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CURRENT APPROACHES AND UTILIZATION OF NEW SCREENING TECHNIQUES FOR EVALUATION OF FHB RESISTANCE AT CIMMYT

ABSTRACT

Fusarium head blight (FHB) is a major disease of wheat in most wheat growing areas of the world. Resistance to FHB is a key trait for CIMMYT and many wheat breeding programs worldwide. New plant phenotyping techniques such as quantification of fungal biomass using real-time PCR have become available recently. CIMMYT's approach is to test new techniques for their feasibility and to apply them in routine disease screening programs if they prove to be valuable.

Two sets of spring wheat genotypes assembled on the basis of low (group 1) and high (group 2) FHB index observed in previous years, were phenotyped and genotyped in CIMMYT's FHB screening program. Phenotyping consisted of visual disease scoring (FHB index), mycotoxin analysis (DON) and quantification of fungal biomass. Apart from the FHB index, the two groups differed slightly in terms of DON accumulation although no differences were observed for average fungal biomass. This observation combined with the lack of correlation between disease symptoms and amount of fungal biomass suggested that some useful information may not be considered to discriminate resistant from susceptible genotypes when field selection is solely based on visual scoring results. DON/biomass-ratio was assessed for all genotypes and was found to be higher in the more resistant group 1 contrary to expectiations. An increase in DON production resulting from a stress or from resistance is discussed as a possible hypothesis. The determination of fungal biomass proved to be potentially valuable as a phenotyping tool. Genotyping results also showed that new genotypes harboring moderate levels of resistance and differing from traditional sources of scab resistance become available.

Key words: DON content, FHB index, fungal biomass, screening techniques, wheat

INTRODUCTION

Fusarium head blight (FHB) is a destructive disease of wheat and remains a major threat to wheat production in hot spot locations worldwide such as of

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the Southern Cone of Latin America, China, Europe and the borders of the Caspian Sea in Iran. Besides direct yield losses, the production of mycotoxins, in particular DON, and their accumulation in grain is of highest concern for food safety and trade (Binder *et al.*, 2007; Pestka, 2007). The recent introduction by the EU of new regulations limiting the level of DON in different grain products places additional pressure to identify solutions to reduce the amount of DON in the food chain in less developed countries and implement better screening techniques that limit scab and DON contamination in wheat improvement programs. After the reemergence of the disease in the mid 90's, considerable effort has been devoted to resistance breeding for FHB resulting in significantly higher levels of resistance (Ruckenbauer *et al.* 2001; Miedaner, 2006). However, immune genotypes are not available and thus breeding for high yielding, broadly adapted germplasm with improved resistance to FHB is still a challenge.

Wheat resistance to FHB is quantitative in nature. Although molecular markers are available for a number of resistance QTLs (Bürstmayr *et al.*, 2009), reliable phenotyping is still the most effective approach to identify resistant germplasm, not least because significant genotype-by-environment (GxE) interactions can be observed (Miedaner *et al.*, 2001). The most common phenotypic screening technique is visual scoring of FHB symptoms. Visual scoring can be done in the field or under more controlled conditions in the greenhouse. Plants are normally inoculated to simulate high disease pressure and provoke an infection. Usually disease incidence (count or estimate of percentage of spikes exhibiting symptoms), disease severity (count or estimate of percentage of spikelets exhibiting symptoms) or FHB index (product of incidence and severity) are determined. Also postharvest examinations such as assessment of visually scabby kernels or kernel weight are used for scoring (Dill-Macky, 2003). Several types of resistance are described for FHB based on the symptom development (Schroeder and Christensen, 1963; Mesterhazy, 2003). Type-I and type-II resistance (resistance against initial infection and against spread of the fungus within the plant, respectively) are nowadays broadly accepted. Depending on the inoculation technique, plants can be screened for different resistance types. If single florets are inoculated the type-II resistance of a genotype can be determined by assessing the disease severity for every spike (number of infected spikelets per total number of spikelets). By spray inoculation and subsequent assessment of disease incidence and/or severity the so called 'field resistance' is determined, which is a combination of type-I and type-II resistance. Recently Gosman *et al.* (2009) developed an assay to test for type-I resistance by inoculating plants with non-DON-producing fungi such as *Microdochium* spp. or Nivalenol (NIV) producing isolates of *F. graminearum*.

Since the accumulation of mycotoxins, especially DON, is of major concern the analysis of DON contamination is considered an important component of phenotyping (Ruckenbauer *et al.*, 2001; Dill-Macky, 2003). However, mycotoxin analysis is costly and labor intensive and thus rarely used as standard

procedure but rather in studies with defined objectives. In order to determine DON accumulation with a high level of accuracy, GC or LC methods can be used. These techniques are highly sensitive but require expensive equipment and experienced users. Hence, in breeding programs, analysis methods based on the ELISA technique serve as a fast and reliable alternative.

Yet, both visual scoring and DON analysis are only indirect methods to determine the resistance of a genotype (Brunner *et al.*, 2009). A more direct approach is the quantification of fungal mycelium *in planta*. One technique to determine the amount of fungal biomass is to quantify the ergosterol content of the infected plant parts (Reid *et al.*, 1999; Dorofeev *et al.*, 2002). However, this method is not practical and time consuming. DNA based assays for quantification of *Fusarium* biomass such as competitive PCR (Nicholson *et al.*, 1998) and quantitative real-time PCR (Waalwijk *et al.*, 2004) provide an alternative and are widely used in research.

Although phenotyping remains the most important approach to determine resistance to FHB, molecular marker techniques can be extremely helpful for screening parental genotypes in order to maintain diversity in terms of sources of resistance. These tools become increasingly useful as new QTL for FHB resistance are identified. Recently, Bürstmayr *et al.* (2009) reviewed 52 studies reporting QTL for FHB resistance.

Due to the reemergence of Fusarium Head Blight (FHB), a research program for FHB resistance has been operational at CIMMYT, Mexico since the mid 1980's. Initially focused on routine screening of conventional and distantly related *Triticeae* germplasm (Mezzalama *et al.* 2008), the program currently incorporates a highly standardized screening system with integrated research. Today, about 4000 genotypes from the different breeding groups of CIMMYT are evaluated each year for FHB resistance in CIMMYT's *Fusarium* screening program. Another 2000 genotypes are tested in the FHB research program for identification of new sources of resistance, studies on the nature of resistance as well as development and improvement of new screening tools and techniques. All materials are visually screened for disease symptoms in the field. After preliminary screening materials with high levels of resistance in the first year are tested again in replicated trials. Promising genotypes in replicated trials (2nd year screening) are analyzed for DON accumulation. If necessary a test for type II resistance can be conducted in the greenhouse. In 2008 and 2009 a collaborative study was conducted with USDA-ARS (Fargo, North Dakota, USA) in order to test the feasibility of genotyping lines for the presence or absence of QTL for FHB resistance. In future, CIMMYT plans to use molecular markers for scab resistance as a standard step to determine which genotypes should be part of future Scab Resistance Screening Nurseries.

Since 2005 real-time PCR has been used as a tool to quantify fungal biomass at CIMMYT (Murakami *et al.* 2008). The aim of this study is to evaluate the possibility of implementing this technique in a breeding program.

Table 1

FHB index, DON content, amount of fungal biomass and DON/Biomass-ratio in 2009 for the wheat lines from the two groups

Group	Genotype	Genotype Code	FHB index [%]	DON [ppm]	Fungal Biomass [pg mg^{-1}]	DON/ Biomass-ratio [pg ng^{-1}]
Group 1	WBLL1*2/TUKURU//KRONSTAD F2004	01	0.5	0.2	4269	0.26
	HEILO/3/SITE/MO//MILAN	02	0.9	0.4	15165	0.11
	QUAIU	03	0.9	1.1	7945	0.30
	PBW343*2/KUKUNA//PBW343*2/ KUKUNA/3/PBW343	04	1.8	1.7	3286	0.29
	CROC_1/AE.SQUARROSA (205)// KAUZ/3/SASIA/4/TROST	05	2.5	2.4	8392	0.45
	MIRIAM 33/KHVAKI/3/BABAX/LR42// BABAX	06	2.8	1.0	10854	0.32
	PARUS/PASTOR//INQALAB 91*2/ KUKUNA	07	2.9	3.8	2896	0.51
	H99326//RDWG/MILAN/3/VARIS	08	3.2	3.5	7289	0.68
	WBLL1*2/KURUKU//KRONSTAD F2004	09	3.6	1.5	6394	0.19
	PBW343/PASTOR//OTUS/TOBA97	10	3.7	4.9	25633	0.25
	SHA7//PRL/VEE#6/3/FASAN/4/ HAAS8446/2*FASAN/5/CBRD/KAUZ/6/ MILAN/AMSEL/7/FRET2*2/KUKUNA	11	3.9	1.2	67540	0.17
	CHIBIA//PRLII/CM65531/7/VEE#8//JUP/ BJY/3/F3.71/TRM/4/BCN/5/KAUZ/6/ MILAN/KAUZ	12	5.0	8.7	17267	0.51
Group 2	SAAR/2*WAXWING	13	10.1	9.8	26490	0.37
	CROC_1/AE.SQUARROSA (205)// BORL95/3/2*MILAN/4/PBW343*2/ KUKUNA	14	10.5	3.5	24013	0.14
	SNB//CMH79A.955/3*CNO79/3/ ATTILA/4/WUH1/VEE#5//CBRD	15	10.8	3.4	13080	0.26
	WEAVER//VEE/PJN/3/MILAN/4/BL 1496/ MILAN/3/CROC_1/ AE.SQUARROSA (205)//KAUZ	16	10.9	2.7	4547	0.59
	WHEAR/BERKUT//ROLF07	17	11.3	3.8	10121	0.38
	PFAU/MILAN//TROST/3/ PBW65/2*SERI.1B	18	11.5	3.0	9371	0.32
	SAAR//PBW343*2/KUKUNA/3/ PBW343*2/KUKUNA	19	12.5	4.2	8320	0.50
	WAXWING*2/KRONSTAD F2004	20	12.5	4.7	15866	0.30
	CAL/NH//H567.71/3/SERI/4/CAL/NH// H567.71/5/2*KAUZ/6/WH576/7/WH 542/8/ SUNSU	21	13.1	3.4	20908	0.16
	CROC_1/AE.SQUARROSA (205)// KAUZ/3/ATTILA/4/PFAU/MILAN	22	13.5	7.3	16220	0.45
	CNDO/R143//ENTE/MEXI_2/3/ AEGILOPS SQUARROSA (TAUS)/4/ WEAVER/5/2*KAUZ/6/TROST	23	13.5	5.6	18851	0.30
	PFAU/SERI.1B// AMAD/3/2*HUW234+LR34/PRINIA	24	14.6	4.9	11993	0.41
	GOROKE//HD29/2*WEAVER/3/ INQALAB 91*2/TUKURU	25	14.7	3.4	16585	0.21
	WHEAR/KIRITATI/3/ C80.1/3*BATAVIA//2*WBLL1	26	15.5	3.5	10024	0.35
Checks	SUMAI #3	Sumai #3	0.2	0.2	645	0.24
	GONDO/CBRD	Gondo/Cbrd	0.5	0.4	1643	0.22
	HEILO	Heilo	4.4	2.7	12781	0.25
	OCORONI F 86	Ocoroni F86	28.7	2.8	17938	0.16

MATERIAL AND METHODS

Plant material and field trial

Twenty-six advanced spring wheat lines from CIMMYT's irrigated bread wheat program were used for the experiments (Table 1). Genotypes were selected based on visual FHB scoring results from 2009 after pre-selection as potentially resistant based on rapid preliminary field observations (resistant versus susceptible) in 2008. Two groups were formed: group 1 characterized by high to moderate resistance (n = 12; FHB index: 0% to 5%) and group 2 showing moderate resistance (group 2; n = 14; FHB index: 10% to 15 %). Four genotypes were used as checks in all experiments: Sumai #3, Gondo/CBRD (both resistant), Heilo (moderately resistant) and Ocoroni F86 (moderately susceptible).
All field experiments were carried out at CIMMYT's headquarters in El Batan close to Mexico City (2,240 masl). Plants were planted in 1m double-row plots. The field experiments were unreplicated in 2008 but replicated thrice in 2009 in an incomplete block design.

Genotyping of wheat lines

All wheat lines were previously genotyped for the presence or absence of ten FHB resistance QTLs in collaboration with USDA-ARS (Fargo, North Dakota, USA) The method and the markers are described in more detail by Chu *et al.* (2008).

Field visual disease scoring

In both 2008 and 2009 plants were inoculated artificially using precision CO_2 backpack sprayers equipped with flat fan nozzles at a defined pressure of 40 psi. The inoculum consisted of a mixture of 5 different *F. graminearum* strains collected during the preceding year in naturally infected fields. All *Fusarium* isolates were of the DON chemotype, confirmed by PCR. The inoculum concentration was adjusted to 50,000 conidia ml^{-1} and 39 ml of inoculum per meter was applied during flowering. Inoculation was repeated three days later. A mist irrigation system (15 min mist irrigation per hour) maintained a humid microclimate which favored infection and development of the fungus within the plant. Thirty days after the first inoculation, the plants were evaluated for disease resistance. In the first year of screening, the whole plots were rated as either susceptible or resistant. In the second year, fifteen flowering spikes per plot were marked prior to inoculation and the marked spikes were evaluated thirty days after inoculation for disease incidence and severity and the FHB index was calculated using the following formula:

$$FHB\ index = \frac{Incidence \times Severity}{100}$$

where incidence is the percentage of spikes exhibiting symptoms and severity is the percentage of spikelets exhibiting symptoms (Dill-Macky, 2003, Kolb and Boze, 2003).

DON analysis

Marked ears in each plot were harvested in bulk and spikes were threshed using a laboratory threshing machine. A tenth of the kernel yield (based on weight) was ground using a coffee grinder. Deoxynivalenol was extracted from two grams of wheat flour by adding 40 ml of distilled water and subsequent shaking for 10 min on a shaker at 400 min^{-1}. One milliliter of the liquid was transferred to a 2 ml reaction tube and centrifuged for 15 min at 14,000 min^{-1} in order to spin down the cell debris. Fifty microliters of the supernatant were used for deoxynivalenol analysis using a Ridascreen® Fast Don ELISA kit (R-Biopharm, Darmstadt, Germany).

Quantification of fungal biomass

For DNA extraction a CTAB method modified from Brunner *et al.* (2009) was used. One hundred fifty milligrams of wheat flour was weighed in to a 2 ml reaction tube. Two steel spheres (3.5 mm diameter) were put in the reaction tube and the sample was ground in a shaker mill at 1,600 min^{-1}. DNA was extracted by adding 750 µl of CTAB extraction buffer (1.4 M NaCl, 2% (w/v) CTAB, 0.1 M Tris-Base pH 8.0, 0.02 M EDTA pH 8, 1% (w/v) polyvinyl pirrolidone 40000) and incubation for 10 min at 65°C. The sample was centrifuged for 10 min at 7,200 x g and the supernatant was transferred to a new 2 ml tube. Sixty microliters of 10% CTAB solution (10% (w/v) CTAB in 0.7 M NaCl) was added followed by the extraction with an equal volume of chloroform:isoamyl alcohol (24:1). After centrifugation for 10 min at 7,200 x g, the upper aqueous phase was collected and transferred to a tube containing three volumes of precipitation buffer (1% (w/v) CTAB, 0.05 M Tris-Base pH 8.0, 0.01 M EDTA pH 8.0). The sample was incubated for 15 min at room temperature and then centrifuged for 15 min at 7,200 g. The formed DNA pellet was washed twice with 70% ethanol, dried for 20 min under a fume hood and resuspended in 100 µl TE-Buffer (0.01 M Tris-Base pH 8.0, 0.001 M EDTA pH 8.0).

DNA solution was diluted tenfold and 1 µl of DNA solution was used for quantification with real-time PCR using the primer pair Fg16NF/Fg16NR (Nicholson *et al.*, 1998). The reactions were carried out in 20 µl volume. The reaction mix consisted of 10 µl iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, USA), 0.4 nM of each forward and reverse primer, 9.5 µl PCR grade water and 1 µl template DNA. The PCR was performed on a Bio-Rad CFX 96 thermocycler with the following protocol: an initial denaturation step of 2 min at 95°C was followed by 35 cycles of 30 s at 95°C, 45 s at 63°C and 45 s at 72°C.

The ratio of DON produced per amount of fungal biomass was calculated using the following formula:

$$DON/biomass-ratio = \frac{Deoxynivalenol\ [ppb]}{Fungal\ Biomass\ [pg \times mg^{-1}]}$$

RESULTS

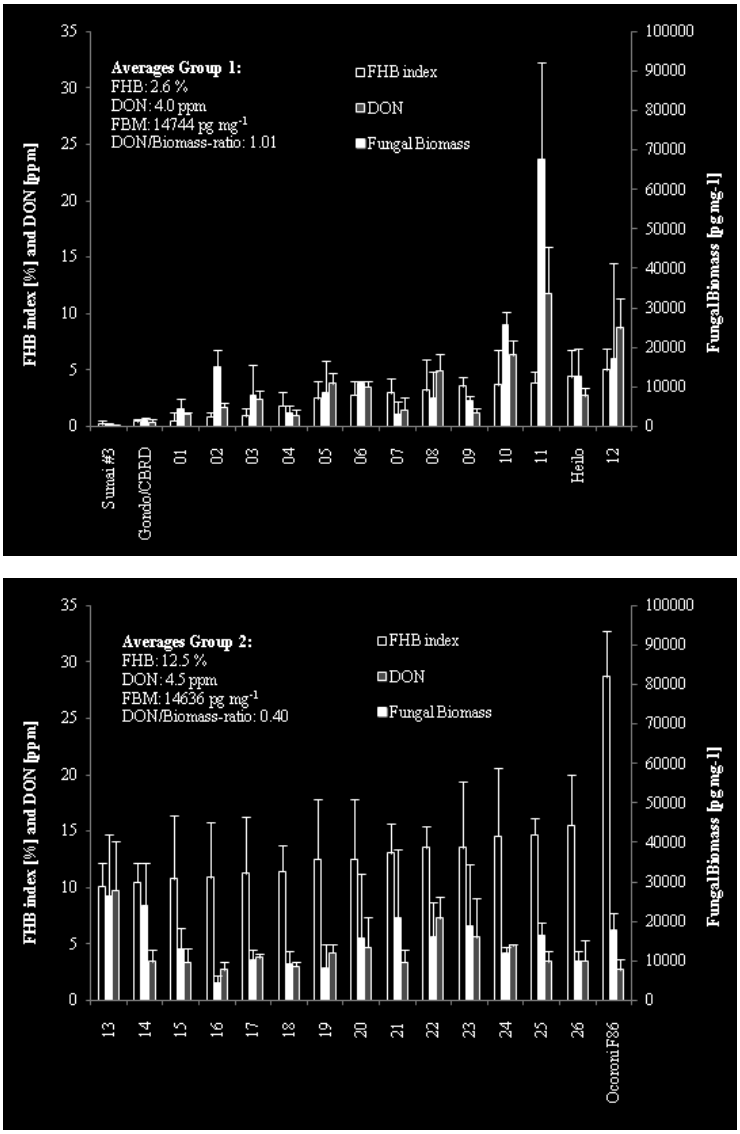

Fig.1. FHB index, DON content, amount of fungal biomass and DON/Biomass-ratio in 2009 for the wheat lines from the two groups; for genotype codes see Table 1

The resistant check Sumai #3 harbored the highest resistance based on all three disease parameters. In contrast, the moderately susceptible check, Ocoroni F86 showed the highest susceptibility in terms of FHB index but comparatively low DON contamination and amount of fungal biomass (Fig. 1). Gondo/CBRD had the second highest level

of resistance close to Sumai #3. Heilo ranked between group 1 and group 2 based on FHB index and also showed moderate resistance in terms of DON contamination and accumulation of fungal biomass. Apart from checks, genotype 01 possessed the highest overall resistance with FHB index, DON content and amount of fungal biomass close to the resistant checks Gondo/CBRD and Sumai #3.

The average FHB index of all genotypes of group 1 and group 2 was 7.9%. The average DON contamination of all genotypes was comparably high with 4.3 ppm. The amount of fungal biomass reached 14,686 pg × mg^{-1}. The two groups of genotypes, assembled on the basis of different FHB indices (average group 1: 2.6%; average group 2: 12.5%), also showed differences in terms of average DON contamination. With 4.0 ppm, the average DON content of group 1 was only slightly lower than that of group 2 with 4.5 ppm. However, no difference between the groups could be observed in terms of average fungal biomass of *F. graminearum* (14,744 pg × mg^{-1} and 14,636 pg × mg^{-1}, respectively).

With 11.8 ppm and 67,540 pg × mg^{-1} , genotype 11 of group 1 had the highest DON content and highest amount of fungal biomass of all genotypes by far. Surprisingly this genotype appeared to be moderately resistant to resistant according to the FHB index (3.9%). Similar results could be observed for genotypes 02, 10 and 12. These genotypes showed relatively high DON contamination and/or amounts of fungal biomass in association with rather low FHB indices.

With FHB indices between 10.1% and 15.5%, genotypes from group 2 varied around the average FHB index of 12.5%. Despite the rather high FHB indices, both DON contamination and amount of fungal biomass were relatively low compared to the higher levels of resistance in group 1. Highest DON content and highest amount of fungal biomass in group 2 was observed for genotype 13. Interestingly, this genotype had the lowest FHB index in this group.

The DON/biomass-ratio ranged between 0.11 pg × ng^{-1} (genotype 02) and 3.41 pg × ng^{-1} (genotype 12). All checks had ratios below one (Table 1). The average DON/biomass-ratio for group 1 was 1.01 pg × ng^{-1}. For group 2 the ratio was much lower and reached only 0.40 pg × ng^{-1}.

Taking into consideration all genotypes of the two groups, DON content and amount of fungal biomass were significantly positively correlated ($r = 0.78$). Correlations between FHB index and DON content ($r = 0.21$) and FHB index and fungal biomass ($r = 0.16$) were weak and not significant. For group 1 all correlations between the different parameters were much stronger than for group 2. FHB index and DON content as well as amount of fungal biomass and DON content were significantly positively correlated ($r = 0.56$ and $r = 0.86$, respectively) in group 1. Interestingly, in group 2 FHB index and DON content were negatively correlated ($r = -0.36$), but not statistically significant as no correlation in group 2 was.

All genotypes were tested for the presence or absence of ten FHB resistance QTL. Five of the genotypes of group 1 carried none of the ten QTLs (Table 2). In group 2 only one genotype carried none of the QTLs. Sumai #3 was the line carrying the highest number of QTLs (6). The QTL from chromosome 2D from Wuhan was the most

frequent QTL and was present in 67% of the genotypes. In contrast, the resistance QTL from chromosomes 3A, 4B and 7A from Frontana, Wuhan and *T.dicoccoides*, respectively, could not be detected in any of the genotypes. Besides Sumai #3, only genotype 16 had the Sumai #3 resistance QTL on chromosome 3BS (syn. Fhb1, Waldron *et al.*, 1999). This genotype showed only moderate resistance in terms of FHB index but DON content and amount of fungal biomass were comparably low. For most genotypes resistance was based on sources other than Sumai #3. This is notably valid for Gondo/CBRD which carried none of the ten resistance QTLs but was the genotype with the highest level of resistance after Sumai #3.

Table 2

Results of genotyping of the wheat lines from the two groups; markers used for genotyping are indicated in the table.

Group	Genotype	Genotype Code	Sumai #3			Frontana		Wuhan		CJ93 06	*T. diccocc.*	
			3B	5A	6B	3A	5A	2D	4B	2D	3A	7A
			1	2	3	4	5	6	7	8	9	10
Group 1	WBLL1*2/TUKURU//KRONSTAD F2004	01						X				
	HEILO/3/SITE/MO//MILAN	02										X
	QUAIU	03										
	PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/PBW343	04										
	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST	05						X				
	MIRIAM 33/KHVAKI/3/BABAX/LR42//BABAX	06						X				
	PARUS/PASTOR//INQALAB 91*2/KUKUNA	07						X				
	H99326//RDWG/MILAN/3/VARIS	08						X				
	WBLL1*2/KURUKU//KRONSTAD F2004	09										X
	PBW343/PASTOR//OTUS/TOBA97	10						X				
	SHA7//PRL/VEE#6/3/FASAN/4/HAAS8446/2*FASAN/5/CBRD/KAUZ/6/MILAN/AMSEL/7/FRET2*2/KUKUNA	11						X				
	CHIBIA//PRLII/CM65531/7/VEE#8//JUP/BJY/3/F3.71/TRM/4/BCN/5/KAUZ/6/MILAN/KAUZ	12						X				

1—umn10; 2—barc186, barc180; 3—gwm133, wmc179; 4—dupw227; 5—barc197, barc186; 6—wmc144, wmc245; 7—wmc238, gwm149; 8—gwm157, gwm539; 9—barc45; 10—barc121, wmc488

Table 2

Results of genotyping of the wheat lines from the two groups; markers used for genotyping are indicated in the table.

Group	Genotype	Genotype Code	Sumai #3			Frontana		Wuhan		CJ9306	*T. diccocc.*	
			3B	5A	6B	3A	5A	2D	4B	2D	3A	7A
			1	2	3	4	5	6	7	8	9	10
Group 2	SAAR/2*WAXWING	13										
	CROC_1/AE.SQUARROSA (205)//BORL95/3/2*MILAN/4/PBW343*2/KUKUNA	14						X				X
	SNB//CMH79A.955/3*CNO79/3/ATTILA/4/WUH1/VEE#5//CBRD	15										X
	WEAVER//VEE/PJN/3/MILAN/4/BL1496/MILAN/3/CROC_1/AE.SQUARROSA (205)//KAUZ	16	X					X				
	WHEAR/BERKUT//ROLF07	17						X				
	PFAU/MILAN//TROST/3/PBW65/2*SERI.1B	18						X				
	SAAR//PBW343*2/KUKUNA/3/PBW343*2/KUKUNA	19						X				
	WAXWING*2/KRONSTAD F2004	20										
	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/WH576/7/WH 542/8/SUNSU	21					X			X		
	CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA/4/PFAU/MILAN	22						X				
	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ/6/TROST	23						X				
	PFAU/SERI.1B//AMAD/3/2*HUW234+LR34/PRINIA	24						X				
	GOROKE//HD29/2*WEAVER/3/INQALAB 91*2/TUKURU	25						X				
	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLL1	26						X				X
Checks	SUMAI #3		X	X	X			X		X		X
	GONDO/CBRD											
	HEILO											
	OCORONI F 86							X				

1—umn10; 2—barc186, barc180; 3—gwm133, wmc179; 4—dupw227; 5—barc197, barc186; 6—wmc144, wmc245; 7—wmc238, gwm149; 8—gwm157, gwm539; 9—barc45; 10—barc121, wmc488

DISCUSSION

Resistant checks Sumai #3 and Gondo/CBRD showed the highest resistance for all assessed disease parameters. Sumai #3 is the reference point for FHB resistance. Nevertheless, promising genotypes such as 01 and 04 performed well and demonstrated that new material with improved resistance in terms of all three diseases parameters is becoming available.

The two groups of genotypes represent two categories of resistance based on FHB index. Both groups also differed slightly in terms of average DON contamination but the difference between the groups regarding this disease parameter was much lower than for the FHB index. Differences in resistance based on symptoms are not necessarily reflected by the DON amount. A genotype with a good resistance based on field visual scoring may show high or low DON contamination (Champeil et al, 2004a). This observation was even clearer for fungal biomass. No difference between the two groups in terms of average amount of fungal biomass could be observed. During the selection process towards FHB resistance, genotypes are usually only selected for low symptoms (field scoring). Since mycotoxin analysis is cost and labor-intensive, normally only advanced materials are tested for resistance against DON accumulation. This may lead to a clear rank in terms of visual symptom formation with a tendency to low DON. The estimation of fungal biomass as a parameter of resistance is a relatively new approach and it is not yet used regularly in breeding programs to select materials. High variation in resistance against accumulation of fungal biomass and a remarkable discrepancy between this resistance parameter and visual symptoms can be observed. For instance, in group 1 some genotypes with high levels of resistance in terms of FHB symptoms also showed good resistance against accumulation of fungal biomass (cf. Sumai #3, Gondo/CBRD, genotypes 01 and 04). On the other hand several genotypes showed good to moderate resistance on the basis of disease symptoms but had much higher levels of fungal biomass (cf. genotypes 02, 10 and 12). Furthermore, extremes can occur such as genotype 11 where fungal biomass and DON content were highly disproportionate to disease symptoms. The discrepancy between these two disease parameters may either mean that fungal biomass is not a reliable parameter for resistance screening or that fungal biomass should be taken into consideration for selections in order to achieve genotypes which carry corresponding resistance genes.

Despite the higher FHB indices for the genotypes of group 2 the resistance against accumulation of fungal biomass and DON of some genotypes was comparable to good genotypes of group 1 (cf. genotypes 15, 16, 17, 18, 19 and 26). This again indicates that a selection solely based on disease symptoms may be misleading and result in a loss of genes responsible for resistance against accumulation of fungal biomass and mycotoxins. In this regard Ocoroni F86 can serve as a negative example. Despite relatively good resistance against accumu-

lation of fungal biomass and DON, this genotype shows a high FHB index and probably would have been discarded in a selection solely based on symptoms. Certainly high resistance in terms of disease symptoms is still important but only in combination with the other resistance types.

The DON/biomass-ratio showed a broad range of variation with values from 0.11 pg × ng^{-1} (genotype 02) to 3.41 pg × ng^{-1} (genotype 12). It is known that strains of *F. graminearum* can differ greatly in their aggressiveness and their ability to produce DON (Cumagun & Miedaner, 2004). Indeed, an infection with a low aggressive strain may generally explain a lower DON/biomass-ratio but cannot explain this observation in this particular study, because the plants were inoculated artificially with highly aggressive high DON producing strains.

The plant can have an influence on DON contamination. Some specific types of resistance allow the plant to detoxify mycotoxins. An example is the FHB resistance QTL on chromosome 3BS (Fhb 1) which co-localizes with the ability to detoxify DON (Lemmens *et al.*, 2005). This resistance mechanism results in lower DON content than expected from the amount of fungal biomass and thus could explain variation in DON/biomass-ratio between genotypes (Champeil *et al.*, 2004b).

Interestingly the DON/biomass-ratio was lower for group 2 than for group 1. In other words, counter-intuitively the amount of mycotoxin per amount fungal biomass was higher in the plants with a higher resistance in terms of FHB index. One explanation could be that the plants of group 2 have higher resistance in terms of detoxification or degradation of mycotoxins (Champeil *et al.*, 2004b). Taking into consideration the higher levels of resistance of group 1 in terms of FHB symptoms and accumulation of DON this explanation seems to be improbable. High levels of resistance most propbably result from a combination of good type-I and type-II resistance (Schroeder & Christensen, 1963; Champeil *et al.*, 2004b). Additionally most of the known resistance QTLs are associated with impeding the spread of the fungus within the rachis (type-II resistance; Bürstmayr *et al.*, 2009). Hence, relatively high levels of type-II resistance are likely to be found in the genotypes of group 1. DON is a pathogenicity factor for the fungus and is essential for the ability to spread within the rachis (Bai *et al.*, 2002). Wheat genotypes with high levels of type-II resistance effectively block or decrease the speed of development of the fungus within the plant (Schroeder & Christensen, 1963; Mesterhazy, 2003). It may be hypothesized that high levels of type-II resistance induce a ‘stress’ on the fungus to which it may respond with an increase in mycotoxin production, resulting in a higher DON/biomass-ratio. However, this hypothesis has to be tested in a more detailed study.

As described by others, the correlation between fungal biomass and DON content is normally stronger than between FHB index (or other visual scoring results) and either of the other disease parameters (Scheider *et al.*, 2009). This confirms that a selection based on low FHB indices doesn’t necessarily lead to

low amounts of fungal biomass or DON content. Since the fungus needs DON for development and spread within the rachis, the fungal biomass is much more closely related to the actual mycotoxin production than the disease symptoms. Thus, the reduction of fungal biomass accumulation may be of higher importance for the reduction of mycotoxin contamination than a reduction of symptoms (FHB index) is.

The much stronger correlations in group 1 in comparison to group 2 may be explained by the extreme values of genotype 11 which together with the low values of Sumai #3 marked the end points of the range for each of the parameters.

Only one genotype (No.16) carried the resistance QTL from chromosome 3BS of Sumai #3 (Fhb 1). Irrespective of the rather high FHB index of this genotype the relatively low levels of fungal biomass and DON content indicate the main effect of Fhb 1: the resistance against spread of the fungus and the co-localized ability to degrade DON (Waldron *et al.*, 1999; Lemmens *et al.*, 2005). Besides the strong effect of Fhb 1 Sumai #3 most probably carries QTL which offer a good type-I resistance. The combination of resistance of type-I and type-II lead to this very resistant phenotype. On the other hand the good performance of Gondo/CBRD demonstrates that very high levels of resistance are achievable without the main QTL from Sumai #3.

CONCLUSIONS

Resistance to FHB remains a challenge for wheat breeding programs. New methods involving the use of molecular markers as a decision support tool have emerged in the last years. However, reliable field screening is still the most important approach for identifying germplasm with high levels of resistance. Besides visual scoring of diseases symptoms and analysis of DON contamination, the quantification of fungal biomass is of increasing importance. Developments in molecular methods in the last decade eased the process of quantification of fungal biomass. In this study the implementation of quantitative real-time PCR as a tool to determine components of resistance to FHB has been tested. It has been shown that the assessment of fungal biomass can give additional information about the resistance of a genotype to FHB. However, in further studies it needs to be clarified if this disease parameter should be taken into consideration for selecting genotypes.

Differences in the DON/biomass-ratio between the genotypes may suggest that some types of resistance (e.g. type-II) induce an increase of DON synthesis by the fungus. If this proves true, countermeasures such as the introduction of other types of resistance have to be taken. The ability of some genotypes to detoxify DON would be a conceivable solution.

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REFERENCES

Bai, G.H., Desjardins, A.E. and Plattner, R.D. (2004): Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia 153:91-98.

Binder, E.M., Tan, L.M., Chin, L.J., Handl, J. and Richard, J. (2007) Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Animal Feed Science and Technology 137:265–282.

Brunner, K., Kovalsky Paris, M.P., Paolino, G., Bürstmayr, H., Lemmens, M., Berthiller, F., Schuhmacher, R., Krska, R. and Mach, L.M. (2009): A reference-gen-based quantitative PCR method as a tool to determine *Fusarium* resistance in wheat. Anal Bioanal Chem 395:1385-1394.

Bürstmayr, H., Ban, T. and Anderson, J.A. (2009): QTL mapping and marker-assisted selection for Fusarium head blight resisatnce in wheat: a review. Plant Bredding 128:1-26.

Champeil, A. Fourbet, J.F., Dore, T. (2004a): Effects of grain sampling procedures on Fusarium mycotoxin assays in wheat grain. J Agric Food Chem 52:6049-6054.

Champeil, A., Dore, T. and Fourbet, J.F. (2004b): Fusarium haed blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. Plant Sci 166:1389-1415.

Chu, C., Chao, S., Cai, X., Zhong, S. and Xu, S. (2008): Haplotype analysis of genes for Fusarium head blight resistance in tetraploid wheat germplasm. Proc. of the 2008 National FHB forum, Indianapolis, Indiana, USA, 2-4 December.

Cumagun, C.J.R. and Miedaner, T. (2004): Segregation for aggressiveness and deoxynivalenol production of a population of *Gibberella zeae* causing head blight of wheat. Europ J Plant Pathol 110:789-799.

Dill-Macky, R. (2003): Inoculation methods and evaluation of Fusarium head blight resistance in wheat. In: Leonard, K.J. and Bushnell, W.R. (eds.): Fusarium head blight of wheat and barley. 184-210.

Dorofeev, D.A.; Artemenko, E.N. and Devyatkina, G.A. (2002): Optimizing the method of determining *Fusarium spp.* biomass based on ergosterol content. Russ. Agric. Sci. 1:10-13.

Gosman, N., Srinivasachary, S., Steed, A., Chandler, E., Thomsett, M. and Nicholson, P. (2009): Evaluation of type I fusarium head blight resistance of wheat using non-deoxynivalenol-producing fungi. Plant Pathology 59:147-157.

Kolb, F.L. and Boze, L.K. (2003): An alternative to the FHB index: incidence, severity and kernel rating (ISK) index. Proc. of the 2003 National FHB forum, Bloomington, Minnesota, USA, 13-15 December.

Lemmens, M., Scholz, U., Berthiller, F. Dall'Asta, C., Koutnik, A., Schuhmacher, R., Adam, G., Bürstmayr, H., Mesterházy, A., Krska, R. and Ruckenbauer, P. (2005): The ability to detoxify the mycotoxin doxynivalenol colocalizes with a major quantitative trait locus for Fusarium head blight resistance in wheat. Mol Plant Microbe Int 18:1318-1324.

Mesterházy, A. (2003): Breeding wheat for Fusarium head blight resistance in Europe. In: Leonard, K.J. and Bushnell, W.R. (eds.): Fusarium head blight of wheat and barley. 211-240.

Mezzalama, M., Duveiller, E., Murakami, J. and Dreisigacker, S. (2008): Fusarium head blight program at CIMMYT: An integrated approach to defeat the disease. Cereal Research Communications 36 (Supplement 6):143-145.

Miedaner, T. (2006): Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breeding 116:201-220.

Miedaner, T., Reinbrecht, C., Laubner, U., Schollenberger, M. and Geiger, H.H. (2001): Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to Fusarium head blight in rye, triticale and wheat. Plant Breeding 120:97-105.

Murakami, J., Lewis, J., Egusa, M., Gargouri, S., Kodama, M., Duveiller, E. and Ban, T. (2008): Evaluation of a real-time quantitative PCR assay for an effective screening of genotypes with FHB resistance and low mycotoxin accumulation in wheat and barley. Cereal Research Communications 36(Supplement 6):147-148.

Nicholson, P., Sampson, D.R., Weston, G., Rezanoor, H.N., Lees, A.K., Parry, D.W. and Joyce, D. (1998): Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol Mol Plant Pathol 53:17-37.

Pestka, J.J. (2007): Deoxynivalenol: Toxicity, mechanisms and animal health risks. Animal Feed Science and Technology 137:283–298.

Reid, L.M., Nicol, R.W., Ouellet, T., Savard, M., Miller, J.D. and Young, J.C.; Stewart, D.W.; Schaafsma, A.W. (1999): Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: disease progress, fungal biomass, and mycotoxin accumulation. Phytopathology 89: 1028-1037.

Ruckenbauer, P., Bürstmayr, H. and Lemmens, M. (2001): Present strategies in resistance breeding against scab (*Fusarium* spp.). Euphytica 119:121-127.

Scheider, N., Guo, J.-R., Verreet, J.-A., Beyer, M. (2009): Assessing the intensity of Fusarium-damage in wheat: A comparison of selected disease parameters during diseases development and the role of fungicides. J Plant Dis Prot 116:118-123.

Schroeder, H.W. and Christensen, J.J. (1963): Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:831-838.

Waalwijk, C., van der Heide, R., de Vries, I., van der Lee, T., Schoen, C., Costrel-de Corainville, G., Häuser-Hahn, I., Kastelein, P., Köhl, J., Lonnet, P., Demarquet, T., Kema, G.H.J. (2004): Quantitative detection of *Fusarium* species in wheat using TaqMan. Europ J Plant Pathol 110:481-494.

Waldron, B.L., Moreno-Sevilla, B., Anderson, J.A., Stack, R.W. and Frohberg, R.C. (1999): RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Sci 39:805–811.