FULL RESEARCH PAPER

Characterisation of kernel resistance against *Fusarium* infection in spring wheat by baking quality and mycotoxin assessments

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Abstract Fusarium head blight (FHB) of wheat heads by Fusarium culmorum causes serious yield losses and compromises the end-use quality by accumulation of mycotoxins and alteration of baking characteristics. The most promising control strategies against the disease combine adequate cropping techniques (i.e. crop rotation avoiding maize as a preceding crop) with the use of resistant varieties. Different types of resistance against this disease have been described such as the resistance to primary infection of the spikelets and the reduction of spread of the infection in other parts of the ear. In recent years, the ability of the kernels to prevent penetration of the fungus and mycotoxin accumulation has received increasing attention. Yet, the detection of kernel resistance for breeding purposes is rather difficult, as the corresponding resistance mechanisms are not fully understood. The aim of the present work is to compare different aspects of kernel resistance in order to define the most significant criteria for breeding purposes. The experimental set up included eight modern Swiss spring wheat varieties grown on small irrigated yield plots (3×1.5 m) inoculated at anthesis with a mixture of Fusarium culmorum isolates. Disease ratings from 7 to 28 days post-inoculation were completed with post-harvest analyses for the

accumulation of the mycotoxin deoxynivalenol and different baking quality parameters. Results indicate that the accumulation of the mycotoxin deoxynivalenol in the kernels is correlated with visible symptoms on the ear before harvest. In terms of baking quality parameters, water absorption, dough softening and dough resistance are impaired in susceptible varieties after FHB infection, while resistant varieties are not affected. The results obtained here indicate that kernel resistance can be defined by low deoxynivalenol accumulation in the kernels and by stability of several baking quality parameters under conditions of high FHB infection pressure.

Keywords Bread · Deoxynivalenol · Flour

Abbreviations

AUDPC area under the disease progress curve

DON deoxynivalenol

FHB Fusarium head blight

Introduction

Wheat scab has become a problem for farmers in all wheat-producing areas of the world and constitutes a risk for the whole food chain (Bottalico and Perrone 2002; McMullen et al. 1997). The disease is caused by fungal pathogens belonging to different species of the genus *Fusarium*. The pathogens infect the ear

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during anthesis. After penetration into the floret, the fungus spreads progressively into the xylem vessels and the phloem tubes and can result in the complete necrosis of the spike (Kang and Buchenauer 1999). Besides directly affecting the yield of the crop, the pathogen contaminates the kernels with different mycotoxins. The ingestion of wheat contaminated with fusariotoxins can seriously affect human and animal health (Cahanier 2001). The predominant mycotoxin produced by both major pathogens, Fusarium graminearum and F. culmorum, is deoxynivalenol (DON; Logrieco et al. 2003). The presence of DON is considered an indicator of mycotoxin contamination in cereal batches. The control strategies against this disease aim at avoiding infection through adequate cultural techniques (i.e. crop rotation avoiding maize as a preceding crop) and the use of resistant varieties that reduce the risk of infection and limit the consequences once the infection occurs (Mascher et al. 2005). There are different resistance types such as type I resistance, able to limit the initial infection of the floret, or type II resistance that limits the spread of the infection into the rest of the spike (Schroeder and Christensen 1963). In recent years, kernel resistance has received increasing attention by breeders. Kernel resistance comprises the mechanisms that prevent the penetration of the fungus into the kernels and impair the ability of the fungus to produce or accumulate mycotoxins (Mesterhazy 1995).

The presence of scabby, misshapen kernels, so-called tombstones, is an important indicator to visually discriminate contaminated from non-contaminated batches after natural infection and artificial inoculation (Jones and Mirocha 1999; our own unpublished results). Such deformed kernels indicate that the fungus affects the filling of the grain. Moreover, several constituents of the grain such as starch, cellulose, hemicellulose, and proteins are modified (Boyacioğlu and Hettiarachchy 1995) explaining the degradation of bread-making quality in fusarium-contaminated wheat (Bockmann 1964).

Little information is available about kernel resistance as part of the varietal resistance in wheat. The present work examines kernel resistance as a function of (a) presence of symptoms on the spike, (b) accumulation of the mycotoxin DON and (c) stability of dough and baking quality. The different analyses are compared and evaluated as selection tools in breeding for kernel-resistant cultivars.



Plant material and seeding conditions

The wheat varieties used in this study are described in Table 1. The varieties were planted in plots of 1.5×3 m and a density of 350 kernels m⁻² using a Hege Seedmatic seeding machine (Hege Maschinen, Eging am See, Germany). The soil was a clay alluvisol. Cultural practices included one herbicide treatment before emergence, fertilization at 120 kg N ha⁻¹ and no straw shortener. The experiment was designed as a split plot with 5 replications per treatment (i.e. inoculation and controls). Each sub-plot was surrounded by a 3 m large spring triticale buffer (variety Trado, DSP/Agroscope Changins-Wädenswil ACW).

Inoculation and measurement of disease incidence

The inoculum consisted of a mixture of $20 \, F.$ culmorum isolates from Switzerland grown for 15 days on oat kernels, dried and ground as described by Saur and Benacef (1993). Each plot was inoculated separately at the beginning of flowering of the variety. The inoculation was repeated two times at 2-day intervals with 5×10^{-6} spores m⁻² of F. culmorum. After each inoculation, the plots were mist irrigated from overhead for 24 h with ~ 2 mm water h⁻¹ m⁻². Symptom development in the plots was recorded at 7, 14 and 21 days after artificial inoculation. Disease severity was recorded as average head blight incidence and severity on a scale of 1 to 9, 1 without symptoms and 9 when all spikelets are blighted.

Table 1 Breeder and year of registration in the national catalogue (Switzerland) of the eight spring wheat varieties used in this study

Variety	Breeder	Year of registration	
Greina	Agroscope/DSP	1994	
Carasso	Agroscope ART/DSP	2005	
Lona	Agroscope/DSP	1991	
Brusino	Agroscope ART/DSP	In test	
Toronit	Agroscope ART/DSP	1996	
Fiorina	Agroscope ART/DSP	2001	
Quarna	Agroscope ART/DSP	2004	
Nadro	Agroscope/DSP	2002	

The variety Brusino is still in the official tests for registration.



Intermediate scores follow a progression scale as shown in Table 2 (Mascher et al. 2005).

Harvest and milling

The crop was harvested with a combine harvester for experimental plots (Wintersteiger, Ried, Austria) reducing the airflow in the machine to recover a maximum number of kernels. The harvested grain was cleaned by hand. Immediately after the harvest, the grain was dried to 13% humidity and stored in linen sacs in a dry and dark room at 13°C until further processing. After rehumidification to 15–16% humidity (*w/w*), the grain of each replication per variety and per treatment was milled separately with a medium size sample mill (Bühler MLU202). Four milling fractions were collected: break flour with an approximate ash level of 0.55, reduction flour, as well as the shorts and the bran from the outer layers of the grain.

Baking quality analyses

Protein and ash content of the break flour was measured by near-infrared spectroscopy (Inframatic 8611, Perten Instruments AB, Huddinge, Sweden). The falling number was determined according to the ICC standard method 107/1: 7 g of break flour was mixed with 25 ml of distilled and heated to boiling. The Zeleny index was determined on whole meal flour (milled with a Brabender Quadrumat Junior mill) according to the ICC standard method 116/1; 3.2 g flour was suspended in 50 ml bromophenol-blue (4 mg l⁻¹) and mixed for 5 min on a laboratory

Table 2 The scale for disease scoring of *Fusarium* head blight used in this study

Score	% of ear affected		
1	0		
2	2.5		
3	12.5		
4	25		
5	50		
6	75		
7	87.5		
8	97.5		
9	100		

The progression scale is a linearized sigmoid curved using a logit transformation.

shaker. After addition of 25 ml lactic acid (2.78 N) and isopropanol, the suspension was mixed for another 5 min and allowed to rest for a further 5 min after which the height of the precipitating sediment was measured.

Dough stability and softening as well as water absorption capacity of straight grade flour (the mixture of break and reduction flour), used in the subsequent baking tests, was analysed with a Brabender farinograph (Brabender, Duisburg, Germany). The official ICC method was followed. The dough was placed in the kneading device and kneading stability is recorded as Brabender units (BU). The optimal volume of added water was obtained when the curve reached the consistency of 500 BUs. The stability of the dough is then equivalent to the time the curve remains at 500 BUs, while the degree of softening corresponds to the decrease in BUs after 12 min.

The rapid mix test, a standardized baking test for determining the baking behaviour of the flour, was performed using 600 g of straight grade flour (Pelshenke et al. 1970). Water was added according to the water absorption capacity of the dough, previously determined with the farinograph. The dough was prepared with 30 g yeast, 9 g salt, 6 g saccharose, 6 g peanut fat and 0.006 g ascorbic acid. Malt was added to complement the lack of free sugars in samples showing high falling numbers (Table 4).

The dough was kneaded for 1 min in a kitchen mixer (Model 4M12D, Stephan, Hameln, Germany), fermented for 20 min at 32°C and rested for 10 min at room temperature. Thirty buns were formed and fermented a second time for 25 min at 32°C before baking at 250°C for 15 min. The volume of the buns was determined using the rapeseed displacement method, when buns had cooled down to room temperature according to the AACC approved method 10-05 (AACC 2002). The length of 25 randomly extracted buns of each sample was recorded.

Deoxynivalenol content in milling fractions and in bread

The concentration of deoxynivalenol (DON) in the milling fractions, (break flour, reduction flour, shorts, and bran) were analysed using an ELISA-kit (Transia Plate, Diffchamb SA, Lyon, France). Routinely, a 1 g sample was suspended in 5 ml double distilled water. Exceptionally, the bran was suspended in 10 ml



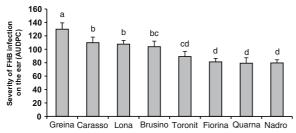


Fig. 1 Fusarium head blight severity of the eight spring wheat varieties in inoculated and control plots in the field experiment

 ddH_2O due to its high water absorption capacity. The suspensions were mixed vigorously for 5 min on a vortex and allowed to precipitate for 2–3 h. The supernatant was used for the subsequent tests. The samples were then diluted 10 or $100\times$ in order to respect the detection limit of the test. The DON concentration in bread was measured in the same way, suspending 1 g of dry milled scabs and crumbs in $10 \text{ ml} \ ddH_2O$.

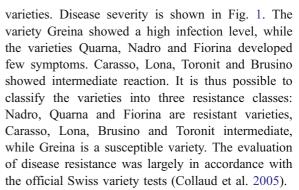
Statistical analysis

The experiment was a completely randomized split plot with five subplots as repetition for each treatment. Each subplot was composed of a randomized pattern of eight spring wheat varieties. Disease assessment was expressed as AUDPC (area under the disease progress curve), that takes into account the disease severity and progression during infection over time (Cambell and Madden 1990).

Differences among subplots, treatments, and varieties as well as interactions among the parameters were verified with ANOVA analysis of variance. Normal distribution of the residues of the data was verified with the Smirnov–Kolmogorov normality test before undergoing the ANOVA analysis. No transformation of data was necessary to achieve normal distribution. ANOVA was performed using the programme Sigma Stat for Windows (vers. 2.03, SPSS, Chicago, USA). The interdependence between the variables was examined with linear regression analysis using the Spearman rank order module of the same programme.

Results and discussion

Artificial inoculation with *F. culmorum* resulted in a differential response in the eight spring wheat



The mycotoxin deoxynivalenol (DON) was detected in the grain of all eight varieties after artificial inoculation (Table 3). Due to the artificial infection with virulent *F. culmorum* strains, the contamination level was very high. The highest amounts of DON were detected in the varieties Carasso and Greina. In the varieties Fiorina, Nadro and Quarna, DON concentrations in the whole grain were just slightly higher than the tolerance limit for food use in Europe (i.e. 1.25 ppm). Lona, Brusino, and Toronit showed intermediate concentrations.

FHB scores on the ear at 21 days after inoculation and DON accumulation in kernels were positively correlated (Fig. 2). Hence, in contrast to other findings (Jones and Mirocha 1999; Snijders and Perkowski 1990), DON accumulation was a function of disease severity. The regression analysis gave the following equation:

$$f(x) = 3.101 d - 10.08$$

$$d = \text{disease severity at 21 days}$$
(i)

Table 3 Deoxynivalenol concentrations before baking in four milling fractions (break flour, reduction flour, shorts and bran) and after baking in the bread

Variety	Break flour (mg kg ⁻¹)	Reduction flour (mg kg ⁻¹)	Shorts (mg kg ⁻¹)	Bran (mg kg ⁻¹)	Bread (mg kg ⁻¹)
Greina	16.2a	16.9a	84.4a	122.0a	12.5a
Carasso	11.0b	14.5a	43.2b	66.6b	9.2b
Lona	7.4c	8.7b	26.4c	43.8c	5.7c
Brusino	5.1d	6.7bc	27.1c	31.4cde	4.6c
Toronit	5.0d	6.9bc	23.5c	26.2cde	4.5c
Fiorina	3.3ef	3.7cd	7.2d	12.4de	2.7cd
Quarna	2.7ef	3.4cd	6.4d	12.3de	3.8cd
Nadro	1.4f	2.5d	12.6d	8.3e	1.5d

Data followed by the same letter are not statistically different.



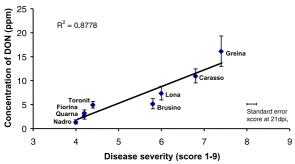


Fig. 2 Correlation analysis of DON content in the grain (whole grain) and the disease score in the field

Applying this model indicated that the variety Brusino was an outlier as the DON concentration was significantly lower than predicted by the model. Brusino may deploy a detoxifying mechanism similar to that described in the spring wheat Frontana (Wang and Miller 1988). Alternatively, it may interfere in the fungal biosynthesis of the mycotoxin.

In order to determine where mycotoxins accumulate in the kernel, the four milling fractions that corresponded largely to the different kernel regions (i.e. teguments and core) were analysed separately. Results showed that DON mainly accumulated in the bran and the shorts, thus the outer regions of the grain (Table 3). The bran contained about five times more mycotoxin than the break flour, and the shorts had still three times more mycotoxin. The proportion of DON accumulated in the different kernel regions was constant and was independent of varietal resistance. As *Fusarium* spp. penetrate into the grain and DON accumulation depends on the degree of colonization of plant tissue by *Fusarium* (Rabenstein et al. 2000),

resistance to the accumulation of mycotoxins in the kernels depends on the resistance against kernel colonization (Mesterhazy 1995) and on detoxifying mechanisms of the kernel tissue (Wang and Miller 1988; Snijders and Krechting 1992).

The second part of this work deals with the alteration of kernel constituents and baking quality after infection with F. culmorum. In the present experiment, ash content increased significantly after FHB infection in the varieties Greina, Carasso and Lona, but in the other varieties, no statistically significant modification was detected (Table 4). Correlation analysis revealed that the ash content in kernels was tightly linked with disease severity (r= 0.93***). Ash is mainly composed of minerals such as potassium and calcium salts of the seed coat (bran and aleurone). The proportion of ash in the flour is therefore an indicator of its purity, as increasing ash content indicates an alteration in the kernel seed coat – volume ratio. It is possible to associate this observation with the presence of shrivelled, misshapen kernels in fusarium-infected batches as observed by other authors (Jones and Mirocha 1999).

A fungal infection is expected to increase the degradation of starch due to the presence of enzymes such as α -amylase in the kernels which is measurable using the falling number method (Hareland 2003). Here, in batches with infection, falling number was significantly higher compared to the control in all varieties, but remained unalterated for Nadro and Quarna, indicating diminishing levels of soluble sugars and the absence of starch degradation in susceptible varieties (Table 4). There are contrasting reports on the occurrence of free sugars and the role

Table 4 Properties of the flour from control batches and from Fusarium-damaged batches

Variety	Control				FHB			
	Protein (%)	Zeleny (ml)	Ash (%)	Falling Number (s)	Protein (%)	Zeleny (ml)	Ash (%)	Falling Number (s)
Greina	13.3c	41b	0.63b	444a	13.0c	34de	0.76a	510a
Carasso	13.4c	35d	0.62b	396c	13.3c	34de	0.73a	444b
Lona	15.0ab	51a	0.62b	413bc	14.6ab	42b	0.69ab	461b
Brusino	14.5b	39c	0.64b	410bc	14.5b	35cd	0.69ab	445b
Toronit	13.2c	33e	0.68ab	365d	13.1c	32e	0.73a	400c
Fiorina	13.6c	37d	0.68ab	394c	14.0c	36cd	0.68ab	442b
Quarna	15.0ab	50a	0.65ab	427ab	15.0a	51a	0.64b	448b
Nadro	15.4a	37cd	0.72a	446a	14.9a	37c	0.72a	454b

Types of flour used for each analysis is described in the text. Data followed by the same letter are not statistically different.



62.14a

Nadro

Variety	Control				FHB			
	Water absorption (%)	Dough softening (BU)	Dough stability (min)	Loaf volume (cm ⁻³)	Water absorption (%)	Dough softening (BU)	Dough stability (min)	Loaf volume (cm ⁻³)
Greina	58.54b	55.7c	7.82a	266.6f	58.28c	141.4b	3.90c	254.6e
Carasso	56.68c	86.4b	6.28b	286.8d	56.06d	127.8bcd	4.58c	292.4c
Lona	61.86a	89.2b	4.80cd	358.4a	60.58b	133.4bc	4.10c	307.2abc
Brusino	58.76b	86.4b	3.92e	320.0c	58.82bc	124.0cd	3.60c	301.0bc
Toronit	57.06c	114.0a	6.74b	304.8c	57.16cd	158.2a	5.66b	321.8a
Fiorina	59.08b	108.6a	5.50c	313.0c	58.8bc	113.4de	5.60b	312.8ab
Quarna	60.30b	35.0d	6.98b	268.4e	60.14b	44.0f	9.54a	271.0d

Table 5 Farinograph and baking quality analyses on the control batches and on the fusarium-damaged batches

Straight grade flour (blend of break and reduction flour) was used. Data followed by the same letter are not statistically different (P<0.02).

336.8b

62.08a

102.6e

of α -amylase in FHB-infected kernels. Boyacioğlu and Hettiarachchy (1995) described the increase of free sugars. Dexter et al. (1996) reported the increase of α -amylase activity in heavily infected kernels. It is, however, well recognized that the falling number depends upon the prevailing climatic conditions. Meyer and Weipert (1986) found the falling number decreased only in one out of 3 years. Conceivably, the infection process was modified by the particularly dry and hot weather conditions during the summer of 2003. In conclusion, in a breeding context, the variation of the falling number after FHB infection, cannot be used as a selection criterion.

91.6b

4.60de

All the varieties tested had a protein content between 13 and 15% and are classified as good, up to very good baking, quality wheat. In the present experiment, the protein content was only slightly reduced by the infection. This observation is con-

Fig. 3 Length of the buns prepared from control (white columns) and infected (grey columns) grain of eight spring wheat varieties. Statistically significant (P<0.02) differences between control and infected batches are marked with a star

firmed by Dexter et al. (1996) while other authors observed an increase in the protein content after infection (Boyacioglu and Hettiarachchy 1995). In these latter works, protein content was determined using the Kjeldahl method. It is not yet clear to what extent fungal proteins contributed to the total protein content. In our case, protein was determined on the break flour using a NIRS technique, calibrated on kernel proteins. As the fungus is mainly located in the external layers of the kernel, the use of break flour should largely exclude the interference of the protein measurements with the fungus.

4.40c

338.0a

Further investigations focused on alteration of protein quality. The Zeleny index measures the swelling potential of the kernel protein. The results show that the Zeleny index was significantly reduced

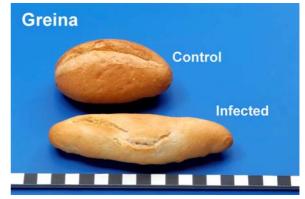


Fig. 4 Comparison of a bun prepared from control and infected grain of the spring wheat variety Greina. The bun from infected grain is elongated, indicating an alteration of the dough structure



in the varieties Greina, Lona and Brusino, but not in the other varieties. Meyer et al. (1986) observed a general reduction of the Zeleny index in wheat after FHB infection. This indicates that although the total amount of protein remains quite stable the infection may alter the quality of the protein in certain varieties.

The farinograph measures the water absorption capacity and the robustness of the dough. Concerning the water absorption capacity, no statistically significant differences between the inoculated and the noninoculated treatment were detected (Table 5). The dough softening, measured after 12 min at 500 BUs, gave an indication of the quality of the gluten responsible for the structure of the bread. The results obtained gave evidence that dough stability is significantly influenced by FHB for the susceptible varieties Greina, Carasso and Toronit. Quarna showed an opposite effect with a slightly but significantly higher dough stability after infection. For the other varieties, the differences between infected and healthy flour were not statistically significant. The results obtained show that Fusarium infection does not influence water absorption capacity in fusariuminfected flour, but does influence the dough stability, confirming the observations of Bockmann (1964), Meyer et al. (1986) and Dexter et al. (1996).

The rapid mix test measures the bread volume and shape. Our results indicated that the infection reduced the volume in the varieties Lona and Brusino (Table 5). In contrast, Toronit showed a higher volume when infected. For the other varieties, no difference in bread volume was detected.

Concerning the shape of the bread, for Fiorina, Quarna and Nadro no difference in shape was observed, while for the other varieties, the breads were longer and flatter in the infected samples than in the control samples, and the dough was sticky in these elongated breads (Fig. 3 and Table 5). Buns produced with infected flour from Greina were particularly long (Fig. 4). These observations are in disagreement with most other studies (Berova and Mladenov 1974; Seitz et al. 1985; Meyer et al. 1986; Moore 1994; Dexter et al. 1996; Protic and Protic 2000), where the bread volume generally decreased after FHB infection. This apparent contradiction in results may be due to the varieties used in this study that have a very good baking quality (Brabant et al. 2006). It is conceivable that, despite infection, the gluten remains strong enough to give the same bread volume. On the other hand, the fermentation time used in the present study was quite short compared to the long processes used by Dexter et al. (1996). Consequently, the time might have been too short for fungal proteases to act. The most susceptible variety Greina, but also Carasso, and Lona, Brusino and Toronit had a sticky dough. This resulted in more elongated breads, as observed by Meyer et al. (1986).

The DON concentration in the breads is given in Table 3. The correlation between DON in breaking flour and in bread was very high, r=0.99. This corresponds to the results of Seitz et al. (1985) and Scott et al. (1983). A tendency of a reduction in DON concentration in the bread was observed. This reduction is more important for the susceptible varieties.

In conclusion, for the spring wheat varieties tested in this work, kernel resistance is congruent with resistance for visible symptoms on the spike. Thus, in a breeding programme, the visual estimation of infection seems to be an adequate criterion for a limited set of varieties in one single environment. On the other hand, kernel resistance in terms of stability of baking quality can be detected through the analysis of mycotoxin accumulation. Future research will question whether this criterion becomes important for evaluating the degradation of quality traits through *Fusarium* spp. infection when screening other spring wheat genotypes, winter wheat or triticale.

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