The influence of *Fusarium* infection and growing location on the quantitative protein composition of (part I) emmer (*Triticum dicoccum*)

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Abstract  The effect of the fungal infection by *Fusarium graminearum* and *Fusarium culmorum* of emmer and wheat cultivars on their total protein content and the protein fractions albumins + globulins, gliadins and glutenins and their different protein types was investigated. In addition, the influence of two different locations on the quantitative protein composition was evaluated. The results showed that *Fusarium* infection changed the content of gliadins and glutenins in emmer and wheat. The wheat glutenin fractions and types were found to be more strongly affected by the *Fusarium* spp. infection than the glutenin fractions and types in emmer cultivars in spite of the wheat’s lower degree of infection. The nitrogen supply at the two locations was associated with an increase in the gliadin content in emmer and an increase in the glutenin content in emmer and wheat. Nitrogen availability, a factor which promotes gene expression, resulted here in a species-specific effect on the gliadin/glutenin ratio.

Keywords  Emmer · *Fusarium* · *Fusarium* protein units (FPU) · ELISA · Deoxynivalenol · LC–MS/MS

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| ALG          | Albumins and globulins |
| FPU          | *Fusarium* protein units |
| HMW-GS       | High molecular weight glutenin subunits |
| LMW-GS       | Low molecular weight glutenin subunits |
| PR proteins  | Pathogenesis-related proteins |
| p            | Significance factor |
| RT           | Room temperature |
| TFA          | Trifluoroacetic acid |

Introduction

*Fusarium graminearum* and *Fusarium culmorum* infection of cereal grains (such as wheat, barley and emmer) leads to pathogenic effects on the plant and spike. These can result in dramatic yield and quality losses [1, 2]. *Fusarium* head blight (FHB) is the visible effect of this fungal infection and is a problem known throughout the world [1, 3, 4]. These two *Fusarium* species are producers of trichothecene mycotoxins such as deoxynivalenol, 3- and 15-acetyldeoxynivalenol (3-Ac-DON and 15-Ac-DON, respectively; major DON precursors) and others [1]. The trichothecene mycotoxins are potential inhibitors of protein biosynthesis. In mammals, they lead to unspecific effects in the intestine causing diarrhoea, vomiting, a reduced food intake and a raised bleeding tendency of the intestines. Their more specific effects include reduced leucocyte content connected with a loss of the immune function and an increase in free radicals in the liver [5–8].

Emmer (*Triticum dicoccum*) cultivars are normally grown in organic farming systems. At the moment, little knowledge exists about the influence of *Fusarium* infection on the protein fractions in this species. The total protein content is documented for emmer at around 12–13% of dry mass [9]. The influence of *Fusarium* infection on grain protein composition has been mainly investigated in wheat cultivars. The results from these investigations have suggested
that depending on infection degree, no impact on total protein content could be observed or just moderately increased concentrations of total protein could be documented [10, 11]. However, an influence on protein fractions, such as an increase in gliadin and a reduction in glutenin content, has also been recorded. A change in the synthesis behaviour at different maturation stages has been postulated as an explanation for this observation [11]. Studies focusing on the synthesis of cereal seed storage proteins did not show any alterations in the synthesis stages during grain maturation; only a belated polymerization of glutenins was noted [12]. An influence of Fusarium infection on LMW glutenin degradation has been shown in a study which focused on sub-optimal storage conditions [13].

The total protein content in wheat cultivars is also increased by increasing the nitrogen supply. The sizes of the gliadin and glutenin fractions are also changed. However, nitrogen has apparently no influence on the albumin and globulin fractions [14, 15]. It is not known how emmer reacts to increasing the supply of nitrogen.

The presented study focuses on the influence of artificial Fusarium infection on the Osborne protein fractions and single protein types in emmer in comparison to naturally infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples. Our investigations were done on harvested grains to determine the conditions in fully developed infected samples.

The location conditions at RH [16] are 152 m above sea level, wind sheltered and dale area near a river border. The Nmin content was 145 kg/ha recorded in 90-cm depth dry soil. At SH, the conditions are 260 m above sea level, hilly and windy. The Nmin content was 95 kg/ha in 90-cm dry soil with an additional fertilization of 40 kg N/ha 2 months after sowing.

Quantitative LC–MS/MS of Fusarium mycotoxin DON and 3-Ac-DON

Whole grain flour (5 g) was extracted with 40 ml of an acetonitrile–water mixture (80:20) over night on a reciprocal shaker. The extracts were centrifuged for 12 min at 5,000g and 4 ml of the supernatant were used for solid-phase extraction (Bond-Elut Mycotoxin, Varian GmbH, Darmstadt, Germany) according to the manufacturer’s instructions. Two millilitres of the cleaned extract were evaporated to dryness under vacuum, redissolved in 200 μl of methanol–water (50:50) containing 0.2 mmol ammonium acetate and 10 μl of the solution was injected onto a C18 column (100 × 2 mm, 3-μm particle size) filled with polar-modified material (Polaris Ether, Varian GmbH, Darmstadt, Germany). The analytes were eluted with a methanol–water gradient (15–70% over 20 min) containing 0.2 mmol ammonium acetate at a flow rate of 0.2 ml/min. DON and 3-Ac-DON were detected by tandem mass spectrometry as described by Adejumo et al. [17].

Quantitative nitrogen analysis

The nitrogen content was quantitatively measured with a C/N analyser (Vario MAX CN Elementar Analysysysteme GmbH, Hanau, Germany). A 100-mg dry sample for each emmer and wheat cultivar was analysed for its N content. This was converted to protein using the factor 5.7 for both the emmer and wheat (ICC No. 105/2).

Quantification of Fusarium protein units (FPU)

Biotin/Avidin ELISA, a double-antibody sandwich with rabbit-anti-fungal antibody, was performed to detect the soluble FPU in mg/kg using 96-well convex-bottomed plates (Immuno Plate Maxisorb, Nunc International, Denmark) and a photometer (Spectra II, SLT Laborinstruments, Austria). The FPU were extracted from the milled grain with 20 times of buffer quantity [0.01 mol/l phosphate buffer + 0.05% (v/v) Tween 20 (PBST) and 1% polyvinyl-polypyrrolidone (PVP) pH 7.2] by shaking for 12 h at 4 °C. The extract was centrifuged (12,000g/4 min) and the supernatant was used for ELISA.
To prepare the micro-wells, 100 μl IgG in 0.05 M carbonate buffer/loading buffer [Na2CO3/NaHCO3] pH 9.6 was placed in each cavity and the antibodies were immobilized on the surface during an incubation time of 12 h at 4 °C. The wells were then washed three times for 3 min with 100 μl washing buffer (1/1 PBST/dest. H2O v/v), and dried by beating the wells gently on a paper towel. To block non-specific binding, 200 μl 1% (w/v) defatted milk powder in loading buffer was added and incubated and incubated for 1 h at 37 °C. The wells were washed again. Following this, 100 μl of sample extract was added and incubated for 12 h at 4 °C. The wells were washed again. In the next step, 100 μl bio-
tinylated antibodies in binding buffer [0.2% (w/v) BSA + PBST, pH 7.2] were added and incubated for 12 h at 4 °C. The wells were then washed as described in the steps before. Subsequently, 100 μl streptavidin alkaline phosphatase [dissolved 1:10,000 (v/v) in binding buffer] was added to each well and incubated for 1 h at room temperature, following which, the washing step was repeated. Finally, 100 μl buffer (1 mg/ml p-nitrophenyl phosphate in diethan-
olamine buffer, pH 9.8) was added and incubated at room temperature for 2 h. The absorption was then measured at 450 nm (with reference at 592 nm). For the external stan-
dard, cleaned soluble Fusarium protein (F. culmorum/F. graminearum) was used to create a standard row as FPU in mg/g sample, with the protein content of the standard being based on a Bradford assay [18]. For analysis of the protein content of the Fusarium standard, cleaned F. graminearum was used and the soluble and insoluble fractions were mea-
sured with C/N analyser.

Quantitative protein analysis with RP-HPLC

Protein extraction from 100 mg flour samples was realized stepwise. In the first step, 1 ml extraction with solution A [HKNaPO4: 97 parts Na2HPO4 (0.067 mol/l) + (0.4 mol/l)
NaCl and 3 parts KH2HPO4 (0.067 mol/l) + (0.4 mol/l)
NaCl; pH 7.6] by vortexing for 2 min and shaking at room temperature for 10 min for the albumin/globulin fractions was repeated twice. The samples were centrifuged for 20 min at 6,000 g and the supernatants were combined and filled up to 2 ml. The pellet was then extracted three times with 0.5 ml extraction solution B [60% ethanol (v/v)], vortexed for 2 min and exposed to 10 min of shaking at room temperature. The samples were subsequently centrifuged for 20 min at 6,000 g and the gliadin-containing superna-
tants were combined and filled up to 2 ml. In the third step, the remaining pellet was extracted twice with 1 ml extraction solution C [50% 1-propanol (v/v)/2 mol/l urea, 0.05 mol/l Tris/HCl (pH 7.5) + 1% dithioerythritol (DTE)] under N2, with 2 min vortexing and 30 min shaking at 60 °C. The samples were centrifuged for 20 min at 6,000 g and these glutenin-containing supernatants were combined and filled up to 2 ml. All the extracts were filtered with a 0.45-μm filter: FP 30/0.45 CA Whatman (schleicher + schuell, Germany) before HPLC injection.

For the RP-HPLC, a Nucleosil 300-5 C8 250 × 4.6 sil-
ica column (Macherey-Nagel, Dueren, Germany) was used. As mobile phases, A = 0.1% TFA in H2O (v/v) and B = 0.1% TFA in acetonitrile (v/v) were applied. The flow rate was 1 ml/min with the column temperature maintained at 50 °C. For the detection of albumins/globulins, 150 μl of sample solution was injected and separated using the following gradient: 0 min, 20% B; 20 min, 60% B; whereas for the gliadins and glutenins, 50 μl and 100 μl were injected, respectively, and the separation was performed by applying the following gradient: 0 min, 24% B; 50 min, 56% B [19]. For the quantification of the protein fractions the external PGW (Prolamin Working Group) gliadin standard was used [20] (Fig. 1).

Statistical analyses were performed using Microsoft Excel 2003 for mean value, standard deviation and signifi-
cance (p).

Results and discussion

The influence of Fusarium infection on the quantitative protein composition of emmer

The detected fungal content expressed as Fusarium toxin (DON and 3-Ac-DON) in the three emmer cultivars and the wheat cultivar showed a significant increase in all the investig-
ted parameters after artificial infection in comparison to the naturally infected cultivars (Tables 1, 2, 3, 4, 5, 6). This confirms that the two cereals and their tested cultivars were susceptible to Fusarium spp. [2, 4, 21]. The total protein content of the two cereals and their cultivars did not change significantly with respect to the Fusarium infection (Table 6). These results are in accordance with an earlier study in which no impact on the total protein content in F. culmorum-infected samples was also found [11]. In contrast, however, older results on F. graminearum found a moderate increase in the total protein content depending on the degree of infection [10].

A comparison between emmer and wheat showed a 28% higher total protein and extractable protein content in emmer (Table 6). A comparison of the total protein content and the amount of extractable protein showed that about 77% of the total protein content can be extracted by the method used in this study (Tables 1, 2, 3, 4, 5, 6). Non-
extractable proteins are membrane associated or integrated basic proteins with a high sugar content [22] and non-
extractable storage proteins. For a more complete extrac-
tion of the plant proteome, special procedures must be used [23].
To quantify the contribution of *Fusarium* protein to the grain’s proteins, the amount of soluble *F. graminearum* protein in the grain was determined as this is the most important fungus in Europe. We found 15% soluble and 85% insoluble *F. graminearum* protein and a total protein content of 18% for the fungus. With this information and the amount of soluble FPU detected by ELISA, we could conclude that the proportion of soluble *Fusarium* protein of the total protein even in strongly infected grains is relatively small: for emmer, a maximum of 0.13%, and for wheat, 0.3% of the protein was of *Fusarium* origin (Table 7). A masking of the absolute grain protein content by *Fusarium* infection and reduced grain protein content due to the *Fusarium* infection can, therefore, be excluded.

The influence of *Fusarium* infection on the Osborne fractions in emmer and wheat showed that the albumin and globulin fraction was not significantly changed by the presence of *Fusarium* infection (Tables 1, 2, 3, 4, 5, 6). Based on the total extractable protein, the albumin and globulin fraction in emmer formed, with 15%, a 6% lower proportion of the total extractable protein than this fraction in wheat. This may be due either to the higher content of this fraction in wheat in comparison to emmer or to there being a better extractability of gliadin and glutenin in emmer.

The gliadin and glutenin fractions and types were influenced by *Fusarium* infection (Tables 1, 2, 3, 4, 5, 6). For gliadins, the content of all types increased after artificial infection in comparison to the naturally infected wheat samples (Table 5). In comparison, this effect was less clear in emmer after artificial infection as only the samples from the location RH with the stronger infection degree (based on the amount of *Fusarium* toxin) showed this effect.
Table 1  Protein concentration (g/kg) in naturally and artificially *Fusarium*-infected grain from the emmer cultivar Linie 9-102 and the relative variance between natural and artificial infection (%) at the locations Sattenhausen and Reinhof as well as the DON and 3-Ac-DON concentration of the naturally and artificially infected grain

| Protein fractions (g/kg) | Type       | Sattenhausen | Reinhof | | |
|--------------------------|------------|-------------|---------| | |
|                          |            | Natural infection | Artificial infection | Variance natural to artificial infection (%) | Natural infection | Artificial infection | Variance natural to artificial infection (%) | | | |
| Albumins + globulins     | Total      | 21.6 ± 0.4 | 21.4 ± 0.0 | −1 | 22.3 ± 1.1 | 22.0 ± 1.0 | −2 | | | |
|                          | Total      | 90.3 ± 0.9 | 89.5 ± 0.6 | −1 | 118.4 ± 0.3 | 119.8 ± 1.9 | 1 | | | |
|                          | o5         | 4.0 ± 0.1  | 3.8 ± 0.1  | −5 | 7.5 ± 0.1  | 7.3 ± 0.2  | −2 | | | |
| Gliadins                 | o1,2       | 0.9 ± 0.1  | 0.7 ± 0.1  | −21 | 1.4 ± 0.1  | 1.2 ± 0.2  | −17 | | | |
|                          | x          | 39.1 ± 0.3 | 38.5 ± 0.6 | −1 | 54.7 ± 0.6 | 55.3 ± 1.3 | 1 | | | |
|                          | γ          | 46.3 ± 0.4 | 46.5 ± 0.8 | 0 | 54.8 ± 0.6 | 55.9 ± 0.2 | 2 | | | |
|                          | Total      | 13.3 ± 0.1 | 11.2 ± 0.5 | −16 | 15.7 ± 0.1 | 13.9 ± 0.0 | −12 | | | |
|                          | o6b        | 0.3 ± 0.0  | 0.3 ± 0.1  | 0 | 0.6 ± 0.0  | 0.4 ± 0.1  | −33 | | | |
| Glutenins                | HMW-GS     | 1.7 ± 0.2  | 1.4 ± 0.1  | −19 | 2.7 ± 0.1  | 2.2 ± 0.2  | −18 | | | |
|                          | LMW-GS     | 11.3 ± 0.3 | 9.5 ± 0.6  | −15 | 12.4 ± 0.1 | 11.3 ± 0.2 | −9  | | | |
| Gluten                   | Total      | 103.6 ± 1.0 | 100.7 ± 0.1 | −3 | 134.1 ± 0.4 | 133.7 ± 2.3 | 0 | | | |
|                          | Ratio      | 6.8        | 8.0        | − | 7.5        | 8.6        | − | | | |
| Extract proteins         | Total      | 125.2 ± 1.4 | 122.1 ± 0.1 | −2 | 156.4 ± 1.5 | 155.7 ± 3.3 | 0 | | | |
| Protein content          | Total      | 159.8      | 152.2      | −5 | 205.8      | 208.7      | 1 | | | |
|                          | DON        | 0.4 ± 0.0  | 2.8 ± 0.9  | − | 1.7 ± 0.3  | 10.3 ± 0.7 | − | | | |
| Toxin (mg/kg)            | 3-Ac-DON   | 0.0 ± 0.0  | 0.1 ± 0.0  | − | 0.1 ± 0.0  | 0.6 ± 0.0  | − | | | |
|                          | Total      | 0.4        | 2.9        | − | 1.8        | 10.9       | − | | | |

Table 2  Protein concentration (g/kg) in naturally and artificially *Fusarium*-infected grain from the emmer cultivar Far-108+Hein-101 and the relative variance between natural and artificial infection (%) at the locations Sattenhausen and Reinhof as well as the DON and 3-Ac-DON concentration of the naturally and artificially infected grain

| Protein fractions (g/kg) | Type       | Sattenhausen | Reinhof | | |
|--------------------------|------------|-------------|---------| | |
|                          |            | Natural infection | Artificial infection | Variance natural to artificial infection (%) | Natural infection | Artificial infection | Variance natural to artificial infection (%) | | | |
| Albumins + globulins     | Total      | 20.7 ± 1.6 | 20.5 ± 2.2 | −1 | 21.6 ± 1.0 | 21.8 ± 0.6 | 1 | | | |
|                          | Total      | 87.1 ± 4.1 | 86.2 ± 2.5 | −1 | 112.7 ± 1.0 | 122.7 ± 0.2 | 9 | | | |
|                          | o5         | 5.2 ± 0.3  | 5.0 ± 0.1  | −4 | 9.1 ± 0.1  | 10.3 ± 0.3 | 13 | | | |
| Gliadins                 | o1,2       | 1.5 ± 0.2  | 1.5 ± 0.3  | 4 | 1.9 ± 0.1  | 2.4 ± 0.3  | 30 | | | |
|                          | x          | 36.8 ± 1.8 | 36.3 ± 1.0 | −1 | 48.9 ± 0.3 | 53.7 ± 0.4 | 10 | | | |
|                          | γ          | 43.7 ± 1.9 | 43.4 ± 1.0 | −1 | 52.8 ± 0.8 | 56.3 ± 0.8 | 7 | | | |
|                          | Total      | 15.7 ± 0.3 | 14.9 ± 0.6 | −5 | 24.8 ± 0.9 | 19.6 ± 0.0 | −21 | | | |
|                          | o6b        | 0.7 ± 0.0  | 0.7 ± 0.0  | 0 | 1.4 ± 0.1  | 1.1 ± 0.1  | −26 | | | |
| Glutenins                | HMW-GS     | 3.0 ± 0.1  | 2.7 ± 0.1  | −9 | 5.6 ± 0.5  | 4.1 ± 0.1  | −28 | | | |
|                          | LMW-GS     | 12.0 ± 0.4 | 11.5 ± 0.6 | −4 | 17.8 ± 0.5 | 14.4 ± 0.0 | −19 | | | |
| Gluten                   | Total      | 102.8 ± 4.5 | 101.1 ± 3.0 | −2 | 137.5 ± 1.9 | 142.3 ± 0.2 | 3 | | | |
|                          | Ratio      | 5.5        | 5.8        | − | 4.5        | 6.3        | − | | | |
| Extract proteins         | Total      | 123.5 ± 2.9 | 121.6 ± 0.8 | −2 | 159.1 ± 0.9 | 164.1 ± 0.8 | 3 | | | |
| Protein content          | Total      | 150.4      | 152.9      | 2 | 209.6      | 211.1      | 1 | | | |
|                          | DON        | 0.2 ± 0.0  | 1.1 ± 0.0  | − | 1.7 ± 0.4  | 5.2 ± 0.7  | − | | | |
| Toxin (mg/kg)            | 3-Ac-DON   | 0.0 ± 0.0  | 0.0 ± 0.1  | − | 0.0 ± 0.0  | 0.4 ± 0.0  | − | | | |
|                          | Total      | 0.2        | 1.1        | − | 1.7        | 5.5        | − | | | |
Table 3  Protein concentration (g/kg) in naturally and artificially Fusarium-infected grain from the emmer cultivar Klein and the relative variance between natural and artificial infection (%) at the locations Sattenhausen and Reinshof as well as the DON and 3-Ac-DON concentration of the naturally and artificially infected grain

| Protein fractions (g/kg) | Type | Sattenhausen | Reinhof |
|--------------------------|------|--------------|---------|
|                          |      | Natural infection | Artificial infection | Variance natural to artificial infection (%) | Natural infection | Artificial infection | Variance natural to artificial infection (%) |
| Albumins + globulins     | Total | 22.3 ± 0.9 | 21.8 ± 2.5 | −3 | 21.0 ± 1.4 | 21.1 ± 0.8 | 0 |
|                          | Total | 81.1 ± 0.4 | 78.9 ± 0.1 | −3 | 103.8 ± 3.6 | 110.4 ± 0.3 | 6 |
| Gliadins                 | o01,2 | 1.4 ± 0.2 | 1.6 ± 0.0 13 | 1.7 ± 0.3 | 2.0 ± 0.1 | 19 |
|                          | χ     | 36.7 ± 0.0 | 36.1 ± 0.1 | −2 | 47.0 ± 0.8 | 50.0 ± 1.5 | 6 |
|                          | γ     | 37.0 ± 0.3 | 35.2 ± 0.8 | −5 | 44.2 ± 2.2 | 46.2 ± 1.3 | 5 |
|                          | Total | 15.2 ± 0.1 | 13.3 ± 0.3 | −12 | 29.6 ± 0.6 | 22.8 ± 0.2 | −23 |
| Gliadin/glutenin         | Ratio | 0.6 ± 0.0 | 0.6 ± 0.0 | 0.16 ± 0.3 |
| Extract proteins         | Total | 96.3 ± 0.3 | 92.2 ± 0.4 | −4 | 133.4 ± 3.0 | 133.2 ± 0.4 | 0 |
| Protein content          | Total | 118.6 ± 0.6 | 114.0 ± 2.9 | −4 | 154.4 ± 1.6 | 154.5 ± 1.3 | 0 |
| DON                      | 0.7 ± 0.1 | 2.6 ± 0.1 | – | 1.8 ± 0.1 | 5.9 ± 1.2 | – |
| Toxin (mg/kg)            | 3-Ac-DON | 0.0 ± 0.0 | 0.1 ± 0.0 | – | 0.0 ± 0.0 | 0.2 ± 0.0 | – |
|                          | Total | 0.7 | 2.7 | – | 1.8 | 6.1 | – |

Table 4  Protein concentration (g/kg) in naturally and artificially Fusarium-infected grain from all three emmer cultivars (Far-108+Hein-101, Klein, Linie 9-102) used in the study and the relative variance between natural and artificial infection (%) at the locations Sattenhausen and Reinshof as well as the DON and 3-Ac-DON concentration of the naturally and artificially infected grain

| Protein fractions (g/kg) | Type | Sattenhausen | Reinhof |
|--------------------------|------|--------------|---------|
|                          |      | Natural infection | Artificial infection | Variance natural to artificial infection (%) | Natural infection | Artificial infection | Variance natural to artificial infection (%) |
| Albumins + globulins     | Total | 21.5 ± 1.0 | 21.2 ± 0.7 | −1 | 21.7 ± 0.7 | 21.6 ± 0.5 | 0 |
|                          | Total | 86.2 ± 3.9 | 84.8 ± 4.7 | −2 | 111.6 ± 7.7 | 117.6 ± 6.4 | 5 |
| Gliadins                 | o01,2 | 5.1 ± 1.1 | 4.9 ± 1.1 | −3 | 9.1 ± 1.7 | 9.9 ± 2.4 | 9 |
|                          | χ     | 37.5 ± 1.1 | 36.9 ± 1.1 | −1 | 50.2 ± 4.2 | 53.0 ± 2.8 | 6 |
|                          | γ     | 42.3 ± 4.5 | 41.7 ± 5.5 | −2 | 50.6 ± 5.7 | 52.8 ± 5.8 | 4 |
|                          | Total | 14.7 ± 1.3 | 13.1 ± 1.9 | −11 | 23.4 ± 7.0 | 18.8 ± 4.5 | −20 |
| Gliadin/glutenin         | Ratio | 0.5 ± 0.2 | 0.5 ± 0.2 | 0 | 1.2 ± 0.5 | 0.9 ± 0.4 | −28 |
| Extract proteins         | Total | 100.9 ± 3.2 | 97.9 ± 4.4 | −3 | 135.0 ± 2.0 | 136.4 ± 4.7 | 1 |
| Protein content          | Total | 117.5 ± 0.5 | 105.5 ± 1.0 | −10 | 174.1 ± 4.8 | 143.3 ± 2.9 | −18 |
| Gluten                   | Ratio | 5.9 | 6.5 | – | 4.8 | 6.3 | – |
| Protein content          | Total | 122.4 ± 2.4 | 119.1 ± 3.7 | −3 | 156.7 ± 2.3 | 158.0 ± 4.9 | 1 |
| Toxin (mg/kg)            | 3-Ac-DON | 0.0 ± 0.0 | 0.1 ± 0.1 | – | 0.1 ± 0.1 | 0.4 ± 0.2 | – |
|                          | Total | 0.5 | 2.2 | – | 1.8 | 7.5 | – |
In contrast to gliadin, the glutenin content in the artificially infected samples showed a significant reduction of its main subunits HMW-GS and LMW-GS in both the emmer (Table 6) and the summer wheat cultivar (Fig. 2). The glutenin-bound \( \textit{o} \text{f} \text{b} \)-gliadins were also reduced, but not significantly. Therefore, we can conclude that a destruction of the glutenin fraction arises related to the infection (Table 4). A comparison between the results for emmer and wheat (Tables 4, 5; Fig. 2) indicates that the glutenin fractions and types are more strongly affected by \textit{Fusarium} infection in wheat than in emmer in spite of the lower degree of infection found in the wheat.

Although the total gluten content did not change significantly, the ratio between gliadin and glutenin content changed according to the extent of the infection in favour of gliadin. The relationship between the ratios of emmer and wheat showed a twice as high ratio for emmer compared to wheat (Table 6). This result is confirmed by an earlier study on wheat which also described an increase in the gliadins and a decrease in glutenin content in this cereal [11]. The explanations given for this include that the changes in glutenin synthesis during the later stages of kernel maturation occur largely due to a reduced protein synthesis invoked by \textit{Fusarium} infection and the inhibition of protein synthesis by the accumulation of DON [5, 8, 11]. In contrast, another study found no gliadin and glutenin monomer synthesis at different maturation stages; just polymeric glutenin formation was detected in the later stages [12].

For the present results, we have assumed an enzymatic degradation of the glutenin subunits by the fungal proteases. Such proteases of the \textit{Fusarium} spp. exoproteome include trypsin-like protease or serine protease, which are known to be protein-degrading enzymes [24–26]. The fragments released by this degradation are most likely to be more soluble in 60% ethanol, so that we could identify the glutenin fragments during the extraction step for the gliadin fraction. This would have led finally to the higher gliadin and lower glutenin contents found in the artificially infected samples in comparison to the naturally infected samples (Tables 1, 2, 3, 4, 5, 6; Fig. 2) [26].

A comparison of the emmer and wheat also indicated that in spite of the stronger infection present in the emmer, the glutenin degradation was not as high as in wheat. The reasons for this effect may be the existence or higher expression of protease inhibitors (serine proteinase inhibitors) in emmer than in wheat [27].

Altogether, the comparison of the emmer and wheat gluten fractions showed that the gliadin fraction is the main fraction in both species (emmer: 71%; wheat: 56%), but that emmer had a 50% higher content than wheat. The
glutenin fractions in emmer are present in the same quantities as in wheat (Tables 1, 2, 3, 4, 5, 6). The higher gliadin content in emmer found in this study may be a result of enhanced gene expression based on upstream factors of the gene, which result in a higher gliadin/glutenin ratio in comparison to wheat [28, 29].

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Effect of N supply at two different locations on the protein content

The emmer cultivars showed at both locations (SH and RH) a 30% higher protein content than in the wheat (Tables 1, 2, 3, 4, 5, 6). The protein contents found in the emmer in this

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**Table 6** Summary of the protein concentration (g/kg) in naturally and artificially *Fusarium*-infected grain from all three emmer cultivars (Far-108+Hein-101, Klein, Linie 9-102) and the wheat cultivar Amaretto with the relative variance between natural and artificial infection (% at both the Sattenhausen and Reinhof locations as well as the DON and 3-Ac-DON concentration of the naturally and artificially infected grain

| Protein fractions (g/kg) | Type | Sattenhausen | Reinhof |
|--------------------------|------|-------------|---------|
|                          |      | Natural infection | Artificial infection | p⁰ | Natural infection | Artificial infection | Variance natural to artificial infection (%) | Natural infection | Artificial infection | Variance natural to artificial infection (%) |
| **Albumins + globulins** | Total | 21.6 ± 0.6 | 21.4 ± 0.5 | −0.8 | 0.18 | 23.4 ± 0.6 | 22.7 ± 0.8 | −2.6 |
|                          | Total | 98.9 ± 14.3 | 101.3 ± 17.9 | 2.4 | 0.25 | 62.2 ± 0.41 | 69.6 ± 2.3 | 11.8 |
|                          | o5   | 7.1 ± 2.5  | 7.4 ± 3.1  | 4.5 | 0.31 | 2.9 ± 0.25 | 3.2 ± 0.4 | 9.9  |
| **Gliadins**             | o1,2 | 1.5 ± 0.3  | 1.6 ± 0.6  | 7.8 | 0.39 | 6.2 ± 0.64 | 8.3 ± 2.7 | 33.4 |
|                          | α    | 43.8 ± 7.1 | 45.0 ± 8.6 | 2.6 | 0.24 | 23.1 ± 0.67 | 26.0 ± 1.0 | 12.5 |
|                          | γ    | 46.5 ± 6.2 | 47.3 ± 7.6 | 1.7 | 0.29 | 30.0 ± 1.15 | 32.1 ± 0.1 | 7.0  |
| **Glutenins**            | Total | 19.1 ± 6.3 | 15.9 ± 4.2 | −16.3 | 0.02 | 25.5 ± 6.6 | 14.3 ± 3.2 | −43.9 |
|                          | o5   | 0.9 ± 0.5  | 0.7 ± 0.3  | −19.4 | 0.10 | 0.8 ± 0.07 | 0.5 ± 0.1 | −33.3 |
|                          | HMW-GS | 3.6 ± 1.7 | 2.9 ± 1.2 | −21.6 | 0.02 | 7.2 ± 2.45 | 3.4 ± 1.2 | −52.5 |
|                          | LMW-GS | 14.6 ± 4.2 | 12.4 ± 2.7 | −14.8 | 0.03 | 17.5 ± 3.48 | 10.4 ± 1.9 | −40.8 |
| **Gluten**               | Total | 118.0 ± 18.0 | 117.2 ± 20.5 | −0.7 | 0.59 | 87.7 ± 7.07 | 83.9 ± 5.5 | −4.3 |
| **Gliadin/glutenin Ratio** | o1,2 | 5.2 | 6.4 | − | 2.4 | 4.9 | − |
| **Extract proteins**     | Total | 139.6 ± 18.0 | 138.6 ± 20.7 | −0.7 | 0.73 | 111.1 ± 6.4 | 106.6 ± 6.3 | −4.0 |
| **Protein**              | Total | 180.2 | 178.8 | −0.8 | 0.54 | 141.1 | 141.2 | 0.1 |
| **Toxin (mg/kg)**        | 3-Ac-DON | 0.0 ± 0.0 | 0.2 ± 0.2 | − | − | 0.0 ± 0.0 | 0.1 ± 0.0 | − |
|                          | Total | 1.1 | 4.9 | − | − | 0.2 | 2.9 | − |

<sup>⁰</sup> p significance of difference between natural and artificial infected emmer cultivars on both locations
The total protein content in emmer seems to be significantly connected to the conditions present at the growing location (Figs. 3, 4, 5), which is supported by the results of the investigated wheat cultivar. The results for the wheat in this investigation confirm the results from a previous study [19].

For the impact of the location on the extracted protein fractions in emmer and wheat, we considered both the

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**Fig. 3** Quantities of protein fractions in emmer (n = 3) and wheat cultivar at two locations (SH Sattenhausen, RH Reinshof) according to the N supply (RH > SH); the significant changes for emmer are denoted as *p* values

**Fig. 4** Quantities of gliadin types in three emmer cultivars and a wheat cultivar at two locations (SH Sattenhausen, RH Reinshof) dependent on the N supply (RH > SH); significant changes for emmer are denoted as *p* values

**Fig. 5** Quantities of glutenin types in emmer (n = 3) and wheat cultivar at two locations (SH Sattenhausen, RH Reinshof) dependent on N supply (RH > SH); significant changes for emmer are denoted as *p* values
naturally and artificially fungal-infected samples together. The emmer showed a 30% and wheat an 8% higher total protein content at RH than at SH (Table 8). The percentage distribution of the different proteins showed that the gluten content rose but the amount of albumins and globulins was just marginally affected by the location—the total content in g/kg was only changed by about 1–3% (Table 8; Fig. 3). This effect has also been described for wheat in the literature [15, 28]. Albumins and globulins are mainly metabolic and structural proteins [30] and have functions in cell metabolism. There is a minimum amount of these compounds needed for these functions, but no increase in content occurs with respect to the N supply.

The gliadin content (g/kg) in emmer changed significantly (Fig. 3): at RH 36% more gliadin was found in the grain than at SH and the glutenin content was also about 50% higher at RH (Table 8). Comparing the emmer to wheat, it can be said that the emmer storage proteins were more affected by the N supply at the location than wheat as the gliadin fraction in wheat was not or just minimally affected (Fig. 4; Table 8). In contrast, the wheat’s glutenin fraction was affected in the same range as in the emmer (Fig. 5; Table 8). A consequence of these species-specific changes on the gluten fractions according to N supply is that the gliadin/glutenin ratio in wheat is more affected than in emmer (Tables 4, 5), which could result in positive effects on its bread-making quality [28, 31]. The reason for this difference in sensibility to N supply is possibly due to a regulation of synthesis. N availability and the resulting higher total protein content have been documented as a factor that can promote gene expression in other cereals such as wheat or maize [29]. Hypothetically, raised N levels in emmer cultivars lead to a more enhanced gene expression of the storage proteins than in wheat.

Conclusions

Emmer, normally grown under organic farming conditions, showed similar characteristics to wheat cultivars in their response to Fusarium spp. infection. In addition to the basic findings, new cognitions on the degradation and changes of protein subunits were found. Therefore, further investigations with a greater focus on protein degradation can be recommended. The influence of the growing location on cereal protein content has been well-documented in previous studies on wheat. The present results showed that this is also true in emmer, although there were differences between emmer and wheat. Further studies in this context may clarify the mechanisms that lead to the irregular synthesis of emmer protein subunits in comparison to wheat as a result of a mixed Fusarium infection and differing nitrogen availability.

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