


Article

Characterization of Agronomical and Quality Traits of Winter Wheat (*Triticum aestivum* L.) for Fusarium Head Blight Pressure in Different Environments

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Abstract: For food security, it is essential to identify stable, high-yielding wheat varieties with lower disease severity. This is particularly important due to climate change, which results in pressure due to the increasing occurrence of Fusarium head blight (FHB). The objective of this study was to evaluate the stability of winter wheat (*Triticum aestivum* L.) grain yield under different environmental conditions. Twenty-five winter wheat varieties were evaluated under two treatments (naturally-disease infected (T1) and FHB artificial stress (T2)) during two growing seasons (2018–2019 to 2019–2020) in Osijek and in 2019–2020 in Tovarnik. The interaction between varieties and different environments for grain yield was described using the additive main-effects and multiplicative interaction (AMMI) effects model. The Kraljica and Fifi varieties were located near the origin of the biplot, thus indicating non-sensitivity to different environmental conditions. Principal component analysis (PCA) was used to understand the trait and environmental relationships. PC1 alone contributed 42.5% of the total variation, which was mainly due to grain yield, 1000 kernel weight and test weight in that respective order. PC2 contributed 21.1% of the total variation mainly through the total sedimentation value, test weight, wet gluten and protein content ratio (VG/P) and wet gluten content, in descending order.

Keywords: environment; fusarium head blight; quality traits; wheat; yield stability



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1. Introduction

Wheat is one of the major grain crops in the world and provides food for about two billion people [1]. It is the primary source of carbohydrates along with essential minerals, vitamins, and lipids [2]. It has been predicted that the needs for wheat production will increase by 2050 due to the development of the industry and the acceleration of urbanization, which could lead to a food crisis considering the fact that global wheat yields have remained constant for more than a decade [3]. Additionally, stressful environmental conditions can contribute to a significant decrease in grain yield and quality. Consequently, biotic stresses such as pests and disease cause considerable damage to the crops. For example, susceptible genotypes with high disease severity have resulted in more than 30% of grain yield losses [4]. Furthermore, it was reported that fungal infestations such as rust, septoria and fusariosis account for nearly 20 to 100% of the losses in wheat production [5]. Therefore, under climate change is very important to maintain grain yield and quality [6]. Fusarium head blight (FHB) is one of the major fungal diseases in temperate wheat growing regions [7]. This is a dangerous disease for cereals, and is mostly caused by *Fusarium graminearum* Schwabe and *F. culmorum* (W. G. Smith) Sacc. [8]. Besides causing significant damage in terms of yield quality and quantity worldwide [9], mycotoxin contamination produced by *Fusarium* spp. can be harmful to both humans and animals [10].

Due to different environmental conditions, wheat grain yield depends on the ability to cultivate a wheat variety suitable for a particular climate in a defined region [11]. One of

the challenges for wheat breeders is how to achieve genetic gain in the yield of wheat under biotic stress. To improve genetic gain when performing phenotypic selection, evaluation of trait stability and the genotype–environment interaction (GEI) could facilitate the breeding of crop varieties with increased tolerance to stressful environments [12]. The additive main-effects and multiplicative interaction effects (AMMI) model is used to analyze the GEI, including identification of mega-environments, determination of varieties to exploit narrow adaptations, and prediction of variety production [13]. A good understanding of the host-pathogen relationship is important in efficient breeding for disease resistance, and therefore the study of GEI interactions is essential. Thus, material for wheat breeding is commonly grown at different locations and years to determine whether different environments affect the quantitative traits of varieties, such as yield and disease severity [14].

Environmental factors influence quantitative traits to a different extent and in different ways [15]. It might be of interest to find the generalities in the interrelations that occur among correlated traits [16]. Principal component analysis (PCA) is often used for this. The main purpose of PCA is to explain the correlation between many variables in terms of a small number of underlying independent factors [17]. Accordingly, the objectives of this study were: (1) to identify winter wheat varieties with stability and higher yield performance in stressful conditions such as high FHB pressure and natural disease infection without usage of fungicides; and (2) to check the association among agronomical and quality traits of winter wheat, along with environment grouping, using PCA analysis. It is essential that wheat breeding programs provide growers with options that fit their local growing conditions and to balance wheat yield and end-use quality. There is a strong need to increase the yield potential and improve wheat quality in respect to climate change and rising demand for healthy wheat products.

2. Materials and Methods

2.1. Field Experiments

The field experiments were conducted in two vegetative seasons: 2018–2019 and 2019–2020 in Osijek (45°27' N, 18°48' E) and in 2019–2020 in Tovarnik (45°10' N, 19°09' E). Those locations are the main wheat growing region in Croatia and have different soil types: in Osijek the soil is eutric cambisol (pHKCl–6.25, humus–2.00–2.20%) and Tovarnik has black soil chernozem (pHKCl–7.42, humus–2.75–3.00%). The climate conditions during the growing season differ significantly in regard to the amount of rainfall in these environments. The total rainfall in Osijek in the growing season during 2018–2019 was 531.3 mm (Figure 1a), while in 2019–2020 in Osijek and Tovarnik it amounted to 408.6 and 448.3 mm, respectively (Figure 1b,c). The average annual temperatures during the growing period were 10.9, 11.1 and 11.7 °C, in Osijek in 2018–2019, and in Osijek and Tovarnik in 2019–2020, respectively. A completely randomized block design was applied with two experimental replications in two treatments (naturally disease infected (T1) and artificially inoculated with *Fusarium* spp. (T2)). Plots consisted of eight row plots, 7 m in length and 1 m wide, at a sowing rate of 330 seeds m⁻². To control seed borne diseases the seed was treated with Vitavax 200 FF (thiram + carboxin) at a rate of 200 g Vitavax for 100 kg of seeds. Standard agrotechnical practice was used as per commercial wheat production (fungicide application was excluded in both treatments in the investigated environments). Weed control was conducted with a herbicide at wheat tillering (GS 31). In Tovarnik, Lancelot 450 WG herbicide (Dow AgroSciences, Indianapolis, IN, USA) was used at a rate of 0.03 L ha⁻¹. In Osijek, Sekator OD herbicide (Bayer Crop Science, Cambridge, Great Britain) was used in both years and applied at a rate of 0.15 L ha⁻¹. Insecticides were sprayed in spring of both growing seasons. In Tovarnik in April 2020, Direkt insecticide (Arysta LifeSciences Benelux, Liege, Belgium) was applied at a rate of 0.12 L ha⁻¹ and in May 2020, Karate Zeon insecticide (Syngenta Crop Protection AG, Waterford, Ireland) was applied at a rate of 0.15 L ha⁻¹. In Osijek, in both years in April and May, Karate Zeon (Syngenta Crop Protection AG, Waterford, Ireland) was applied at a rate of 0.15 L ha⁻¹. Fertilization varied during this research (N:P:K 120–140:80–100:120–150 kg ha⁻¹).

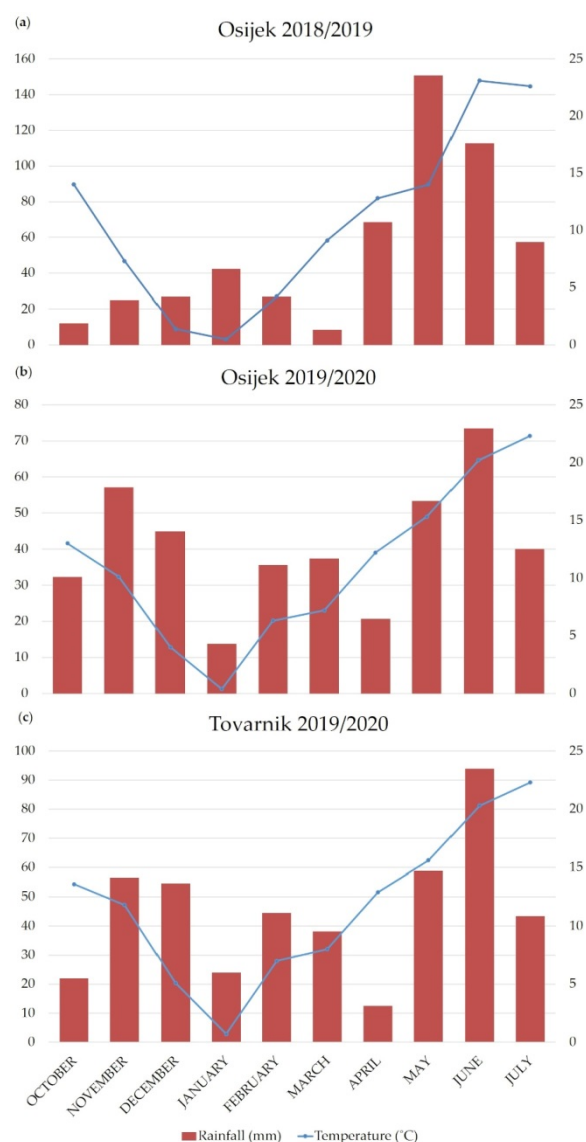


Figure 1. Climate diagram for vegetation season 2018–2019 (a) and 2019–2020; (b) in Osijek and for 2019–2020; (c) in Tovarnik, Croatia.

A Wintersteiger cereal plot combine-harvester was used for harvesting the grains from the whole plot. The grain yield was measured by harvesting the whole area of each plot and then corrected to 14% moisture (on a wet basis) and converted into dt ha^{-1} . A measure of density (mass/volume; test weight (kg hl^{-1})) was obtained by using the GAC 2100 (DICKEY-john) analyzer. A MARVIN grain analyzer was used to calculate the 1000 kernel weight. Protein content was measured by Infratec 1241, Foss Tecator. Wet gluten content and the gluten index were obtained by ICC method No. 155. ICC method No. 116/1 and ICC method No. 107/1 were used for measurement of the Zeleny sedimentation volume and falling number.

2.2. *Fusarium* Inoculum Production, Inoculation Procedure and Disease Assessment

Fusarium graminearum strain (PIO 31), obtained from the wheat in East Croatia and *F. culmorum* strain (IFA 104), obtained from IFA, Tulln (Austria), were blended together (1:1) and used for inoculum production in three different experiments. Conidial inoculum of *Fusarium* spp. spores were produced by a blend of wheat and oat grains (3:1 by volume) [18]. Firstly, grains were saturated with water overnight, then seeds were filtrated and autoclaved. The *Fusarium* strain was added into seeds, which were kept for 2 weeks at

25 °C in the dark. Hemocytometer was used for determination of conidial concentrations + which were set to $10 \times 10^4 \text{ mL}^{-1}$. *Fusarium* inoculum (100 mL) was sprayed on an area of m^2 . One treatment was grown according to standard agronomical practice with no usage of fungicide and without misting treatment, while another treatment was subjected to two inoculation events using a tractor-back (Osijek) and hand sprayer (Tovarnik) with *Fusarium* spp. at the time of flowering (Zadok's scale 65) [19]. Misting was done by spraying several times with a tractor back-sprayer. On days 10, 14, 18, 22 and 26 after inoculation the percentage of bleached spikelets per plot was estimated according to a linear scale (0–100%) which showed general FHB resistance of wheat varieties. According to modified formula from Shaner and Finney [20] the area under the disease progress curve (AUDPC) for FHB severity was calculated.

2.3. Statistical Analysis

Statistical R package version 1.3-3 (R Core Team, 2020) [21] was used to conduct the AMMI model [22] and principal component analysis (PCA), while R package version 0.55 [23] was used to produce PCA figure.

The data were subjected to analysis of variance (ANOVA) using an appropriate model, followed by Fisher's Least Significant Difference (LSD) test ($\alpha = 0.05$) by Statistica version 12.0 (Statsoft Inc., Tulsa, OK, USA). The LSD value was used to evaluate whether the observed difference in performance between environments or varieties was significant.

3. Results

3.1. *Fusarium* Head Blight (FHB) Scoring in *Fusarium* Artificially Inoculated Treatment

No visible disease symptoms were found in the varieties from the naturally infected plots, and without usage of fungicide. Therefore, the FHB assessment was not made in those plots. FHB severity, expressed as general resistance, varied across both environments (Table 1) with FHB artificially inoculated treatment. Overall, the area under the disease progress curve (AUDPC) for FHB severity per variety in Osijek in 2019 ranged from 17 (Apache) to 392 (Feliks). The AUDPC for disease severity per variety at Osijek in 2020 ranged from 0.3 (Rujana) to 250 (Srpanjka). At Tovarnik, in 2020, it ranged from 0.4 (Rujana) to 455 (Feliks). In Osijek in 2019, the AUDPC for disease severity for all varieties on average was significantly higher (192), compared with 2020 in Osijek and Tovarnik (66 and 91, respectively).

3.2. Stability of Wheat Grain Yield in Six Environmental Conditions

The combined analysis of variance for the grain yield revealed that mean squares (MS) due to variety and two treatments (natural disease infection-T1 and artificial inoculation-T2) in different environments were highly significant for grain yield ($p < 0.001$) (Table 2). In general, environment showed the largest effect followed by variety. The interaction of variety*environment (GE) was highly significant ($p < 0.001$).

The stability of investigated wheat varieties can be evaluated according to the biplot for grain yield (Figure 2). When applying the AMMI analysis, the two first PCs explained 77.8% (61.4 and 16.5% by PC1 and PC2, respectively) of the variance in the original variables. The PC1 axis separated three environments with natural disease infection treatment and three environments with FHB artificial infection treatment. Therefore, all environments in natural infection had positive PC1 values and were located at the right side of the biplot.

Table 1. The area under the disease progress curve (AUDPC) calculation for 25 winter wheat varieties in *Fusarium*-inoculated treatment in three investigated environments.

Varieties	AUDPC		
	Osijek 2018/19	Osijek 2019/20	Tovarnik 2019/20
FELIKS	392	203	455
DEMETRA	369	92	150
SRPANJKA	362	250	291
KATARINA	348	83	158
FIFI	339	101	159
SOFRU	331	94	151
GOLUBICA	298	104	93
BUBIMIR	263	90	34
TATA MATA	256	56	159
EL NINO	217	137	213
ANDELKA	216	67	76
OS OLIMPIJA	198	60	49
PEPELJUGA	189	53	14
ANTONIJA	179	49	33
KRALJICA	139	72	18
TIKA TAKA	129	43	70
FICKO	125	29	37
VULKAN	117	36	34
SILVIJA	95	13	39
BUBNJAR	62	10	19
GALLOPER	59	1	18
BOLOGNA	55	16	3
FOXYL	33	3	0.8
RUJANA	17	0.2	0.4
APACHE	17	7	1
AUDPC	91b *	66c	192a

* Different lower-case letters represent significantly different values ($p < 0.05$) within each environment.

Table 2. Analysis of variance for grain yield in six different environments.

Source of Variation	Df	MS	F-Value
Variety (G)	24	860.06	14.68 ***
Environment (E)	5	26,390.00	450.35 ***
G × E	120	154.00	2.62 ***
Residuals	150	59	

*** = significant at $p < 0.001$, respectively; Df = degrees of freedom, MS = mean square.

The wheat varieties with the most similar yield across environments, which were grouped in the middle of the biplot and which were found to be the most stable, were Kraljica and Fifi. Varieties Sofru, El Nino and Golubica appeared to be very unstable over both the environments based on their significant distance from the origin of the biplot graph. Both Osijek in 2019–2020 and Tovarnik in 2019–2020 with natural disease infection had the highest positive PC1 score and were the main contributors to the phenotypic stability of the varieties. Other environments made a significant contribution to the G × E interaction, since they were positioned further from the origin.

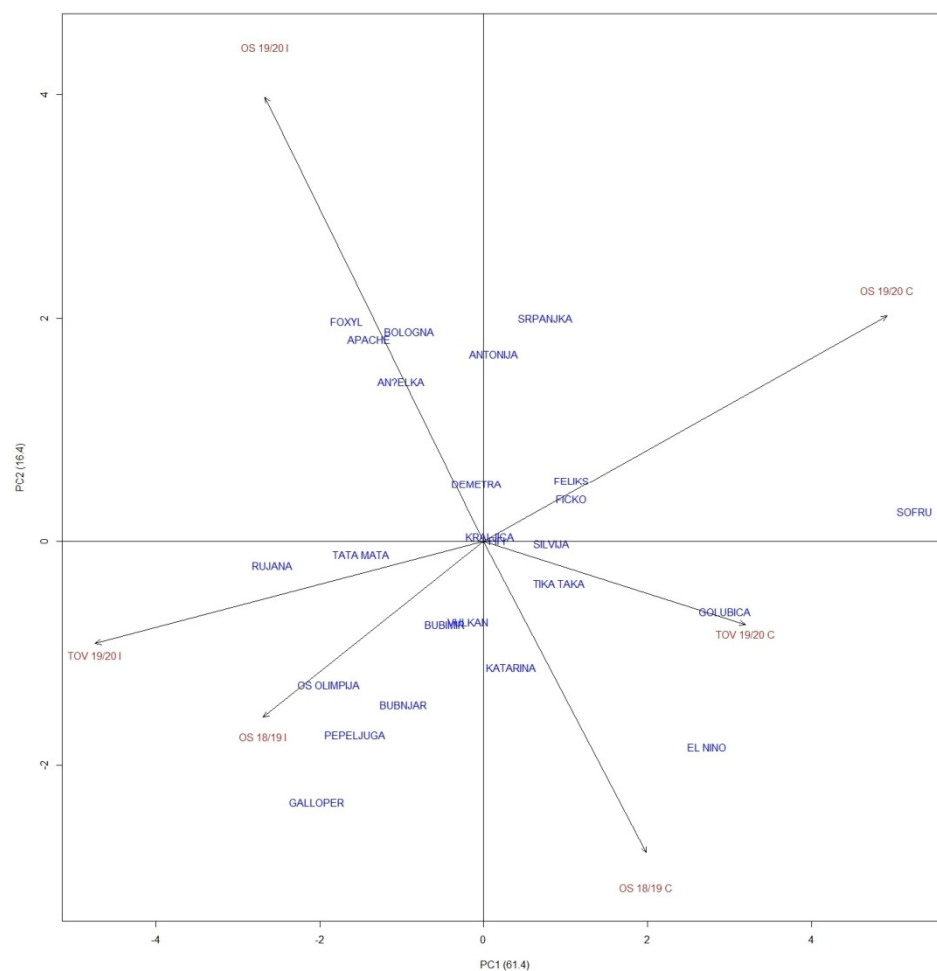


Figure 2. Additive main-effects and multiplicative interaction (AMMI) biplot showing two main axes of interaction (PC1 vs. PC2) in 25 winter wheat varieties from six environments. OS 18/19 C, OS 19/20 C, TOV 19/20 C—Osijek in 2018–2019, Osijek and Tovarnik in 2019–2020 in natural infection, respectively; OS 18/19 I, OS 19/20 I, TOV 19/20 I in FHB inoculated treatment, respectively.

Varieties at the highest point in certain sections of the graph had the highest grain yield values in environments located in the same section. The Feliks and Ficko varieties interacted positively with the Osijek location in 2019–2020 with natural infection, but negatively with Tovarnik in 2019–2020 and Osijek 2018–2019 in FHB inoculated treatment areas. Golubica was adapted to the Tovarnik environment in 2019–2020 in a naturally infected environment, but not with other environments. Tovarnik in 2019–2020 and Osijek in 2018–2019 with FHB inoculated treatment had the highest negative PC1 score and negative interaction with El Nino and positive interaction with Galloper, Pepeljuga, Bubnjar and Os Olimpija varieties. Other lower specific associations were revealed for varieties, FoxyL, Apache and Bologna in Osijek 2019–2020 with FHB inoculated treatment.

Grain yield in naturally infected treatment areas varied from 76.2 dt ha⁻¹ (Olimpija) to 109.9 dt ha⁻¹ (Sofru) in three environments on average. In Fusarium inoculated treatment areas, it ranged from 40.5 dt ha⁻¹ (Golubica) to 94.6 dt ha⁻¹ (FoxyL). The relative decrease in grain yield in inoculated treatment areas, compared to natural infection for 25 winter wheat varieties in three environments, was on average up to 28.4%. The reduction in grain yield in FHB inoculated treatment compared to the naturally infected treatment in three environments, was on average, the highest in varieties, Sofru (57.1%), Golubica (53.9%) and El Nino (45.8%). The lowest reduction in grain yield was obtained in Rujana (10.3%), FoxyL (11.8%), Apache (14.0%) and Galloper (17.7%) varieties (Figure 3).

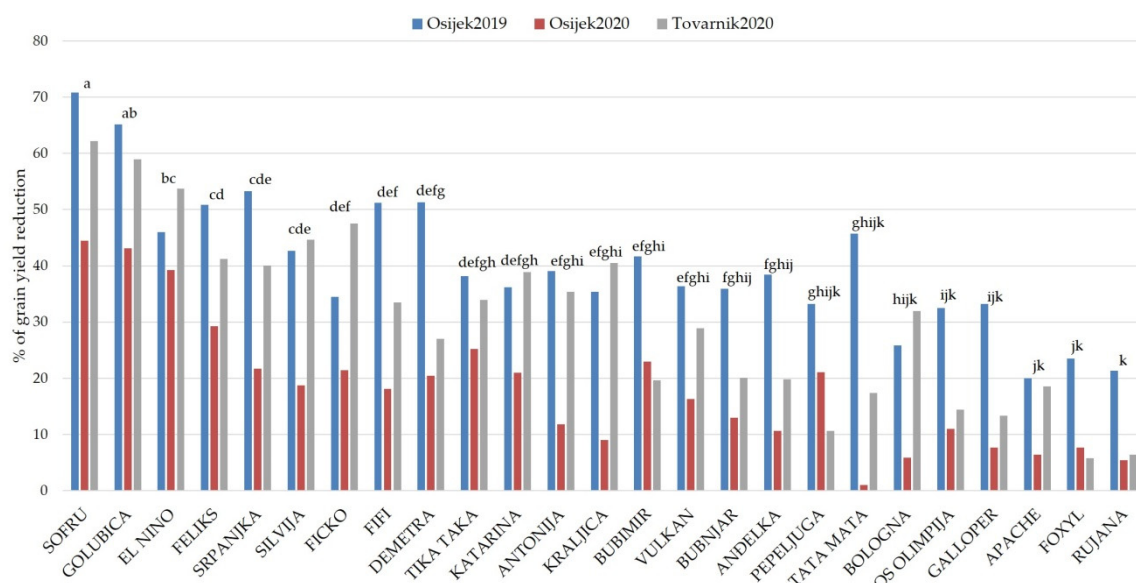


Figure 3. Percentage of grain yield reduction in FHB inoculated treatment compared to naturally infected treatment in three environments. Different lower-case letters represent significantly different values ($p < 0.05$) within wheat varieties in three environments on average.

3.3. Relation among Important Agronomical and Quality Traits in Six Environments

Principal component analysis was performed for nine traits of winter wheat in six different environments. Out of nine traits, nine principal components (PCs) exhibited 1.000 SS loadings (Table 3), and showed a maximum variability of about 99.9% among the traits studied. PC1 had the highest variability (42.5%) followed by PC2 (21.1%), PC3 (12.4%), PC4 (9.2%), PC5 (7.5%), PC6 (4.3%), PC7 (1.5%), PC8 (1.4%) and PC9 (0.1%).

Table 3. Vector loadings and percentage of variation explained by the nine principal component axes (PCAs). *GY-Grain yield, TW-test weight, TKW-thousand kernel weight, P-protein content, SED-sedimentation value, VG-wet gluten content, GI-gluten index, FN-falling number, SD-standard deviation, % of TV-% of total variation.

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
*GY	0.464	0.160	0.000	0.141	0.132	0.000	0.294	0.794	0.000
TW	0.359	0.456	0.000	0.103	0.137	−0.110	0.561	−0.552	0.000
TKW	0.399	0.344	0.108	0.000	0.222	−0.335	−0.737	0.000	0.000
P	−0.414	0.186	0.146	0.366	−0.158	−0.56	0.000	0.126	0.523
SED	0.000	0.556	−0.152	0.322	−0.489	0.529	−0.191	0.000	0.000
VG	−0.427	0.359	0.000	0.000	0.106	−0.303	0.000	0.165	−0.738
GI	0.274	0.000	−0.506	−0.382	−0.601	−0.395	0.000	0.000	0.000
FN	0.000	0.143	0.778	−0.485	−0.368	0.000	0.000	0.000	0.000
VG/P	−0.245	0.393	−0.270	−0.587	0.376	0.178	0.000	0.114	0.425
SS loadings	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SD	1.955	1.377	1.056	0.909	0.822	0.622	0.373	0.360	0.083
% of TV	42.487	21.067	12.383	9.185	7.510	4.304	1.549	1.437	0.077

The PCA classified the estimated wheat variables into two main components: PC1 accounted for about 42.5% of the variation and PC2 for 21.1% (Figure 4). The traits that contributed most positively to PCA1 were grain yield (0.464), 1000 kernel weight (0.399) and test weight (0.359). The other trait that contributed positively to PC1, was gluten index (0.274). Wet gluten content (VG) (−0.427), protein content (P) (−0.414), and VG/P (−0.215) had negative contributions to the observed variations in PC1. However, sedimentation value and falling number had no contribution to PC1. The observed variations in PC2 were mainly due to sedimentation value, test weight, VG/P and wet gluten content, which

contributed 0.556, 0.456, 0.393 and 0.359, respectively. The third PCA contrast variable is related solely to α -amylase enzyme activity (falling number), while a negative contribution came from gluten index, VG/P and sedimentation value. The variations for PC4 were mainly due to protein content and sedimentation value, which were 0.366 and 0.322, respectively. Likewise, 1000 kernel weight and test weight in PC5; sedimentation value in PC6; test weight in PC7; grain yield in PC8; and protein content in the PC9 were the major contributing traits for variability in those principal components (Table 3).

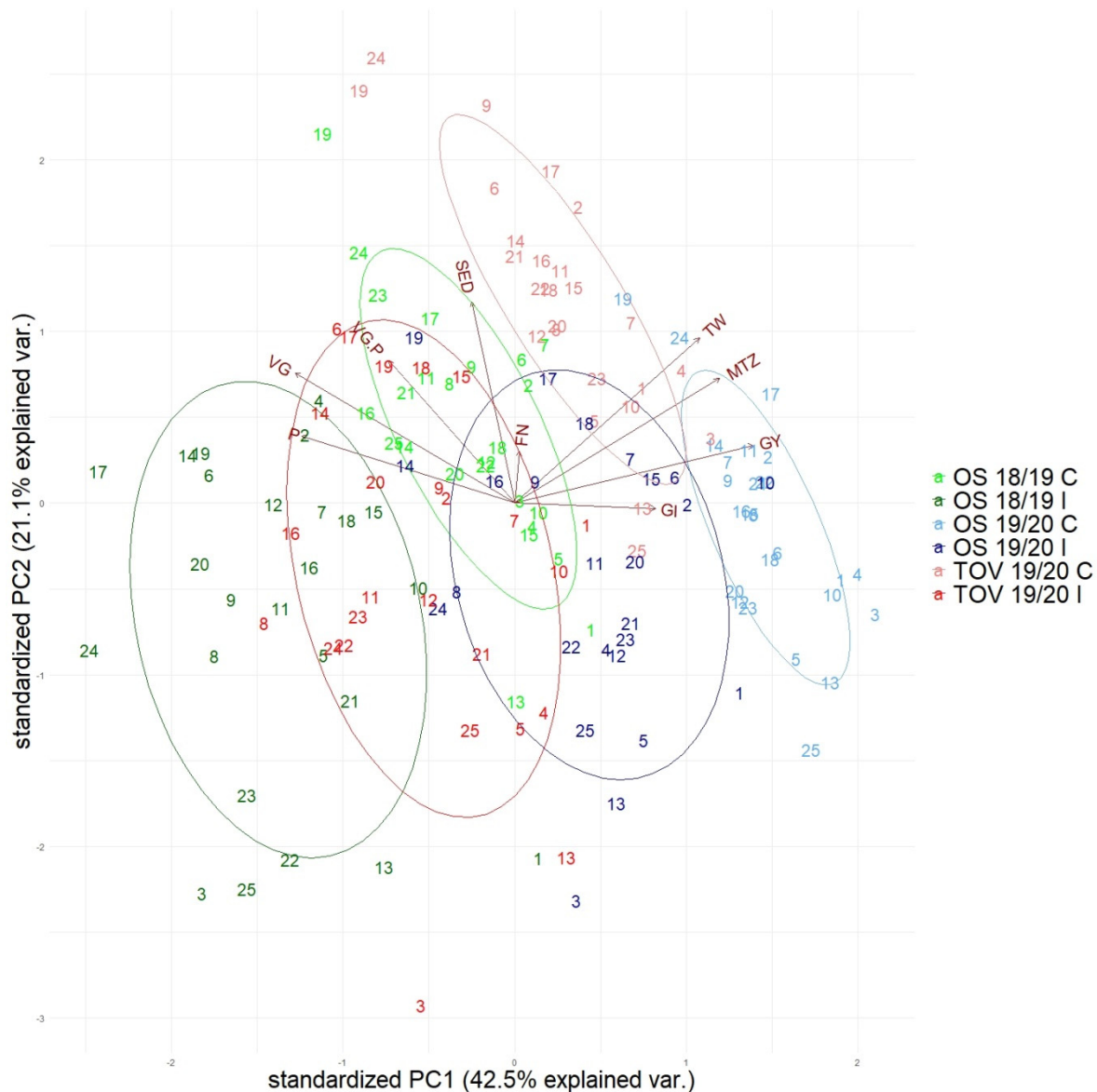


Figure 4. Plot of the first two PCAs showing the relationship among investigated wheat traits and different environments. GY-Grain yield, TW-test weight, MTZ-thousand kernel weight, P-protein content, SED-sedimentation value, VG-wet gluten content, GI-gluten index, FN-falling number, VG.P-ratio of wet gluten and protein content. OS 18/19 C, OS 19/20 C, TOV 19/20 C—Osijek in 2018–2019, Osijek and Tovarnik in 2019–2020 in natural infection areas, respectively; OS 18/19 I, OS 19/20 I, TOV 19/20 I in FHB inoculated treatment areas, respectively.

The first two principal components contributed more than half of the variance and were plotted to observe the relationship between the measured wheat traits and different environments (Figure 4). The position of the varieties on the PCA plot indicated the tendency of relative grouping in six distinct groups. The ordination was mainly determined

by yield related traits on PC1, and to a lesser extent by yield related traits on PC2. The most prominent relationships shown were: a strong positive association between grain yield, test weight and 1000 kernel weight; and between protein content (P), wet gluten content (VG) and VG/P. The PCA plot also showed that varieties depicted on the right part of the plot had high values of grain yield, 1000 kernel weight and test weight; and those depicted on the left side of the plane had higher values for quality traits.

Strong associations could be seen between grain yield and varieties from natural infection areas in Osijek in 2019–2020 (Figure 4). Those varieties on average had significantly higher grain yield, 1000 kernel weight and gluten index compared with the other two environments (Table 4). The nearest group contained varieties from Tovarnik in 2019–2020 with natural infection. From both of those environments, varieties subjected to FHB inoculation were located at the middle part of the plot and less tightly clustered than either group. The low-yielding varieties from Osijek in 2018–2019 from FHB inoculated treatment areas were located on the left side of the plot, exhibiting increased quality traits. The lowest grain yield in the inoculated treatment area was recorded for varieties at Osijek in 2018–2019, along with the lowest test weight and 1000 kernel weight. However, varieties from this location had the highest significant protein content, sedimentation value and wet gluten content (Table 4). The highest sedimentation level was found at Osijek in 2018–2019 and Tovarnik 2019–2020 in natural infection areas, where varieties from the Osijek environment obtained the lowest yield. As yield increased over the environments, the sedimentation value decreased, reaching a minimum at Osijek 2019/2020 with natural infection. In the inoculated treatment, the significantly highest sedimentation value was obtained at Osijek in 2018–2019, compared with other two environments in this treatment.

Table 4. Significant differences between three environments considering nine studied traits in natural infection and FHB treatment. *GY-Grain yield, TW-test weight, 1000 KW-thousand kernel weight, P-protein content, SED-sedimentation value, VG-wet gluten content, GI-gluten index, FN-falling number, different lower-case letters represent significantly different values ($p < 0.05$) within the environment with naturally infected and FHB inoculated treatment.

Naturally Infected		Trait							
Environment	GY*	TW	1000 KW	P	SED	VG	GI	FN	VG/P
Osijek 2018/2019	80.4 c	78.0 c	33.9 c	13.2 a	38.5 a	28.8 a	89.5 c	335.2 a	2.2 b
Osijek 2019/2020	112.5 a	83.6 b	44.4 a	10.7 c	30.9 b	22.0 b	98.8 a	347.3 a	2.0 c
Tovarnik 2019/2020	87.2 b	85.2 a	43.0 b	12.8 b	38.2 a	29.1 a	94.5 b	338.5 a	2.3 a
FHB Inoculated									
Osijek 2018/2019	48.4 c	64.7 c	26.8 c	14.1 a	32.8 a	31.1 a	86.4 a	327.2 b	2.2 b
Osijek 2019/2020	91.8 a	78.2 a	38.3 a	12.6 b	30.4 b	24.9 c	89.5 a	370.7 a	2.0 c
Tovarnik 2019/2020	60.2 b	73.9 b	32.5 b	12.5 a	29.1 b	28.8 b	88.3 a	351.8 a	2.3 a

3.4. Impact of FHB Inoculation on Investigated Traits in Relation to Natural Infection Treatment

In naturally infected treatment, wheat varieties had higher test weight compared to artificially inoculated treatment. In general, significantly highest reduction in test weight in average at three locations had Sofru (28.9%), followed by Golubica (20.5%) and Feliks (19.1%). The lowest reductions of test weight were obtained by varieties Apache (3.4%) and Rujana (4.3%) (Figure S1a). The highest significant reduction in 1000 kernel weight in FHB inoculated compared to natural infection treatment included Sofru (41.3%), Golubica (32.0%) and El Nino (30.8%). The lowest reductions were for had Apache (6.9%) and Rujana (7.7%) (Figure S1b). Golubica (30.5%) had the highest significant reduction in sedimentation value in inoculated treatment compared to naturally infected treatment. This reduction was significantly different from Foxyl (12.3%). A significant increase in sedimentation value occurred in Pepeljuga (−3.5%) and Tata Mata (−4.2%) (Figure S1c). There were no significant differences between varieties in reduction of gluten index in inoculated treatment compared to natural infection (Figure S1d). Varieties Foxyl, Feliks, Antonija and

Sofru had the highest significant reduction in wet gluten and protein content ratio (VG/P) in FHB inoculated treatment compared to natural infection (Figure S1e).

The highest protein content in the three naturally infected environments, on average, was recorded in Os Olimpija (14.5%), Golubica (13.3%), Kraljica (13.1%), Fifi (13.1%) and Bubimir (13.0%). In *Fusarium* inoculated treatment, the lowest protein content was observed in Apache (11.3%) and Antonija (11.8%). A significant increase in protein in inoculated treatment compared to natural infection, was evident in the Sofru variety (12.8%) in contrast to Bologna with the lowest increase (0.2%) (Figure S2a). Bubimir and Fifi had a significantly high increase of wet gluten content (16.0 and 15.2%, respectively) in inoculated environments compared to naturally disease infected. There were few varieties in specific environments with a decrease in inoculated treatment compared with natural infection (Figure S2b). The highest falling number increase in naturally infected treatment compared to FHB inoculated treatment, was Tika Taka (23.1%) in three environments, on average. Some wheat varieties showed reductions in some environments (Figure S2c).

4. Discussion

4.1. Grain Yield Stability of Winter Wheat Varieties under Increased FHB Pressure and Natural Disease Infection

A total of 25 winter wheat varieties were assessed in three yield trials in two treatments in Croatia to determine the effects of different environmental conditions on FHB resistance together with the impact on different agronomical and quality traits. There were no visible disease symptoms on the wheat heads in naturally infected treatment areas and therefore no FHB scoring was done for that treatment. The first FHB scoring in *Fusarium* inoculated wheat was done once the first symptoms occurred at 10 days post inoculation (dpi) in three environments. In general, FHB severity had a linear increase during the time-course experiment. Excessive, prolonged wet weather during anthesis favored the development of higher levels of FHB disease in the vegetation season in Osijek in 2018–2019. This was expected because the yields of cereals vary according to the distribution of seasonal rainfall [24]. Grain yield is a quantitative trait, which is controlled by minor genes, under the strong influence of environmental factors with low to medium heritability. [25]. So therefore, its expression is the result of genotype (G), environmental factors (E) and GE interaction. In the current study, environment showed the highest variability. Previously, it was reported that only genotype and GE interaction are relevant to the evaluation of genotypes in multi-environment trials, although environment is responsible for about 80% of the total variability [26]. Therefore, multivariate procedures such as the additive main-effects and multiplicative interaction (AMMI) model can obtain the multi-dimensionality of GE interactions [27]. According to Lin and Binns [28], to obtain the most stable and adaptable varieties it is necessary to do an analysis of GE interaction. In the current study, significant GE interaction suggested that the yield of varieties varied across different environments with variable disease pressure. It was concluded that dissimilarity in the genetic systems, which control the physiological processes with yield stability in different environments, is caused by the large magnitude of GE interaction [29]. Therefore, it is not easy to select the best yielding and most stable genotypes [30]. In the current study, the reduction of grain yield in inoculated treatment compared to the naturally infected treatment in three locations, on average exceeded 70% in some varieties. In a previous study *Fusarium* infection reduced grain yield of some wheat varieties up to 64% [31]. Furthermore, according to some research, yield reduction was even up to 80% [32].

The main purpose of stability analysis (AMMI model) was to identify winter wheat varieties with better stability and higher yield performance, both in a natural disease environment and in highly stressful conditions with artificially increased FHB pressure. Although this might lead to controversial results in the estimation of the grain yield stability of winter wheat varieties, some varieties had a score near to zero and a grain yield near to the grand mean, and hence can be considered as stable. It was previously concluded that varieties with wide adaptation have quite high yield potential and good stress tolerance, whereas varieties adapted at individual locations have maximum levels of

either yield potential or stress tolerance [33]. In contrast to varieties near to zero, varieties further from the origin of the plot may either differ in yield or show a different response to different environments. The Sofru variety reacted positively to lower moisture and water resources from precipitation in April, May and June in 2020 in both the Osijek and Tovarnik locations and that is why the AMMI model revealed a positive interaction of Sofru with those two environments. The same variety had the highest grain yield reductions on average in FHB inoculated treatment compared with natural infection, followed by Golubica and El Nino. A similar pattern of reductions was obtained for 1000 kernel weight and test weight. In the AMMI biplot, these three wheat varieties with the highest grain yield reductions were located the furthest from the inoculated environments, showing instability due to increased variability over environments. So therefore, according to some studies the least stable genotypes have the highest yield productivity in specific environments [34]. Only the Foxy1 variety exhibited superiority in grain yield in more than one location (Osijek 2018–2019 in inoculated treatment and in all environments with natural infection). Also, grain reductions of Foxy1 in inoculated treatment compared to naturally infected, were significantly lower than in Sofru. According to Rodriguez et al. [35] the best ranking varieties at a majority of locations across a production area are characterized by considerable variation in different environments with yields substantially above the environmental means. Based on the cosine of angles of environment vectors, environments with natural infection were located at the opposite side of PC1 from the environments with FHB artificial inoculation. Environments with the same treatment were located relatively closer on the biplot. This was expected as the angle between the vectors of two environments is related to the correlation coefficient between them [36]. In general, varieties and environments in close distance on the plot graph had positive associations. The Osijek environment in 2019–2020 had the lowest amount of rainfall, which resulted in lower disease pressure and that is why that environment with natural infection is located on the positive PC1 and PC2, and is the most yielding environment (112 dt ha^{-1}), followed by the same location with FHB artificial inoculation (91.8 dt ha^{-1}) on the opposite side of the biplot. Furthermore, the FHB severity was three-fold higher in 2019 compared with 2020 in Osijek, and two-fold higher compared with 2020 in Tovarnik, for 25 wheat varieties on average. Increased disease pressure in Osijek in 2018–2019 resulted in smaller grains of uneven seed size. Due to decreased 1000 kernel weight and test weight, the yields in Osijek 2018–2019 in FHB inoculated treatment were about 47% lower compared to Osijek 2019–2020 in the same treatment environment.

4.2. Principal Component Analysis for Nine Agronomical and Quality Traits in Six Different Environmental Conditions

Different grain characteristics and quality traits were studied through principal component analysis (PCA) to get an outline of the relationship among these traits. Taken together, the PCA method indicated which components of grain yield and quality traits were the most important under different environments. Similar studies with different traits were previously performed in wheat [37]. The positive and negative correlation trends between the components and the variables were obtained by positive and negative loadings. Therefore, the investigated traits that differentiated the grouping of wheat varieties in different environments had a high positive or negative load. The PC1 containing grain yield, 1000 kernel weight and test weight contributed more to the variation. That means that yield genetic potential was reflected by PC1 through some aspects of yield components. Each trait of the PCA is able to serve as a basis for establishing the pattern of varieties clustering based on traits. On the right side of the plot were mainly located varieties from Osijek and Tovarnik in 2019–2020 with natural infection, resulting in significantly high grain yield on average, which showed a negative relationship with quality traits. This was expected because the association of grain yield is positive with the test weight and 1000 kernel weight, while it is negative with quality traits as the angle between the vectors in the PCA shows their approximate correlation [38]. The significant positive relationship between 1000 kernel weight and grain yield has been reported previously [39]. Also, for the

selection of high yielding wheat varieties, 1000 kernel is often used as it is closely related to grain yield [40]. Protić et al. [41] concluded that components such as heads per plant, spikelets per spike, grains per spike and the 1000 kernel weight can be considered part of grain yield of bread wheat. Besides, the fact that 1000 kernel weight and test weight are components of yield means these traits can be used to determine yields of flour and in the milling industry as major quality determinants [42]. That means that higher 1000 kernel weight and higher test weight will give an increased proportion of endosperm in the grains, and thus a greater flour yield.

In the current study, along with test weight and 1000 kernel weight, the traits which contributed positively to PCA2 were sedimentation value, protein content (P), wet gluten content (VG) and VG/P, suggesting that these components reflected the quality potential of each variety. To a lesser extent, grain yield positively contributed to PC2. This was expected, as it is known that there is a negative correlation between grain yield and quality, thus making it very difficult to obtain good quality varieties with high yields [43]. The group with varieties in Osijek in 2018–2019 from FHB inoculation areas was located on the left side of the plot but further from the quality traits, and also showed significantly high quality traits. The same environment had a significantly low grain yield, test weight and 1000 kernel weight. It is well known that higher grain yields are associated with lower protein concentration [44]. In spite of that, wheat breeding efforts have made a great impact on grain yield and quality together [45]. In the current study, the grain protein content and wet gluten content varied considerably, depending on wheat variety, with an increased value on average in *Fusarium* inoculated treatment compared with natural infection. FHB inoculation increased protein content, as proteins were formed in the early stage of development of the grain, but endosperm reserve protein (gluten) was degraded. FHB infected seeds were smaller with lower 1000 kernel weight, and had less endosperm, which resulted in increased protein content due to carbohydrate utilization by pathogens.

4.3. High FHB Pressure Impact on Quality Traits

Different research has indicated that climatic conditions during the reproductive period when protein reserves are formatted, have a direct impact on wheat quality [46]. Also, the period from flowering until grain maturity (from April–May to July in Croatia) matches a time of high disease severity. The formed proteins are transferred to the grain after wheat deflowering [47]. Wheat proteins are of high importance to dough properties, thus influencing bread loaf volume and pasta production. Sedimentation value is the most important indirect quality trait [48]. In the current study, the sedimentation value was significantly lower in the group with the varieties with natural infection treatment in Osijek in 2019–2020, and with *Fusarium* inoculated treatment in Osijek and Tovarnik in 2019–2020. While protein content, test weight and 1000 kernel weight are affected by environmental conditions, it is reported that the Zeleny sedimentation value is highly affected by genetic factors [48]. Sedimentation values reflect the quality of gluten proteins that are influenced by the environment [49]. Almost all varieties had higher protein content, wet gluten content and falling number under artificial infection with FHB. Similar results of an increase of those traits were obtained previously [50,51]. Wet gluten content analysis shows a variety of responses similar to those exhibited by protein content. The valuable indicator of gluten strength, the gluten index, varied in naturally infected treatment across all environments, mostly resulting in reductions with inoculated treatment, thus indicating the significant influence of the environments. Gluten content and composition are the main determinants of the end-use wheat quality [52], and therefore, it is important to monitor those traits under high FHB pressure.

5. Conclusions

In general, simultaneous selection for agronomical and quality traits is very difficult since FHB resistance, agronomical and quality traits are all controlled by polygenes. Although significant progress has been made in understanding FHB, environmental changes

are highly challenging for disease control. The results of this research indicate the importance of multi-environment experiments to obtain reliable data on quantitative characteristics of a particular wheat variety. Although 21 varieties that originated from Croatia were well adapted to the region, there were different GEI responses, with changes in ranking across environments. Increased FHB pressure on wheat production affected both the yield and quality of wheat production. Furthermore, rheological analysis should be combined with this kind of experiment to reveal the influence of FHB on baking quality. In future, more and different locations should be included in the field experiments. Overall, experiments with different environments, if coupled with provoked biotic or abiotic stress, will help to improve cereal production as well as to secure yield stability.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4395/11/2/213/s1>, Figure S1: % of test weight (a), 1000 kernel weight (b), sedimentation value (c), gluten index (d) and wet gluten to protein ratio (e) reduction in FHB inoculated treatment compared to naturally infected treatment at three environments, Figure S2: percentage of total protein content (a), wet gluten content (b) and falling number (c) reduction in FHB inoculated treatment compared to naturally infected treatment in three environments.

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