (2000, 2004)
3	*F. boothii*	O'Donnell et al. (2000, 2004)
4	*F. mesoamericanum*	O'Donnell et al. (2000, 2004)
5	*F. acaciae-mearnsii*	O'Donnell et al. (2000, 2004)
6	*F. asiaticum*	O'Donnell et al. (2000, 2004))
7	*F. graminearum*	O'Donnell et al. (2000, 2004)
8	*F. cortaderiae*	Ward et al. (2002); O'Donnell et al. (2004)
9[a]	*F. brasilicum*	O'Donnell et al. (2004)
	F. vorosii[b]	Toth et al. (2005)
	F. gerlachii[b]	Ward et al. (2005)

[a] proposed lineage designation.
[b] species name informally proposed at time of this writing.

Fusarium graminearum clade have been proposed informally (Tóth et al., 2005; Ward et al., 2005) and a total of 13 phylogenetic species have been suggested to exist (Starkey et al., 2005).

With the exception of SNPs, differences between the nine phylogenetic species or lineages appear to be few. Ward et al. (2002) reported that trichothecene mycotoxin chemotype (nivalenol, 3ADON, or 15ADON) did not correlate well with phylogenetic lineage. O'Donnell et al. (2004) found overlapping ranges for conidial characters such as length, width, and widest region of the conidium. They claimed that a combination of conidial characters could be used to recognize three species. Even these modest claims are questionable due to the very small number of strains examined.

The pathological uniqueness of these newly described species also appears to be low. Cumagun et al (2004) tested progeny from a cross between lineage 6 (*F. asiaticum* O'Donnell, T. Aoki, Kistler et Geiser) and lineage 7 (*F. graminearum* Schwabe *sensu stricto* O'Donnell et al. 2004) and found no transgressive segregation for aggressiveness to wheat. This result suggests that aggressiveness factors were similar in both parents. Goswami and Kistler (2005) tested nine phylogenetic species for pathogenicity to wheat and found that aggressiveness and production of trichothecene mycotoxins were strain-specific rather than a species-specific character. Tóth et al. (2005) tested 20 wheat genotypes with differing resistance against eight members of the *F. graminearum* species complex. They found no evidence of differential resistance reactions.

Despite the phylogenetic evidence for genetic isolation of the lineages, there is clear evidence that hybrids between lineages occur in the field. O'Donnell et al. (2000) reported a naturally occurring hybrid between lineage 2 and lineage 6 in a collection from Nepal. Evidence for the hybrid nature of the strain was very strong, including an intragenic recombination event in the *TRI101* gene. Leslie et al. (2005) reported a few putative hybrids from Brazil and Uruguay between lineages 1 and 7 as well as 2 and 7 based on AFLP markers and sequences of four nuclear genes. One significant problem with detecting natural hybrids is the small number of loci for which lineage-specific alleles have been described. In addition, many of the characterized loci are closely linked, which reduces genome coverage. The result is that the power to detect interlineage hybrids is low using current methods and the frequency of naturally occurring hybrids is still largely unknown.

The ability of different lineages of *F. graminearum* to hybridize in the laboratory is well established. Bowden and Leslie (1999) described a crossing procedure using nitrate non-utilizing (*nit*) mutants to distinguish recombinants. For additional discussion of genetic methods in this fungus see Bowden and Leslie (2004). Cross fertility was demonstrated between lineages 3, 6, and 7 in laboratory studies (Bowden and Leslie, 1999). Jurgenson et al. (2002) constructed an entire genetic map based on 99 progeny from a cross between lineage 6 and lineage 7 strains. Commenting on this cross, O'Donnell et al. (2004) suggested that it was not surprising that morphologically indistinguishable sister species can form hybrids, albeit with a reduction in fertility.

Although hybrids among these lineages clearly occur in the field and the laboratory, our understanding of potential genetic exchange among lineages is incomplete. It is unknown whether the expectation of low fertility in pairings between lineages is correct. It also is unknown whether cross fertility between lineages is the exception or the rule in the *F. graminearum* clade. We assessed cross fertility between nine lineages of *F. graminearum* in laboratory crosses using two different methods.

RESULTS

In the first test, three strains of *F. graminearum* lineage 7 with an insertion in the mating (MAT) locus that renders them heterothallic (Lee et al., 2003) were used as females and standard tester strains of each of the nine lineages were used as males. Ascospore production was variable, but depended on particular combinations of strains rather than on lineage. All males from all lineages produced viable progeny with at least two of the lineage 7 female strains. At least one male of each lineage had high fertility with at least one female of lineage 7.

In the second test, strains representing nine lineages were crossed as complementary *nit* mutants in a diallel design. Every lineage was able to pair with at least two other lineages (Table 2). Surprisingly, many of the lineages failed to pair with themselves. These negative results are most likely due to poor female fertility of some strains and/or the small number of strains tested. Therefore, the results in this table probably represent an underestimate of the amount of potential cross fertility between lineages. Nevertheless, the results from this experiment confirmed that lineage 7 is highly cross fertile with all other lineages.

Table 2. Summary of diallel cross fertility test of lineages of *F. graminearum* using nitrate non-utilizing (*nit*) mutants.

Lineage (# of strains)	1 (1)	2 (2)	3 (3)	4 (2)	5 (2)	6 (3)	7 (5)	8 (4)	9 (2)
1 (1)	-[a]	-	-	-	-	-	++	-	-
2 (2)	-	-	-	-	-	-	++	-	-
3 (3)	-	-	+	-	+	++	++	-	+
4 (2)	+	+	+	++++	-	+	+++	-	-
5 (2)	-	-	-	-	-	-	+++	-	-
6 (3)	-	-	+++	++	-	++	++++	-	-
7 (5)	+++	+++	++	++	+++	++++	++++	++++	+++
8 (4)	-	-	++	+	-	+	+++	++	-
9 (2)	-	-		-	-	-	++	-	-

[a] "–" = no recombinants produced; "+" = 1-5 recombinant colonies per plate; "++" = 6-25 recombinants; "+++" = 26-100; "++++" = >100 recombinants. The highest rating among the individual lineage pairings is shown.

DISCUSSION

Phylogenetic analyses indicate a history of genetic isolation among the lineages of *F. graminearum* (O'Donnell et al., 2000, 2004). This isolation may be relatively recent, since the phylogenetic lineages apparently differ very little from each other. Nevertheless, according to the genealogical concordance phylogenetic species recognition concept, these lineages merit species rank (O'Donnell et al. 2004).

Limited field surveys and extensive laboratory crosses reveal that genetic exchange can occur between these lineages. Every single lineage was able to cross with two or more other lineages. In many cases, interlineage pairings were highly fertile (Table 2). Lineage 7 could serve as a universally cross-fertile lineage. Furthermore, many progeny of an interlineage cross (6 x 7) were highly aggressive on wheat heads and were sexually fertile (Cumagun et al, 2004; Jurgenson et al., 2002) indicating that interlineage progeny may have adequate fitness. According to the biological species concept, these lineages would not merit species rank.

Remarkably, two different ways of looking at genetic isolation resulted in opposite conclusions about species limits in the *F. graminearum* clade. Opinions about species definitions differ and there is no universally accepted methodology for recognizing and delimiting species (Sites and Marshall, 2004). In our opinion, these morphologically and pathologically indistinguishable phylogenetic lineages should be classified as separate species only if we can reasonably expect them to remain genetically distinct when they co-occur in the field. Unfortunately, neither species concept is capable of reliably predicting genetic exchange in this case.

Phylogenetic analyses provide an objective, but retrospective, assessment of genetic exchange or isolation. Historical genetic isolation should be predictive of future genetic isolation for sympatric (overlapping ranges) lineages. However, it is difficult to determine if sympatry is an historical or recent situation as strains from some lineages may have moved recently in global trade. If sympatry is recent, there may not have been sufficient time for hybrids to proliferate. Evidence for historical genetic isolation of allopatric (non-overlapping ranges) lineages is not necessarily a good predictor of cross fertility when geographically isolated lineages are reunited because the isolation mechanism could be solely geographic.

Laboratory cross fertility studies provide an objective, but artificial, assessment of genetic exchange or isolation. If laboratory test crosses are consistently negative while appropriate controls are consistently positive, then the lab tests should be predictive for genetic isolation under field conditions. However, consistently positive laboratory crosses are not necessarily a good predictor cross-fertility under field conditions since undiscovered genetic or ecological isolating mechanisms may be inhibiting genetic exchange or selecting against hybrids in the field.

The conflicting conclusions about species boundaries in the *F. graminearum* clade may indicate that these lineages are in the early stages of speciation, but we do not know the mechanisms of genetic isolation that

allowed them to diverge. Laboratory crossing results do not support fertility barriers as a mechanism, especially not for lineage 7. Neither is there evidence for ecological separation since all appear to be good pathogens, at least on wheat (Goswami and Kistler, 2005; Tóth et al., 2005). All are presumably good saprophytes on crop residue, which is where the sexual stage occurs. A reasonable working hypothesis is that geographic isolation allowed the lineages to develop independent evolutionary trajectories. Migration of strains, probably through the global grain trade, may have changed the situation. The situation may be complicated because evolutionary trajectories of lineages may now vary in different locations.

The question of genetic exchange among lineages in this group will probably best be resolved empirically through intensive surveys of field populations in areas of geographic overlap among lineages. There are several places in the world where multiple lineages of *F. graminearum* are known to co-occur (Jeon et al, 2003; O'Donnell et al., 2000, 2004; Toth et al., 2005; Vargas et al, 2001). If significant frequencies of hybrids are found in the field, then lineage distinctions may be blurred and species rank may not be justified. Accurately determining the hybrid frequency is problematic with current methods, but this may be solved with new population genomics tools.

Pending resolution of the conflicting conclusions regarding species boundaries, researchers have a choice of concepts for *F. graminearum*. Researchers should not feel compelled to use one concept or the other, as long as they are clear which one they are using. Each choice has advantages and disadvantages.

O'Donnell et al. (2004) advocated formal recognition of the lineages in the *F.graminearum* clade as species. An advantage of the new species names is that they promote collection of data that is specific for each phylogenetic lineage. Currently there are no significant morphological, sexual, pathological, ecological, or toxicological phenotypes that are lineage-specific. The main disadvantage is that assigning a strain to species requires amplifying and sequencing diagnostic genes or using primers targeting specific single nucleotide polymorphisms (SNPs). This method of identification may be daunting for many applied researchers. A high throughput multilocus genotyping assay for identification of species and chemotypes has been described (Ward et al., 2005), which could help ameliorate the problem. A second disadvantage is that hybrids between lineages may easily escape detection because only a few loci can be assayed with current technology. If hybrids are misidentified as pure species, then information about the phylogenetic species may be incorrect. A third disadvantage is that using species names for phylogenetic lineages could promote erection of quarantines that serve as non-tariff trade barriers. At this time, there are no data that justify erecting quarantines based on these lineages. Use of a precautionary principle for quarantines of cryptic species/lineages is ultimately a philosophical, economic, and political issue.

Leslie and Summerell (2006) recommended using the single name, *Fusarium graminearum*, for all of the phylogenetic lineages/species associated with this group. The main advantage is simplicity and ease of identifying strains. This polytypic view of *F. graminearum* is justified by the high genetic similarity

Table 3. Correspondence of names for different concepts of *Fusarium graminearum*.

	Phylogenetic lineage view[a]	**Phylogenetic species view**[a]
Narrow sense	***F. graminearum* Schwabe lineage 7** or ***F. graminearum* Schwabe lineage 7 sensu O'Donnell et al., 2000** or ***F. graminearum sensu stricto***	*F. graminearum* Schwabe or ***F. graminearum* Schwabe sensu O'Donnell et al. 2004** or ***F. graminearum sensu stricto*** or *Gibberella zeae* (Schwein.)Petch
Broad sense	*F. graminearum* Schwabe or ***F. graminearum* clade** or ***F. graminearum sensu lato*** or *Gibberella zeae* (Schwein.)Petch	***F. graminearum* species complex** or ***F. graminearum* clade** or ***F. graminearum sensu lato***

[a] Designations that are NOT ambiguous are in bold.

and monophylly of the phylogenetic lineages. The main disadvantage is potential loss of lineage-specific information. For many studies, this may not be critical. In cases where lineage could be important, such as resistance screening nurseries, researchers are strongly encouraged to report the lineage designations. The second disadvantage is that O'Donnell et al. (2004) abandoned the lineage designations when naming the new species. There is no problem correlating lineages and species for the first nine lineages (Table 1). However, there could be ambiguity for future lineages/species. Lineages are informal taxa and this can be easily solved by numbering lineages in the order in which the corresponding species are described.

A problem generated by the new species descriptions is the potential ambiguity of the names *Fusarium graminearum* Schwabe and *Gibberella zeae* (Schwein.) Petch. O'Donnell et al (2004) emended the concept of *Fusarium graminearum* to encompass only phylogenetic lineage 7, but changed neither the species epithet nor authority. Unfortunately, this allows for ambiguity of the species concept being used. Gale et al. (2005) suggested that the name *Gibberella zeae* (Schwein.) Petch was implicitly emended by O'Donnell et al. (2004) to represent only *F. graminearum* lineage 7, leaving none of the other species/lineages with a defined sexual stage name. To avoid confusion, they recommended that the *Fusarium* designations be used rather than *Gibberella zeae*. Of course, that does not solve the problem for *F. graminearum*.

Accordingly, researchers should strive to be clear which concept of *F. graminearum* is being used. A suggested correspondence of names is given in Table 3 for the phylogenetic lineage view and the phylogenetic species view of the *F. graminearum* clade. Tóth et al (2005) used *F. graminearum sensu stricto* and *F. graminearum sensu lato* to represent the narrow and broad interpretations, respectively. Although this lacks formality, at least for the moment it is unambiguous. When a formal name is required, the full species epithet and authority should be used.

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FUSARIUM CHEMOTYPES IN THE UK AND CHEMOTYPE-HOST INTERACTIONS

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ABSTRACT

Prior to 1998, the predominant trichothecene-producing species associated with Fusarium head blight on wheat in the UK was *Fusarium culmorum*. Since that time, the occurrence of *F. graminearum* has increased year-on-year relative to *F. culmorum* and it is now the more prevalent. We have developed a series of PCR-based assays to characterise isolates with respect to trichothecene biosynthetic genes and infer their chemotype. Two chemotypes of *F. culmorum* are present in the UK. One type produces predominantly nivalenol (NIV), while the other produces deoxynivalenol (DON) and 3-acetyl DON (3ADON). The latter is a consequence of the deletion of a region of the principal trichothecene gene cluster encoding *TRI7* and the promoter region of *TRI8*. Whereas a single chemotype of *F. graminearum* predominates in the USA producing DON and 15-acetyl DON (15ADON), three chemotypes are present in the UK. These are NIV producers, DON/3ADON producers and DON/15ADON producers. It is unknown why, despite the very recent appearance of *F. graminearum* in the UK, there should be more apparent chemotype diversity here than in the USA.

We have been carrying out a series of experiments to establish whether chemotype affects interaction with host plants or has other biological consequences. Preliminary results indicate that different chemotypes interact differently with wheat and other cereal hosts. These results will be presented and discussed in relation to host resistance and epidemiology.

RELATIVE PATHOGENICITY OF 3-ADON AND 15-ADON ISOLATES OF *FUSARIUM GRAMINEARUM* FROM THE PRAIRIE PROVINCES OF CANADA

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ABSTRACT

Eighteen isolates of *Fusarium graminearum* Schwabe, 3 producing 15-ADON and 3 producing 3-ADON from each of the Canadian provinces of Manitoba, Saskatchewan and Alberta, were tested for relative pathogenicity and consistency of production of toxin, on two Canadian spring wheat cultivars, 'Roblin' (S) and '5602 HR' (MR). The experimental design was a 3-replicate randomized complete block. Each replicate consisted of a pot containing 2 or 3 plants of one cultivar, and were grown in a cooled greenhouse. At anthesis, 2 to 5 heads per pot were inoculated with one isolate and heads covered with glassine bags for 48 h to promote a favourable environment for disease development. Disease was scored at 14 d and 21 d after inoculation and recorded as percentage infected spikelets. These preliminary results showed no significant differences in pathogenicity among isolates from the three provinces and producing either 3-ADON or 15-ADON. Toxin analysis by GC/MS of seeds from the inoculated heads found higher levels of DON in the susceptible (45 ppm) vs resistant (14 ppm) wheat. All isolates formed their respective 3-ADON or 15-ADON analogs. The 3-ADON isolates formed, on average, 16.1 ppm DON on 5602HR and 56.9 ppm on Roblin, higher than the 15-ADON isolates which averaged 11.9 ppm and 33.6 ppm respectively.

COMPARISON OF INOCULUM SOURCES IN DEVELOPMENT OF FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL CONTENT IN WHEAT IN A DISEASE NURSERY

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OBJECTIVES

The objectives of this study were to compare three inoculum sources for their efficacy in FHB induction and progression and, in addition, to determine if disease development and DON accumulation in wheat grains were affected by the inoculum sources and inoculation treatments used in a disease nursery.

INTRODUCTION

Fusarium head blight (FHB), caused by *Gibberella zeae* (Schwein.) Petch (anamorph = *Fusarium graminearum* Schwabe) is the most important disease of wheat (*Triticum aestivum* L.) in North America (Gilbert and Tekauz 2000; McMullen et al. 1997). Infection of wheat by the pathogen results in reduced grain yield and quality, and kernel contamination with deoxynivalenol (DON) and other mycotoxins, which are harmful to livestock and pose a safety concern in human food (Miller 1995; Placinta et al. 1999). Currently, there is no satisfactory cultural or chemical control for FHB (McMullen et al. 1997; Shaner 2003; Tekauz et al. 2003).

The use of genetic resistance is considered one of the most practical and environmentally safe measures to control FHB (Bai and Shaner 1996; Gilbert and Tekauz 2000). High levels of resistance to FHB have been identified in wheat germplasm (Fedak et al. 2001; van Ginkel et al. 1996; Wilcoxson et al. 1992) and considerable breeding has been conducted to develop resistant cultivars and lines (Comeau et al. 2003; Pandeya et al. 2003; van Ginkel et al. 2003). Resistance to *F. graminearum* has been characterized by a typical quantitative reaction (Mesterhazy 1995; Schroeder and Christensen 1963) and evaluated in FHB nurseries at multiple locations and years (Dill-Macky 2003). Since FHB does not always appear in consecutive years, a disease nursery has become an essential part of most wheat breeding programs aimed at FHB resistance across Canada and the United States (Evans et al. 2003; Humphreys et al. 2001; Pandeya et al. 2003). However, there has been considerable variation among breeding programs in the use of inoculum source, inoculation treatment, and assessment method (Dill-Macky 2003; Evans et al. 2003). As a result, breeders have frequently encountered inconsistent disease reactions in their breeding lines, leading to significant loss of time and resources (Bockus et al. 2001; Campbell and Lipps 1998). Although both conidial suspension and infested barley, corn or wheat kernels are commonly used inoculants in FHB nurseries (Dill-Macky 2003; Evans et al. 2003; Groth et al. 1999), there has been little information available on the relative efficacy of these methods.

MATERIALS AND METHODS

Three single-spore isolates of *F. graminearum*, DAOM 178148, DAOM 212678, and AC Taho P2G1, obtained from the Canadian Collection of Fungal Cultures at the Eastern Cereal and Oilseed Research Centre (ECORC), Ottawa, Canada, were used. These isolates were chosen because they are known to be aggressive (Xue et al. 2004). Conidial suspensions were made separately for each isolate. The final spore suspension used for field inoculation consisted of a 1/3 volume of each of the three isolates.

Infested kernel inoculum was prepared by soaking barley and corn kernels in water separately overnight. Equal volumes of both were mixed into aluminum trays (21" x 12.5" x 3.5") and autoclaved. Each tray was then inoculated with 100 ml of a spore suspension

at 5 x 10^4 spores ml^{-1} under sterile conditions, labeled, and incubated at ambient room temperature (20-25 °C) to allow the kernels to be fully covered by mycelium. The infested kernel inoculum was made separately for each isolate and equal volumes of the three isolates were used for field inoculation. Inocula were produced in April each year in preparation for field inoculations in June.

Wheat debris consisted of FHB-infected wheat spikes and peduncles collected from the previous years' FHB nursery, resulting from artificial inoculation with the infested barley and corn kernels. Debris was air-dried, cut into 2 cm pieces, and stored at 4 °C until used.

Field experiments were conducted at ECORC, Ottawa, Ontario from 2001 to 2003. Three spring wheat genotypes, including a FHB susceptible cultivar AC Foremost and two moderately resistant lines CIMMYT 11 (Cross: SHA4/3/2*CHUM18//JUP/BJY; Selection History: CMBW91Y02946M-030TOPM-1Y-010M-010Y-015M-10Y-0M; Origin: PTB96E1 #107) and HY644 (Fedak et al. 2001), were used. Experiments were arranged in a split plot design with inoculum source and inoculation frequency assigned to the main plots and wheat genotype assigned to the subplots. Plants were grown in two-row plots, 1.5 m long with 18 cm row spacing. There was 20 cm between subplots and 3.0 m between main plots, with four replicates in 2001 and 2002 and three in 2003. To prevent inter-plot interference, a four-row plot of barley was seeded between the main plots and as a border around the experiment. Plants were hand-harvested at maturity, air-dried, and threshed.

Inoculum from each of the three sources was applied two and three times in each growing season. For the treatment of two applications of conidial suspension, inoculations were conducted at 50% anthesis (Zadoks scale 65) and 3-4 days later. Treatment of three applications of conidial suspension included these two applications plus an additional application 3 days after the second application, when plants were at the end of their flowering stage (Zadoks scale 69). At each inoculation, approximately 50 ml of the conidial suspension was sprayed evenly onto the spikes in each plot with an ultra low-volume Microfit Herbaflex inoculator (Micron Sprayers Ltd., Bromyard, UK).

For treatments with two applications of infested barley and corn kernels and two applications of infested wheat debris, inoculations were conducted about three and two weeks before anthesis (Zadoks scale 15-30). Treatments of three applications of the respective inocula were these two applications and a third inoculation at one week before anthesis (Zadoks scale 47). At each inoculation, approximately 50 g of the infested kernels or 45 g of infested debris was scattered evenly by hand between the two rows of each plot. Control plots were not inoculated. A non-inoculated control treatment with two applications of Folicur®432F (tebuconazole, Bayer CropScience Inc., Calgary, AB) was added to the experiments in 2002 and 2003 because of the expected high disease pressure in the FHB nursery. The fungicide was applied at 50% anthesis and seven days after the first application, at the recommended rate for cereals using a polyethylene compressed air sprayer (Chapin Manufacturing Inc., Batavia, NY). Sprinkle irrigation was applied each year for about 0.5 h each in the morning and afternoon (excluding rain days), starting at the first inoculation with infested kernels and debris and continuing until about 3 wk after anthesis, when plants were at the soft dough stage.

The development of FHB was monitored by visually estimating disease severity on ten randomly selected plants from each plot using a 0-9 scale described by Xue et al. (2004). The ten plants were labeled at the heading stage when disease symptoms were absent. Assessments were carried out six times during each growing season at 5-7 day intervals starting at 50% anthesis (first inoculation date of conidial suspension) and ending at the early maturity stage (3-4 wk after the final date of inoculation with conidial suspension). Severity of FHB over time was summarized as area under the disease progress curve (AUDPC) for each plot using the formula described by Wilcoxson et al. (1975). In addition, critical-time disease severity (DS) and percentage of infected spikelets (IS) of the 10 selected plants were rated at the soft dough stage, which occurred 21-25 days after the final date of inoculation with conidial suspension depending on genotype maturity. At the soft dough stage, plot disease severity for a population of approximately 200 spikes per plot was estimated for both incidence (percentage of infected spikes) and severity (percentage of infected spikelets of the diseased spikes). An FHB index (incidence x severity)/100 was derived to give an assessment of plot severity (Groth et al. 1999).

Percentage of FDK (kernels with a chalky white appearance) was determined for 300 randomly selected seeds per plot with a magnifying lamp. Analyses for DON were carried out at the Mycotoxin Research Laboratory, ECORC. Each DON analysis was conducted using a 30-g seed sample from each plot. The concentration of DON was determined by the competitive direct enzyme-linked immunosorbent assay (CD-ELISA) procedure using monoclonal antibodies (MABs) as described by Sinha et al. (1995).

RESULTS AND DISCUSSION

Symptoms of FHB were observed on all wheat genotypes each year (Figure 1). The level of FHB in the nursery was considered adequate as demonstrated by the fast disease progression and severe infection of the susceptible cultivar 'AC Foremost' in all three years. Disease development was slower and final disease severity was notably lower for wheat genotypes CIMMYT 11 and HY644 than cultivar 'AC Foremost'. High levels of natural inoculum were present as indicated by the development of FHB in the uninoculated and fungicide treated controls. It was not known whether the infection of uninoculated controls was from the ground applied inocula or residues of adjacent fields. Although FHB was observed in the fungicide protected control treatment in 2002 and 2003, the disease symptoms appeared about 7-10 days later, had slower disease progression and lower final disease severity than other treatments in both years.

Analysis of variance indicated highly significant differences among the inoculation treatments and among the genotypes ($P < 0.01$) for all parameters in all three years except for FDK in 2003 where the differences among inoculation treatments were not significant (Data not shown). Inoculation treatment × genotype interaction was not significant for any parameters in 2003, but a significant interaction was observed for AUDPC and DS in 2001, for FHB index in 2002, and for percentage of IS in both years. The combined analyses over years indicated heterogeneity of variances for all variables, thus a model with heterogeneous residual errors (a different residual error for each year) was used for significance testing and drawing inferences over years. Overall, there were significant differences among inoculation treatments, genotypes, and genotype × year interaction (Table 1). The interaction of inoculation treatment × genotype was significant only for percentage of IS and that of inoculation treatment × year for DON only. There were no significant three-way interactions. This implies that the results of the experiments in all three years were consistent with respect to the inoculation treatments for all but one of the parameters. The lack of a significant interaction effect of inoculation treatment × genotype for most of the parameters indicates that the genotypes responded similarly to the different inoculation treatments. We conclude that the experimental protocol and disease assessment parameters provided conditions allowing the effects of the treatments to be observed and rated consistently and satisfactorily.

Table 1. Mean squares from three years of test of fixed effects of inoculation treatment, genotype, and inoculation × genotype interaction and selected variance components for area under the disease progress curve (AUDPC), critical-time disease severity (DS), Fusarium head blight (FHB) index, percentage of infected spikes (IS), Fusarium damaged kernels (FDK), and deoxynivalenol (DON) content in spring wheat.

Source of variance	df	AUDPC	DS	FHB index	IS	FDK	DON
Fixed effect F values[a]							
Inoculations (I)	6	6.7**	4.0**	5.9**	5.9**	10.1**	3.1*
Genotype (G)	2	16.9*	9.9*	44.2**	59.8**	36.1**	20.4**
I x G	12	1.1	0.8	1.1	3.4**	0.6	1.1
Variance component χ^2 values[b]							
G x Y	1	87.6**	88.7**	37.5**	19.3**	11.0**	431.8**
I x Y	1	1.1	1.3	2.1	1.6	1.3	10.7**
G x I x Y	1	0.0	2.1	0.0	0.0	0.4	0.1

Note: FHB index, IS and FDK were arcsine square root transformed and DON was log transformed prior to analysis to stabilize variances. [a]Tests for fixed effects were Type III F tests in a mixed model with random effects of year, replicate, genotype by year, for log DON only, and inoculation treatment by year. Residual variances were heterogeneous over years and modeled as such. [b]Variance components were tested with likelihood ratio tests and where nonsignificant were not included in the final model. *,**significant at $P < 0.05$ and $P < 0.01$, respectively.

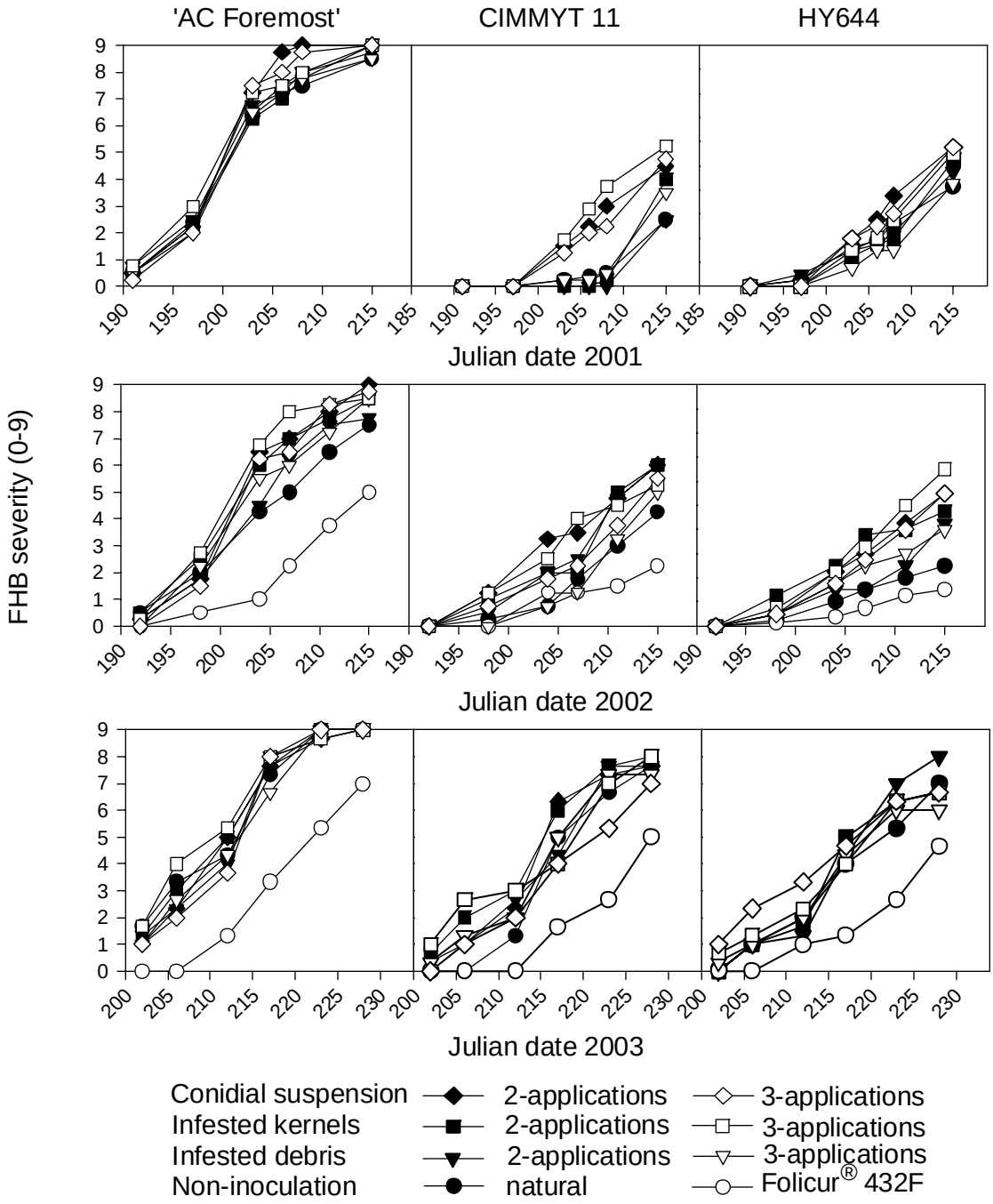

Figure 1. Fusarium head blight disease progress curves for seven inoculation treatments in 2001 and eight treatments in 2002 and 2003 on three wheat genotypes in a disease nursery in Ottawa, Ontario. Each point is the mean of four replications in 2001 and 2002 and three replications in 2003.

All inoculated treatments had significantly greater AUDPC and DS in 2002, FHB index and percentage of IS in 2001 and 2002, and FDK in all three years than the non-inoculated control (Table 2). Overall, inoculation treatments were highly significantly ($P < 0.01$) greater than the non-inoculated control in all parameters except DON in the combined analyses. The non-inoculated treatment with two applications of Folicur®432F was significantly lower in all parameters than the mean of the inoculated treatments in 2002 and 2003, when the fungicide treatment was used. There were significant differences among the inoculation treatments for all parameters. However, the contrast comparing the two numbers of applications (two applications versus three applications) was not significantly different for any parameter in any year or in the combined analysis ($P > 0.15$). This result indicates that two inoculations produced as much FHB symptoms (AUDPC of 18.3 versus 18.4 over all genotypes and years; DS of 5.6 versus 5.6; FHB index of 48.7 versus 48.0; and, percentage of IS of 32.4 versus 32.0), FDK (36.8 versus 34.5), and DON (25.9 versus 24.8) as three inoculations, regardless of inoculum source. The means shown in Table 2 have been averaged over the two numbers of applications for simplicity and clarity of presentation. Infections following inoculation with conidial suspensions or infested kernels were significantly greater than infection following application of the infested debris for all parameters except FDK, where there was no difference between infested kernels and infested debris. Inoculation with conidial suspensions also produced higher percentages of IS than the infested kernels. These results indicated that infested debris, although a natural source of inoculum, is less effective than the use of conidial suspension or infested kernels for inoculation in FHB nurseries.

AC Foremost was the most susceptible of the three wheat genotypes to FHB as measured by the parameters in all three years (Table 2). All assessment parameters identified that CIMMYT 11 and HY644 were less susceptible than AC Foremost by any inoculation treatment. Genotype CIMMYT 11 had significantly lower FDK than HY644, but there was no significant difference between the two genotypes for all other parameters. The genotypical reactions were in agreement with previous field observations that CIMMYT 11 and HY644 were moderately resistant and AC Foremost was susceptible (Fedak et al. 2001).

Although the present study demonstrates that both conidial suspension and infested kernels are equally effective inoculants in detecting genotypical differences in reaction to FHB in a disease nursery, there are considerable differences in efficacy of the inoculum production and application between the two inoculation methods. The approach of spraying conidial suspension onto flowering spikes requires that plots must be carefully scouted for anthesis prior to inoculation and inoculated individually. Since wheat genotypes vary considerably in anthesis date, and this critical time lasts for only 3-5 days, inoculation must be carried out daily for as long as 3 weeks, to screen the 10,000 to 20,000 lines in a typical wheat breeding program for FHB resistance (Campbell and Lipps 1998; Evans et al. 2003). The procedure is time-consuming and labor-intensive. Results sometimes vary considerably because of different environmental conditions at the time of inoculation, uneven flowering as a result of late tillers or late emergence, types of plant resistance, and a possible variation in the pathogen spore viability (Dill-Macky 2003). In contrast, the use of infested barley and corn kernels approaches the natural situation. Under moist conditions in a nursery, the infested kernels produce ascospores about 7-10 d after application, releasing spores over a 2-3 wk period (Paulitz 1996). Infested barley and corn kernels are simple and inexpensive to produce and store, require less time for application in the nursery, and can be applied to all genotypes in a single application regardless of anthesis date. Therefore, the infested barley and corn kernel method is less labor intensive, more economical, and suitable for screening large numbers of genotypes in a FHB nursery.

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Table 2. Effects of inoculation treatment and genotype on area under the disease progress curve (AUDPC), critical-time disease severity (DS), fusarium head blight (FHB) index, percentage of infected spikes (IS), fusarium damaged kernels (FDK), and deoxynivalenol (DON) content in spring wheat in 2001, 2002, and 2003.

	AUDPC					DS (0-9)					FHB index (0-100)				
				Mean					Mean					Mean	
	2001	2002	2003	3-years	2-no. appl. [a]	2001	2002	2003	3-years	2-no. appl. [a]	2001	2002	2003	3-years	2-no. appl. [a]
Inoculation treatment															
Conidial suspension 2-appl.	17.6 a	18.8 a	22.4 a	19.6 a	19.2 a	5.3 a	5.7 a	7.4 a	6.1 a	6.0 a	35.7 ab	50.3 a	73.3 a	53.1 a	52.5 a
Conidial suspension 3-appl.	16.6 a	16.6 ab	22.9 a	18.7 ab		4.7 a	5.3 a	7.4 a	5.8 a		37.5 a	43.2 ab	74.8 a	51.8 a	
Infested grains 2-appl.	12.7 b	18.3 a	23.8 a	18.3 ab	19.3 a	3.3 b	5.6 a	7.9 a	5.6 a	5.8 a	31.6 ab	35.3 bc	82.2 a	49.7 a	49.4 a
Infested grains 3-appl.	17.3 a	19.9 a	23.9 a	20.4 a		4.8 a	5.9 a	7.3 a	6.0 a		30.1 b	40.9 ab	76.7 a	49.2 a	
Infested debris 2-appl.	13.1 b	15.0 b	22.8 a	17.0 bc	16.5 b	3.4 b	4.9 ab	7.3 a	5.2 b	5.1 b	25.5 c	29.7 c	74.6 a	43.3 b	43.1 b
Infested debris 3-appl.	12.4 b	14.3 b	21.5 a	16.1 c		3.3 b	4.5 b	7.4 a	5.1 b		25.6 c	32.5 bc	70.8 a	43.0 b	
Non-inoculation	13.0	12.0 **	20.9	15.3 **		3.5	3.8 **	7.0	4.8 **		22.2 **	22.0 **	73.3	39.2 **	
Folicur 2-appl.	-	6.1 **	9.2 **			-	2.2 **	3.6 **			-	3.1 **	35.9 **		
Genotype															
AC Foremost	29.8 a	24.3 a	27.6 a	28.6 a		8.1 a	7.2 a	8.4 a	8.2 a		72.2 a	70.2 a	92.2 a	82.6 a	
CIMMYT 11	5.3 c	11.0 b	18.6 b	12.5 b		1.5 c	3.8 b	6.7 b	4.3 b		5.1 c	11.1 b	62.0 b	28.3 b	
HY644	8.8 b	10.0 b	16.7 c	12.6 b		2.6 b	3.3 b	5.7 c	4.1 b		12.0 b	15.1 b	56.5 c	30.3 b	
	IS (%)					FDK (%)					DON (ppm)				
Inoculation treatment															
Conidial suspension 2-appl.	32.1 ab	33.3 a	47.9 ab	37.8 a	38.0 a	25.8 a	19.6 a	72.0 a	39.1 a	39.1 a	4.6 a	19.8 a	60.4 ab	28.3 a	26.9 a
Conidial suspension 3-appl.	37.1 a	26.1 ab	51.4 ab	38.2 a		29.2 a	14.3 ab	73.4 a	39.0 a		5.1 a	16.6 ab	54.8 ab	25.5 a	
Infested grains 2-appl.	24.7 bc	20.5 bc	61.0 a	35.4 a	33.7 b	14.9 bc	13.7 b	82.6 a	37.1 a	34.9 b	1.5 b	15.6 ab	68.3 ab	28.5 a	28.4 a
Infested grains 3-appl.	25.4 bc	20.2 bc	50.0 ab	31.9 ab		19.2 b	10.6 b	68.6 a	32.8 ab		1.8 b	13.9 bc	68.8 a	28.2 a	
Infested debris 2-appl.	23.8 c	13.5 cd	43.9 b	27.1 b	26.6 c	16.2 bc	9.6 bc	77.0 a	34.3 ab	33.0 b	1.4 b	7.9 d	53.3 bc	20.9 c	20.9 b
Infested debris 3-appl.	23.1 c	12.3 d	42.7 b	26.0 b		12.0 c	10.0 bc	73.0 a	31.7 bc		1.3 b	12.5 cd	48.5 c	20.8 c	
Non-inoculation	21.1 *	10.7 **	51.9	27.9 **		14.5 *	7.3 **	51.7 *	24.5 **		1.5	7.8	54.3	21.2	
Folicur 2-appl.	-	2.2 **	14.4 **			-	3.1 **	39.2 **			-	2.5 **	20.4 **		
Genotype															
AC Foremost	47.5 a	40.7 a	72.2 a	57.3 a		39.8 a	22.0 a	87.2 a	51.5 a		5.6 a	24.2 a	90.5 a	43.9 a	
CIMMYT 11	13.4 c	5.4 b	29.1 c	17.3 b		8.5 b	4.4 c	48.7 c	22.1 c		0.9 b	4.5 c	36.9 b	15.3 b	
HY644	19.4 b	5.9 b	34.9 b	21.6 b		8.1 b	6.7 b	65.7 b	28.7 b		0.8 b	7.5 b	33.5 b	15.1 b	

Note: Means followed by the same letter within a column under each inoculation treatment or genotype are not significantly different at $P = 0.05$ (LSD).
*,**significant at $P < 0.05$ and $P < 0.01$, respectively (controls versus all inoculation treatments).

[a]Means are the average of two numbers of applications tested with three wheat genotypes in each of three years.

DEVELOPMENT OF NEW TOOLS TO DISSECT FUNGAL VIRULENCE AND PLANT RESISTANCE COMPONENTS IN A PROJECT FUNDED BY THE AUSTRIAN GENOME PROGRAMME GEN-AU

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OBJECTIVES

The progress made in a three-year, interdisciplinary, nationally-funded project will be reviewed. We suggest that the most important task for the future is to set up internationally-coordinated collaborative efforts to maintain central repositories for *Fusarium* genomics data and for mutant strains created in various genomics projects, and to establish improved links between the fungal research community, phytopathologists and plant breeders.

INTRODUCTION

In recent years enormous progress has been made in *Fusarium* genomics. The most significant step was the generation of a high quality genome sequence of *F. graminearum,* funded by the National Research Initiative of USDA (USDA/NSF Microbial Genome Sequencing Project. Principal investigators: Corby Kistler, USDA, ARS Cereal Disease Lab, University of Minnesota; Dr. Jin-Rong Xu at Purdue University; Dr. Frances Trail at Michigan State University; Dr. Bruce Birrene, Whitehead Institute, Boston). The genome sequence and an automated annotation is now publicly accessible at the Broad Institute web site together with sequences of several other fungal species (Fungal Genome Initiative, see: http://www.broad.mit.edu/annotation/fgi/, and for the *Fusarium graminearum* database see: http://www.broad.mit.edu/annotation/fungi/fusarium/).

With the help of the genome sequence it should be possible to identify fungal genes required for full virulence, to determine the virulence mechanisms, and to dissect plant resistance components which are at least partially able to antagonize such fungal virulence factors and contribute to the complex trait *Fusarium* resistance in crop plants. The long term goal is to use the tools of genomics to dissect plant resistance into measurable resistance components, improving the mapping and utilization of such genes quantitatively increasing resistance of crop plants. This task most likely requires the interdisciplinary collaboration of plant breeders, chemists and fungal researchers.

RESULTS AND DISCUSSION

Within the Austrian genome program GEN-AU a project (coordinated by G. Adam) was funded aiming to improve the understanding of *Fusarium* virulence and plant resistance mechanisms. The first goal was to establish a user friendly bioinformatics resource to allow straightforward utilization of the genome sequence by the international research community. Consequently MIPS (Munich Information Center for Protein Sequences) was included as subcontractor to establish the Fusarium Genome Database (FGDB: http://mips.gsf.de/genre/proj/fusarium/).

As the current computer algorithms used to predict fungal genes from DNA sequences are still imperfect, independent automated annotations by Broad and MIPS using different software frequently led to

alternative gene models. Meanwhile a significant portion of the *F. graminearum* genome is also manually annotated. More detailed information on features of the FGDB is available in Güldener *et al.*, 2006a. The MIPS gene predictions were also very important for the design of gene specific oligos for the now commercially available Affymetrix gene chip for *F. graminearum* (Güldener *et al*., 2006b).

The GEN-AU/MIPS database was also very helpful in identifying candidate target genes with a putative role in virulence. We have, for instance, identified phosphopantetheinyltransferase (*PPT1*) as a new virulence factor (Peruci *et al*., unpublished). Many fungal secondary metabolites are produced by polyketide synthases (PKSs) or non-ribosomal peptide synthases (NRPSs), both of which require posttranslational attachment of the prosthetic group 4′-phosphopantetheine to the respective acyl- or peptidyl-carrier domains. Disruption of the *PPT1* gene, which is equivalent to a loss of all PKSs and NRPSs leads as expected to a dramatically reduced virulence on wheat, indicating that potentially new or overlooked *Fusarium* metabolites could play an important role in disease development. The challenge for future work is to dissect the possibly redundant role of the multiple PKS and NRPS genes of *Fusarium*. To this end we currently develop new tools for *Fusarium* functional genomics. The *Cre*/*lox* system should allow removal of the transformation marker and its subsequent reuse. The *Cre* recombinase gene was cloned behind the *Trichoderma* xylanase promoter and placed together with a hygromycin B resistance marker between repeated *loxP*-sites. This cassette should be stable on glucose medium, but allow self-excision on xylose medium.

Another problem is to detect quantitative effects of gene disruptions on virulence. We are developing competitive virulence assays, which should reduce the problem of large variation due to failed inoculations into wheat heads ("disease escape"). In brief, two unique 20 bp tags (uptag and downtag sequences) are introduced during the gene disruption. The distribution of tags is coordinated by MIPS (FGDB). The tag sequences can be amplified from infected plant tissue by unique primers, labeled and hybridized to immobilized tag sequences. Shifts in the relative amount of tags reamplified by unique primers should reveal differences in virulence between mutants present in the mixed inoculum. We are currently performing reconstitution experiments with *tri5* and other mutants of *F. graminearum*.

As biosynthesis of deoxynivalenol (DON) was previously the best known virulence factor we started to characterize mechanisms in plants relevant for DON resistance. By heterologous expression in yeast we identified a UDP-glucosyltransferase from the model plant *Arabidopsis thaliana (*Poppenberger *et al*., 2003*)*. The chemists in our team developed reference materials and analytical techniques based on LC-MS/MS (Berthiller *et al*, 2003) and for the first time identified DON-3O-glucoside also in naturally *Fusarium* infected wheat (Berthiller *et al*., 2005). Meanwhile it was possible to show that DON resistance co-localizes with the main previously identified spreading resistance QTL derived from Sumai-3 (Lemmens *et al*., 2005). Much higher ability to form the glucoside-conjugate of DON was shown to be present in plants containing the 3BS *Fusarium* resistance QTL.

Other lines of research in the GEN-AU project deal with the possible role of the estrogenic *Fusarium* metabolite zearalenone. We have evidence supporting the hypothesis that zearalenone (ZON) may also be relevant for virulence on plants by interfering with ethylene signal transduction (Werner, 2005). On the other hand, ZON is rapidly metabolized into ZON-4O-glucoside in most plants (Berthiller *et al*., 2006) and is therefore most likely a nearly defeated virulence factor. We also succeeded in cloning a ZON-glucosyltransferase from *Arabidopsis* (Poppenberger *et al*., 2006).

In summary, the *Fusarium graminearum* genome sequence and *Fusarium* genomics tools, together with the advances in metabolomics should lead to the identification of new relevant virulence genes and mechanisms. A challenge for the future will be to maintain the MIPS FGDB and support a curator for community annotation efforts, and to incorporate the growing set of data from microarray experiments after the end of the GEN-AU project. Another challenge for the future is to coordinate the production of *Fusarium* knock-out strains and to preserve the generated strains for easy access by other groups.

Our Austrian project has showed that by bringing together researchers with complementary expertise (fungal genetics, chemistry, phytopathology and plant breeding) significant progress can be made in a short time. The main challenge for the future will be to establish ways to fund internationally coordinated efforts to solve the *Fusarium* problem.

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IMPLICATIONS OF POPULATION VARIABILITY ON THE MANAGEMENT OF FUSARIUM HEAD BLIGHT

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ABSTRACT

The principal pathogens associated with Fusarium head blight (FHB or scab) of wheat and barley are *Fusarium graminearum* (Schw.) (teleomorph *Gibberella zeae* (Schw. & Petch) and *F. culmorum* (W.G. Smith) Sacc.. Other species including *F. avenaceum* (Corda: Fr.) Sacc., *F. crookwellense* Burgess, Nelson & Toussoun, *F. equisiti* (Corda) Sacc., *F. poae* (Peck) Wollenw., F. sporotrichioides Sherb. and *Michrodochium nivale* (Fr.) Samuels & I.C. Hallett (teleomorph *Monographella nivalis* (Schaffnit) E. Müller) may also be associated with FHB. The frequency of the association of these species with FHB varies among geographical locations over time. The taxonomy of the genus *Fusarium* was originally based on morphological traits and has been revised following studies of anamorph-teleomorphs connections and DNA sequences. These studies have demonstrated the immense diversity within the taxonomic groupings within the genus *Fusarium*, and indeed within *F. graminearum*. Historically scientists interested in taxonomy have constructed, deconstructed and reconstructed taxonomic groupings. In recent years there has been much debate over the taxonomic classification of the genus *Fusarium*. For researchers focused on the management of FHB the genetic diversity of the pathogens which incite FHB is important only as it effects the biology of the pathogen and thus impacts the efficacy of disease control practices.

Host genetic resistance is considered the most effective and economic means to control plant diseases. Although resistance to FHB is obviously highly desirable, immunity to *Fusarium* has not been identified in wheat or barley. The development of wheat and barley with improved resistance to FHB currently relies on the introgression of multiple genes conferring partial resistance. To aid the genetic resistance already deployed and control FHB prior to the deployment of resistance, fungicides are being used in the United States. It has been demonstrated that fungicide applications can reduce the severity of epidemics although again disease control is only partial. Cultural control options appear limited given the current constrains, both agronomic and economic, on crop selection, crop rotation sequence and tillage practices. It would appear that rather than elimination of this disease that we will likely manage FHB through the deployment of the best host resistance available, the judicious use of fungicides and the implementation of available cultural control practices. The use of these disease management options are likely to be facilitated by forecasting systems being developed for use in the United States.

The use of an integrated approach to FHB management will reduce the chance that any single control option will influence the genetic structure of *Fusarium* populations and should be cognizant that other forces will influence the population structure of *Fusarium* spp. which spend much of their life as saprophytes on the residues of both gramineous and non-gramineous plants.

FUSARIUM HEAD BLIGHT IN ARGENTINA: A LOCAL COMPANY APPROACH TO BREEDING FOR SCAB TOLERANCE

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ABSTRACT

FHB has become a disease of increasing importance in most wheat-producing countries. Annual wheat production in Argentina may vary between 13 and 16 MMT and nearly 60% is exported. Wheat production is marketed in three commercial grades. The commercial standard allows up to 3% damaged kernels (which include damage by FHB), but the Alimentary Codex establishes no regulations on mycotoxin levels in cereal or cereal products. Thus, local end users such as millers and bakers purchase according to their requirements, usually having to implement prior testing for DON in years of high scab incidence. Foreign customers may expect variations in grain quality and toxin levels of shipments according to year and shipment port. Due to lack of segregation into different end use quality classes, Argentine wheat competes in the international market mostly by price, its buyers often being low resource countries.

Fusarium resistant wheat should not only prevent farm production losses but also contribute to protect consumers' health, especially in less developed countries with no regulations on toxin content.

Buck Semillas S.A. is a local private bread and durum wheat breeding company; data on work aiming to improve *Fusarium* tolerance will be presented. Crosses between elite lines and scab tolerant materials derived from Chinese and local germplasm are made regularly. Field evaluations of segregating material and stabilized lines are carried out in several locations. Natural field infections show wide yearly variations according to weather conditions, location and tillage system. Promising advanced lines are artificially infected using *Fusarium* conidia under partially controlled conditions. Visual notes are taken on artificially infected spikes and also on the development of *Fusarium* on their kernels in humid chamber. Results obtained in the last two years—some of them not easy to interpret—will be discussed and materials showing stable tolerant behavior will be mentioned.

In recent years a joint project between the Instituto de Recursos Biológicos at INTA Castelar and three private sector breeding companies has been implemented, aiming to develop enhanced germplasm for scab resistance using molecular marker assisted backcross selection.

Buck Semillas strongly supports the development of an International Interactive Scab Resistance Screening Nursery within the framework of the Global Fusarium Initiative and would be willing to collaborate on field screening and/or germplasm exchange. Given the importance of local private breeders—whose varieties cover around 90% of Argentina's wheat planted area—their inclusion in this project should eventually be taken into consideration.

CURRENT STATUS OF FHB RESEARCH IN ROMANIAN BREAD WHEAT BREEDING PROGRAM

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OBJECTIVES

The objectives of this study were to review the latest results obtained in nationally funded projects on improvement of resistance to Fusarium head blight (FHB) in winter bread wheat. Part of our breeding efforts are devoted to cooperation in international FHB wheat nurseries.

INTRODUCTION

Winter bread wheat and maize are the main food crops in Romania. An increasing interest is currently shown in durum wheat and in some extent in triticale. Under favourable conditions for epidemical development of *Fusarium* disease(s), all these crops may be damaged in both conventional and organic farming systems by yield reduction and contamination with mycotoxins (Ittu, 2001a, Ittu et al., 2004).

The spread of intensive wheat cultivars, not necessarily very resistant, in parallel with an expansion of wheat/corn rotation, has emphasized in the past decades the need for a multidisciplinary approach to *Fusarium* research, focused on successful management of risks.

In wheat, breeding cultivars that combine higher resistance to FHB (mainly Type II) and low content of DON, with other desirable agronomic traits, is considered, the most reliable strategy of control, in terms of efficiency, costs and protection of environment (Bai and Shaner, 1996). This is also the main breeding approach for the development of resistance to FHB in Romanian winter bread wheat. We have developed a multi-environment field procedure (year/location) of phenotypic assessment, under artificial inoculation (point/single head method) (Ittu et al, 1992, Ittu et al, 2001c).

As a result of continuous breeding efforts to improve resistance to FHB in bread winter wheat at ARDI Fundulea and others of its regional breeding centres, some valuable genotypes with a high and stable level of type II resistance and lower DON content have been obtained. One of these achievements is Fundulea 201R, not related to Chinese sources of FHB resistance, previously reported (Ittu et al., 1998, 2001b). Four quantitative trait loci (QTLs) for resistance to FHB were found on chromosomes 1B, 3A, 3D and 5 A (Shen, Ittu & Ohm, 2003). F 201R is characterized also by a multiple resistance to the main foliar diseases (powdery mildew, leaf and stripe rusts and septoriosis) and good winterhardiness.

CURRENT GOALS

Continuous improvement of screening techniques, selection of genotypes that combine a higher resistance to FHB than that of Fundulea 201R, with resistance to other pathogens and better agronomic traits are our current research goals.

At least two prerequisite selected *Fusarium* isolates and combined pre and post-harvest criteria are used per genotype/year combination for inoculation and assessment, respectively. For a better interpretation of experimental field data, we use classification of the entries according to their inoculation day. Between groups inoculated on different days the influence of climatic factors could also be very informative.

Recently, two three-year, multidisciplinary, nationally-funded projects have been initiated: (1) MAS for FHB resistance and DON contamination in winter wheat (BIOTECH-4545/2004) and (2) Neutralization of harmful effect of *Fusarium* mycotoxins in the entire food chain (CEEX-25/2005).

SELECTION FOR RESISTANCE

Trials performed in recent years demonstrated that a higher level of resistance to FHB than that identified in *Fundulea 201R* could be achieved, concomitantly with

a better combination of desirable traits: bread making quality, preharvest sprouting resistance, Al tolerance, BYDV resistance etc. The newest Romanian advanced lines are derivatives of bread wheat/triticale crosses (*F 00628G34-1)* and of bread wheats with complementary levels of resistance to FHB.

The use of microsatellite markers associated with resistance to FHB has been used particularly to validate the resistance derived from crosses among Romanian and Asian sources of resistance.

These results represent a good premise for further progress on improvement of both reliable methods of assessment and the level of resistance to FHB in Romanian winter bread wheat, durum wheat and triticale.

INTERNATIONAL COOPERATION

Firmly convinced about the global potential of ring trials for improved resistance to FHB, in the rational and accelerated selection of more resistant and adapted winter wheat genotypes, we have maintained and developed an active partnership in international/ European/ bilateral trials with CIMMYT, United States Wheat and Barley Scab Initiative (USWBSI) etc. Beginning approximately two years ago the European Fusarium Ringtest (EFR) was founded, and includes, besides the Czech Republic and Romania (initiating countries), Germany, Switzerland and France. We are very interested to cooperate with other countries within the framework of the Global Fusarium Initiative, for the creation of an International Scab Nursery Consortium and the Development of a new International Interactive Scab Resistance Screening Nursery (IISRSN) for germplasm enhancement and global compilation of data on Genotype x Environment x Management effects on resistance to FHB (Ban, 2005). Although Romania only grows winter wheat, we can grow spring wheat nurseries. We would, however, like winter wheats also to be included in this project.

Possible ways for interfacing CIMMYT's 11th Scab Resistant Screening Nursery with existing regional FHB nurseries should be explored.

Table 1. New lines of bread winter wheat with improved resistance to FHB, expressed as area under disease progress curve (AUDPC) and relative head weight (RHW), as % of control, plus other desirable traits

Line	Polymorphism linked to FHB on chromosomes			Resistance to FHB		Other desirable traits
	3A	3B	6B	AUDPC	RHW (%)	
F 00146G3-2 (95584G1/Debut/96831)	X			135	53	
F 99051G3-3 INC2 *(91375G4-13/135U2-103)*	X			139	63	Preharvest sprouting resistance
F 01459G4-1 *(BYDV-Scott2/Boema)*	X			146	72	BYDV resistance
F 00628G34-1 *(191Tr2-1221Fuz/Bucur//pol.lib)*			X	149	65	
F 01096G2-2 *(96915G1-1/96869G1-1)*				151	100	
F 99419G4-1Al1-1 *(Colonia/Bucur)*		X		161	57	Al tolerance

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ADVANCEMENT IN FHB RESISTANT WINTER WHEAT CULTIVAR DEVELOPMENT USING FRONTANA AS THE RESISTANCE DONOR PARENT

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ABSTRACT

Fusarium head blight (FHB) has become a national problem, and continues to cause economic and product quality/safety damage to the Canadian wheat crop. Continued development of FHB resistant winter wheat is of vital importance to Canada, including Ontario, Quebec and Atlantic Canada and the prairie provinces. Despite the likelihood of escape in western Canada, incorporation of FHB resistance into winter wheat is important, particularly for the moist eastern prairies. Furthermore, the incorporation of FHB resistance into new CWRW cultivars may allow national registration for all parts of Canada. Food/feed safety and sustainability of the overall wheat agri-economy in Canada (and other major wheat producing regions in USA, Asia, Europe and Australia) have been affected by mycotoxin residues on grains and grain products due to the frequently-occurring fungal disease FHB, caused by *Fusarium graminiarum*.

Agriculture and Agri-Food Canada has successfully developed genetic stocks (FHB 143, FHB 147, FHB 148 and FHB 161) from the crosses between Frontana (Brazilian FHB resistance donor) and standard commercial cultivars, namely Harus, Augusta and Frederick winter wheat cultivars. Backcrosses to the respective F1s were conducted to restore plant types and winter wheat growth habits. Using these resistant genetic stocks in crosses with the commercial cultivars, namely Augusta, Casey, Diana and Augusta, several thousand doubled haploids (DH) were generated via wheat-maize pollination and embryo rescue techniques. All DH lines were screened for their resistance to FHB in the *Fusarium* epiphytotic nursery. Selected DH were evaluated for agronomic, disease and quality parameters.

We have made progress at AAFC-ECORC, in cooperation with Hyland Seeds of W.G. Thompson Limited, and developed and released a soft red winter wheat in 2001-02. This was FT Wonder, the first FHB resistant/tolerant wheat in North America that has the lowest DON and visual symptoms of all winter wheat cultivars grown in Eastern Canada. Commercial seed sale and production has already begun. In 2005, AAFC-ECORC registered FT-Action and Ashley, the two first soft white winter wheat cultivars resistant to FHB in Canada (and anywhere in the world), in cooperation with Hyland Seed.

INTRODUCTION

Wheat (of all classes) is a very important crop in the Canadian agri-economy with a contribution of 12% or $3-4 billion towards the total Farm Cash Receipt (from animals and crops) of $36 billion. Western Canadian provinces are the main producing region, accounting for well over 90% of wheat. Amongst the eastern provinces, Ontario produced 4.7% of the total wheat cash receipt, accounting for well over $150 million in 2005. Ontario mainly produces soft white and soft red winter wheat; lately there has been a surge of interest in soft red and hard red winter wheat.

Over the past 20 years, Fusarium head blight caused by *Fusarium graminearum,* has gained national and international prominence as one of the most devastating cereal diseases. FHB affects almost all wheat growing regions of the world where warm, moist conditions prevail during the flowering and grain-filling period (Mesterhazy, 1983). In Canada, the estimated losses to the cereal grain industry from 1993 to 2000 are in excess of $1 billion (Fernando, 1999) through impacts on every aspect of the grain industry (Gilbert and Tekauz, 2000):

- Fusarium-damaged kernels (FDK) may contain high concentrations of deoxynivalenol (DON) which makes the grain unfit for human and animal consumption (Charmley et al., 1994).
- Yields are reduced.
- The presence of FDK results in downgrading and lost quality premiums (McMullen et al., 1997).
- Milling, baking, and pasta-making properties are altered (Dexter et al., 1996) due to the destruction of starch granules, cell walls, and endosperm proteins (Bechtel et al., 1985; Nightingale et al., 1999).
- Seed producers must deal with reduced germination (Gilbert and Tekauz, 1995).
- Lost sales in the domestic and export markets due to low FDK tolerances (Charmley et al., 1994).

FHB has moved progressively westward from eastern Canada since the 1980s, and was detected in Alberta in 1994 (Clear and Patrick, 2000). Major epidemics occurred in Ontario in 1982 and 1986, causing severe damage to soft white wheat production and overall usability. The most recent epidemic in 1996 almost destroyed the entire wheat crop in Ontario and north-eastern USA. The main reason for the escape of winter wheat in western Canada is that the crop usually reaches anthesis before temperature and humidity conditions are favourable for infection (Brule-Babel and Fernando, 2001).

Several types for resistance to FHB have been characterized: 1) resistance to initial infection, 2) resistance to spread following infection, 3) resistance to kernel infection, 4) reduced mycotoxin production, 5) yield maintenance in the presence of disease (Mesterhazy, 1995; Chen, 1996). These components have been related to various morphological or physical factors (Schroeder and Christensen, 1963; Mesterhazy, 1995) including head type, spike density, plant phenology, trapped anthers, plant height, and rate of grain fill (Stack, 1999). Based on the complexity of the resistance mechanisms, it is not surprising that numerous studies have shown that FHB resistance is conditioned by multiple genes with dominant or additive effects. Pandeya (1998) concluded that genes conferring FHB resistance in wheat were located on most of the chromosomes of the A, B and D genomes.

Genetic variability is essential for the development of FHB resistant cultivars. Sources of resistance/ tolerance have been introduced to North America from China, Brazil, Europe and Japan (Fedak et al., 2001). Canada's first FHB-resistant wheat cultivar, a soft red winter wheat named 'FT Wonder' was registered in 2002, through the collaborative efforts of AAFC and Hyland Seeds. Two new cultivars of soft white winter wheat were registered in January 2003. These cultivars derive their resistance from the Brazilian cultivar 'Frontana'. Resistance from other germplasm is in various stages of development.

Continued development of FHB resistant winter wheat is of vital importance in Ontario, Quebec and Atlantic Canada. Despite the likelihood of escape in western Canada, incorporation of FHB resistance into winter wheat is important, particularly for the moister eastern prairies. Furthermore, the incorporation of FHB resistance into new CWRW cultivars may allow national registration for all parts of Canada.

MATERIALS AND METHODS

Fusarium resistance breeding in winter wheat began in the early 1980s. Frontana (a Brazilian spring wheat) was found to be a tolerant variety, and it had the tolerance to initial infection (Type I resistance). Frontana was then crossed with our standard winter wheat cultivars, such as Fredrick, Harus and Augusta in 1984. Individual plants in the F1 were back-crossed to the three commercial cultivars to restore phenotype and restoration of winter wheat growth habits. Plants from the F2 generation were planted in the greenhouse for seed increase, and 625 individual plants were selected from the BC1-F4 generation in 1986-87. These 625 plants were grown in the greenhouse and inoculated with a FHB culture suspension with a spore-concentration of 50,000 per ml. Individual heads were rated three weeks after inoculation. Head-row lines of the selected individuals were planted. A suitable method of inoculation, to assess the tolerance of different winter wheat lines, was developed. Injection and spray methods of inoculation were compared for pedigree derived and doubled haploid lines. The results are presented for the formative phase of germplasm development and the actual utilization for cultivar development as follows:

A pedigree population consisting of 625 lines was sprayed with Fusarium head blight spores (50,000 spores per ml of suspension) under the field and/or greenhouse epiphytotic nursery conditions in 1990-91. Lines were rated visually on a 1-10 scale (where higher numbers indicate susceptibility). Samples of selected lines with rating score of less than or equal to four were analysed for the mycotoxin deoxynivalenol (DON) by GC-MASS SPEC method.

Based on their visual symptom ratings (VSR), 336 lines (100 with rating zero, 64 with 1, 48 with 2, 56

with 3 and 68 with 4) were advanced for the next cycle of epiphytotic evaluation and selection in 1991-92. Further inoculation and selection were conducted and a total of 176 lines were advanced for the third cycle of selection in 1992-93. We determined DON in the 1992-93 selections (total 176) by monoclonal antibody base technique developed at ECORC

RESULTS AND DISCUSSION

The formative phase for germplasm development

Over a three year period, continuous selection pressure was applied in favour of low visual rating and/or DON. By the 1992-93 crop season, a large number of lines were identified, with DON values ranging from 3 ppm to 43 ppm. We succeeded in transferring genes for resistance from a spring to winter wheat. The data distribution for disease rating suggested multigenic inheritance control. Correlation analysis revealed no definite physical associations between visual rating and DON content (Table 1). This indicated that symptom expression and DON are under separate genetic controls. Low or no correlation between DON and VSR appeared to be compatible with a discrete class distribution (suggesting few major genes with modifiers may be controlling the characteristics).

Figures 1 and 2 depict the results of three cycles of inoculation and selections. One of the main findings was that the visual symptom rating and DON contents were not related as presented in the Table 1.

Lines with LOW-LOW, LOW-HIGH, HIGH-LOW and LOW-LOW combinations of DON and VSR were identified. The inference that DON and VSR were independently inherited appeared justified. Based on low DON and VSR, 27 lines were advanced for final evaluation in 1993-94 (Table 2).

The lines **FHB 143, 147, 148 and 161** were chosen as winter wheat lines with FRONTANA type resistance as donor parents in our current FHB resistance breeding for soft winter pastry wheat. These pedigree lines exhibited low VSR and low DON. Also, the sub-lines of the four FHB lines showed stable performance for VSR (see FHB Index, Figure 3). Doubled haploids (DH) were generated via maize pollination and embryo rescue technique from the F1 between the four FHB lines and current standard pastry wheat. Our primary goal was to develop a resistant variety.

Cultivar development study

The doubled haploids were evaluated for their agronomic traits and disease (FHB) resistance. Over the past three years, we have developed three cultivars for release.

Table 1. Inoculation procedures and correlations in doubled haploids population

	Correlation
DON vs visual rating	0.39 SPRAY 0.31 INJECTED
VSR spray vs VSR injected	0.92
DON injected vs DON spray	0.67

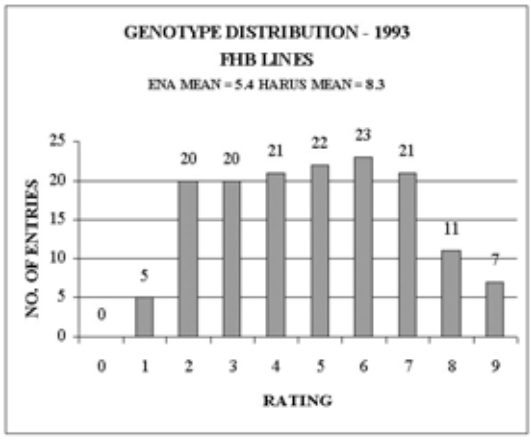

Figure 1. Third cycle selection for VSR

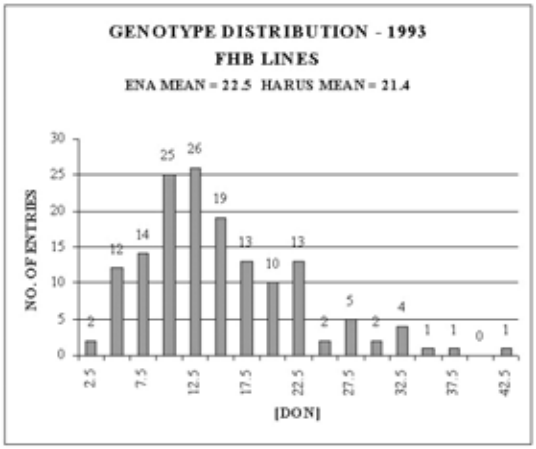

Figure 2. Third cycle selection for DON

Table 2. Number of entries for VSR and DON: Independent factors

Visual rating	DON concentration	Number of entries
HIGH	HIGH	25
HIGH	LOW	55
LOW	HIGH	41
LOW	LOW	53

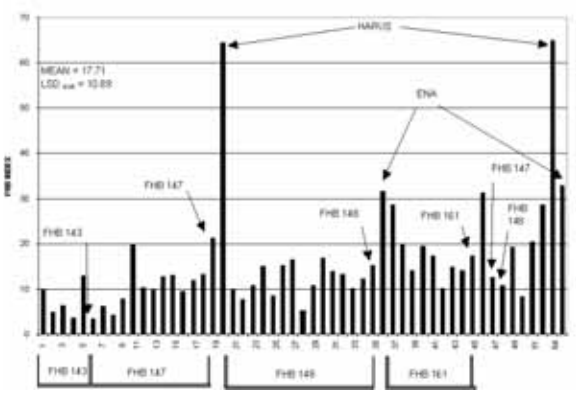

Figure 3. FHB resistance stability of the sub-lines selected from the four main genetic stocks according to FHB index (y-axis)

The line OTH 017-033, from the cross FHB 147 and Casey, was released and registered as FT Wonder. FT Wonder consistently showed resistance in terms of VSR and DON (Table 3). The commercialization of FT Wonder began during the fall of 2005 (Table3).

We have also developed two first soft-white winter wheat cultivars (FT Action and Ashley, Tables 4 and 5). Seed increase and commercialization process have begun.

Our results clearly indicate that the most suitable procedure for the inoculation was the spore suspension spray. The method closely mimics the natural infection process. The results from the point inoculation were inconsistent in delineating susceptible and resistant genetic lines. Fusarium-damaged kernels were much more closely related to the DON content of the lines.

Table 3. Comparative FHB Index and DON ratings for FT-Wonder (OTH 017-033), a soft red winter wheat cultivar in inoculated nurseries in Nairn and Ottowa over multiple years.

	97-98 Nairn FHB Index %	**98-99 Nairn FHB Index %**	**99-00 Ottawa FHB Index %**	**Nairn FHB Index %**	**Nairn DON (ppm)**	**00-01 Nairn FHB Index %**	**Nairn DON (ppm)**	**Ottawa FHB Index %**
AUGUSTA	25.0	62.5	47.5	65.0	6.0	30.0	0.64	30.0
AC Ron	25.0	80.0	54.0	70.0	6.0	40.0	0.47	22.5
Freedom	15.0	52.5	43.7	45.0	4.0	25.0	0.43	25.0
2540	25.0	80.0	44.0	70.0	5.6	32.5	1.60	15.0
OTH017-033	*5.0*	*20.0*	*10.0*	*25.0*	*1.9*	*7.5*	*0.23*	*12.5*
Check Mean	15.0	52.5	43.7	45.0	4.0	32.5	0.43	25.0

The columns have values for the FHB indices and DON toxin level under inoculated nursery conditions

Table 4. Comparative FHB performance of OTH 013-081 (FT Action), a soft white winter wheat (DH from the cross Augusta x FHB 148) in inoculated (Inocu.) and natural conditions over multiple years.

	2000	**2001**		**2002**		**Overall mean**	
	Inocu.	**Inocu.**	**Natural**	**Inocu.**	**Natural**	**Inocu.**	**Natural**
Station year	3	3	1	6	5	12	6
Varieties							
	FHBI	**FHBI**	**0-9**	**FHBI**	**0-9**	**FHBI**	
Augusta	52.3	34.2	2.2	24	2.6	36.8	2.4
AC Ron	45.8	35	2.2	26.7	4.7	35.8	3.5
Freedom	26.8	32.5	3	21.3	1.5	26.9	2.3
P2540	17.3	36.7	4.8	19.7	4.2	24.6	4.5
OTF 013-081	*12*	*5.8*	*0.7*	*8.5*	*1.4*	*8.76*	*1.1*

FHBI is the FHB Index = (% infected head X % infected spikelet)/100. 'Station year' is the number of sites evaluated under inoculated or natural conditions in the given year. The means in the Table 4 (and 5) are presented as these were measured. The CV and LSD are not provided, because generally CV for FHB resistance are very high due to strong environmental and local plot effects. The absolute values are taken as indicator of FHB resistance

Table 5. Comparative FHB performance of Ashley (TWF 020-038), a soft white winter wheat cultivar (DH from the cross Diana x FHB 147) for FHB Indices under inoculated conditions in Nairn and Ottawa.

	2000	2001		2002		**Overall mean**
	Nairn	Nairn	Ottawa	Nairn	Ottawa	**Inocu.**
Station year	1	1	1	1	1	**4**
Varieties						
Augusta		30	42.5	21	27.5	**36.20**
AC Ron	68	40	30	3.5	30	**42.00**
Freedom	35	32.5	32.5	9	25	**31.25**
P2540	60	45	20	22.5	25	**34.00**
TWF020-038	30	7.5	22.5	0.3	15	**18.75**
Mean of check	35	32.5	32.5	9	2025	
CV	8.05	19.22	44.1	51.96	29.4	
LSD	10.8	13.7	11.83	16.8	8.6	

'Station year' is the number of sites evaluated under inoculated or natural conditions in the given year. The means in the Table 5 are presented as these were measured. TWF 20-38 is approved for registration and named as 'Ashley'.

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PROGRESS IN IMPROVING FUSARIUM HEAD BLIGHT RESISTANT WHEAT IN HOKKAIDO, JAPAN

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ABSTRACT

Fusarium head blight (FHB) is the most serious disease for wheat production in Hokkaido Island, northern Japan. Wheat production in Hokkaido accounts for about 60% of domestic wheat in Japan and the yield losses caused by rain damage, pre-harvest sprouting and FHB are estimated at 10–20% of total production. Exposure to continuous rain conditions during the wheat maturing stage has become more frequent in Hokkaido. In 2002 the Japanese government applied maximum limits of 1.1 mg/kg for deoxynivalenol (DON) in raw cereals for human consumption and feed material, 1.0 mg/kg in feed for various animal species and 4.0 mg/kg for cattle older than 3 months. The FHB resistance of Hokkaido wheat varieties has not been sufficient to avoid contamination with mycotoxins produced by some *Fusarium* pathogens.

Evaluation and selection of FHB resistant wheats are conducted by several methods, such as spray inoculation of *Fusarium* spores onto wheat heads or spreading *Fusarim* inoculum using oat cultures with mist irrigation in the field nursery, and a single floret injection method in the greenhouse. For developing resistant wheat lines, we use Asian resources, such as Sumai 3, Japanese landraces, and breeding materials developed at Kyushu Agricultural Research Center, as well as Brazilian varieties and European materials. These materials have many inferior characters under Hokkaido conditions, such as low yield potential, low quality, lodging, and susceptibility to cold and snow mold. Since 2004, we started marker-assisted selection to introduce FHB resistance quantitative trait loci (QTLs) into spring wheat germplasm. We are actively screening and developing good resistant germplasm and lines with excellent resistance to FHB in both winter and spring wheat. These lines show low DON accumulations. There was significant correlation between Fusarium-damaged kernels (FKD) and DON content in inoculated field experiments at Kitami Agricultural Experiment Station. The yield potential of these breeding FHB resistant lines is lower than Hokushin, a leading variety in Hokkaido and they are still inferior in some traits: snow mold in winter wheat, lodging and quality.

FUSARIUM HEAD BLIGHT (FHB), AN EMERGING WHEAT DISEASE IN TUNISIA

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ABSTRACT

Fusarium spp. are ubiquitous plant pathogens causing seedling blight, root and foot rot and head blight in wheat (*Triticum aestivum* L.) over a broad range of environmental conditions. The most economically important disease is head blight (FHB), resulting in yield losses, a severe reduction in grain quality, and mycotoxin contents.

Wheat crops produced in Tunisia are often susceptible to root and foot rot diseases caused by *F. culmorum* and *F. pseudograminearum.* An outbreak of FHB occurred in 2004, localised around regions where higher rainfall occurred during anthesis in April. A collection of *Fusarium* isolates obtained from foot and head of wheat from different locations in Tunisia were identified using a combination of morphological and molecular criteria. Results showed that *Michrodochium nivale*, (syn. *F. nivale*), *F. culmorum, F. pseudograminearum* and *F. avenaceum* are a dominant pathogenic species isolated from both foot and head of durum wheat. FHB is a disease of growing concern since several species that cause the disease can produce trichothecene mycotoxins such as nivalenol (NIV) and deoxynivalenol (DON). ELISA analysis of naturally contaminated grains (23%) revealed the presence of 0.053 ppm of DON mycotoxin.

Trichothecenes also are phytotoxic and act as virulence factors on sensitive host plants. Recent reports suggest that strains of *Fusarium* that produce DON may be more aggressive toward hosts than NIV-producing strains. A total of 90 isolates of *F. culmorum* and *F. speudograminearum* were chemotaxonomically classified into DON and NIV chemotypes based on specific amplification of *Tris7* and *Tri13* genes involved in trichothecene biosynthesis. Only three isolates of *F. culmorum* were of the NIV chemotype while the other 87 isolates were of the DON chemotype. Our results suggest that strains of *Fusarium* prevailing in Tunisian cereal growing regions belong to DON chemotype and stress the importance of considering FHB as an emerging disease of wheat.

SOURCES OF "ENVIRONMENTAL INTERACTIONS" IN PHENOTYPING AND RESISTANCE EVALUATION; WAYS TO NEUTRALIZE THEM

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OBJECTIVES

Our objective is to give an overview of the environmental influences in *Fusarium* resistance research. It is often suggested that inconsistent results are a consequence of the environment. We will show that most deviations are not of an environmental nature: they have genetic and methodic causes, and the heterogeneity of the population tested and differences in epidemic severity are also responsible for the problems. At the end several recommendations are given to improve phenotyping or resistance evaluation and research.

INTRODUCTION

The high level of environmental influence in *Fusarium* research has been often pointed to as the most important source of the frequently inconsequent results (Wilde and Miedaner 2006, Chen et al. 2003, Buerstmayr et al. 2003). The interactions may be so strong that that genotype ranking may be very different between tests. The problem is especially great for the inoculations and spraying tests similar to natural conditions; whereas the problem is less for the more stable point inoculation results. It is necessary for us to determine the content of the environmental interaction. In the past decades we have performed many experiments with results that support the statement that the 'environmental effects' contain many very different influences which are not of environmental origin. We should produce comparable data, but in most cases data cannot be compared as conditions producing these data are different. In a quantitative trait locus (QTL) analysis this is a serious problem. The problem is less pronounced in practical resistance breeding where highly productive and less exact methods are used that correspond to the need of handling thousands of lines. However, when the amount of resistance is to be determined, for example for registration of cultivars, the problem is greater. Therefore, this is also a problem in breeding, though less central.

The experimental material used in this study originates from our breeding and research program, the testing of DH population within FUCOMYR FP5 projects CM82036/Remus and Frontana/Remus, the screening nursery from the FUCOMYR project, and fungicide tests from the Szeged program. Inoculation methods were described by Buerstmayr et al. (2003) and Mesterházy (1995, 1999).

PASSIVE INFLUENCING AGENTS

Morphological traits

Plant height significantly influences disease severity. In 1985 we had a severe FHB epidemic, where four replicated trials provided natural FHB data. Parallel with this about 100 genotypes included in these trials were tested using the bagging method. The plant height classes revealed significant differences: when 90 cm is considered as standard, the dwarfs (60-70 cm) had 20-30 % more disease severity and the tall genotypes 20-30 % less FHB. However, the means of the same groups did not differ significantly under artificial infection (Mesterházy 1987). This means that plant height has nothing to do with FHB resistance; for further details see Parry et al. (1995), Miedaner (1997), Mesterházy et al. (2005) and Steiner et al. (2004). Therefore plant height is a pseudo resistance factor. The story is similar for awns: awned lines showed on average up to 80 % more natural infection than awnless plants, but means for artificial inoculation data were the same (Mesterházy 1987). Higher head density (compact heads) is also an infection severity increasing factor, as demonstrated by Steiner et al. (2004), but its influence was moderate.

Long flowering period

In mapping populations or breeding materials flowering time differences might be two weeks or more. Ecological conditions over this long period are never stable. In Table 1 the Frontana/Remus results

show that earlier inoculation dates under bagging and mist irrigation resulted in significantly higher FDK and yield loss than later dates. The earliest materials were exposed to four times as much misting as the latest Table 2 shows the results of this population from 2004. Here the FHB AUDPC data show a more than five-fold difference between inoculation dates, but FDK is relatively stable. Here the earliest genotypes showed the least infection. In 2004 the warm period, with higher infection severity, was in June, whereas in 2002 the earliest inoculation was made in warmer weather, followed by a cooler period. Table 3, with data from a cultivar resistance test of over 100 genotypes, shows a similar picture. In each case FDK shows much less deviation. It seems that FDK is much more suitable to characterize DH populations than FHB AUDPC data, and that that infections in the palea or glume do not automatically cause higher grain infection.

Leaf diseases

In some cases (Mesterházy 1987) leaf diseases significantly increase FHB severity. We found up to a 40% increase in FHB severity compared with the severity on healthy plants .We observed this also in artificial stem rust infected plants compared to plants that were not infected. For this reason it is reasonable to control leaf diseases, at the latest when the flag leaf emerges.

Epidemic severity

Epidemic severity has a significant impact on cultivar differences. Isolates of low aggressiveness will not differentiate between medium and highly resistant genotypes, as both will be nearly without symptoms. The most aggressive isolate will present differences between these two groups, but all medium susceptible to highly susceptible genotypes will show the maximum level of disease (Figure 1). Therefore a mean of different epidemics gives more precise information on the amount of resistance than any single epidemic. Isolates used in parallel can replace multilocation tests so that the environmental interaction is near zero as all epidemics generated by the different isolates have the same environmental determination.

Table 1. Frontana/Remus population data grouped according to inoculations dates, Szeged, 2002

Important traits grouped according to inoculation dates						
Inoculation date May	Plant height cm	Lodging %	Leaf diseases mean	FHB AUDPC	FDK % Mean	Relative Yield % Mean
10	82	2.2	22	368.4	34.9	30.8
13	103.4	0.8	19.8	248.8	31.4	48.7
15	109.8	4.3	24.1	303.2	25.8	54.8
21	115.9	10.6	13.8	315.2	19.4	61.6
Mean	102.8	4.5	19.9	308.9	27.9	49

Table 2. AUDPC and FDK values for the Frontana/Remus population in 2004, Szeged

Means for genotypes according to inoculation time

Inoculation date May/June	AUDPC	FDK %	n
19	151	37	29
21	173	40	72
24	169	31	29
26	440	44	49
7	832	35	27

Table 3. Mean AUDPC and FDK of the genotypes of the screening nursery by inoculation time, Szeged nursery, 2003-2005

2003	AUDPC	FDK	2004	AUDPC	FDK	2005	AUDPC	FDK
21 May	475.25	25.05	25 May	96.3	6.1	20 May	229.8	15
24 May	772.41	30.71	30 May	73.8	6.7	26 May	361	15.5
26 May	885.14	23	6 June	139.29	9.22	28 May	576.9	22.5
2 June	2284.37	47.28				2 June	788.7	23.8
7 June	1710.3	43.18						

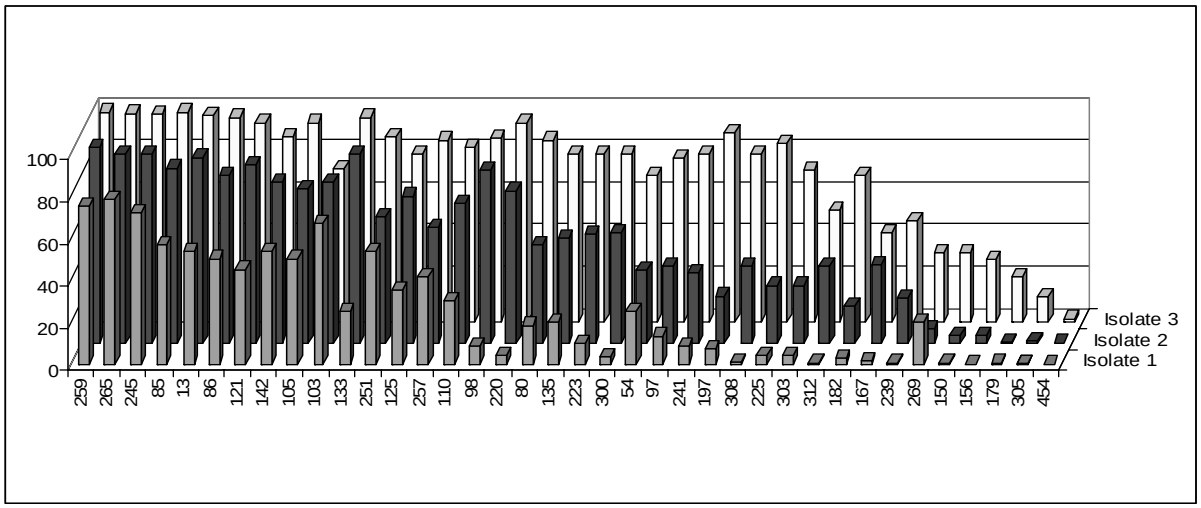

Figure 1. FDK data (%) from the 2002 resistance tests with three differently aggressive isolates, i.e. three different epidemic severities.

METHODOLOGICAL CONSIDERATIONS

The main stream in the methodology is the application of methods that simulate natural infection. In the simplest case the experimental field is sited where high humidity often occurs and helps the infection process.

Corn stubble, infected maize or other cereal grains can serve as inoculum source, and mist irrigation helps to ensure high humidity to initiate infection. As heading takes about two weeks, several waves of infection occur. Additional rains initiate further infections. The early genotypes receive much more inoculum than the late ones; therefore large differences in infection severity may develop, even when ecological conditions are relatively stable. With respect to flowering time, the duration of humid conditions is much longer for the early than for the later ripening groups, so an over-infection of the early materials is a real problem, but it cannot be solved within this methodological framework.

Spray inoculation regimes

Spray inoculation regimes always involve some means of securing high humidity. Many authors believe that with this method both Type I and Type II resistance can be tested together; we support this view.

The sprayer goes every 3-4 days through the test field, inoculating the whole area. After each inoculation mist irrigation is applied for two days. Misting regimes differ: it may be continuous for 8-10 hours, or equipment may be controlled by computer to mist 2-3 times an hour. This is clearly a problem in obtaining comparable data. However, this method gives higher disease pressure, so selection for higher resistance is easier, which works well for breeding purposes.

A more sophisticated version is when the spraying occurs only once for each plot and the 3-5 inoculations are made according to flowering. However, mist irrigation is used after each inoculation; therefore the humid period will be 3-5 times greater in the earliest

than in the latest group. Previous results showed that a two-day humid period increased infection severity by 30% over a one-day period, and the increase varied between 0 to 100% in individual genotypes (Mesterházy 1978). The data show that each genotype may have a distinct optimal humid period. Even where we cannot secure this, a stable humid period length is much better than any significant change during the vegetation period. Using mist irrigation this problem cannot be solved. Mist irrigation has also a disease development modifying effect. In cool weather it significantly cools the temperature of the heads, especially when the weather is windy, and has a negative influence on disease development. On hot days the cooling effect is positive, but the water drops can function as a glass lens and the plant tissue can be damaged. The result is that even within one test, disease development can be modified in both directions.

A solution is to spray the heads at flowering, and ensure humidity using plastic bags (Mesterházy 1978, 1983, 1995). At spraying, 15-20 heads are sprayed from every side, than bagged for 48 hrs. The bagging has the advantage that the wet period is the same for all inoculations. In cool weather, through the greenhouse effect in the bag, the temperature is higher than outside, therefore under these conditions this method provides better disease development. On hot days the disease development is slower than optimal, but heat damage is seldom seen; perhaps 1 in 100 genotypes shows susceptibility. Two assistants can inoculate 4-500 groups of heads in a morning.

The results show very similar tendencies using both methods, single spraying and misting and single spraying and bagging (Table 4). The agreement between FDK values is much better, indicating the less sensitive nature of the FDK values. AUDPC values seem to be less informative under mist irrigation. However, the results from bagged tests give AUDPC values that relate more closely to FDK than in the data from the mist irrigation regime. Large effect QTLs are more stable under different inoculation regimes.

Point inoculation

The method is well known, and tests Type 2 resistance. Most tests are done in a controlled environment (growth chamber, greenhouse), therefore the quality of the data is mostly satisfactory. When it is done in the field, the changing environment over the inoculation period can influence disease development differently.

Symptoms to be evaluated

Most papers deal with visual FHB evaluation, and neglect other traits. Disease evaluation stops when heads turn yellow, but 3-4 weeks remain from this time until harvest. In this period significant changes may occur in FDK and DON contamination. These are both key traits, and the value of the lot depends on the DON contamination. It is important that FDK is much more stable than FHB (Tables 2, 3, 4). In the CM82036/Remus population the correlation between the mist-irrigated and bagged regimes is 0.6352 for AUDPC and r = 0.8284 for FDK, therefore FDK is more valuable for important research tasks.

Pure isolates or mixtures

There has long been discussion over whether to use isolates or their mixtures. Mesterházy (1977) compared the effect of pure isolates and their mixtures. In all cases the mixture had lower aggressiveness than the arithmetical mean of the participant inocula alone. As its effect could not be forecasted, we did not use this approach. In the rusts, where different specialized races exist, a mixture is inevitable when a field test is

Table 4. Comparison of spray inoculation + mist irrigation and spray inoculation + bagging methodical regimes according to QTL groups, CM82036/Remus DH population, 2002-2004

BAGGING	AUDPC	AUDPC	FDK %	FDK %
QTL group	Check	Mean	Check	Mean
3B/5A	0	44.7	0	2.3
3B	0	153.4	0	8.5
5A	0	164.6	0	10.3
no QTL	0	313.8	0.1	15.4
LSD 5 %	0	79.4	0	2.6
MIST IRRIGATION	AUDPC	AUDPC	FDK %	FDK %
QTL group	Check	Mean	Check	Mean
3B/5A	19.7	783.3	0.3	10.1
3B	23.3	932.4	4.2	25.4
5A	32.9	1215.9	1	19.8
no QTL	47.5	1434.5	4.4	35.7
LSD 5 %	13.8	145.3	2	4.4

done. However, in cereal *Fusaria* no races exist, so this argument does not apply. The data in figures 1 and 2 data demonstrate that different aggressiveness levels cause different disease levels, with varying usefulness in differentiating levels of resistance. A mixture is not better or worse than a single isolate, and provides only one epidemic situation. For this reason we believe that the use of separate isolates increases greatly the exactness of the work without increasing environmental influence.

Conidium concentration and aggressiveness

The aggressiveness of the isolates is not stable (Mesterházy 1977). Tests over a number of years have demonstrated that inocula from the same isolate may change their aggressiveness markedly; the isolate/year interactions show this clearly (Mesterházy 1987, 1995, Mesterházy et al. 1999, 2003). The aggressiveness and conidium concentration are not closely correlated when different inocula are compared, since all inocula react differently to dilution. However, excellent correlations are observed when the same inoculum is diluted to different concentrations (Mesterházy 1977). The consequence is that changing the inoculum for different inoculation dates in an experiment is not recommended, as it may result in additional variation. Enough inoculum should be produced for all inoculations. In regulating aggressiveness, the conidium concentration is of secondary importance. We use an aggressiveness test to give us direct information about the aggressiveness of a given inoculum.

Experimental design

In a number of experiments the number of replicates is low, sometimes only one. From these tests no correct estimation of the environmental effect is possible. It is clear that cost of the experiment should be kept to a minimum, but 2-3 replicates per treatment per location or year is unavoidable to achieve a comprehensive data set and to present fair conclusions.

Variation width of the population in resistance

When the variability in the population is narrow, every small influence can severely affect the picture. Larger genetic differences help genotype ranks to remain more stable. This is one source of the phenotyping errors in mapping populations where low or medium effective QTLs are tested. When large effect QTLs are in the test, the resistance differences are larger and these can be easier identified than the smaller deviations.

PHYSIOLOGICAL RESISTANCE TRAITS

The *Fusarium* QTLs determining different components or types of resistance are the most important resistance components we have to deal with. A given resistance level is a combined effect of several QTLs. When this combined effect results in a high level of resistance, the disease severity remains low in any situation, as shown in figure 1. The higher the resistance level, the greater the stability of the resistance (Mesterházy 1995). All the influences we have discussed affect the disease severity in the genotypes. As susceptibility increases, the greater the potential influence of these effects on disease development, and so the probability of possible errors increases sharply. The highly effective QTLs are more stable (Table 3). The medium or small effect QTLs can easily be misidentified (Table 5). Some markers are more stable: GWM293 gave log of odds (LOD) values greater than 2.0 in three of the four data sets. If the data were more precise, the matrix would probably be more complete.

Table 5. LOD values for the Frontana/Remus DH population in different years and traits, 2002-2004, Szeged.

Szeged genotyping and phenotyping		FHB AUDPC CRC 2002	FDK 2002	FHB AUDPC *F. c.*2004	FHB AUDPC *F. gr.* 2004
Chromo-some	Marker	LOD	LOD	LOD	LOD
3A	*DuPw227				
3A	*GWM1121				3.92
3A	*GWM1110				
3A	*GWM720				3.16
5A	*BARC197				
5A	*GWM293	2.19	2.56	2.15	
5A	*GWM156			2.43	
5A	*GWM304				
2D	*GWM261				
2D	*A66	3.56	5.93		
2D	*A169				
2D	*GWM614B			2.47	3.67

INFLUENCE OF ENVIRONMENTAL CONDITIONS

The influence of warm and rainy weather has been understood since Atanasoff (1920). They are even now the most important epidemiological factors; recent forecasting systems use the functions very effectively. Many factors that influence these traits, such as location near a lake or river, soil type, etc., influence disease development significantly. The influence of temperature and precipitation on FHB AUDPC, FDK, DON, and yield loss based on a seven-year fungicide test is shown in Table 6. The correlations between traits are very close, indicating that the different traits are strongly interdependent. High May temperatures had the strongest effect on FDK; the correlation between DON and mean temperature was not significant. Interestingly, the June and July temperatures were almost neutral in their effect on the FHB traits. The rains in the inoculation period were not significant as the 48 hr polyethylene bag coverage ensured the humidity. However, the rains before inoculation were important, because after the removal of the bags the higher soil humidity contributed to dew development and so indirectly enhanced infection severity. The June rains had, against expectations, no significant influence. However, the July rains were very influential and correlated strongly with DON. This means the rains before harvest are the most important for toxin accumulation. Harvest time was normally at the end of July. The columns of the correlation matrix also show that the different traits are influenced somewhat differently, and this may be one cause of the different responses in different experiments.

HOW LARGE IS THE ENVIRONMENTAL INTERACTION?

In the CM82036/Remus population the FDK data present remarkable results (Table 7). The genotype, isolate, year and location effect are highly significant. There are large differences in disease severity between genotypes, isolates, years and locations. However, the interactions including genotypes (bold printed in Table 7) show significances at 0.001 level, but their value is very small compared to the main genotype effect and in each case differ significantly from the main genotype effect. Therefore, the genotype ranking is surprisingly stable in different environments. For the Frontana/Remus population the data are somewhat different (Table 8). The extent of the test was smaller; only 16 rather than 64 data points were behind each genotype. The trend is similar. The main effects are

Table 6. Correlation coefficients between FHB traits and weather parameters, 1998-2004

Trait group	Traits	DON mgkg-1	FHB %	Yield red. %	FDK %
FHB traits	FHB %	0.8612**			
	Yield red. %	0.8368**	0.9802****		
	FDK. %	0.6943o	0.8807**	0.9030***	
Mean temperature	May	-0.4553	-0.6646o	-0.7004o	-0.8338***
	June	-0.4379	-0.1183	-0.1019	-0.3201
	July	0.2618	0.2126	0.2013	0.138
	Mean	-0.4696	-0.493	-0.5141	-0.7174o
Rain	May 20-31	-0.0094	0.032	-0.0951	-0.2468
	May	0.7114o	0.7701*	0.6357	0.5077
	June	0.1506	-0.1732	-0.1521	0.1199
	July 1-20	0.7322o	0.7224o	0.7177o	0.6856o
	July	0.9635****	0.8525**	0.7858*	0.6899o
	Total	0.7854*	0.5327	0.4785	0.5697

**** P = 0.001, *** P = 0.01, ** P = 0.02, * P = 0.05, o = P = 0.10,

Bold printed: significant correlations

Table 7. ANOVA of the Szeged-Tulln resistance tests on the DH population CM82036/Remus for FDK, 2002-2004.

Source of variance	SS	df	MS	F	F AxBxC
Genotype A	516296	95	5434.7	54.02***	27.21***
Isolate B	987904	8	123488	1227.51***	618.36***
Year C	73138	1	73138	727.02***	366.24***
Location D	320301	1	320301	3183.90***	1603.91***
AxB	257093	760	338.3	**3.36*** **	1.69***
AxC	74391	95	783.1	**7.78*** **	3.92***
AxD	125158	95	1317.4	**13.09*** **	6.59***
BxC	257743	8	32217.9	320.25***	161.33***
BxD	102493	8	12811.7	127.35***	64.15***
CxD	27953	1	27952.7	277.85***	139.97***
AxBxC	133930	760	176.2	**1.75*** **	0.88ns
AxBxD	140742	760	185.2	**1.84*** **	0.92ns
AxCxD	75562	95	795.4	**7.90*** **	3.98***
BxCxD	101247	8	12655.8	125.80***	63.37***
AxBxCxD	151776	760	199.7	**1.98*** **	
Within	347666	3456	100.6		
Total	3693392	6911			

*** P=0.001, ns=not significant

Table 8. ANOVA of the FDK values for the Frontana/Remus population, 2002-2005, Szeged data

Source of variance	SS	df	MS	F	F AxBxC
Genotype A	289017	210	1376	**19.94*** **	2.71***
Isolate B	58516	1	58516	848.05***	115.41***
Year C	275882	2	137941	1999.14***	272.07***
AxB	62114	210	296	**4.28*** **	0.58ns
AxC	226330	420	539	**7.80*** **	1.06ns
BxC	861812	2	430906	6245.01***	849.91***
AxBxC	212825	420	507	**7.34*** **	
Within	87554	1266	69.16		
Total	2074050	2531			

*** P = 0.001

highly significant, but the isolate and year effects were many times larger than genotype effects. The genotype effect dominated over interactions, but these were relatively larger than presented in Table 7. The genotype main effect and its interactions are printed in bold.

OPERATING A PHENOTYPING SYSTEM

The most important source of variation is the long flowering time, with changing weather causing very large deviations in the infection severity of the groups inoculated on different days. For this reason we should decrease the variability in the mapping populations. We can reduce existing populations so that the flowering period should not be more than 4-5 days, so the inoculation for all genotypes can be made on the same day. Care should be taken to work with plants that do not differ in height by more than 15-20 cm. When mapping populations are large enough, a reduction of the population to 130-160 members presents no problem. In developing new ones, selection can secure the necessary homogeneity to achieve good quality phenotypic data. It is more important to collect good data than to improve them using statistical programs. An early spraying against leaf diseases is suggested. Mist irrigation should be avoided in phenotyping unless the population has been reduced so that one inoculation is enough for the whole population. Even in this case the bagging method might be more suitable when the population is morphologically variable. Instead of using several locations, the use of more parallel isolates helps to test a larger number of epidemics without increasing environmental instability. One site is enough each year with three to five isolates over a two- to three-year testing period.

It seems to be helpful to include FDK in serial phenotyping work as this is much more stable than visual assessments of the spike, such as AUDPC. In FDK evaluation threshing is the key challenge, to keep every grain for further analyses.

CONCLUSIONS

We should conclude that the environmental influence is not as large as often presented in the literature. However, many methodological and other causes have been identified that contribute to often inconsequent results. We have outlined ways to increase the reliability of the data and so lessen the possibility of misidentifying QTLs or resistance levels. Highly effective screening methods are not suitable to generate precise data; therefore the methodology used in these fields should be rethought. The homogeneity of mapping populations should be increased and more precise methods should be applied.

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STRATEGIES AND CONSIDERATIONS FOR MULTI-LOCATION FHB SCREENING NURSERIES

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ABSTRACT

Screening of cereals for reaction to Fusarium head blight (FHB) occurs world-wide and employs diverse methods, or, at best, variations on a basic method. Is there one protocol that is suitable/appropriate for all environments? Or is it best to create an epidemic in any way that can be certain of success, but attempt a uniform analysis to compare reactions of genotypes within and across specific environments? The FHB index commonly used and developed by Charles Snijders of The Netherlands in the 1990s has served us well, but it incorporates only visual symptoms of the disease, i.e. incidence and severity. In societies becoming increasingly conscious of food safety and security, should we consider including additional factors such as Fusarium-damaged kernels (FDK) and deoxynivalenol (DON) as part of the determination of a genotype's reaction to FHB? For a screening nursery to work well there must be a knowledge base of both the pathogen and the host within a specific environment, in order to manipulate factors to create optimal conditions for disease to occur. Some factors to discuss include inoculum, inoculation method (what types of resistance are we screening for?), timing and number of inoculations, application of misting/irrigation, rating (field/lab – single/multiple), and incorporation of FDK and DON into the analysis. One method of analysis (ISK, Kolb, Illinois) proposes that a proportion of the incidence (I), severity (S), Fusarium-damaged kernels (FDK or K) be added to give a ranking of genotypes. To this we should also consider including DON evaluations. Alternatively, we have been experimenting in Canada with 'GGEbiplot' (Genotype – Genotype X Environment), a software package developed at the AAFC Eastern Cereals and Oilseeds Research Centre in Ottawa, Canada. Biplots are used to visualize relationships among genotypes, environments and traits. It appears that either of these methods might be an improvement on the screening method originally proposed by Snijders, and they could also incorporate measures of damage caused by toxin accumulation and FDK.

CORN KERNEL INOCULUM PRODUCTION METHODS FOR A LARGE UNIFORM FHB DISEASE NURSERY

Background and Production Requirements

The following method for corn inoculum is used by pathologists at the Cereal Research centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada. This method allows us to prepare and freeze the corn kernel inoculum in the off season. We can then apply it over a 1-2 day period in the early summer at end of tillering (3 weeks prior to flowering). With respect to labour, the corn method is complementary to the spray inoculation method. We begin to prepare the conidial inoculum 2-3 weeks before anthesis which usually occurs after the corn inoculum has been applied.

The original application rate prescribed for corn kernel inoculum was 40 g/m^2 or 161 Kg/acre. From 2000 to 2005 we have used a rate of about 20 g/m^2 with good success.

Preparation of corn for FHB Inoculation

Use 26.4 liter Rubbermaid tubs. Into each tub pour ~8-9 Kg of dry corn then add tap water to a level of 4-6 inches above the corn (Figure 1). We usually do this step mid day as 16 hours of imbibition seems to provide for a better substrate for fungal colonization than 24 or even 48 hours of imbibition.

The following morning we drain the tubs, and cover them with 2 layers of industrial strength aluminum foil beneath the plastic lid. We sterilize the corn by autoclaving at 10 to 15 atmospheres for 1 ½ to 2 hours (Figure 2).

We allow the autoclave to cool overnight and remove the sterile corn the following day. We inoculate the corn mixture in a laminar flow hood (or clean room).

Inoculation of sterile corn kernels

Fungal cultures from potato dextrose agar (PDA) plates or millet, pre-inoculated and colonized with *Fusarium graminearum* can be used.

PDA Plate Method
Isolates are sub-cultured on to PDA from master synthetic nutrient agar (SNA) plates (Figure 3). The isolates will take about 1 week to colonize the entire Petri dish (Figure 4). Good culture growth occurs under fluorescent lighting at room temperature.

Using aseptic techniques blend 3-5 plates per tub of sterile corn in 150 ml sterile water with 0.2 grams of streptomycin sulfate and thoroughly mix into the corn.

Millet Method
Fusarium graminearum-infected millet is prepared in much the same the way as the corn kernel inoculum via the PDA plate method. To a large Mason jar add approximately 350-400 g millet and soak in water overnight. Drain the millet through a single layer of cheese cloth or muslin. Replace the lid of the jar loosely, but do not tighten! Choose the wet cycle and autoclave the millet for about 1 hour. Allow to cool. Add a single isolate of *F. graminearum*, about 30-50 ml of sterile water and 0.1 g of streptomycin sulphate to a blender and mix for about 30 seconds. Pour this mixture into the millet and shake to mix. Do not seal the jar when incubating the millet. The incubation period for millet is about 1 week. To dry the millet we empty the jar into a 6 or 8 litre sterilized Rubbermaid container and leave the sample in a laminar flow hood overnight.

The timeline for preparation of millet inoculum is as follows:

Day	Time	Procedure
Day 1	Afternoon	Soak millet in Glass Mason Jar
Day 2	Morning	Autoclave millet and allow to cool
Day 2	Afternoon	When cool inoculate millet with *F.graminearum*. Cover loosely with lid during incubation
Day 6 to Day 8	Afternoon	Layout millet in laminar flow hood and allow to dry.

Before using the colonized millet for corn inoculum, we test its viability by placing 50 kernels directly onto PDA and observe the colony development around the millet seed after 3-5 days (Figure 5).

Millet Inoculation
Corn kernel inoculation with millet: To each tub of corn add 30-60 ml (~25-50 g) of millet (Figure 6). Because the millet is dry it adheres to the individual corn kernels and colonizes uniformly throughout the corn.

Incubation, Drying, and Storage

After inoculation in the laminar flow hood the corn medium is then re-covered and allowed to incubate for no less than 2, and no more than 3 weeks. The FHB requires an aerobic environment, so tightly sealing or stacking the tubs will inhibit development. Room temperature (20-25 ˚C) is excellent for fungal development and the FHB will tolerate low (10 ˚C) overnight temperatures as long as the daytime temperatures get above 20 ˚C.

The end product after 2-3 weeks of standing is a white/pink/yellow mycelial mass that is surprisingly dense (Figure 7). At this point the sample should also be purely *F. graminearum.* However, *Rhizopus, Cladosporium,* and *Penicillium* are secondary invaders which may occur. If a secondary infection should occur, try to remove the growth from the culture as this is tolerable at this stage.

Following the incubation period, the corn kernel inoculum is thinly spread in the greenhouse to dry for 3-8 days depending on the amount of corn, kernel water content, green house temperature and humidity (Figure 8). The drying period is when most secondary fungal infections occur. A strong secondary infection can over-run and spoil an entire batch. The best way to minimize secondary infections is to dry the corn as rapidly as possible. Possible solutions include spreading the corn very thinly (only 1-2 kernels thick), using a fan, increasing temperature and lowering humidity. For our specific situation we mix the corn and break up newly formed mycelium corn aggregates daily for 2-3 days. This helps dry the corn more rapidly and evenly. After the corn has dried it is packed in mesh bags and stored in a cold room at 0 ˚C (or below) until it is required (Figure 9).

Figure 1. Corn soaking in 26.4 litre Rubbermaid tubs.

Figure 2. The soil sterilizer (autoclave) holds four 26.4 litre Rubbermaid tubs

Figure 3. *Fusarium graminearum* isolates grown on Synthetic Nutrient Agar are used as "master" cultures to produce many second generation subcultures on PDA.

Figure 4. *Fusarium graminearum* subcultures (from SNA master cultures) growing on PDA. 12-20 plates are used per batch of corn (4 Rubbermaid tubs).

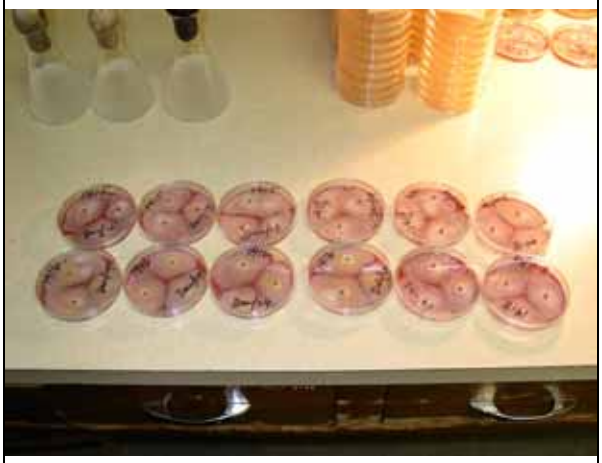

Figure 5. Plate to check level of successful millet inoculation with *Fusarium graminearum*

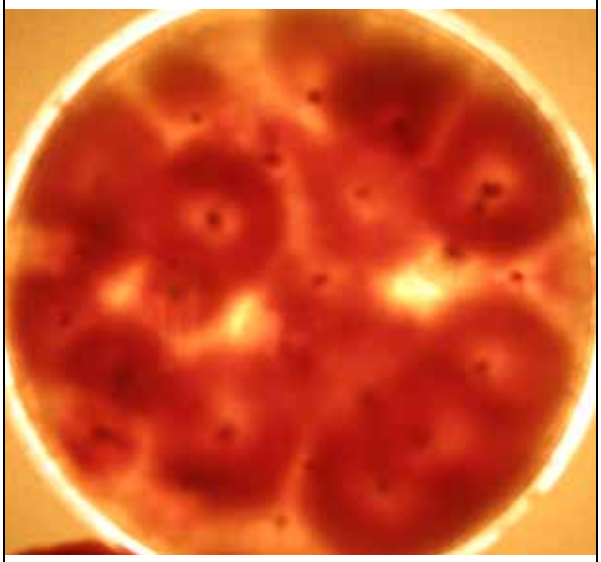

Figure 6. Corn is inoculated with dry *Fusarium graminearum*-infected millet in a laminar flow hood.

Figure 7. Corn kernel inoculum after 2 weeks incubation.

Figure 8. 32 kg (dry weight) of corn inoculum spread over a 2.5 X 0.75 m2 area. A medium weight polythene sheet is surface-sterilized with 95% alcohol. Mycelium continues to develop on kernels. Aggregates of corn are broken up daily

Figure 9. Dried *Fusarium*-infected corn kernels ready to be stored in a cold room or freezer.

Figure 10. Macroconidial suspension of *Fusarium graminearum* attached to vacuum line.

MACROCONIDIAL INOCULUM PROTOCOL FOR FUSARIUM GRAMINEARUM

New isolates of *Fusarium graminearum* are collected annually during late summer in southwestern Manitoba. Isolates are collected from fields that are randomly inspected for fusarium head blight (FHB). Diseased heads are threshed and the Fusarium-damaged kernels (FDK) are surface sterilized using a 0.3% NaCl solution and allowed to dry. These kernels are then plated on streptomycin-amended potato dextrose agar (PDA/S) and placed under cool white light at room temperature for 5-7 days. Isolates are then identified to the species level. Colonies are started from a single germinated conidium, which ensures a pure culture (single spore culture), and grown. The isolate is then tested for pathogenicity by inoculating healthy plants at anthesis with a liquid macroconidial suspension at a standard dilution of 50,000 spores/ml.

The CMC medium for *Fusarium graminearum* inoculum increase consists of:

$NH_4 NO_3$ (ammonium nitrate)	1.0 g
$KH_2 PO_4$ (potassium phosphate)	1.0 g
$MgSO_4$-7H2O (magnesium sulphate)	0.5 g
Yeast extract	1.0 g
CMC (carboxymethyl cellulose)	15 g
H_2O (distilled)	1 litre
Streptomycin sulfate (once solution cools to 50 °C)	0.2 g

Pour about 1/3 of the distilled water into a blender. While the blender is running at LOW speed, SLOWLY add the CMC. Pour this mixture into a 2 litre Erlenmeyer flask. The rest of the dry ingredients may now be added to this mixture. Pour half of the remaining water into the blender and run at low speed for a few seconds to remove any of the mixture that has adhered to the sides of the blender, then add to the first mixture in the flask. Pour the remaining amount of water into the blender & repeat as before. Cover with foil or cotton plug and autoclave for 30 minutes.

After autoclaving, cool medium to 50 ˚C or cooler and add 0.2 g streptomycin sulfate which has been added to 5 ml sterile water.

Add 1 Petri-plate fresh *Fusarium graminearum* culture (shredded). Sterilize a scalpel and cut or shred the culture into 1cm pieces or smaller and add to the CMC. Place a sterile rubber stopper with 2 sterile glass tubes inserted. One tube should reach almost to the bottom of the flask, the other should be well above the solution itself. The other ends of the tubes should be protruding several inches beyond the top of the rubber stopper (Figure 10). On the long tube, place a wad of sterile cotton batting, cover with a double thickness of cheese cloth and secure with an elastic band. On the short tube, attach a piece of flexible hose and connect to a vacuum line. Allow this to bubble gently and continuously for about 4-7 days. Perform a spore count, using a hemacytometer. We use a standard solution of 50,000 spores / ml. For inoculation, add 0.2 ml Tween 20 per 100 ml inoculum. **This inoculum should be used within 2 weeks and stored in a fridge or cold room.**
Conidial suspension can be inoculated 2 ways:

- Single or Double Floret Injections (SFI, DFI)
- Spray inoculations

DFI is used to measure Type-2 resistance (resistance to spread of infection) of the plant. The top third of the spike is referred to as the "Inoculation Zone". The remainder of the spike is rated, on a percent basis, for infection. Using an Eppendorf pipette, 10 µl of inoculum is injected between the lemma and palea of the florets located at the bottom of the top third: one on either side of the main spike (Figure 11).

Spray inoculation is used to measure Type-1 resistance (resistance to initial infection) of the plant. Between 3-5 ml of inoculum are sprayed onto the spike via a pump-spray bottle or by an atomizer.

Inoculated plants are placed into a humidity chamber (100% RH) for 24 hours to enable the pathogen to colonize the host. Infected heads are rated 21 days post inoculation. **Check the plants around 7-10 days post inoculation to observe whether infection is taking place.**

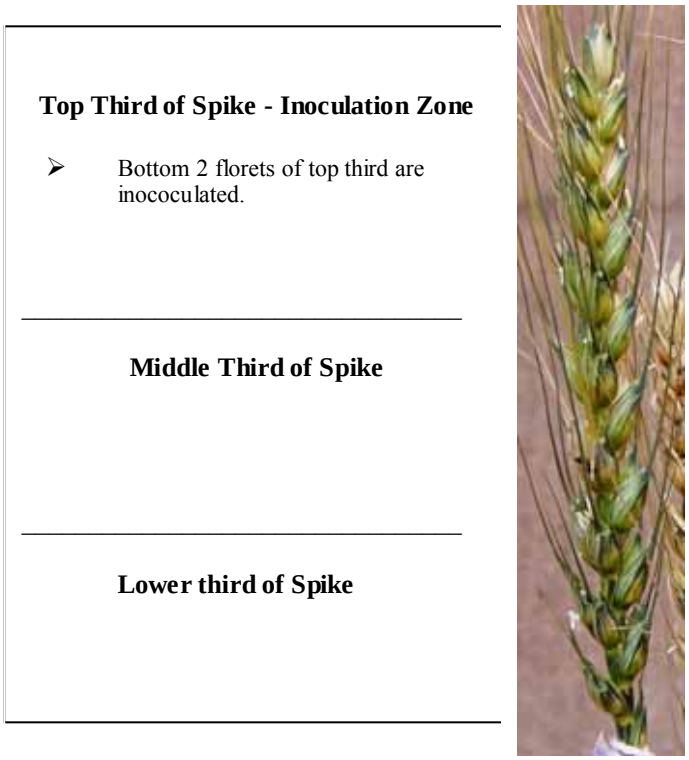

Figure 11. Location of florets for single or double floret inoculation

ANALYSIS OF FUSARIUM HEAD BLIGHT NURSERY DATA

The fusarium head blight (FHB) index, in one form or another, has been used since the 1990s to express the reaction of wheat lines to FHB (Snijders 1990). A typical method has used the product of scores for percent incidence and severity divided by 100 to express the index on a scale of 1-100. However, there are advantages to including measures of damage to the grain and levels of deoxynivalenol (DON) accumulation that would provide an overall indication of the potential problems for grain end-use.

Kolb and Boze (2003) suggest using Fusarium-damaged kernels (FDK) in addition to incidence and severity (or ISK - Incidence, Severity, and Kernel damage). ISK uses a weighted mean with weights of 0.3, 0.3, and 0.4 for incidence, severity, and kernel damage respectively.

ISK= (0.3 Inc(%)) + (0.3 Sev(%)) + (0.4 FDK(%))

If we add a DON measure to make it DISK we might assign the following proportions:

(0.2 Inc(%)) + (0.2 Sev(%)) + (0.3 FDK%) + (0.3 DON(ppm))

We used the data from the 2003 Uniform Regional Scab Nursery (URSN) grown at Glenlea, Manitoba and obtained the following correlations based on genotype means (Table 1):

Table 1. Pearson Correlations among traits for FHB reaction USRN 2003 in MB (n=40)

	FDK(A)	INC(labA)	SEV(labA)	INC(fldA)	SEV(fldA)
DON (log)	0.88	0.77	0.80	0.77	0.69
FDK (A)		0.71	0.83	0.72	0.69
INC(labA)			0.80	0.79	0.50
SEV(labA)				0.75	0.83
INC(fldA)					0.62

log – logarithmic, A – arcsin square root transformed to stabilize variances, lab – lab, fld – field.

The experiment, consisting of 40 genotypes, was grown in in single row plots in a randomized complete block design with 4 replicates. Rows were rated for incidence and severity in the field and the harvested grain samples were returned to the lab for further testing.

In the preceding table the components of the FHB Index, incidence and severity, based on counts made in the lab (lab) were compared to field (fld) ratings, which were based on a visual estimate of incidence and severity. The correlation between DON and FDK is relatively high. Incidence and severity between field and lab are less strongly correlated.

In the data set, DON was measured only on bulk samples from reps 1 and 2 and reps 3 and 4. Incidence, severity, and FDK were measured on 4 replicates, but means of reps 1 and 2 and reps 3 and 4 were used in computing ISK and DISK (Table 2) and in the ANOVA (Table 3).

At Glenlea we use the term visual rating index (VRI) to differentiate between an index based on field ratings versus lab ratings (FHB index). The VRI is an estimate of incidence and severity of FHB in a row or plot. The FHB index is a counted value for incidence and severity based on a random sample taken from the field and counted in the lab. Comparing just the field ratings of incidence and severity with the combined measure of VRI, and ISK, the correlation between VRI and ISK is high (Table 2)

The addition of DON values to the equation results in very high correlations among the VRI, ISK and DISK (Table 2). Further consideration might be given to identifying the most appropriate weights to be assigned parameters that comprise ISK and DISK.

In the ANOVA, DISK was the variable with the highest F value, indicating the greatest discrimination among genotypes (Table 3).

Table 2. Pearson Correlations among traits for FHB reaction USRN 2003 using DISK (n= 40)

	AFDK	AINC	ASEV	AVRI	AISK	ADISK
LDON	0.88	0.77	0.69	0.80	0.85	0.90
AFDK		0.72	0.69	0.80	0.84	0.88
AINC			0.62	0.86	0.89	0.88
ASEV				0.92	0.90	0.88
AVRI					0.99	0.98
AISK						0.99

L – logarithmic, A – arcsin square root transformed to stabilize variances

Table 3. ANOVA of 2003 URSN based on 2 combined replicates.

Name	F value
LDON	4.8
AFDK	9.8
AINC	5.9
ASEV	9.1
AVRI	10.1
AISK	11.1
ADISK	12.4

L – logarithmic, A – arcsin square root transformed to stabilize variances

2D-biplots

2D-biplots can be used to provide a visual representation of data (Yan and Tinker 2006). Practically, it is a two-dimensional display of a two-way table by both row and column. Singular value decomposition (SVD) is used as in principal component analysis. In the biplots presented here the singular values are partitioned with the columns making it easier to see relationships among the columns or traits. The biplot visualizes patterns among row factors and patterns among column factors and patterns of interactions underlying the row and column patterns.

Any 2-way table can be represented using a 2D-biplot if it can be sufficiently approximated by a rank 2 matrix.

In figure 12 below, the data for the 2003 URSN grown at Glenlea, Manitoba, in 2003 are presented. The genotypes are in mixed case (or blue) and the parameters in upper case (or red). Principal component 1 (PC1) versus PC2 form the primary biplot and in this case explain a large proportion of the variation, 86.3%.

The biplot is based on the data in Table 1. Genotypes such as Oslo that are closest to the traits (incidence and severity from the field and from the lab ratings, FDK, and DON content) are more susceptible to FHB than those that are a greater distance from the traits, such as ND2710. Genotypes such as SD3739 have lower incidence, but higher severity, while 98S003-12 has higher incidence and lower severity.

Figure 13 provides information on fewer parameters, including incidence and severity from the field, FDK and DON. Just the checks and one Canadian cultivar are named to make the picture easier to see. The cosine of the angle between parameters gives information on their relatedness. Acute angles show a positive correlation, obtuse angles show a negative correlation, and right angles no correlation. The length of the vector describes the discriminating ability of the parameter. A short vector may indicate that the trait is not related to other parameters, that there is a lack of variation or that it is not well represented in the biplot. For these data, all traits are positively correlated.

The biplot in figure 14 represents 23 variety means for 4 traits (incidence, severity, FDK and DON) for each of two seeding dates in 2003 in the Ottawa (OT) FHB nursery. The measurements are coded as 31, 32 for the first and second seeding dates in 2003. The first principal component is closely related to the average of the standardized traits (mean and standard deviation standardized) ranging from low to high incidence, severity, DON, FDK on the left to the right, respectively. The second principal component relates to consistency across the traits where the most consistent entries are closer to zero. The bold uni-directional line indicates the means of the standardized traits while the line at right angles relates to consistency.

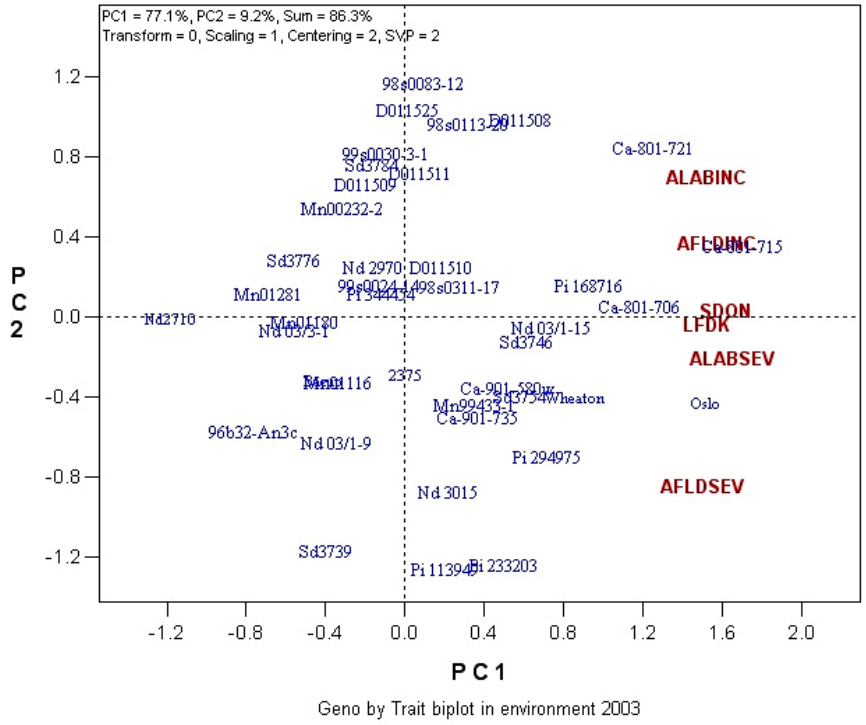

Figure 12. Genotype by trait biplot of 2003 URSN grown at Glenlea, Manitoba.

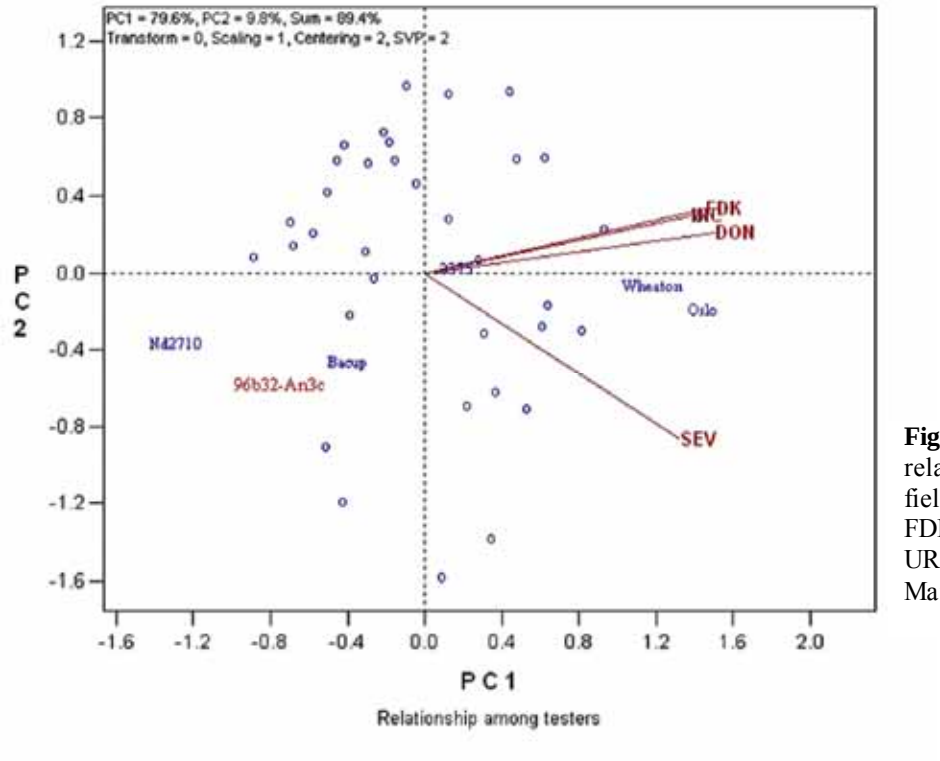

Figure 13. Biplot showing relationship among traits of field incidence and severity, FDK and DON for 2003 URSN grown at Glenlea, Manitoba.

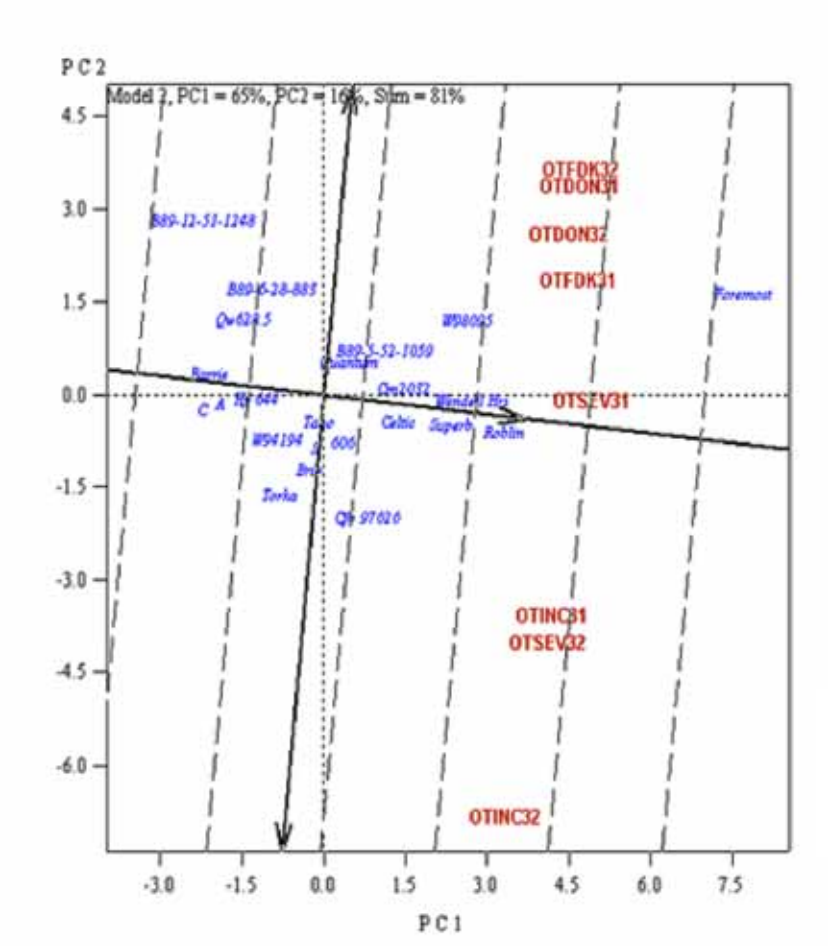

Figure 14. Biplot showing effect of two seeding dates on incidence, severity, FDK and DON in 23 genotypes in the 2003 FHB nursery in Ottawa, Ontario.

SUMMARY

Using ISK and DISK, other important effects of FHB damage, other than incidence and severity, can be considered when making decisions concerning advancement of lines with resistance to FHB. The biplots can show how well a genotype performs against different traits, in different environments or for different seeding dates. All these measures provide more information for genotypes than the reliance on incidence and severity alone.

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GERMPLASM EXCHANGE IN THE SOUTHERN CONE OF LATIN AMERICA

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OBJECTIVES

To review antecedents and the products of the exchange of germplasm resistant to *Fusarium* spp. in the Southern Cone of South America.

INTRODUCTION

Fusarium head blight (FHB) caused mainly by *Fusarium graminearum* (Schw.) is an important disease of wheat in Paraguay, Brazil, Uruguay and Argentina. Fusarium crown root is present in Chile, while in Bolivia, another head disease (Blast) caused by *Pyricularia grisea* is more important than FHB. In Argentina, the first report of the FHB pathogen was in 1927 while the first FHB epidemic was reported in 1945 (Galich, 1989). In Uruguay the first report of the pathogen was in 1928 (Boerger, 1928) while the first FHB outbreak was reported in 1977 (Tavella et al., 1979). In Brazil the first epidemic was in 1939 (Luzzardi and Pierobom, 1989) and in Paraguay in 1972 (Viedma, 1989). Although FHB has been a sporadic disease in the past, its occurrence in the region has increased since the 1970s.

Several wheat landraces from Argentina, Brazil, and Uruguay have been reported as highly resistant in the greenhouse and moderately resistant in the field (Weng et al., 2001; Zang and Jin, 2002; Weng et al., 2003).

Generally, FHB epidemics occur in years with humid and warm weather conditions at flowering time. In recent years, FHB has been more frequently related to the increase of zero tillage practices, a practice recommended for sustainable agricultural systems. Consequently breeders in South America have selected regional and international germplasm with acceptable levels of resistance in the region.

MATERIALS AND METHODS

In order to identify sources of resistance to FHB countries in the Southern Cone of Latin America have exchanged germplasm continuously since 1975. The first nursery (the Southern Cone Cooperative Nursery – Scab), was coordinated by Dr. J.F. Sartori, (CNPT, EMBRAPA, Brazil) and financed by IICA/PROCISUR. (IICA-Cone Sul / BID, 1981). The nursery was planted each year at appropriate dates to coincide flowering with the time of highest probability of occurrence of favorable climate conditions in Bolivia, Paraguay, Brazil, Chile, Argentina and Uruguay. Plants were inoculated at flowering time and three or four days later. The percentage of spikes affected with FHB was evaluated in the field, and the percentage of tombstone kernel affected was evaluated after harvest.

Since 1986, others nurseries have been tested in the region such as the Scab Screen Nursery (SCABSN), Advanced Lines from Yangtze (AL Yangtze), Scab Resistant Screen Nursery (SRSN), and Fusarium Head Blight Screen Nursery (FHBSN), all organized and distributed by CIMMYT. The Southern Cone *Fusarium* Resistant Nursery (FUCOSUR) was organized and distributed by Paraguay.

From 2000 to 2002 FHB Resistant Nursery (VIRFET) coordinated by Dr. M.M. Kohli, CIMMYT representative in Uruguay, and financed by FONTAGRO, was planted in South America and Mexico. The VIRFET was planted by different cooperators having used different methodologies for developing infection. The methodologies used were the following: natural infection under field conditions, artificial infection for Type I resistance in greenhouse, and artificial infection to study Type II resistance under a shaded house. For evaluating Type I resistance inoculation was conducted using a pressurized sprayer

at CIMMYT, Mexico, and a hand sprayer in INTA Marcos Juárez. Type II resistance was studied using single floret inoculation with inoculum soaked cotton in Mexico and by pipette injection in all other locations. Related to the inoculation methodology, different scales for evaluation were used by different cooperators. In the field, the scales used were as follows: double digit scale 0-9/0-9 (the first digit shows the spikes affected and the second digit the spikelets affected but in terms of 0-9), regional scale 0-5/0-5 (same as previous scales but in terms of 0-5) and percentage of diseased spike. For evaluation of Type I resistance, scale 0/5 and percentage of infected spikelets were used. For evaluation of Type II resistance, percentage of spikelets infected per spike from point of inoculation and scale 0/5 were used.

Data of infection levels were provided by INTA Marcos Juárez (shaded house and greenhouse), INTA Pergamino (field), DIA Capitan Miranda (shaded house), INIA La Estanzuela (field) and CIMMYT-Mexico (field). The analysis was based on two years for the majority of the locations (2000 and 2001) except for the Estanzuela where also year 2002 was considered. Data collected by collaborators were purified (put them into a common scale and remove suspicious data) and classified according to the types of resistance considered (IICA-BID ATN/SF 6486 RG., 2003).

Since 2006, a new nursery coordinated by INIA Uruguay, with sources of resistance already identified in the Southern Cone will be tested also for DON production in Argentina, Brazil, Paraguay and Uruguay. The nursery includes 85 lines, 60 lines that have been reported as stable sources of resistance in Southern Cone in the FONTAGRO Project: *Development of technologies for the integrated management of the Fusarium Head Blight of wheat,* one susceptible check and 24 lines with different reaction to FHB. Isolates of *Fusarium* spp. will be obtained from this nursery in the different locations with the objective of studying the diversity of the pathogen causing FHB in cooperating countries. For the present year, three nurseries have been sent to Paraguay, Brazil and Argentina, two for Mexico, and three were planted in Uruguay. Each cooperator will plant the entries under at least two conditions: one that promote natural infection (planting date, infested residue, etc.), and one that is artificially inoculated. Harvested grain from the naturally infected nursery would be sent to Uruguay and Mexico. *Fusarium* species present in the grain would be identified and quantified in Uruguay. *F. graminearum* isolates will be used for studies of population diversity. DON content will be analyzed in Uruguay by Fluoroquant® method and in Mexico by several methods which will be compared. The artificially inoculated nursery will be used to confirm stability of the reported resistance. Each entry will be planted in a single one-meter three row plot, (6g/plot, 2 g/row), in order to obtain enough grain for the toxin analysis and pathogen isolation. Scales used to evaluate the disease will be standardized (Regional Wheat Project; INIA Spain, CIMMYT, INIA Uruguay, PROCISUR).

RESULTS AND DISCUSSION

Sources of resistance reported from the first regional Southern Cone Cooperative Nursery – Scab nursery (Viveiro Cooperativo do Cone Sul – Giberela) were the following: Pergamino Gaboto MAG, Buck Napostá, B. Namuncurá and B. Cimarrón in Argentina; Nobeoka Bozu, Nyu Bay, Abura, Pekin#8, Ynayama, Minami Kyushi 69, Norin 34, Toropí, Encruzilhada, Cinqüentenário, Pel 73007, Pel 73081, Pel 73151, Pel 74142 and Pat 19 in Brazil; Itapua 1 and Itapua 25 in Paraguay; and Toropí, Encruzilhada, E. Young, Abura, Toropí/N.Bozu, Nyu Bay and Pel 74142 in Uruguay (IICA-Cone Sul /BID, 1981). The use of these sources of resistance in national breeding programs resulted in several lines with different levels of resistance to FHB.

From other nurseries tested in the region since 1986, such as the Scab Screen Nursery (SCABSN), the Advanced Lines from Yangtze (AL Yangtze), the Fusarium Head Blight Screen Nursery (FHBSN), and the Southern Cone *Fusarium* Resistant Nursery (FUCOSUR), no new sources of resistance were reported. From the Scab Resistant Screen Nursery (SRSN), Guam92 // PSN / Bow (6SRSN 01-22), Frontana, NG8675 / CBRD (7SRSN 02-05), Milan / Sha7 (7SRSN 02-07), Catbird, NG8201 / Kauz (7SRSN 02-26) and Sumai#3 (7SRSN 02-49) were reported as resistant. From the SCABSHA, Zuo1330 was reported as resistant.

The results from VIRFET are presented by the type of resistance:

Type I resistance

In order to study Type I resistance, data from M. Juárez (Argentina) and CIMMYT (Mexico) were used. A great variability in the classification of the data between both locations of evaluation was observed; allowing identification of about 10% of what was evaluated like resistant or moderately resistant. Sha5/Weaver was the most resistant in both locations having a significantly smaller severity than the rest. Although Pergamino and Estanzuela did not perform artificial inoculation to evaluate this type of resistance, the frequency of spikes infected in the field was

considered a good indicator for being included in the grouping of the materials in the Type I resistance analysis.

Using the canonical analysis to cluster the data, the entries fitted into five independent groups (Table 1). The groups with greater indexes of infection (2, 4 and 5) had smaller coefficients of variation. As well, groups 1 and 3, with smaller indexes of infection, were more dispersed with higher coefficients of variation indicating the difficulty in the evaluation of the resistance. Group 3 includes the most resistant germplasm in all locations, except Mexico. Group 2 was susceptible in all locations. It is important to indicate that the difference of the evaluations between Mexico and the rest of the locations of the Southern Cone observed by this classification could be due to differences in the pathogen population.

Type II resistance

In order to study the Type II resistance, data from La Estanzuela (Uruguay), I. Pergamino (Argentina), M. Juárez (Argentina), C. Miranda (Paraguay) and CIMMYT (Mexico) were used. There was a greater coincidence among locations, and 10 materials were identified as consistently resistant: Frontana, Br 32 / Itapua 35, CEP 24, V.Sol / N.Bozu // Pel73101 / LRInta, Sha5 / Weaver, E.Pelon 90 / Suzhoe F3#8, Buck Charrúa, Frontana / Remus, Remus / CM 82036 and Neixiang 184. Half of the selected lines categorized as resistant had a long maturity cycle, which could have some influence in the expression of the disease.

Using canonical analysis to cluster the data, the entries fitted into four independent groups (Table 1). Group 1 with the lower level of infection had the highest coefficient of variation, as was also observed for Type I resistance. Group 2 was the most susceptible in all locations. In spite of its good level of resistance through locations, group 1 had moderate infections in Paraguay and M. Juárez (Argentina). These differences in the behavior of the resistant entries were not known previously, it is suspected that they could be due to the interaction pathogen population x temperature differential with respect to other locations.

Types I and II resistance

Considering the differences of evaluation among locations of the Southern Cone and Mexico, data from Mexico were not included in the combined analysis. The combined analysis of Type I and II resistance data clustered the entries in five independent groups (Table 1). Groups 1 and 3 had low levels of infection through locations, a great dispersion of the data, and high coefficients of variation. Overlapping of groups 1 and 3 in the graph would be related to the differences in the maturity cycles of the involved materials. Group 2 included the most susceptible entries, except for those that were most susceptible in Paraguay, confirming once again the interaction or difference in the pathogenic population that could exist in this location. Group 3, considered as the most resistant in all locations, showed the maximum level of infection in Paraguay. This information is considered critical for future research on the variability of the pathogen population in the region.

Table 1. Characteristics of the clusters organized on the base of the type of resistance

Resistance type	Group	Obs.	Average	Std. Dev.	C.V.
Type I	1	29	45.7	40.2	88.0
	2	23	67.9	25.7	37.9
	3	24	50.6	41.4	81.9
	4	26	67.4	27.5	40.8
	5	16	73.0	18.4	25.2
Type II	1	32	43.2	40.5	93.8
	2	51	69.6	26.7	38.2
	3	19	52.5	35.6	67.9
	4	16	67.3	27.8	41.3
Type I and II	1	17	2.1	1.4	86.8
	2	38	4.5	1.1	25.4
	3	17	1.4	0.6	98.6
	4	23	2.7	1	50.3
	5	23	3.5	0.9	35.1

Identification of stable resistance germplasm

On the basis of the global analysis of the resistance and the statistical classification of germplasm in the nursery (VIRFET) in different groups for Types I and II resistance, it was possible to identify genotypes that were stable throughout locations (Table 2 and 3). From all the materials included in the nursery, only three (Frontana, Sha5/Weaver and Sha3/Catbird showed both types of resistance. It was interesting to observe that PROINTA Granar, a new variety with high grain yield potential in Argentina was selected for Type I resistance. Varieties Klein Cacique, Buck Charrúa (Argentina), and CEP 24 (Brazil) were good for Type II resistance and also have high grain yield potential and good adaptation. An additional group of 38 lines of well-known resistance to FHB in the region was selected by several collaborators, but they are not stable (IICA-BID ATN/SF 6486 RG, 2003 – Project).

Some of advanced lines with resistance to FHB include in their crosses sources of resistance like the following: LAJ 1409 (NAD//BB/INIA), Nobeoka Bozu, Pekin8, Nyu Bay, Ning 8343, Ning 82109, and Sumai #3 in Argentina. Advanced lines with Type II resistance to FHB include in their crosses Ning 8331, Catbird, Shangai#4 in Paraguay and regional germplasm, Sagvari-NB/MM-Sumai#3, Frontana, Sumai#3, Shangai#3, Catbird, YMI#6 and Suzhoe in Uruguay. Commercial cultivars with some Type II resistance are Buck Guapo, B. Poncho, Klein Cacique, K. Escorpion, K. Sagitario, PROINTA Granar, P. Molinero and P. Quintal from Argentina; Itapúa 45, Cordillera 3 and Itapúa 40 from Paraguay and commercial cultivars with resistance or moderate Type I and II resistance are INIA Torcaza, I. Churrinche, I. Tijereta, I. Gorrión and I. Caburé from Uruguay.

Due to the presence of the disease in the region for many years, all the breeding programs participating in the exchange of germplasm have selected germplasm with variable levels of genetic resistance. Nevertheless, the diversity of location, methodologies used for the evaluation, and rating scales made it difficult to confirm the stability of the resistance.

The regional nursery and its evaluation in different locations through the FONTAGRO project allowed identification of a reduced group of lines with specific characteristics for different types of resistance. Among them, Frontana (used world-wide), Sha5/Weaver (CM95103-25Y-0M-0Y-2M-0RES-5PZ-0Y-10PZ-0Y-2SCM) and Sha3/Catbird (CMSS92Y00595S-1SCM-0CHN-015Y-3SCM) showed stable resistance for both types of resistance (I and II). This information is of great value for the regional breeding programs and enlarges the variability of resistance sources in the world.

Table 2. Stable sources of Type I resistance

Source of resistance	**Type of reaction**
SHA5/WEAVER CM95103-25Y-0M-0Y-2M-0RES-5PZ-0Y-10PZ-0Y-2SCM	R
NING 8331 BC-207	MR
SHA3/CBRD CMSS92Y00595S-1SCM-0CHN-015Y-3SCM	MR
FRONTANA (CHECK MR) -0BRA	MR
PROINTA GRANAR MJI//PAK3563/CHAP70/3/DEI	MR
BOW´S´/´NOBEOKA BOZU//CEP75203/VEE´S´ LFJ-I-7	MR
KLAT/PEL74142//LRI/NYUBAI/3/KLAT/CEP75203//LAJ1409/PF7815 LFJ-IV-59	MR

Table 3. Stable sources of Type II resistance

Source of resistance	**Type of reaction**
SHA5/WEAVER CM95103-25Y-0M-0Y-2M-0RES-5PZ-0Y-10PZ-0Y-2SCM	R
VILELA SOL/NOBEOKA BOZU//PEL73101/LAS ROSAS INTA LFJ-III-38	R
CEP24 BR 3/CEP 7887//CEP 7775/CEP 11	R
EPELON90/SUZHOE F3#8 31B-0Y (PLANTA 2)	R
BUCK CHARRUA RAF/K.PET//K.REN/3/K.IMP//RAF/K.PET/4/LOV/5/RAF/K.PET//K.REN/3/K.IMP	R
REMUS/CM 82036 E1	R
FRONTANA/REMUS E4	R
NEIXIANG 184	R
KLEIN ATLAS (CHECK MR) K.LUCERO/K.157//K.RENDIDOR	MR
CC/PTES LAJ2231	MR
CEP24/PF87107//PVN/ANI´S´ B36293-B-0A-1A-2A-0A-0V	MR
COOPERACION CABILDO BATL/4/KLAN/BLACKHULL/PENTAD/3/MQ/G.ROCA/5/VMAR//VSOL/JAR"S"	MR
KLEIN CACIQUE BCIM/25348//VEE"S"	MR
LI 107/YMI#6 BC-208	MR
SHA3/CBRD CMSS92Y00595S-1SCM-0CHN-015Y-3SCM	MR

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CONSIDERATIONS IN DESIGNING NURSERIES FOR SCREENING FHB RESPONSE IN WHEAT AND BARLEY

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ABSTRACT

Fusarium head blight (FHB or scab), caused primarily by *Fusarium graminearum*, has become the principal production constraint on wheat and barley production in the Upper Midwest of the Unites States since the resurgence of this devastating disease in the early 1990s. In Minnesota a large collaborative initiative was established in 1993 in response to the reemergence of FHB, aimed at the management of FHB. Central to this initiative has been the effort to develop wheat and barley with improved genetic resistance to FHB. Field screening wheat and barley lines for reaction to *Fusarium* spp. has been essential in the development of wheat and barley germplasm with improved levels of resistance to FHB. Although the use of marker-assisted selection for major QTLs is becoming widespread in breeding programs in the U.S. field screening nurseries remain a vital tool for the identification and incorporation of genetic resistance. Our small grains pathology laboratory at the University of Minnesota has conducted numerous studies exploring new inoculum production systems, inoculation techniques, irrigation systems, nursery management options, and disease assessment methods to facilitate the development of FHB epidemics. Although *F. graminearum* is undoubtedly a difficult and often frustrating pathogen to work with, it is possible to manipulate FHB epidemics to establish screening nurseries with good prospects of yielding data useful for selection on plant breeding programs. Using the knowledge accumulated from this research we have been able to support the wheat and barley breeding programs establish screening nurseries at multiple sites, both irrigated and dryland, within Minnesota for over a decade.

CIMMYT AND TURKEY'S INTERNATIONAL SHUTTLE BREEDING PROGRAM TO DEVELOP WHEAT LINES WITH FUSARIUM CROWN ROT AND OTHER SOIL BORNE PATHOGEN RESISTANCES

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ABSTRACT

CIMMYT International in collaboration with The Turkish Ministry of Agriculture and Rural Affairs have established an international field and laboratory screening program for identifying spring and winter wheat accessions with resistance to SBPs (soil borne pathogens) including dryland root rot and cereal nematodes. Several screening protocols for assessing resistance to both cereal root rots and nematodes have been modified and optimized. Known resistance sources to SBPs from other regions of the world have been tested against Turkish isolates of SBPs and several of these have been shown to be effective in the region. In addition new sources of resistance with genetic variability have been identified against the prevalent SBPs. These diverse genes for resistance are being pyramided into both spring and winter bread wheat backgrounds using both conventional and molecular tools where feasible. The integrated coordinated screening and breeding program involving pathologists, breeders and molecular biologists is described to illustrate the approach in developing multiple disease resistant germplasm to SBPs.

INTRODUCTION

Soil borne pathogens (SBPs), including dryland cereal root rots and cereal nematodes are a major constraint to cereal production worldwide, particularly where cereals dominate rotations, and sub-optimal growing conditions and or cultural practices are common. Dryland root rots, also commonly known as root, crown, or foot root rots, include a complex of fungi with several species of crown rot (CR) (*Fusarium* spp.) and common root rot (CRR) (*Bipolaris sorokiniana* (syns. *Helminthosporium sativum*, *H. sorokiniana*, Teleomorph *Cochliobolus sativus* (Ito &Kurib.) Dresch.ex Dast.)). The two most reported *Fusarium* species are *F. pseudograminearum* (formerly *F. graminearum* Group 1, teleomorph *Gibberella coronicola*) and *F. culmorum*. Furthermore two groups of microscopic nematodes are commonly found on wheat roots and include several species of the cereal cyst nematode (CCN) *Heterodera* spp. and at least two important species of the root lesion nematode (RLN) *Pratylenchus thornei* and *P. neglectus*. Frequently two or more SBPs can occur in the soil at one time, making a disease complex and hence a holistic approach in management principally based primarily on resistance but where possible integrated with rotational options is required.

Yield loss caused by these SBPs has been reviewed and documented in many regions of the world including Europe, America and in particular the more marginal cereal production areas of West Asia, North Africa, Australia and Canada with losses reported between 3-50% (Diehl *et al*., 1983; Singh *et al*., 2005; Nicol *et al*., 2001; Nicol *et al*. 2004a; McDonald and Nicol, 2005). Recent yield losses studies in Turkey have confirmed that cereal root rots and cereal nematodes are associated with yield losses of 42% and

45% in commonly cultivated winter wheats (Hekimham *et al.,* 2004; Nicol *et al*., 2005). Considering the similarity in WANA (West Asia and North Africa), parts of South America, South Africa and other parts of the world in relation to cropping patterns and climate, it is likely that soil borne pathogens could cause similar economic losses in these regions.

Resistance, which is defined as a reduction in the multiplication of the pathogen, is one of the best methods to control these diseases. Although these nematodes and fungi have been considered important for several decades in certain countries, little advancement in breeding has been made. This is due to the difficulties of screening for these pathogens under field and greenhouse conditions. Currently there are very few known effective sources of resistance against these pathogens available in commercially grown wheat varieties, and many of the identified resistant sources are found in unadapted germplasm which will require considerable breeding investment to produce commercial varieties. Hence a precise laboratory/field breeding strategy has been established by Turkish and CIMMYT scientists in Turkey with CIMMYT Mexico to identify and incorporate new sources of resistance, particularly those identified in well adapted backgrounds.

The integrated coordinated screening and breeding program involving pathologists, breeders and molecular biologists is described to illustrate the approach in developing multiple disease resistant germplasm to SBP. CIMMYT's experience with the preliminary shuttle screening to identify multiple Fusarium (Fusarium head blight and CR) resistant germplasm between CIMMYT Mexico and CIMMYT Turkey will be described, in addition to the importance of special hot spot international disease nurseries and global networks for the validation, usability and adoption of disease resistance germplasm.

MATERIALS AND METHODS

International linkages

A strategy for screening, validating and disseminating germplasm has been developed linking CIMMYT Mexico and the International Winter Wheat Improvement Program (IWWIP) in Turkey which is a tripartite breeding effort between the Turkish Ministry of Agriculture and Rural Affairs, CIMMYT and the sister center ICARDA. Spring wheats are developed in Mexico at CIMMYT headquarters and winter wheats in Turkey under the IWWIP program (Nicol *et al.*, 2004b, Figure 1).

Field screening for cereal root rots to identify resistance

A clear strategy for germplasm screening, validation and subsequent incorporation into both spring and winter wheat breeding programs has been developed (Figure 2). Germplasm entering these nurseries is sourced from several breeding programs around the world including CIMMYT, IWWIP, Turkish national materials and a number of advanced research institutes and national programs. In all cases the observation plots are inoculated under field conditions in 1.5m pairwise plots, one with inoculum and the other without. In inoculated plots, monosporic conidial suspensions of Turkish isolates of *F. pseudograminearum* (F4), *F. culmorum* (F2) and *B. sorokinana* (B1) are mixed with the seed before planting at a concentration of 2 x 10^5 spores/ml. The yield trial seed is similarly inoculated. Confirmation of the inoculation effectiveness is determined during the growing season by collected selected plants from inoculated and non-inoculated plots and extracting the root-rotting pathogens from the root and crown sections.

The plants are scored twice, comparing with and without inoculation plots, side by side for the symptom development of these root pathogens (Nicol *et al.*, 2004a). After heading on two occasions at ripening scoring white head development and (Zadoks growth stage 91-94) and at full maturity (Zadoks growth stage 99) scoring the growth reduction with inoculation. These two scores are used to rank the material for subsequent promotion.

Greenhouse screening for cereal root rots and cereal nematodes

Pre-germinated seeds are grown in open-ended electrical conduit tubes which are placed in large trays at 22°C (±5°C), 16 hour day 8 hour night. With RLN, a 70: 29: 1 (Sand: field soil: organic matter) soil mixture placed in 12.5cm x 3 cm diameter tubes whilst a 90: 10 (sand: field soil) is used for CCN. RLN are reared on carrots and CCN is extracted by hatching cysts collected from a naturally infested field site in Haymana-Ankara, population *Hf1*. Each plant is inoculated at a rate of 100 juveniles for CCN or 400 nematodes (juveniles and adults) for RLN in a 1 ml aliquot of water per plant, immediately or 1 week after planting germinated grains, respectively (Yorgancilar, 2006). The same monosporic cultures of CR and CRR fungi used in field inoculation are assessed individually by pipetting a conidial suspension on the stem base of the seedlings medium one week after planting at 250µ (1x10^6 spore/ ml) spore suspension is following a modified method of Vivek *et al.* (2006). Water is supplied in adequate amounts for plant

growth using a capillary bottom up by capillary reaction.

All greenhouse tests use a randomized complete block design with seven replicates per genotype. For all pathogens a range of known check lines are included. After two months the lesions on the roots are scored for both CR and CRR on a qualitative scale of lesion development (Nicol *et al*., 2001). Similarly after two months the plants are harvested for nematodes. In the case of CCN the number of cysts per plant are counted under a stereomicroscope after washing the roots and collecting on a 250µm sieve. For RLN, the number of nematodes per plant is determined by extracting nematodes from the root system and surrounding soil and counting them microscopically. It should be noted that CCN comprises a complex group of several closely related species with varying pathotypes as reviewed by Nicol *et al*., (2004a). However, presently only the Turkish population of *H. filipjevi Hf1* has been screened.

Field tolerance screening for dryland root rot and cereal cyst nematode

The tolerance of the best resistant germplasm is assessed in Cumra for dryland root rot using the same field protocol as with the observation plots, however with yield plots being used instead. In the near future tolerance against the Cereal Cyst Nematode will be screened under natural field populations near Ankara (Haymana) with and without the application of the nematicide Aldicarb (Temik® 15G).

Molecular validation with markers

Crosses segregating for sources of root disease resistance that have molecular tags are firstly screened in the F1 top cross or F2 generation. This is a process of allele enrichment as most of the available markers are dominant and heterozygotes cannot be identified. Once gene frequency has been influenced in this way, markers are not applied again until fixed lines have been developed. Presently three molecular markers are routinely used in CIMMYT Mexico including *Cre1*, *Cre3* for CCN and *rln1* for RLN – *P. neglectus*. Those lines expressing the desired combination of resistance genes are then sent to Turkey for confirmation under field conditions.

Integration of resistance and the development of advanced lines

Currently, around one third of all spring crosses made at CIMMYT in Mexico for the drier areas segregate for soil borne pathogen resistance. New advanced materials with improved root disease resistance have shown excellent yield performance even in environments where root diseases do not occur (Table 1). More than 300 targeted winter wheat crosses have been made since 2001 and in 2006 these will undergo a validation process using this strategy. Since 2002 this spring wheat germplasm has been sent out with the international Semi-Arid Wheat Screening Nurseries from CIMMYT Mexico and the frequency of these entries has continually increased from 3% in 2002 to 16% in 2005. In addition more than 20 special disease nurseries have been sent to collaborators working with these soil borne pathogens, particularly in West Asia and North Africa and also Advanced Research Institutions in Australia and America.

RESULTS AND DISCUSSION

As clearly illustrated in Table 1 more than 24 spring wheat lines from CIMMYT Mexico have been validated with resistance to one or more SBP. One quarter of these represent synthetic derivatives which have also provided excellent sources of other biotic and abiotic resistances. It is very reassuring to note that resistant sources identified from other countries such as Sunco and Silverstar provide the same resistant reaction in Turkey and Mexico, suggesting the pathogen complexity of some of the SBPs is relatively conserved. Within winter wheat, 16 sources have been identified, 4 of which are released Turkish cultivars and the others represent Turkish National and IWWIP sources of advanced highly adapted lines. Tolerance work in Mexico has revealed that several of the sources of confirmed spring resistance against specific soil borne pathogens are in significantly higher yielding backgrounds (from 10 up to 90%) than the parental source without disease pressure. These are now being validated under disease pressure in Turkey.

As mentioned, CCN is much more complex than the other SBPs and will require more extensive studies to understand the regional complexity of the different species and their relative importance, but work with the other resistant sources for RLN and CR seems to be transferable between countries. As molecular tools develop further they will aid the development of advanced germplasm with greater efficiency.

Through integrated efforts of CIMMYT Mexico, the Turkish Ministry of Agricultural and Rural Affairs and CIMMYT Turkey substantial progress has been made in developing SBP disease resistant germplasm. We welcome collaboration with any other scientific groups.

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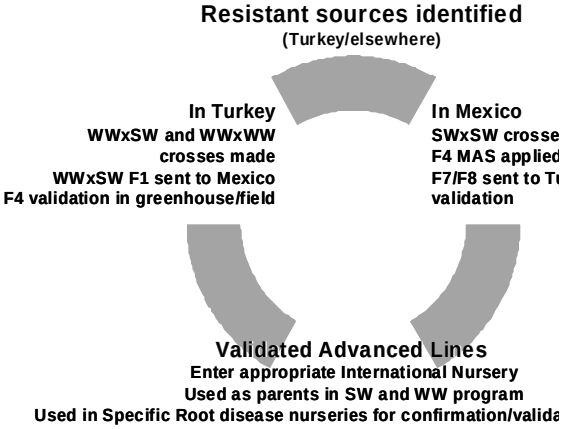

Figure 1. International breeding strategy to develop dryland root rot and cereal nematode resistant wheat germplasm. (WW = winter wheat, SW = spring wheat, MAS = marker assisted selection).

FIELD Dryland Root Rot, Cumra, Central Anatolian Plateau, Turkey

NEW ACCESSIONS

KNOWN SOURCES OF RESISTANCE
(Principally from Mexico and Australia)

Years 1 and 2
RRPN - Root Rot Probe Nursery
Consists of National Breeding Material + IWWIP advanced nurseries + spring wheat (Mexico/other)
2 yr field screening observation plots, unreplicated, 2000 lines
Lines promoted again for testing in 2nd year with 5% lines selected
Root rot complex inoculum (*Fc* + *Bs*)

Approx. 10% of lines selected that have performed well for 2 years in RRPN

Years 3 and 4
RREL - Root Rot Elite Lines
Lines that have performed well for 2 years in RRPN and additional lines with proven resistance from elsewhere.
2 year field screening observation plots, lattice design, 3 reps, 121 lines
Root rot complex inoculum (*Fc* + *Bs*)

Approx. 40% of lines selected

GREENHOUSE Dryland Root Rot and Cereal Nematode Screening, Eskisehir, Turkey

NEW ACCESSIONS

KNOWN SOURCES OF RESISTANCE
(Principally from confirmed data in Turkey, Mexico and Australia)

CEREAL NEMATODES PGS - Preliminary Greenhouse Screen
Screened for one or more nematode *Hf, Pt* and *Pn* individually based on pedigree
7 replicates RCBD

Lines with resistance promoted for cereal nematodes from greenhouse and from field root rot screening

CEREAL NEMATODES AND ROOT ROTS AGS – Advanced Greenhouse Screen
Screened individually against *Fc, Hf, Pt, Pn*
7 replicates RCBD

Lines providing best resistances and adapted phenotype promoted – emphasis placed on multiple root disease resistance

Field Yield Trial – Tolerance test
Root rot +/- inoculum, *Fc, Bs*
Yield plots, Cumra, 5 reps, RCBD
Cereal nematode *Hf*
Yield plots, Haymana, 4 reps, lattice

Soil Borne Pathogen Crossing Block
National/International Program
CIMMYT /IWWIP/Turkey

International Nurseries
Spring/Winter/Special

Figure 2. International screening program to identify resistance and tolerance in bread wheat against soil borne pathogens, including cereal root rots (*Fc = F. culmorum, Bs = Bipolaris sorokiniana)* and cereal nematodes (cereal cyst *Hf = Heterodera filipjevi*, root lesion *Pt = Pratylenchus thornei & Pn= P. neglectus*).

Table 1. Summary of the confirmed spring and winter wheat lines identified from the joint Turkey/CIMMYT screening program with resistance to one or more soil borne pathogen(s).

	[1]Characterised Cereal Cyst Nematode Gene	Greenhouse Cereal Cyst Nematode *Heterodera filipjevi*	Greenhouse Root Lesion Nematode *Pratylenchus thornei*	Greenhouse Root Lesion Nematode *Pratylenchus neglectus*	Greenhouse Crown Rot *Fusarium culmorum*	Field Screening Crown Rot & Common Root Rot *F. culmorum & Bipolaris sorokiniana*	SW Spring Wheat WW Winter Wheat	Cross Name	IWWIP ACCESSION #	CIMMYT MEXICO CID	CIMMYT MEXICO SID	CIMMYT MEXICO SELECTION HISTORY	COUNTRY OF OCCURRENCE
1			1		1*	1*	SW	CROC_1/AE.SQUARROSA (224)//OPATA	020615	72726	531	CMBW91Y00935S–80Y-11KBY-1KBY-010M-1Y-2M-	MX
2					1*	1	SW	CROC_1/AE.SQUARROSA (224)//OPATA	020616	72726	532	CMBW91Y00935S-80Y-11KBY-1KBY-010M-1Y-3M-	MX
3					1*	1	SW	CROC_1/AE.SQUARROSA(224)//OPATA	030825	72726	530	CMBW91Y00935S	MX
4					1	1	SW	SABUF/7/ALTAR 84/AE.SQUARROSA (224)//YACO/6/CROC_1/AE.SQUARROSA (205)/5/BR12*3/4/IAS55*4/CI14123/3/IAS55*4/EG, AUS//IAS55*4/ALD	020632	167180	136	CASS94Y00045S	MX
5					2	1	SW	[2]YAV79//DACK/RAB1/3/SNIPE/4/AE.SQUARROSA (460)	020635	154092	7	CIGM88.1348 MX BW30157	MX
6		2			2		SW	[2]MAYOOR//TK SN1081/AE.SQUARROSA (222)	031035	167144	420	MX	MX
7					1*		SW	[2]ALTAR 84/AE.SQUARROSA (224)//YACO/6/CROC_1/AE.SQUARROSA (205)/5/BR12*3/4/IAS55*4/CI14123/3/IAS55*4/EG, AUS//IAS55*4/ALD	031037	152384	14	MX	MX
8					1*		SW	[2]CATBIRD	031031	21597	2279	MX	MX
9	*Cre3*		1*	2			SW	VP1620 (VF304/TTAU.69.5-33//YANAC)	030901				AUS
10	*Cre1*	1	1*				SW	SILVERSTAR	031017				AUS
11	*Cre2*	1*	1*	1			SW	ID-2150	020626				SP
12	*Cre7*	1*	2		2		SW	T-2003	020628				SP
13						1	SW	302-5	020637	221383	0		AUS
14		1*	2		1	2	SW	SUNCO	020650	76058	0		AUS
15	*Cre8?*				1*	2	SW	SUNCO/FRAME//PASTOR		394740	50	CMSS99M01589T-040Y-0P0M-16SY-010M-010Y-5M	MX
16		2			1*	2	SW	SUNCO/PASTOR		429528	44	CMSS98GH00077S-0100M-040Y-020M-040SY-23M-0Y	MX
17					2	2	SW	SUNCO/2*PASTOR	030802	431763	51	CMSS99Y05530T–10M-1Y-010M-3SY-0B	MX
18		2			1*	1	SW	SUNCO/3/URES/JUN//KAUZ/4/URES/JUN//KAUZ		394737	15	CMSS99M01586T-040Y-0P0M-040SY-040M-040SY-10M	MX
19		2	1*			1	SW	AUS GS50AT34/SUNCO//CUNNINGHAM	030799	431762	31	CMSS99Y05529T–12M-6Y-010M-3SY-0B	MX
20	*Cre?*	2	2				SW	AUS4930 5.3/Spear DH#44	030921				MX
21	*Cre?*	1	2				SW	AUS 4930.7/2*PASTOR	030857	431784	83	CMSS99Y05544T - -9M-10Y-020M-9Y	MX
22			1		1	2	SW	CANADIAN/2*PASTOR		394714	20	CMSS99M01563M-040Y-0P0M-040SY-040M-040SY-	MX
23					2	1	SW	CANADIAN/CUNNINGHAM//KENNEDY		394715	8	CMSS99M01564T-040Y-0P0M-040SY-040M-040SY-3M	MX
24					1*	1	SW	CANADIAN/CUNNINGHAM//KENNEDY		394718	21	CMSS99M01567T-040Y-0P0M-040SY-040M-040SY-16M	MX
25		2				1*	WW	ALTAY 2000	010627				TK
26					1*	1	WW	BAGCI 2002	030845				TK
27					1*	1	WW	SONMEZ	950193				TK
28					1*	2	WW	KATEA-1	950590				TK
29					2	1*	WW	MVR27-82//LI7/LE2062	000406				TK
30					1*	1*	WW	TAM201/4/BL/AU/3/AGRI//HYS/7C/5/F134.71/NAC	000240				TK
31		1*	1*				WW	BEZ/HAWK//ES14	030788				TK
32					1*	2	WW	SKP35/SAM2/4/55-1744/D101//MAYA.S/3/MUS.S/DRM.MAYA/ALD.S	030791				TK
33				1*		1	WW	ES84-24/DYNASTY	030793				TK
34		1*				1	WW	BILINMIYEN96.7	000064				IWWIP
35					1*	1*	WW	LOV41//LI7/LE2062	000161				IWWIP
36				1*		1*	WW	BILINMIYEN96.7	000330				IWWIP
37					1*	1	WW	JING411//PLK70/LIRA/3/GUN91	000393				IWWIP
38					1*	1	WW	F130L1.12/ATTILA	980872				IWWIP
39					2	1*	WW	BURBOT-6	990857				IWWIP
40					1	1	WW	ZANDER-39	010221				IWWIP

Score of 1* indicates a higher level of resistance than the best known resistant check, 1 indicates resistance equivalent to the best known resistant check line, 2 level of moderate resistance not as high as best known check, but still effective. Partially resistant (PR) and Suscpetible (S) check lines used for each soil borne pathogen. [1]refers to characterized single gene for resistance against different various pathotypes of the cereal nematode *Heterodera avenae* (see Nicol et al., 2003). [2]these sources also have known resistance against Fusarium head blight (*Fusarium graminearum*) from CIMMYT Mexico. AUS = Australia, IWWIP = International Winter Wheat Improvement Program, MX = Mexico, SP = Spain, TK = Turkey

Table 2. The yield of soil borne pathogen spring wheat resistant lines compared to their genetic source parents in drought or reduced irrigation (Irr) in northwestern Mexico

CROSS	SELECTION HISTORY	Drought YIELD T/HA	Drought % OF THE ROOT ROT RESISTANT PARENT	Reduced Irr YIELD T/HA	Reduced Irr % OF THE ROOT ROT RESISTANT PARENT
TRIAL 1					
SILVERSTAR (RESISTANT TO CCN pathotype *Ha13*)		3.621	100.0	3.429	100.0
FRAME (TOLERANT & RESISTANT TO CCN AND BORON TOLERANT)		3.450	100.0	3.191	100.0
FRAME*2/3/URES/JUN//KAUZ	CMSS98Y03487F-040M-0100M-040Y-020M-040SY-13M-0Y	5.109	148.1	5.820	182.4
FRAME*2/3/URES/JUN//KAUZ	CMSS98Y03487F-040M-0100M-040Y-020M-040SY-22M-0Y	5.038	146.0	6.147	192.6
CROC_1/AE.SQUARROSA (205)//KAUZ/3/FRAME	CMSS98Y04515S-020Y-030M-040SY-020M-29Y-010M-0Y	4.813	139.5	5.604	175.6
CROC_1/AE.SQUARROSA (205)//KAUZ/3/FRAME	CMSS98Y04515S-020Y-030M-040SY-020M-32Y-010M-0Y	4.588	133.0	5.559	174.2
CROC_1/AE.SQUARROSA (205)//KAUZ/3/FRAME	CMSS98Y04515S-020Y-030M-040SY-020M-36Y-010M-0Y	4.878	141.4	5.880	184.3
PASTOR/SLVS	CMSS98Y04337S-0100M-040Y-020M-040SY-6M-0Y	4.612	127.3	4.942	144.1
PASTOR/SLVS	CMSS98Y04337S-0100M-040Y-020M-040SY-28M-0Y	4.961	137.0	5.348	156.0
HXL7573/2*BAU//SLVS	CMSS98Y04486S-0100M-040Y-020M-040SY-10M-0Y	4.426	122.2	4.992	145.6
TRIAL 2					
PASTOR		4.219	140.8	6.046	139.4
SILVERSTAR (RESISTANT TO CCN pathotype *Ha13*)		2.966	99.0	4.336	100.0
SLVS*2/PASTOR	CMSS98Y03489F-040M-0100M-040Y-020M-040SY-14M-0Y	5.618	187.5	6.435	148.4
SLVS*2/PASTOR	CMSS98Y03489F-040M-0100M-040Y-020M-040SY-21M-0Y	5.577	186.1	6.376	147.1
SLVS*2/PASTOR	CMSS98Y03489F-040M-0100M-040Y-020M-040SY-27M-0Y	5.168	172.5	6.152	141.9
SLVS*2/PASTOR	CMSS98Y03489F-040M-0100M-040Y-020M-040SY-28M-0Y	5.190	173.2	6.540	150.8
SLVS//HXL7573/2*BAU/3/HXL7573/2*BAU	CMSS98Y03490T-040M-0100M-040Y-020M-040SY-5M-0Y	4.673	156.0	5.839	134.7
AMSEL/2*SLVS/3/HD29/2*WEAVER//2*SLVS	CMSS98M00985D-1KBY-040M-040Y-020M-040SY-2M-0CRE-0M	4.657	155.4	5.219	120.4
T.TAU.83.2.29/3/PRL/SARA//TSI/VEE#5	CMSS98M00147S-0100M-040Y-020M-040SY-8M-0Y	4.847	161.8	6.082	140.3
TRIAL 3					
PASTOR		4.519	95.7	5.362	102.3
WORRAKATTA (RESISTANT TO RLN Pn, TOLERANT TO BORON)		4.724	100.0	5.242	100.0
WORRAKATTA/2*PASTOR	CMSS99Y05557T-3M-11Y-010M-010SY-8M-0Y	4.471	94.6	4.953	94.5
WORRAKATTA/2*PASTOR	CMSS99Y05557T-3M-12Y-010M-010SY-5M-0Y	4.568	96.7	5.621	107.2
WORRAKATTA/2*PASTOR	CMSS99Y05557T-3M-19Y-010M-010SY-5M-0Y	4.478	94.8	4.939	94.2
WORRAKATTA/2*PASTOR	CMSS99Y05558T-1M-18Y-010M-010SY-3M-0Y	4.947	104.7	5.227	99.7
TRIAL 4					
WORRAKATTA (RESISTANT TO RLN Pn, TOLERANT TO BORON)		4.772	100.0	5.240	100.0
KRICHAUFF (RESISTANT TO RLN Pt, TOLERANT TO BORON)		4.112	100.0	4.665	100.0
WORRAKATTA/2*PASTOR	CMSS99Y05558T-1M-18Y-010M-010SY-8M-0Y	5.029	105.4	5.050	96.4
WORRAKATTA/2*PASTOR	CMSS99Y05558T-1M-19Y-010M-010SY-3M-0Y	5.009	105.0	5.025	95.9
KRICHAUFF/2*PASTOR	CMSS99Y05560T-2M-1Y-010M-010SY-3M-0Y	5.476	133.2	5.638	120.9
KRICHAUFF/2*PASTOR	CMSS99Y05560T-2M-1Y-010M-010SY-6M-0Y	5.434	132.2	5.762	123.5
KRICHAUFF/2*PASTOR	CMSS99Y05560T-2M-1Y-010M-010SY-8M-0Y	5.317	129.3	5.522	118.4
WORRAKATTA/PASTOR	CMSS98GH00023S-0100M-040Y-020M-040SY-20M-0Y	5.022	105.2	5.542	105.8
WORRAKATTA/PASTOR	CMSS98GH00023S-0100M-040Y-020M-040SY-22M-0Y	5.574	116.8	6.007	114.6
WORRAKATTA/PASTOR	CMSS98GH00023S-0100M-040Y-020M-040SY-27M-0Y	5.003	104.8	5.928	113.1
KRICHAUFF/2*PASTOR	CMSS99Y05559T-9M-4Y-010M-010SY-5M-0Y	5.163	125.6	5.209	111.7

FHB SCREENING NURSERY IN WESTERN CANADA: CRITICAL CONTROL POINTS FOR LARGE-SCALE FUSARIUM HEAD BLIGHT FIELD SCREENING TRIALS

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ABSTRACT

Fusarium head blight (FHB) continues to be a serious disease of wheat in western Canada especially the Red River Valley in Manitoba. In 2001, breeders and pathologists entered into a collaborative agreement to establish a common FHB screening nursery at Carman, Manitoba funded by Western Grains Research Foundation. In the last five years in operation, the screening of lines/cultivars has increased with 12,000 lines evaluated in 2005 for FHB resistance. The results from lines evaluated from 2001 to 2004 were analysed with the objective of identifying critical control factors that could improve large-scale FHB screening trials and to characterize variation observed in different years and different environmental conditions. These were done either in replicated or non-replicated trials. In each year, five checks with known reactions to FHB were included for every 50 plots. The factors analysed were variation for disease incidence, severity and FHB Index measurements within and among years (nurseries), the effect of different evaluators on these measurements, and frequency of change in ranking or classification among the checks. Environmental factors were also assessed. The results from this analysis assisted in the interpretation of data from large field screening nurseries and comparison of results between years.

SEARCHING FOR NOVEL SOURCES OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN BARLEY

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ABSTRACT

ICARDA, in cooperation with CIMMYT, has been producing barley with enhanced resistance to Fusarium head blight (FHB) in its Mexico-based program since the early 1980s. ICARDA's germplasm bank in Syria offers a diverse reservoir of genes that can be explored as possible new sources of resistance for this devastating disease. The crop's wild relatives represent even richer reservoirs of genes for stress tolerance and adaptation, as their history in the Central and West Asia and North Africa (CWANA) region is very long and includes periods with very harsh climate in the Pleistocene Era.

The ICARDA/CIMMYT barley breeding program started research on FHB resistance in response to the needs of the Andes countries. In 1986, a total of 5,000 barley accessions were screened in Mexico; of these, 23 were found with some level of resistance, and were subsequently intensively introgressed into the main program. Resistance sources were shared with programs worldwide, especially after the FHB outbreaks of the 1990s. Collaboration and cooperative efforts with advanced research institutions, such as the US Wheat and Barley Scab Initiative (USWBSI), allow the project to make germplasm sources with enhanced levels of resistance widely available. Environmental conditions at CIMMYT's Toluca Experiment Station in Mexico in earlier years and at El Batán since 2006 are ideal for FHB development and evaluation. In addition, the project obtains data through collaboration with programs in the USA, Canada, China, Ecuador, Brazil and Uruguay. A recent initiative to comprehensively screen ICARDA's gene bank for unique and undiscovered sources of resistance has identified some potentially promising barley sources.

BREEDING APPROACH

The approach of combining different sources of resistance, followed by selection at Toluca and El Batán, to accumulate resistance genes, has proven effective in enhancing the resistance to FHB and other important diseases. Sources of resistance were used as parents in resistant x susceptible as well as resistant x resistant crosses, producing a core of germplasm through recurrent selection in which 1–3 or more resistance sources are present.

COLLABORATION WITH USWBSI

The program's formal collaboration with the USWBSI started in 2000 with the delivery of putative resistant germplasm to participants. When the 1993 FHB outbreak occurred in the Midwest, all the germplasm previously tested in the ICARDA/CIMMYT program was made available to the US programs. Since 2000, several previously evaluated genotypes were sent to the US programs. Important elite lines and cultivars were also introduced from the USA and extensively used in the ICARDA/CIMMYT program crosses to introgress germplasm adapted to specific conditions. Among the introduced material, several lines already had 1-2 cycles of selection for FHB in the USA. This special USWBSI breeding program is expected to increase the probability of finding FHB resistant lines adapted to their target area. Besides adaptation, special emphasis is placed on malting quality.

The USWBSI collaboration is helping to synchronize efforts among the participating programs, avoid duplication, and to generate more meaningful multi-environment stepwise data. Three key collaborative research activities have been:

1. Participation in the early screening nurseries in the USA with up to 300 new putative resistant lines every year.
2. Participation through germplasm in the China nursery, providing up to 180 research plots, and collaborating through technical visits and annual evaluation of experiments in China, and
3. Participation in the North American Barley Scab Elite Nursery (NABSEN) with up to 8 elite FHB resistant lines and with the establishment and evaluation of a replication of the nursery at the Toluca Experiment Station.

THE STRATEGY

Recently, additional emphasis has been given to the need to assure that all sources of resistance are being used in the breeding programs. Presently "a large number of programs in the USA are involved in developing FHB resistance", but are "hindered by a lack of adequate resistant sources" (FY04_RFPP-Kit, 2004). Identifying, confirming through validation experiments and making such diversity available to USWBSI will immensely expand the foundation of resistance on which US barley germplasm rests and so strengthens its insurance against FHB. This project aims to both 'identify new sources of FHB resistance and to facilitate the utilization of resistant barley germplasm' using conventional and, where available, modern tools. Discussions with scientists collaborating with the USWBSI raised the need to conduct a final thorough search and identification of new sources of resistance to FHB in barley, especially in the six-row pool. An additional effort to cover all possible global sources of resistance has yet to be accomplished.

RESEARCH METHODS

Hypotheses

The research undertaken focuses on the following questions:

1. Have we collected most of the potentially resistant stocks from barley improvement programs and gene banks around the world, mostly in developing countries?
2. Are these stocks genetically distinct from other known sources?
3. Do these stocks express their resistance in a stable reliable fashion under different production and ecological conditions around the world?
4. How can these resistances most effectively be introgressed into US commercial cultivars.

Activities

The following activities constitute the research program:

1. ICARDA and CIMMYT regional staff and selected national program breeding and gene bank staff mostly in developing countries are directly contacted by the project. We request sharing of the latest barley resources for FHB resistance, to complement materials presently available to USWBSI collaborators. As FHB globally increases in importance, direct links with barley improvement programs in FHB-prone areas in developing countries must be maintained. We expect progress to be made and novel resistance to be identified on a continuing basis as overall research effort increases (Table 1). Active contact with global colleagues was a continuous CIMMYT activity under past USWBSI projects and will be for potential future projects.
2. A systematic search will be conducted for resistance sources from the ICARDA and CIMMYT gene banks (Tables 1 and 2).
3. Potentially new resistant barley sources are tested against local *Fusarium* isolates in Toluca and El Batán to determine the types of FHB resistance that are present.
4. Promising sources are multiplied for dissemination to USWBSI.
5. The most resistant entries are crossed with commercial US barley varieties.
6. Newly developed barley stocks are shared with USWBSI researchers.

Table 1. Summary of nurseries and entries screened at Toluca from the ICARDA Gene Bank and the number of lines found resistant to different diseases and FHB.

Name Nursery	No. of Genotypes Total	Resistant to Stripe Rust	Resistant to Leaf Rust	FHB Range (1-5)	FHB Range (%)	Heading Date Range
Introductions ICARDA	277	45	2	0 - 3	-	Jul 19 - Aug 9
Collection Eritrea	295	34	5	-	1.0 - 28.6	Jul 11 - Jul 28
Collection Palestine	19	11	-	2 - 5	3.1 - 34.5	Jul 18 - Aug 8

Table 2. Collection of barley genotypes introduced from Palestine and screened for FHB and barley stripe rust on 2004 and 2005 at Toluca.

Plot	ig	tax_name	lat_dd	Toluca 2005			Toluca 2004	Selections
				Heading Date	Stripe Rust	FHB Type I (1-5)	FHB Type I (%)	
1	25445	Hordeum vulgare subsp. vulgare convar. vulgare	33.01806	25-Jul	S	-	34.5	
2	25530	Hordeum vulgare subsp. vulgare convar. vulgare	31.78333	18-Jul	S	2	12.3	
3	27377	Hordeum vulgare subsp. vulgare convar. vulgare		18-Jul	S	3	19.8	
4	125765	Hordeum vulgare subsp. vulgare convar. distichon		8-Aug	MR	2	15.5	S
5	125766	Hordeum vulgare subsp. vulgare convar. vulgare		18-Jul	MS	2	20.0	
6	125767	Hordeum vulgare subsp. vulgare convar. vulgare		25-Jul	MS	3	16.3	
7	125768	Hordeum vulgare subsp. vulgare convar. vulgare		25-Jul	MR	3	27.5	
8	125769	Hordeum vulgare subsp. vulgare convar. vulgare		25-Jul	MR	2	7.1	S
9	125770	Hordeum vulgare subsp. vulgare convar. vulgare			R	-	-	
10	125771	Hordeum vulgare subsp. vulgare convar. vulgare		25-Jul	MR	4	17.3	
11	125772	Hordeum vulgare subsp. vulgare convar. vulgare		18-Jul	R	2	11.7	S
12	125773	Hordeum vulgare subsp. vulgare convar. vulgare		25-Jul	MR	3	17.0	
13	125774	Hordeum vulgare subsp. vulgare convar. vulgare		25-Jul	R	4	15.9	
14	125775	Hordeum vulgare subsp. vulgare convar. vulgare		8-Aug	MS	2	-	
15	125777	Hordeum vulgare subsp. vulgare		25-Jul	R	5	8.8	
16	125778	Hordeum vulgare subsp. vulgare convar. vulgare		18-Jul	MS	3	9.8	
17	125779	Hordeum vulgare subsp. vulgare convar. vulgare		8-Aug	R	3	3.1	S
18	125780	Hordeum vulgare subsp. vulgare convar. vulgare		25-Jul	R	5	19.2	
19	125781	Hordeum vulgare subsp. vulgare convar. distichon		18-Jul	MS	4	6.9	

CHARACTERIZATION AND DEVELOPMENT OF ARGENTINEAN WHEAT GERMPLASM WITH RESISTANCE AGAINST FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB), induced by *Fusarium graminearum* Schwabe, is a devastating disease affecting wheats worldwide, including central/south-east Argentina, where optimal conditions for disease development are more frequent. In this region, yield losses of 20-50% have been reported in years of severe epidemics like 1945, 1978, 1985, 1993 and 2001.

Breeding for resistance to FHB is complex task as several loci are involved in the character. No immune genotypes are currently available and the best sources of partial resistance come from old genetic pools from (1) East-Europe, (2) China and Japan and (3) Brazil and Italy. Since 1982, INTA Marcos Juarez has been working in the development of wheat breeding lines with improved resistance against FHB incorporated to local germplasm. The program is based in two main activities, (1) Identification of effective sources of genetic resistance to FHB, by artificial inoculation using local *F. graminearum* isolates (2) Incorporation of genetic resistance to FHB in adapted germplasm of higher agronomical value by cycles of recurrent selection in an environment appropriated to select resistance to FHB using germplasm of 5 origins: (1) Introductions from China and Japan highly resistant to FHB; (2) Germplasm developed by CIMMYT and South Cone countries; (3) Advanced breeding lines from the INTA Wheat Breeding Program; (4) Old and recent local cultivars; (5) Breeding lines obtained with this methodology to feedback into the program.

Up to date, 79 lines with long cycle and 77 with short cycle have been obtained and 29 are in latest stages of evaluation. All these lines showed similar or superior resistance than the best controls (Sumai3, YMI 6 and Klein Atlas) but many of them did not showed optimal performance in quality and/or agronomical traits.

Since 2002 to date, important efforts in the characterization of storage proteins of lines with better agronomic performance and good resistance to FHB have been done. This information is currently being used to solve deficiencies in quality by the selection of best combinations of storage proteins in segregating populations. Microsatellites *Xgwm493* and *Xgwm533* linked to the most important QTL linked to the resistance of Sumai3 to FHB are currently used to introgress this source of resistance in local germplasm with good resistance against FHB like ProINTA Granar.

Workshop Summary

Fusarium Head Blight (FHB) and Fusarium Crown Rot (FCR) are two major wheat diseases caused by *Fusarium* fungi. FHB is one of the most destructive diseases of small grain cereals in areas where the weather is warm and humid during the heading to harvesting period. FHB not only reduces grain yield and quality, but also produces mycotoxins in the grain that are harmful to animal and human health. FCR is prevalent in sub-optimal growing condition and results in dead heads and major yield losses. *Fusarium* diseases pose grave threats to wheat and barley industries around the world. During this workshop, progress was made principally on the development of a concrete base for research operations for facilitating international collaboration on *Fusarium* research.

Fifty participants from 17 countries and relevant *Fusarium* research programs (Argentina, Australia, Austria, Canada, China, Germany, Hungary, Iran, Japan, Mexico, Romania, South Africa, Tunisia, Turkey, UK, Uruguay and USA) presented their latest research progress and ideas for future prospects for the global community. It was an invaluable opportunity to exchange ideas regarding the current status of *Fusarium* research around the world and to develop action plans for collaborative efforts to combat FHB and FCR.

The Global Fusarium Initiative endorsed by this workshop provides a mechanism for creating a virtual community of *Fusarium* researchers with which to identify and pursue critical research objectives on a global scale that can only be accomplished through collaboration. The accomplishment of such objectives will allow the *Fusarium* community, as a whole, to more effectively understand the nature of FHB and FCR and combat the effects of these diseases around the world through a greater understanding of host resistance and the pathogen, and exchange of resistance germplasm. Coupled with international, comparative studies of the interaction between resistance genes and pathogen diversity, these collaborations will enable accelerated development of solutions to the problems of FHB/FCR.

Participants discussed and agreed upon the most critical research needs, opportunities for web-based knowledge, opportunities for international collaboration, and action plans in three areas which collectively cover the research issues of FHB/FCR.

The workshop highlighted the important status of collaborative efforts to address the disease, and future prospects for international collaboration in developing the following specific consortiums:

1) *FHB-QTL consortium:*
   Wheat FHB/FCR-QTL comparative study for the deployment of resistance genes, including the analysis of the bases of resistance, the development of an effective MAS system and the pursuit of germplasm enhancement,

2) *Fusarium consortium*:
   Global compilation/monitoring system of genetic diversity, pathogenicity, and toxigenicity from studies on *Fusarium* fungi, to control FHB/FCR,

3) *International Fusarium nursery consortium*:
   Development of new international interactive *Fusarium* Resistance Screening Nurseries for germplasm enhancement and global compilation of genotype x environment x management effects on resistance to FHB/FCR.

The outcomes compiled the most critical research needs, opportunities for international collaboration, and areas for web-based knowledge sharing. Action plans were drafted and a resolution to coordinate international research, with endorsement of the research priorities, was agreed upon. We endorsed in principle the concept of and need for the Global Fusarium Initiative at the end of the workshop. The Global Fusarium Initiative provides a platform to fight these grave threats which require all our wisdom and expertise to overcome.

Outcomes and Strategic Action Plans

Session 1: FHB-QTL Consortium

1. **Most Critical Research Needs**
   - Validation [effect of quantitative trait loci (QTLs)] and diagnostic markers (closely linked, polymorphic)
     i. Consensus map with QTL locations from different populations (heat map view)
     ii. Are there any epistatic effects?
   - Source of immunity reaction type
   - More QTLs
     i. For Type I (initial penetration resistance)
     ii. Resistance to deoxynivalenol (low DON accumulation in the grains)
   - Marker assisted selection (MAS) protocols
   - Resistance phenotyping/scoring protocols

2. **Opportunities for Web-based Knowledge Sharing**
   - Germplasm resources information (e.g. origin, nomenclature, known QTLs)
   - Mapping/QTL information
     i. Published and pre-published/unpublished (curated)
     ii. Include a simplified summary
   - Current and future mapping plans
   - Phenotyping protocols
   - MAS protocols

3. **Opportunities for International Collaboration/Activities**
   - Review paper and FHB QTLs consensus map
   - Coordinate future mapping efforts
   - Phenotyping protocols
   - MAS protocols
   - Germplasm sharing (with QTL information, if known).
     i. International screening nursery (40-50 entries)
     ii. Screen QTL-near isogenic lines (NILs).
   - Curation of key resistant germplasm (primary, secondary, tertiary and others)
   - Sharing mapping parents
     i. Opportunities to map other traits.
   - Cloning resistance genes/QTLs
   - Cloning targets, chromosome regions

4. **Action Plan**
   - Review paper for FHB-QTLs, resistance germplasm
   - Initiate web database
     i. Input initial QTL information for database
     ii. Add information from web-based knowledge list
   - Initiate international screening nursery
   - Curation of key resistant germplasm
   - Share mapping parents
     i. Opportunities to map other traits

Session 2: Fusarium Consortium

1. **Most Critical Research Needs**
   - Characterization of FHB/CR isolates using screening from global locations (natural and inoculated)
   - Investigate the question of whether or not the pathogen can adapt to resistant varieties:
     i. Are there "resistance breaking" new strains?
     ii. Are different resistant varieties effective on all species/lineages/chemotypes?
     iii. Are different QTL NILs effective on all species/lineages/chemotypes?
     iv. Determine fitness costs and selection pressures; importance of saprophytic stage and migration from other crops.
   - Identify mechanisms that control production, transport, and accumulation of trichothecenes in grain (including sympotomless).
   - Establish a clearer understanding of the life cycle and epidemiology of *Fusarium* – on different parts of the plant and also the 'saprophytic' stage.
   - Information and recommended protocols (internet interface)
     i. Identification of isolates and chemotypes
     ii. Aggressiveness testing.
   - What is the full extent of pathogen diversity of *F. graminearum*, *F. pseudograminearum*, and *F. culmorum* – especially in centers of diversity? What is the significance of that diversity (species/lineage/chemotype) with respect to aggressiveness to hosts and toxicity to animals or humans?
   - What is the importance of international movement of strains?
   - Can the pathogen adapt to triazole fungicides?
   - What are the mechanisms of pathogenicity and aggressiveness in the pathogen that could be targeted for control?

2. **Opportunities for Web-based Knowledge Sharing**
   - Identification of isolates and chemotypes
     i. List the methods for species (morphological/molecular) and chemotype
     ii. Ultimately provide comparative information and standards needed for global acceptability.
   - Aggressiveness testing
     i. List the various methods.

3. **Opportunities for International Collaboration/Activities**
   - Coordinated effort to collect isolates from around the world. These would be characterized for a number of species, chemotypes and aggressiveness.
   - Preparation of an international nursery to establish whether the pathogen can adapt to resistant varieties (should be similar/same as other international nurseries proposed).
   - Investigate the mechanisms that control production, transport, and accumulation of trichothecenes in grain including FHB-symptomless high mycotoxin accumulation.
   - Establish a clearer understanding of the life cycle and epidemiology of Fusarium on different parts of the plant and also the 'saprophytic' stage.
   - Information and recommended protocols
     i. Identification of isolates and chemotypes
     ii. Aggressiveness testing

4. **Action Plan**
   1. Execute "Opportunities for International Collaboration"

Session 3: International Scab Nursery Consortium

1. **Most Critical Research Needs**
   - Vehicles to make useful materials available throughout the world (winter and spring wheat for FHB and FCR)
     i. Elite material (registered and near-registered cultivars)
     ii. New sources of resistance
     iii. Genetic stocks which enable validation of effects of known QTL and identification of vulnerabilities from pleiotropy, linkage or new strains.
   - Haplotyping of resistance sources and inoculum (artificial and natural infection)
   - Vehicles to monitor pathogen diversity with emphasis on changes affecting host plant resistance, and mycotoxin accumulation.
   - Clear and uniform evaluation protocols to be proposed and actual standardized protocols deployed.
   - Data on Genotype x Environment x Management interaction
     i. Pathogen focus
     ii. Germplasm focus

2. **Opportunity for Web-Based Knowledge Sharing**
   - Data on germplasm linked to agronomic information.

3. **Opportunities for International, Collaboration/Activities**
   - Internationally coordinated nurseries
     i. Host plant resistance perspective
     ii. Mycotoxin perspective
     iii. Pathogen perspective
   - Exchange of knowledge generated in the diverse ongoing FHB/FCR nurseries
   - Resolution of best case protocols
     i. Nursery management
     ii. Data acquisition
     iii. Isolate collection/characterization
   - Calling to identify ideal evaluation methods including inoculation methods using an expert panel.

4. **Action Plan**
   - Two types of winter and two types of spring wheat nurseries.
     i. Elite cultivars (or near release cultivars) with FHB resistance that has been verified regionally. These should have good agronomic traits. The nursery should contain some local checks from each contributor.
     ii. Experimental materials (i.e QTLs, genetic resources, synthetics, mapping population parents). Some purposes for various QTLs in the nursery are risk identification/stability of resistance, surveillance for isolates which are new/problematic, vulnerabilities due to linkage and pleitropy. The nursery should contain some local checks from each contributor.
   - We should have additional trait data maintained with the germplasm when possible.
   - Winter Wheat Nurseries:
     The extension of existing regional/local cooperation in FHB/FCR nurseries, with the aim of further global integration of these efforts, is strongly encouraged. The GFI should facilitate further bridges between existing nurseries through information integration & web-based sharing.
     Initial work on winter wheat nurseries is planned for the following regions:
     i. North America
     ii. Europe
     - Western Europe:
       Germany, France, UK, Austria, Switzerland

- Eastern Europe:
  Romania, Czech Republic, Poland, Hungary, Russia
- European Fusarium Ringtest (EFR, since 2002)

◆ Spring Wheat Nurseries:

- CIMMYT program will coordinate the increase and distribution of these nurseries. The following is a description of the two spring wheat nurseries:
  - Elite Spring Wheat Fusarium Nursery.: The specific objective of this nursery is to enable contributors to know the performance of their entries across environments, and allow participants to identify useful sources of resistance in entries from other programs. Regional resistant and susceptible checks from each contributor are important to facilitate interpretation of the results. The nursery will include two types of entries:
    1. Elite FHB/FCR resistant spring wheats (registered or near-registered resistant cultivars) that have performed well in regional FHB/FCR nurseries,
    2. Regional FHB/FCR resistant and susceptible reference/standard checks.

  - Preliminary Spring Wheat Fusarium Nursery:.The purposes of this nursery include identification of new sources of resistance, examination of stability of QTL for FHB/FCR resistance, surveillance for new and/or problematic pathogen strains, and development of knowledge or solutions in regard to other issues such as negative correlations between resistance QTL and other traits. The nursery can include:
    1. Any materials which address the objectives listed above including near isogenic lines (NILs) of FHB/FCR QTLs,
    2. Parents of mapping populations.

Resolutions from meeting

The Global Fusarium Initiative (GFI), is an international group of public institution researchers and private industry individuals actively working to combat the prevalence and impact of Fusarium head blight (FHB) and Fusarium crown rot (FCR) diseases in wheat and small cereal. We assembled in Mexico in March, 2006 to address opportunities for global collaboration. At the completion of the meeting we endorsed, in concept, the following draft resolutions:

We:

1. *Declare* that Fusarium and the diseases it causes in wheat warrant concerted international research to combat yield losses and threats to food safety;

2. *Endorse* the research priorities identified during the CIMMYT Workshop on the Global Fusarium Initiative for International Collaboration (Mexico, March 14-17, 2006);

3. *Endorse* in principle the concept of and need for a Global Fusarium Initiative, which will be facilitated by CIMMYT and informed by technical advisory committees.

"The mission of the Global Fusarium Initiative is to provide a platform for international collaboration on *Fusarium* research projects and to function as a world wide facilitator of information exchange, germplasm enrichment, development of breeding methods and materials, and to encourage communication and cooperation between individuals, institutions and governments focusing on this disease."

Attachment 1: Agenda for the Workshop

CIMMYT Workshop on the Global Fusarium Initiative for International Collaboration

March 14-17th, 2006: Sasakawa Room, CIMMYT, El Batan, Mexico

Agenda

March 14th Tuesday

Opening session

8:30 Opening address **Masa Iwanaga** Director General, CIMMYT

8:40 Workshop orientation talk: Tomohiro Ban (Co-organizer)

8:55 ***Session-1: FHB-QTL consortium***

FHB-R germplasm, QTLs, Markers, MAS

Chair: Tomohiro Ban

8:55 Speaker-1* **James Anderson**, Marker-assisted selection for FHB resistance in wheat.

9:25 Speaker-2 **Viktor Korzun**, EUREKA Project on Fusarium head blight - results and perspectives.

9:45 Speaker-3 **Guihua Bai,** Molecular mapping of QTLs for resistance to Fusarium head blight in Asian wheat.

10:05 Speaker-4 **Hongxiang Ma**, QTL for the resistance to Fusarium head blight and DON accumulation in Wangshiubai/Annong 8455 under field conditions.

10:25 Coffee break/ Photo shot of participants

11:00 Speaker-5 **Hermann Buerstmayr**, Genetic mapping of FHB resistance at IFA-Tulln, Austria.

11:20 Speaker-6 **JianRong Shi**, DNA marker analysis for FHB-resistance pyramiding from different genetic germplasms.

11:40 Speaker-7 **Chunji Liu**, Evidence that resistance to Fusarium head blight and crown rot are controlled by different mechanisms.

Pursuit New R-germplasm, Technique

12:00 Speaker-8 **George Fedak**, Fusarium head blight resistance from wide crosses in bread wheat and durum.

12:20 Speaker-9 **Masahiro Kishii**, Utilization of wild genetic resources for the improvement of FHB resistance in wheat breeding.

12:40 Lunch break (Cafeteria)

14:30 Speaker-10 **Daryl Somers**, Mapping FHB resistance in wheat: an update from AAFC-Cereal Research Centre (Winnipeg).

14:50 ***Discussion***

Moderators: James Anderson, Hermann Buerstmayr and Tomohiro Ban

Topic-1: Merge global status on FHB-Resistance germplasm, and construct update/database system.

Topic-2: Tracking FHB-QTLs effects/location with DNA marker information, and construct update/database system.

Topic-3: FHB-QTL comparative study for the deployment of resistance genes, and construct FHB-QTL Consensus Map.

Topic-4: Collaborative operation on exchanging materials (mapping population) for multiple-environmental FHB evaluation for G x E effect to confer the QTLs performance.

Topic-5: Share information and protocols for effective MAS.

16:20 ***Wrap up***

Moderators: James Anderson, Hermann Buerstmayr and Tomohiro Ban

Outcomes and action plans

17:50 End of the session

19:00 **Workshop Reception in the "Rincon"**

March 15th Wednesday

Session-2: Fusarium consortium

8:30 Session-2 orientation talk: Paul Nicholson

Fusarium genetic diversity/ pathogenicity/ toxigenicity

Chair: Robert Bowden

8:40 Speaker-1 **Thomas Miedaner**, Global biodiversity in *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum* and implications for resistance breeding.

9:00 Speaker-2 **Silvia Pereyra**, Diversity of fungal populations associated with Fusarium head blight in Uruguay.

9:20 Speaker-3 **Sukumar Chakraborty**, *Fusarium* pathogens of wheat in Australia.

9:40 Speaker-4 **Julie Nicol**, Turkish isolates of Fusarium crown and head blight from wheat and preliminary data on their pathogencity on crown tissues.

10:00 **Coffee break**

Chair: Thomas Miedaner

10:30 Speaker-5 **Dilantha Fernando**, Genetic diversity of *Gibberella zeae* isolates from Manitoba.

10:50 Speaker-6 **Akos Mesterhazy**, Present status of the *Fusarium graminearum* clade in Europe and possible development strategies.

11:10 Speaker-7 **Robert Bowden**, Cross fertility of *Gibberella zeae*.

Fusarium chemotype, epidemiology and control

11:30 Speaker-8 **Paul Nicholson**, *Fusarium* chemotypes in the UK and chemotype-host interactions.

11:50 Speaker-9 **Jeannie Gilbert**, Relative pathogenicity of 3-ADON and 15-ADON isolates of *Fusarium graminearum* from the prairie provinces of Canada.

12:10 Speaker-10 **Allen Xue**, Comparison of inoculum sources on development of Fusarium head blight and deoxynivalenol content in wheat in a disease nursery .

12:30 Speaker-11 **Hermann Buerstmayr**, Development of new tools do dissect fungal virulence and plant resistance components in a project funded by the Austrian genome programme GEN-AU.

12:50 Speaker-12 **Ruth Dill-Macky**, Implications of population variability on the management of Fusarium head blight.

13:10 **Lunch break (Cafeteria)**

15:00 ***Discussion***

Moderators: Paul Nicholson and Julie Nicol

Topic-1: Relationship among *Fusarium* genetic diversity/ pathogenicity/ toxigenicity.

Topic-2: Global compilation of *Fusarium* status, and collaboration strategy/structure.

Topic-3: Development of Global *Fusarium* Monitoring System.

16:30 ***Wrap up***

Moderators: Paul Nicholson and Julie Nicol

Outcomes and action plans

18:00 End of the session

19:00 **Cafeteria open for dinner**

March 16th Thursday

Session-3: International scab nursery consortium

8:30 Session-3 orientation talk: Tom Payne

Present status and future prospects of FHB breeding progress in the world

Chair: Viktor Korzun

8:40 Speaker-1 **Hilda Buck,** Fusarium head blight in Argentina: a local company approach to breeding for scab tolerance.

9:00 Speaker-2 **Mariana Ittu**, Current status of FHB research in Romanian bread wheat breeding program.

9:20 Speaker-3 **Radhey Pandeya**, Advancement in FHB resistant winter wheat cultivar development utilizing Frontana as the resistance donor parent.

9:40 Speaker-4 **Yasuhiro Yoshimura**, Progress in improving Fusarium head blight resistant wheat in Hokkaido, Japan.

10:00 Speaker-5 **Mohamed Rabeh Hajlaoui**, FHB in Tunisia: An emerging wheat disease.

10:20 Speaker-6 **Akos Mesterhazy**, Sources of "environmental interactions" in phenotyping DH and other populations in QTL research and resistance evaluation; ways to neutralize them.

10:40 **Coffee break**

Possible strategies for global screening of Fusarium resistance germplasm

Chair: Dave Van Sanford

11:10 Speaker-7 **Jeannie Gilbert**, Strategies and Considerations for multilocation FHB screening nurseries.

11:30 Speaker-8 **Martha Diaz de Ackermann**, Germplasm exchange in South America.

11:50 Speaker-9 **Ruth Dill-Macky**, Considerations in developing screening nurseries for FHB resistance in wheat and barley.

12:10 Speaker-10 **Julie Nicol**, CIMMYT Mexico and TURKEY/CIMMYTs International shuttle breeding program to develop wheat lines with Fusarium crown rot and other soil borne pathogen resistances.

12:30 **Lunch break (Cafeteria)**

14:20 ***Discussion***

Moderators: Rick Ward and Thomas Payne

Topic-1: Global compilation FHB/FCR epidemics with mycotoxin awarness, management system for understanding Genotype x Environment x Management effects, and update present status.

Topic-2: Standardization of evaluation/scoring methods, data management, and construct/assemble international standard/reference sets for FHB evaluation and comparative studies.

Topic-3: Development of a new International Interactive Scab Resistance Screening Nursery for germplasm enhancement & exchange.

Topic-4: Knowledge sharing and dissemination for global community.

15:50 ***Wrap up***

Moderators: Rick Ward and Thomas Payne

Outcomes and action plan

17:20 End of the session

18:30 **Buses depart for dinner at Dr. Iwanaga's Home (Front of the Cafeteria)**

18:45 - 21:00 **Mexican dinner**

March 17th Friday

Session-4: Plans for global collaboration on Fusarium research

8:30 Session-4 orientation talk: Rick Ward

Review and Discussion of Outcomes and Action plans

Moderators: Rick Ward and Tomohiro Ban

8:40 Session-1: **James Anderson**

9:10 Session-2: **Paul Nicholson**

9:40 Session-3: **Thomas Payne**

10:10 **Coffee break**

10:30 ***Structure and operation of a Global Fusarium Initiative***

Moderators: Rick Ward and Tomohiro Ban

12:00 ***Closing session***

Closing remarks: **John Dodds**, Deputy Director General, Research, CIMMYT

12:15 End of Workshop

14:00 **Optional pyramid tour (those who are interested)**

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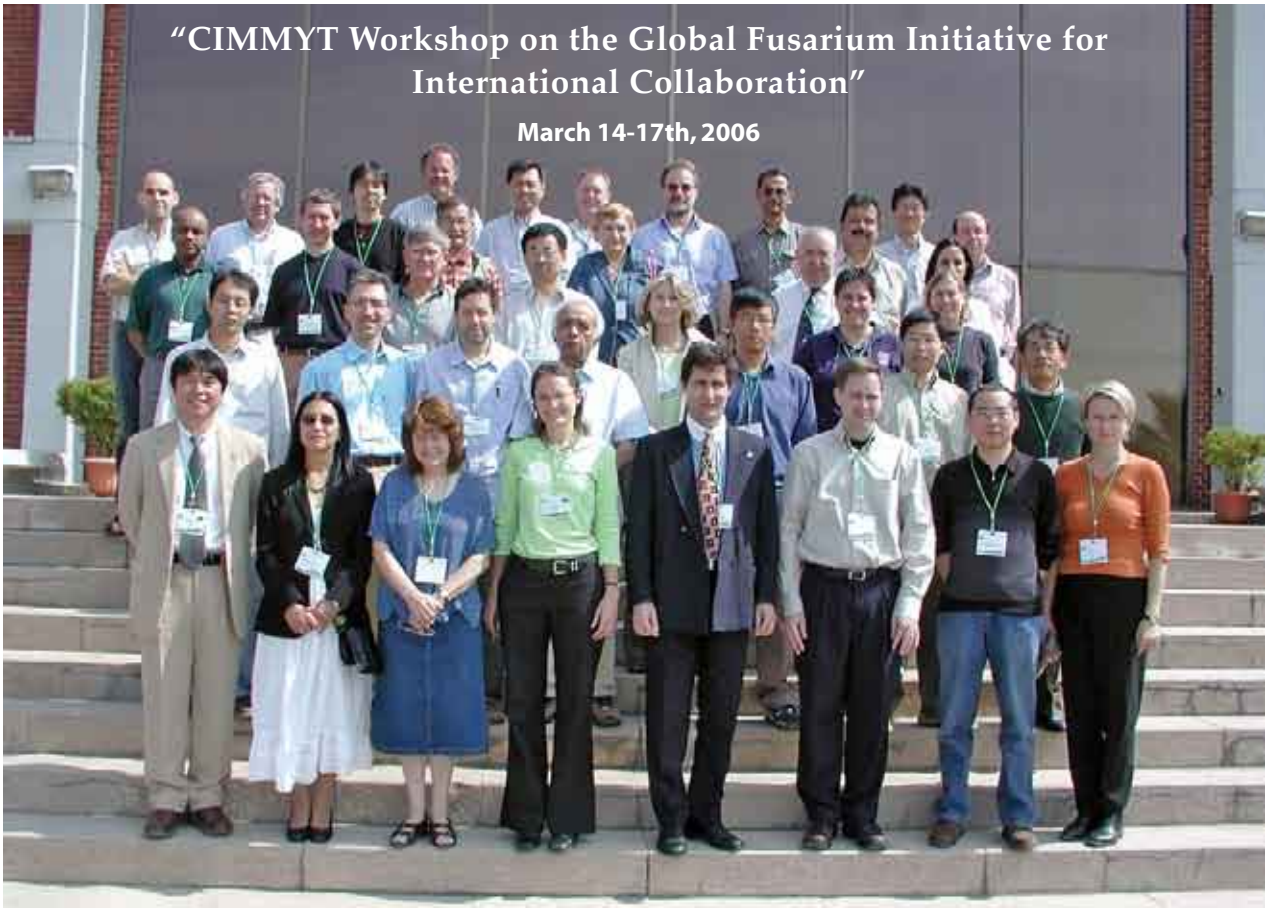
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