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Changes of Agronomic and Quality Traits in Fusarium-inoculated Wheat Genotypes

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Summary

Wheat is an important crop grown in Croatia, with production approximately 4 t ha^{-1} in average from 1996 till 2006. The main growing areas for wheat production in Croatia are situated in the eastern part of Croatia. Wheat plants are attacked by several Fusarium species responsible for diseases, such as seedling blight, crown or foot rot. The aim of this paper was to test agronomic and quality traits of wheat genotypes under Fusarium infection. In total 24 genotypes were evaluated in 2008/09 at the experimental field of Agricultural Institute Osijek (Croatia). Wheat plants were inoculated with F. culmorum (first treatment), and the second treatment were control plots which were left to natural infection. Spray inoculations were performed individually for each genotype at flowering (Zadok's scale 65) using a hand-held-sprayer. The genotypes Libellula, Divana, Soissons and Srpanjka showed smallest grain yield reduction in inoculation treatment as compared to the control treatment. Almost all genotypes had higher protein content, sedimentation value and wet gluten content under infection with F. culmorum (inoculation treatment). Low differences between control and inoculation treatments in quality traits had genotypes Sirban Prolifik, Pipi and Super Zitarka. Also it is important to check dough reheological properties and baking performance in inoculation treatment. The obtained results in combination with phenotypic selection could be a strategy to develop genotypes with improved Fusarium resistance.

Keywords: Fusarium, wheat, grain yield and quality

Sažetak

Pšenica je važan ratarski usjev u Hrvatskoj, sa prosječnim prinosom od 4 t ha^{-1} u prosjeku od 1996 do 2006. Glavne proizvodne površine u Hrvatskoj nalaze se u njenom istočnom djelu. Pšenica može biti napadnuta sa nekoliko Fusarium vrsta odgovornih za različite bolesti, a među njima i za palež klasa te trulež stabljike i korijena. Cilj ovoga istraživanja bio je testirati agronomska svojstva, kao i svojsta kvalitete prilikom infekcije Fusariumom. Tijekom 2008/09 testirano je 24 genotipa na eksperimentalnom polju Poljoprivrednog instituta Osijek. Biljke u parceli su inokulirane s Fusarium culmorum (inokulacijski tretman), a u kontrolnom tretmanu su prepuštene prirodnim uvjetima. Inokulacije rasprskivanjem izvedene su indivudalno na svakom genotipu u vrijeme cvjetanja (Zadoksova skala 65) upotrebom leđne prskalice. Genotipovi Libellula, Divana, Soissons i Srpanjka imali su najmanje smanjenje prinosa zrna u inokulacijskom tretmanu u usporedbi s kontrolnim tretmanom. Gotovo svi genotipovi su imali veći sadržaj proteina, sedimentacijske vrijednosti i vlažnog glutena u inokulacijskom tretmanu. Promatrajući sva tri svojstva kvalitete, male razlike između tretmana imali su genotipovi Sirban Prolifik, Pipi i Super Zitarka. Potrebno je provjeriti reološka svojstva, kao i karakteristike pečenja istraživanih genotipova u inokulacijskom tretmanu. Dobiveni rezultati u kombinaciji s fenotipskom selekcijom mogli bi biti dobra strategija za razvoj genotipova s poboljšanom Fusarium otpornosti.

Ključne riječi: Fusarium, pšenica, prinos zrna i kvaliteta

Introduction

Wheat (*Triticum aestivum* L.) is an important crop grown in Croatia, with production approximately 4 t ha^{-1} in average from 1996 till 2006 (Croatian central bureau of statistics in RH, 2007). The main growing areas for wheat production in Croatia are situated in the eastern part of Croatia. Wheat plants are attacked by several *Fusarium* species responsible for diseases, such as seedling blight, crown or foot rot. Fusarium head blight (FHB), also called ear blight or scab, is economically one of the most serious fungal diseases of wheat in many producing regions of the world. We can also find it as a widespread disease in Croatia (Ćosić et al., 1997; Spanic et al., 2010). The main causative agents of FHB in the world are *Fusarium graminearum* (teleomorph *Gibberella zeae*), *F. culmorum* (teleomorph unknown) and *F. avenaceum* (teleomorph *Gibberella avenacea*) (Lemmens at al., 2004). Infection of grain with FHB decreases kernel quality, and several causal *Fusarium* species produce toxic mycotoxins that contaminate the grain. Wheat is most susceptible around anthesis, probably because pollen provide nutrients which stimulate fungal growth. The optimal temperature for infection is 20-25°C, with 80 % air humidity. Typical FHB symptoms are water soaked spots on the glumes followed by discoloration which spreads from the point of infection to the whole spikelet and the neighbouring spikelets. The most effective strategy for controlling FHB in wheat is through the development of resistant cultivars. The aim of this paper is to test agronomic and quality traits of wheat genotypes under Fusarium infection. It is important for us to see the impact of inoculation on the grain yield, which is one of the main criteria for determining the genetic potential of the investigated genotype.

Materials and Methods

The survey was conducted during 2008/09 using 24 genotypes of winter wheat (Table 1). The experiment was set up as completely randomized block in four replications in two treatments at Osijek (45°27' N, 18°48'E). The area of one experimental plot was 7.56 m^2. To control seedborne diseases the seed was treated with Vitavax 200 (thiram+carboxin) at a rate of 200 g 100 kg^{-1}. To produce macroconidia of *F. culmorum*, we used the method described by Snijders and Van Eeuwijk (1991). Concentration of the conidial suspension was 10x10^4 ml^{-1}. Plots were inoculated with *F. culmorum* (one treatment), and the second treatment were control plots which were left to

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Table 1. *Origin and pedigree of the examined genotypes*

Nr	Genotype	Origin	Pedigree
1	Srpanjka	HR, PIO*, 1989	Osk.4.50-1/Zg.2696
2	Zitarka	HR, PIO, 1985	Osk.6.30-2/Slavonka//Osk.6.78-1-73/Kavkaz
3	Golubica	HR, PIO, 1998	Slavonija/Gemini
4	Super Zitarka	HR, PIO, 1997	GO3135/Zitarka
5	Janica	HR, PIO, 2003	Osk.5.36-9-91/Srpanjka
6	Lucija	HR, PIO, 2001	Srpanjka/Kutjevcanka
7	Osk.388/00	HR, PIO, 2003	Osk.5.140-22-91/Sana
8	Divana	HR, Jost, 1996	Favorit/5/Cirpiz/4/Jang/Kwang/2/A+66/ Comanche/3/Velvet
9	Lela	HR, PIO, 2006	Srpanjka/Super Zitarka
10	Pipi	HR, PIO, 2006	Soissons/Osk.6.83/5-91
11	Katarina	HR, PIO, 2006	Osk.5B.4-1-94/Osk.5.140-22-91
12	Osk.102/03	HR, PIO, 2006	Zitarka/Osk.7.5-4-82/Kom.Bg.160/86//Srpanjka
13	Aida	HR, PIO, 2006	Srpanjka/Rialto
14	Seka	HR, PIO, 2006	Srpanjka/Demetra
15	Osk.108/04	HR, PIO, 2007	Srpanjka/Kom.Bg.160-86
16	Soissons	FR, 1987	Lena/HN-35
17	Renan	FR, 1991	Mironovskaya 808/Maris Huntsman//VPM1/Moisson/3/Courtot
18	Sirban Prolifik	HU, 1905	Unknown pedigree
19	U1	HR, PIO, 1936	Marquis/Carlotta Strampelli
20	Libellula	IT, 1965	San Pastore//Tevere/Guiliani
21	Bezostaja	Former USSR, 1963	Skorospelka 2/Lutenscens 17
22	Zlatna Dolina	HR, Bc, 1971	Leonardo/ZG 414-57
23	Tena	HR, PIO, 1973	Libellula/Bezostaja 1
24	Osjecanka	HR, PIO, 1989	Tena (EMS1.5%)

*PIO-Agricultural Institute Osijek, Croatia; Bc-Bc Institute, Croatia

natural infection. Spray inoculations were performed individually for each genotype at flowering (Zadok's scale 65) (Zadoks et al., 1974) using a back-pack sprayer. Inoculations were performed in the late afternoon and repeated two days later. To maintain moisture at ears we sprayed water with tractor backsprayer on several occasions during the day. After harvest the following traits were analyzed: grain yield (dt ha^{-1}), test weight (kg hl^{-1}), 1000 kernel weight (TKW, g), protein content (%), zeleny sedimentation value (ml) and wet gluten content (%). Analysis of variance was performed using the GLM procedure

Table 2. *Analysis of variance for grain yield, test weight, 1000 kernel weight, protein content, sedimentation value and wet gluten content in treatments (control and inoculation)*

Source of variation	df	F-value					
		GY	TW	TKW	P	SED	WGC
Genotype (G)	23	10.55***	15.70***	10.54***	19.48***	17.30***	17.21***
Replication	3	1.58 ns	2.71*	5.04**	5.83***	2.90*	5.21*
Treatment (T)	1	216.46***	1216.46***	335.76***	133.95***	65.33***	101.5***
G x T	23	4.50***	20.46***	4.67***	1.42 ns	1.48 ns	1.42 ns
Error	141						

GY-grain yield; TW-test weight; TKW-1000 kernel weight; P-protein content; SED-zeleny sedimentation value; WG-wet gluten content; ***,**,*=significant at $P<0.001$, 0.01 and 0.05, respectively; ns=not significant ($P>0.05$)

of SAS 9.1. Stat Softwer (SAS Institute, 2004). Differences between genotype means were tested using the Least Significant Difference (LSD) procedure at 0.05 probability level.

Results and Discussion

Analysis of variance revealed significant differences between genotypes and treatments for all traits, whereas the genotype-by-treatment interaction effect was significant for all traits except for protein content, sedimentation value and wet gluten content (Table 2).

Grain yield in control treatment varied from 59.90 dt ha^{-1} (U1) to 94.22 dt ha^{-1} (Renan), and in inoculation treatment from 26.83 dt ha^{-1} (Golubica) to 81.93 dt ha^{-1} (Renan). The reduction of grain yield in inoculation treatment as compared to the control treatment was the highest in genotypes Golubica (63.82%) and Super Zitarka (48.72%), and the lowest in genotypes Libellula (0.45%), Divana (2.40%), U1 (3.26%) and Sirban Prolifik (6.88%) (Table 3). Previous studies showed that Fusarium infection could reduce the grain yield of some wheat genotypes up to 20% (Spanic et al., 2008). Martinčić and Kozumplik, (1996) indicate that in favorable conditions reduction of yield can be between 50 and 80%. Grain yield of wheat is a complex quantitative trait, with low to medium heritability, which is controlled by minor genes and is under the strong influence of environmental factors (Drezner et al., 2007). One could say that genotypes with good grain yield potentially posses a Type V resistance (Mesterhazy, 1995, Mesterhazy et al., 1999), if they retain grain yield in the presence of infection at a level that is above the average of other genotypes. Grain yield was well-preserved in some older genotypes (Table 3), which could be a source of Fusarium resistance genes or older

Table 3. *Mean values of grain yield (dt ha^{-1}), test weight (kg hl^{-1}), 1000 kernel weight (g) in treatments (control and inoculation), and their relative measure*

Genotype	Grain yield (dt ha^{-1})			Test weight (kg hl^{-1})			1000 Kernel weight (g)		
	CT*	IT	RGY (%)**	CT	IT	RTW (%)	CT	IT	RKW (%)
Renan	94.22	81.93	-13.04	81.15	76.43	-5.82	49.33	44.90	-8.98
Katarina	92.18	61.82	-32.94	80.50	74.18	-7.85	43.33	35.83	-17.31
Soissons	89.23	81.90	-8.21	81.18	76.33	-5.97	41.00	35.63	-13.10
Aida	88.73	54.73	-38.32	81.58	74.38	-8.83	44.68	34.88	-21.93
Srpanjka	87.95	80.63	-8.32	81.08	78.88	-2.71	38.08	39.15	2.81
Osk.388/00	87.85	66.97	-23.77	80.58	74.48	-7.57	44.68	36.08	-19.25
Seka	86.44	70.04	-18.97	79.23	75.65	-4.52	45.68	38.88	-14.89
Osk.108/04	86.36	70.81	-18.01	82.80	77.40	-6.52	48.35	37.90	-21.61
Osk.102/03	85.92	71.82	-16.41	81.68	77.55	-5.06	49.35	43.80	-11.25
Pipi	85.25	59.35	-30.38	81.78	72.35	-11.53	44.45	32.58	-26.70
Zitarka	81.64	54.66	-33.05	82.10	74.68	-9.04	47.20	35.83	-24.09
Osjecanka	80.82	67.92	-15.96	81.38	76.60	-5.87	47.15	41.75	-11.45
Janica	78.25	58.89	-24.74	80.73	76.15	-5.67	41.03	36.45	-11.16
Bezostaja	76.42	67.39	-11.82	80.40	75.93	-5.56	48.68	41.28	-15.20
Lucija	76.28	70.25	-7.91	79.55	75.93	-4.55	43.28	38.03	-12.13
Divana	75.51	73.70	-2.40	81.43	78.78	-3.25	48.23	42.88	-11.09
Super Zitarka	75.15	38.54	-48.72	83.13	70.60	-15.07	49.60	32.55	-34.38
Lela	74.23	56.64	-23.70	83.80	77.05	-8.05	45.50	35.35	-22.31
Golubica	74.15	26.83	-63.82	82.63	65.90	-20.25	44.68	26.83	-39.95
Tena	73.90	58.08	-21.41	80.13	75.80	-5.40	43.93	41.90	-4.62
Zlatna Dolina	72.29	50.01	-30.82	78.43	69.75	-11.07	42.05	30.98	-26.33
Sirban Prolifik	72.19	67.22	-6.88	78.73	77.08	-2.10	44.33	42.48	-4.17
Libellula	64.56	64.27	-0.45	78.58	77.25	-1.69	45.45	40.38	-11.16
U1	59.90	57.95	-3.26	78.65	77.35	-1.65	47.95	45.30	-5.53
Average	79.98	63.01	-20.97	80.88	75.27	-6.90	45.33	37.98	-16.07
LSD (P<0.05)	12.63	8.72		0.99	1.98		4.32	3.34	

* CT-control treatment; IT-inoculation treatment; RGY-relative grain yield; RTW-relative test weight; RTKW-relative 1000 kernel weight

** the percentage change in each trait, relative to the control treatment

Table 4. *Mean values of protein content (%), sedimentation value (ml), wet gluten content (%) in treatments (control and inoculation), and their relative measure*

Genotype	Protein content (%)			Sedimentation value (ml)			Wet gluten content (%)		
	CT*	IT	RPC (%)**	CT	IT	RSV (%)	CT	IT	RWGC (%)
Sirban Prolifik	16.23	16.48	1.52	59.15	58.90	-0.42	40.28	40.03	-0.62
U1	15.58	16.55	5.86	56.68	58.25	2.70	38.35	40.08	4.32
Tena	14.58	15.35	5.01	57.63	61.30	5.99	35.55	37.80	5.95
Osjecanka	13.45	14.03	4.13	47.90	50.33	4.83	33.00	34.85	5.31
Zitarka	13.35	14.90	10.40	44.50	55.68	20.08	31.83	36.13	11.90
Super Zitarka	13.20	13.83	4.55	45.73	43.90	-4.17	32.28	33.35	3.21
Renan	13.18	14.20	7.18	41.05	50.78	19.16	31.13	33.95	8.31
Divana	13.03	16.08	18.97	42.68	60.45	29.40	30.90	39.58	21.93
Golubica	12.98	14.93	13.06	48.25	55.80	13.53	31.45	35.68	11.86
Libellula	12.98	14.13	8.14	41.48	49.20	15.69	31.95	35.35	9.62
Osk.108/04	12.90	14.20	9.16	38.05	48.53	21.59	29.93	34.08	12.18
Bezostaja	12.88	14.48	11.05	41.03	51.43	20.22	29.48	34.35	14.18
Pipi	12.73	13.28	4.14	36.70	36.88	0.49	30.00	30.53	1.74
Janica	12.65	13.50	6.30	37.48	42.13	11.04	29.78	32.28	7.75
Lela	12.65	14.08	10.16	38.20	47.90	20.25	29.18	33.83	13.75
Osk.102/03	12.58	14.13	10.97	36.35	48.85	25.59	29.43	34.15	13.82
Zlatna Dolina	12.40	13.08	5.20	35.93	37.98	5.40	29.80	31.05	4.03
Seka	12.28	13.53	9.24	32.20	40.68	20.85	29.58	33.43	11.52
Katarina	12.15	13.43	9.53	36.48	42.10	13.35	28.10	31.83	11.72
Lucija	12.03	12.73	5.50	34.48	36.48	5.48	27.05	30.20	10.43
Srpanjka	12.03	13.50	10.89	31.30	38.33	18.34	28.75	33.18	13.35
Aida	11.78	13.73	14.20	33.55	44.48	24.57	24.53	30.25	18.91
Osk.388/00	11.45	12.33	7.14	30.58	33.93	9.87	25.75	28.50	9.65
Soissons	11.43	12.40	7.82	28.98	34.85	16.84	26.40	28.70	8.01
Average	12.94	14.12	8.34	40.68	47.05	13.36	30.60	33.88	9.70
LSD (P<0.05)	1.13	0.63		8.54	5.20		3.55	2.04	

*CT-control treatment; IT-inoculation treatment; RPC-relative protein content; RSV-relative sedimentation value; RWGC-relative wet gluten content
** the percentage change in each trait, relative to the control treatment

genotypes have the passive mechanisms of resistance (longer stems). Test weight in control treatment varied from 78.43 kg hl^{-1} (Zlatna Dolina) to 83.80 kg hl^{-1} (Lela) and in inoculation treatment it varied from 65.90 kg hl^{-1} (Golubica) to 78.88 kg hl^{-1} (Srpanjka).

The highest reduction of test weight in inoculation treatment as compared to the control treatment had genotypes Golubica (20.25%), Super Žitarka (15.07%), Pipi (11.53%) and Zlatna Dolina (11.07%), whereas the smallest reduction showed genotypes U1 (1.65%), Libellula (1.69%), Sirban Prolifik (2.10%) and Srpanjka (2.71%) (Table 3).

In control treatment TKW varied between 38.08 g (Srpanjka) and 49.60 g (Super Zitarka), and in inoculation treatment it varied between 26.83 g (Golubica) and 45.30 g (U1). The highest reduction of TKW in inoculation treatment as compared to the control treatment had genotypes Golubica (39.95%), Super Zitarka (34.38%), Pipi (26.70%) i Zlatna Dolina (26.33%), and the smallest genotypes Srpanjka (-2.81%), Sirban Prolifik (4.17%), Tena (4.62%) and U1 (5.53%) (Table 3). A higher TKW and higher test weight means a greater proportion of endosperm in the seed and thus a better yield of flour.

Protein content in control treatment ranged between 11.43% (Soissons) and 16.23% (Sirban Prolifik) and in inoculation treatment it ranged between 12.33% (Osk.388/00) and 16.55% (U1) (Table 4). Protein content is a complex trait with relatively low heritability, strongly influenced by environment and is negatively correlated with grain yield. In control treatment the highest sedimentation value had genotype Sirban Prolifik (59.15 ml), and the lowest Soissons (28.98 ml), whereas in inoculation treatment sedimentation value ranged between 33.93 ml (Osk.388/00) and 61.30 ml (Tena). Wet gluten content in control treatment varied between 24.53% (Aida) and

40.28% (Sirban Prolifik), and in inoculation treatment it ranged from 28.50% (Osk.388/00) and 40.08% (U1). In all three quality parameters small differences between the two treatments had genotypes Sirban Prolifik, Pipi and Super Zitarka (Table 4).

In this study almost all genotypes had higher protein content, sedimentation value and wet gluten content under infection with *F. culmorum* (inoculation treatment). Similar results were obtained by Pawelzik et al., (1998) and Matthäus et al., (2002). Proteins are formed in early stage of the development of grain. Inoculation with *Fusarium* species will increase protein content, but will degrade endosperm reserve protein (gluten). Infected seeds were smaller, and had smaller endosperm, what had increased protein content, and what is result of carbohydrate utilization by pathogen. Wang et al., (2005) determined that the protein content wasn't affected by infection with *F. culmorum*. To obtain reliable information on the impact of artificial inoculation on quality of wheat genotypes, it is necessary to study the primary structure of gliadins and glutenins, the components of gluten. It is well known that gliadins affect the dough viscosity while glutenins provides strength and elasticity to the dough (Branlard et al., 2001).

Conclusions

The results of this research showed in the investigated wheat genotypes a large variability in agronomic and quality traits reduction in inoculation treatment as compared to the control treatment. Some of genotypes could be suitable for cultivation under heavy infection pressure (Libellula, Divana, Srpanjka and Soissons), because they showed smallest grain yield losses between those two treatments. The variety Golubica showed very heavy grain yield losses (>60%). In avearge higher protein content, sedimentation value and wet gluten content is made on samples of winter wheat genotypes in treatment which was artificially infected with isolates of *F. culmorum* than in control treatment. On this basis, we can only get an indication of quality reduction, and according to our research genotypes Sirban Prolifik, Pipi and Super Zitarka had low differences between control and inoculation treatment. Further research should go in direction of study gliadins and glutenins. Also it is important to check dough reheological properties and baking performance in inoculation treatment. The obtained results in combination with phenotypic selection could be a strategy to develop genotypes with improved Fusarium resistance.

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