Journal of Elementology ISSN 1644-2296

Wachowska U., Konopka I., Tańska M., Korzeniewska E. 2017. *Biochemical features of winter wheat grain affected by biological and chemical control treatments*. J. Elem., 22(4): 1453-1461. DOI: 10.5601/jelem.2017.22.1.1250

ORIGINAL PAPER

BIOCHEMICAL FEATURES OF WINTER WHEAT GRAIN AFFECTED BY BIOLOGICAL AND CHEMICAL CONTROL TREATMENTS*

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Abstract

A new trend in plant protection consists in the integration of biological and chemical control treatments. Unfortunately, the biological control agents for winter wheat are still in short supply. Bacteria of the genus *Sphingomonas* have a unique ability to produce prolyl endopeptidases. Those enzymes are capable of hydrolyzing the peptide PQPQLPYPQPQLP. During the growing season, the bacteria may be used to protect winter wheat against infections caused by fungi of the genera *Fusarium*. The objective of this study was to evaluate the effectiveness of bacterial isolates in protecting field-grown winter wheat plants against spike infections and to assess the effect of bacteria on the chemical composition and microbiological purity of winter wheat grain. The effects of bacteria of the genus *Sphingomonas* as biological control agents against Fusarium head blight (FHB) of winter wheat were evaluated in a three-year field experiment. For comparative purposes, the fungicides propiconazole at the elongation stage (BBCH 31) and fluoxastrobin + prothiconazole at the heading stage (BBCH 55) were applied. In 2010 and 2011, the application of cell suspensions of bacteria alleviated the symptoms of disease by 27.3% and 75.8%, respectively in comparison with control. Wheat grain yield was higher in plots subjected to the biological and chemical treatment (by an average of 9.5 and 13.6%, respectively). For the first time, we observed that biological control modified the chemical characteristics of wheat grain. In control grain, the content of gluten proteins was 7.9% higher than in grain treated with the biocontrol agent. In wheat grain treated with the biocontrol agent, the highest decrease was observed in the concentrations of alpha/beta-gliadins (10.59%), but grain quality was most affected by an estimated 8% decrease in the content of HMW glutenins. Biological treatment inhibited the growth pathogens of *F. culmorum*, *F. poae*, *F. sporotrichiodes* and *F. avenaceum*. A cell suspension of bacteria did not inhibit the growth of yeasts and epiphytic bacteria of the genus *Azotobacter* on grains.

Keywords: biological control, *Sphigomonas* sp., protein, microbiota, *Fusarium* spp.

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* This research was supported by the Ministry of Science and Higher Education of Poland as part of statutory activities.

INTRODUCTION

Biochemical characteristics of wheat grain could be changed by a different of phosphorus and potassium fertilization level, pathogenic infections and various chemical treatments (Gaj et al. 2013, Rodrigo et al. 2015). Symptoms of Fusarium head blight (FHB) are observed on wheat spikes in epidemic years. Those pathogens contaminate grain with mycotoxins, and the predominant metabolites accumulated in wheat grain infected by *F. culmorum* and *F. graminearum* are deoxynivalenol (DON) and other trichothecenes as well as zearalenone (Stępień, Chełkowski 2010). Effectiveness of synthetic fungicides in inhibiting the proliferation of *Fusarium* fungi is often limited, and they do not reduce toxin concentrations in grain (Pirgozliev et al. 2002). The integration of biological and chemical control treatments is a new trend in plant protection which receives legal support (Directive 2009/128/EC). Despite efforts, biological control agents for winter wheat are still in short supply. During the growing season, bacteria have been used to protect winter wheat against infections caused by fungi of the genera *Fusarium* (Schisler et al. 2006, Khan, Doohan 2009, Wachowska et al. 2013). Bacteria of the genus *Sphingomonas* have a unique ability to produce prolyl endopeptidases, which hydrolyze the peptide bond on the carboxyl side of a proline residue (Kabashima et al. 1998). These enzymes are capable of hydrolyzing the peptide PQPQLPYPQPQLP, and they have been proposed as oral treatment for celiac disease (Osorio et al. 2012). The objective of this study was evaluate the effectiveness of bacterial isolates in protecting field-grown winter wheat plants against spike infections, and to evaluate the effect of bacteria on the chemical composition and microbiological purity of winter wheat grain.

MATERIAL AND METHODS

Potential biocontrol agents

Most isolates of *Sphingomonas* sp. were washed off from the surface of wheat grains (cv. Tonacja) with sterile water. The isolate of *Sphingomonas* S 11 was obtained from the rhizosphere soil of winter wheat. The isolates were identified based on sequences of the ITS rDNA region (Wachowska et al. 2013). The source of carbon and the enzymatic activity of isolates were determined with the API 20NE microtest system (bioMérieux) in accordance with the manufacturer's instructions. Bacterial isolates used in biological control treatments were identified as belonging to the genus *Sphingomonas*. All isolates formed smooth, bright yellow or bright orange-colored colonies with a diameter of up 5 mm on the PDA. All isolates identified as belonging to the genus *Sphingomonas* (S1, S2, S3, S4, S6, S11) were gram-negative. The remai-

ning isolates of *Sphingomonas* sp. assimilated most tested substrates and reduced nitrates to nitrogen.

Preparation of bacteria cell suspensions for biological control treatments

Bacterial suspensions were prepared twice in each winter wheat growing season. Isolates were cultured for seven days in Petri dishes on solid potato dextrose agar (PDA) medium with pH 7.2, in the dark, at 27°C. After incubation, bacterial colonies were removed from the medium (50 Petri dishes for each isolate) with an inoculation loop and placed in 100 cm ^{3} sterile water. The bacterial suspension was a mixture of 6 isolates with cell density of 10^9 CFU per 1 cm^3 water. The suspension was applied to plants at the stem elongation stage (BBCH 31) and the heading stage (BBCH 55) (Meier 2003). Prior to application, one liter of bacterial cell suspension was diluted in 10 dm^3 of water and sprayed over an area of 80 m^2 (four plots of 20 m^2 each).

Field experiment

A field experiment was established at the experimental station in Tomaszkowo (53.71°N, 20.41°E) in 2009-2011. The experiment had a randomized block design with four replications. Winter wheat (*Triticum aestivum* L.) cv. Bogatka, a bread wheat cultivar, was sown in 20 m^2 plots. Control plots were sprayed with water twice. For comparative purposes, the fungicides Bumper 250 EC (propiconazole) and Fandango 200 EC (fluoxastrobin and prothiconazole) were applied at the stem elongation stage (BBCH 31) and the heading stage (BBCH 55), respectively. The fungicides were used at the manufacturer's recommended dose of 1 dm^3 ha^{-1}. Plants were fertilized with nitrogen (N) at 100 kg ha^{-1}, potassium (K) at 70 kg ha^{-1} and phosphorus (P) at 26 kg ha^{-1}.

Evaluation of the health status of spikes and grain yield

The intensity of FHB was expressed in terms of the average percentage area of 100 spikes showing symptoms of the analyzed diseases. Spikes was evaluated in the watery ripe stage (BBCH 71).Wheat was harvested at the fully ripe stage from an area of 15 m^2 with a plot combine. Yield was determined as the weight of grains harvested from an area of 1 m^2.

RP-HPLC analysis of wheat grains proteins

Three protein fractions were extracted from purified grains ground to particle size of 300 mm: 1) albumins and globulins, 2) gliadins, 3) glutenins. The extraction process was performed with the use of solvents described by Wieser et al. (2000). For the determination of protein content, grain was harvested in 2010 from control and protected plots. It was ground to produce flour with the particle size below 300 μm. The protein content and composition were determined by the RP-HPLC technique described by Konopka

et al. (2007), at the following parameters: a RP-18 Vydac 218TP54 column with 5 µm bead size and 300 Å pore size, 250 x 4.6 mm; a Zorbax 300SB-C18 pre-column, 4.6 x 12.5 mm; a column temp. of 45°C, a mobile phase flow rate of 1 cm^3 min^{-1}, and an injection volume of 20 µl. A two-component gradient was used. A component: 0 min 75%, 5 min 65%, 10 min 50%, 17 min 25%, 18 min 15% and 19 min 75%. The first component (A) was water with 0.1% of TFA and the second (B) was ACN with 0.1% of TFA. The absorbance spectra of eluted proteins were determined by a diode-array detector (HP 1050). Quantification of proteins was done by UV absorbance at 210 nm. The integration procedure was performed using HPLC 3D ChemStation software. The content of every protein fraction was expressed in terms of peak area (mAU x s) per mg of grain. Proteins were detected at 210 nm wavelength. Bovine serum albumin (BSA) manufactured for Bio-Rad (Bradford Protein Assay) and gliadins isolated from the grain of wheat were used as the standards.

Evaluation of microbiological grain features

Grain samples for microbiological analyses were obtained immediately after threshing and after six months' storage at 11°C. To produce microbial suspensions, grain samples of 10 g were placed in 250 cm^3 flasks filled with 90 cm^3 sterile water. The flasks were shaken for 30 min on a shaker table (358 S). Microbial suspensions were twice diluted: pseudomonads – at 10^{-3} and 10^{-4}, and the remaining microorganisms – at 10^{-1} and 10^{-2}. Cell suspensions at two dilutions were cultured by the pour plate method in Petri dishes with the respective media cooled to 42°C. The pseudomonads were isolated from King's B medium, atmospheric nitrogen (N_2) fixing bacteria – from a nitrogen-free medium, and yeast and filamentous fungi – from Martin's medium (Wachowska et al. 2013). Bacterial and fungal colony forming units were counted on Petri dishes. The structure of fungal communities was analyzed by transferring fungal colonies from Martin's medium onto agar slants. Filamentous fungi were determined under a light microscope (Nikon E 200) based on sporulation characteristics.

Statistical analysis

An analysis of variance (ANOVA) was performed using Statistica 12.0 software. The significance of differences in wheat yields and in the content of protein fractions between treatments was determined by the Student-Newman-Keuls test. Bacterial and fungal colonies were counted, and their numbers were estimated based on the following formula: total number of colonies in Petri dishes from two successive dilutions of microbial suspensions / number of plates containing the first and second dilutions x 0.1. The results were log transformed according to the log(CFU+1) formula, and the significance of differences between means was evaluated by the Student-Newman-Keuls test. The structure of filamentous fungal communities was described as the number of colonies identified on Martin's medium.

RESULTS AND DISCUSSION

In 2010 and 2011, when the severity of FHB was low, the application of cell suspensions of bacteria alleviated the symptoms of disease by 27.3% and 75.8%, respectively, in comparison with control (not significantly) – Table 1.

Table 1

Severity of FHB (percentage of spikes' area) and grain yield of winter wheat plants protected with biocontrol and chemical agents

Treatment	Year	Severity of Fusarium head blight		Grain yield from 1 m^2	
		mean	mean 2009-2011	mean	mean 2009-2011
Control	2009	2.31^a	0.92	489.0^a	524.0^c
	2010	0.11^b		422.7^a	
	2011	0.33^b		660.3^b	
Bacteria	2009	2.53^a	0.90	500.7^a	573.7^b
	2010	0.08^b		437.1^a	
	2011	0.08^b		783.3^b	
Fungicides	2009	4.40^a	1.69	515.0^a	595.4^a
	2010	0.17^b		460.5^a	
	2011	0.50^b		810.7^c	
2009		3.08^x		501.56^y	
2010		0.12^y		440.10^z	
2011		0.30^y		751.44^x	

Mean values assigned the same letter differ not significantly within columns according to SNK-test at $p < 0.01$ (*a - g* – means for years and pathogens, *x-z* – means for years).

Khan and Doohan (2009) demonstrated that selected bacterial isolates of *Pseudomonas fluorescens* inhibited the progression of FHB by 44% in field-grown wheat inoculated with *F. culmorum*. In our studies, treatments involving a mixture of bacterial isolates of the genus *Sphingomonas* were more effective than Fandango 200 EC. Likewise, Schisler et al. (2006) observed that under field conditions, pseudomonads isolated from wheat (*Pseudomonas* sp. AS 64.4) reduced the severity of FHB more effectively than the Folicur 3.6 F fungicide (tebuconazole).

In successive years of the experiment, biological control increased winter wheat yield by 2.3%, 3.4% and 18.6% (not significantly), respectively, as compared with the control treatment (Table 1). A significant average increase in grain yield was observed in treated plots during the three-year experiment. In plots treated twice with fungicides, yield increased by 5.3%, 8.9% and 22.8% in successive years of the study, respectively, in comparison with control, but the noted increase was significant only in 2011.

Table 2

Content of the main protein fractions in wheat grain in 2010 expressed as the peak area (mAU x s mg^{-1} grain)

Protein fraction	Control	Bacteria	Fungicide
Albumins+globulins	$1.15 \cdot 10^{4\,a}$	$1.09 \cdot 10^{4\,ab}$	$1.09 \cdot 10^{4\,ab}$
Gliadins	$3.37 \cdot 10^{4\,b}$	$3.07 \cdot 10^{4\,a}$	$3.13 \cdot 10^{4\,ab}$
omega	$3.70 \cdot 10^{3\,a}$	$3.59 \cdot 10^{3\,a}$	$3.61 \cdot 10^{3\,a}$
alfa/beta	$1.89 \cdot 10^{4\,c}$	$1.69 \cdot 10^{4\,d}$	$1.74 \cdot 10^{4\,d}$
gamma	$1.11 \cdot 10^{4\,e}$	$1.02 \cdot 10^{4\,ef}$	$1.03 \cdot 10^{4\,ef}$
Glutenins	$1.83 \cdot 10^{4\,bc}$	$1.72 \cdot 10^{4\,c}$	$1.80 \cdot 10^{4\,c}$
HMW	$5.70 \cdot 10^{3\,a}$	$5.26 \cdot 10^{3\,a}$	$5.52 \cdot 10^{3\,a}$
LMW	$1.26 \cdot 10^{4\,d}$	$1.20 \cdot 10^{4\,e}$	$1.25 \cdot 10^{4\,d}$

a - f – values assigned the same letter differ not significantly in rows according to SNK-test at $p < 0.01$.

Albumins and globulins accounted for 18.3% of wheat grain proteins on average (Table 2). The remaining 81.7% proteins were gluten proteins, mostly gliadins which had a 64.2% share of that fraction. Gliadins consisted mostly of alpha/beta-gliadins as well as gamma- and omega-gliadins with a 55.5%, 33.1% and 11.4% share, respectively. In the glutenin fraction, aggregates of high molecular weight (HMW) accounted for 30.8%. The analyzed plant protection agents lowered the content of most protein fractions. The only exceptions were omega-gliadins and low molecular weight (LMW) glutenins, whose concentrations remained constant. In control grain, the content of gluten proteins was 5.22% higher than in fungicide-treated grain and 7.9% higher than in grain treated with the biocontrol agent. In wheat grain treated with the biocontrol agent, the highest drop was observed in the concentrations of alpha/beta-gliadins (10.59%), but grain quality was most affected by an estimated 8% decrease in the content of HMW glutenins. The protein fractions of the analyzed wheat grain were within the reference ranges (Singh, Macritchie 2001, Shewry et al. 2002, Konopka et al. 2007). Gliadins had a very high share of gluten proteins, whereas according to published sources, they do not account for more than 60% of gluten proteins in most wheat cultivars. Based on the above protein profile, cv. Bogatka was classified as a bread wheat cultivar with average baking properties.

Higher bacteria and yeast counts on harvested grain of wheat plants protected with a cell suspension of bacteria probably contributed to changes in the grain's biochemical characteristics. The biocontrol agent reduced the content of alpha/beta gliadins and HMW glutenins, which determine the baking quality of wheat grain (Wieser, Zimmerman 2000, DuPont et al. 2004). There are no studies documenting the effect of bacteria of the genus *Sphingomonas* as a biocontrol agent on the biochemical characteristics of wheat grain, including reserve, structural and metabolic proteins. The applied bac-

Average abundance of epiphytic fungi and bacteria colonizing winter wheat grains in conditions of experiment in 2009-2011

Treatment	Date of analysis	Yeasts		Bacteria				Number of filamentous fungi colonies					
				growth on nitrogen--free medium		pseudomonad group		total		percentage of species (min-max)			
		Log(CFU+1) on 1 g grain								*A.strictum*	*A.alternata*	C.herbarum	Fusarium spp
Control	H	2.78^{c}	3.20^{A}	3.13^{b}	3.53^{A}	5.24	5.15	2.95^{a}	2.85^{B}	0.00	0.47	0.00	2.60
										-	(0-1.42)	-	(1.84-3.40)
	S	3.62^{ab}		3.93^{a}		5.06		2.75^{a}		0.85	0.09	4.25	2.31
										(0-1.98)	(0-0.14)	(0-10.2)	(1.56-3.40)
Bacteria	H	2.64^{c}	3.18^{A}	3.22^{b}	3.64^{AB}	5.27	5.25	2.77^{a}	2.77^{B}	0.14	0.19	1.46	2.26
										(0-0.43)	(0.14-0.28)	(0-4.39)	(1.84-2.97)
	S	3.72^{a}		4.06^{a}		5.24		2.77^{a}		1.12	2.03	0.00	1.04
										(0-3.23)	(0-5.52)	-	(0-2.83)
Fungicide	H	2.22^{d}	2.88^{B}	3.32^{b}	3.70^{B}	5.23	5.23	2.31^{b}	2.56^{A}	0.05	0.38	0.00	1.89
										(0-0.14)	(0-0.85)	-	(0.42-3.26)
	S	3.43^{b}		4.09^{a}		5.24		2.81^{a}		0.00	1.32	0.66	1.28
										-	(0-3.82)	(0-1.98)	(0-2.55)
2009		2.53^{z}		3.02^{x}		4.85^{y}		2.41^{z}		0.21	0.16	0.42	1.96
2010		3.24^{y}		3.62^{y}		4.66^{x}		3.09^{y}		0.02	1.66	0.00	2.45
2011		3.41^{x}		4.24^{z}		6.12^{z}		2.67^{x}		0.95	0.40	2.76	1.55
	H	2.55**		3.23**		5.17		2.67**		0.13	0.52	0.48	2.24
	S	3.59		4.02		5.24		2.77		0.75	1.15	1.67	1.73

H – after harvest, S – after storage. Mean values assigned the same letter differ not significantly within columns according to SNK-test at $p < 0.01$ (a - d – means for microorganisms and date of analysis, A – B – means for treatments, x-z – means for years), ** means for date of analysis (H-S) differ significantly at $p < 0.01$, *A. strictum – Acremonium strictum, A. alternata – Alternaria alternata, C. herbarum – Cladosporium herbarum, Fusarium* spp. – sum of *F. culmorum, F. poae, F. sporotrichiodes, F. avenaceum*. Other designations see Table 2.

teria could produce extracellular enzymes or stimulate the activity of endogenous proteolytic enzymes in grain. Bacterial strains of the genus *Sphingomonas* produce specific proline endopeptidases that hydrolyze gluten proteins, including alpha/beta gliadins which are toxic for people suffering from celiac disease, and the most significant decrease was noted in the content of this protein fraction. Thus, although the applied biological control decreased the quality of standard bread wheat, it contributed to the production of grain with a unique biochemical protein profile.

The abundance and structure of filamentous fungi colonizing grains were determined by the dates of microbiological analyses and the applied control treatments (Table 3). In comparison with control samples, chemical treatment suppressed the growth of filamentous fungi immediately after harvest. The structure of filamentous fungi colonizing winter wheat grains was typical of that environment. In total, 706 colonies were identified with the predominance of species of the genus *Fusarium* (*F. culmorum, F. poae, F. sporotrichiodes*, *F. avenaceum*), which accounted for 34.1% of all colonies. In 2010-2011, less abundant communities of *Fusarium* fungi were isolated from threshed grain of wheat plants treated with the biocontrol agent and fungicides, especially stored for six months, in comparison with control. A cell suspension of bacteria did not inhibit the growth of yeasts and epiphytic bacteria of the genus *Azotobacter* on grains (Table 3), which improved the health status of wheat plants as yeasts are considered to be potential antagonists of winter wheat pathogens (Zhang et al. 2007).

CONCLUSIONS

1. The biological control by bacteria modified the biochemical characteristics of wheat grain.

2. Biological treatment increased winter wheat grains yield and inhibited the growth pathogens of *F. culmorum*, *F. poae*, *F. sporotrichiodes* and *F. avenaceum*.

REFERENCES

Directive 2009/128/EC of the European Parliament and of the Council, 2009.

DuPont F. M., Vensel W., Encarnacao T., Chan R., Kasarda D.D. 2004. *Similarities of omega gliadins from Triticum urartu to those encoded on chromosome 1A of hexaploid wheat and evidence for their post-translational processing*. Theor. Appl. Genet., 108: 1299-1308. DOI: 10.1007/s00122-003-1565-9

Gaj R., Górski D., Przybył J. 2013. *Effect of differentiated phosphorus and potassium fertilization on winter wheat yield and quality*. J. Elem., 18(1): 55-67. DOI: 10.5601/jelem.2013.18.1.04

Kabashima T., Fujii M., Meng Y., Ito K., Yoshimoto T. 1998. *Prolyl endopeptidase from Sphingomonas capsulata: isolation and characterization of the enzyme and nucleotide sequence of the gene*. Arch. Biochem. Biophys., 358: 141-148. DOI: 10.1007/s00018-007-7270-0

Khan M.R, Doohan F. 2009. *Bacterium-mediated control of Fusarium head blight disease of wheat and barley and associated mycotoxin contamination of grain.* Biol. Control, 48: 42-47. DOI: 10.1016/j.biocontrol.2008.08.015

Konopka I., Fornal Ł., Dziuba M., Czaplicki S., Nałęcz D. 2007. *Composition of proteins in wheat grain obtained by sieve classification.* J. Sci. Food. Agr., 87: 2198-2206. DOI: 10.1002/ jsfa.2900

Meier U. 2003. *Phenological growth stages.* In: *Phenology: an integrative environmental science*: Springer Netherlands, pp. 269-283.

Osorio C., Wen N., Gemini R., Zemetra R., Von Wettstein D., Rustgi S. 2012. *Targeted modification of wheat grain protein to reduce the content of celiac causing epitopes.* Funct. Integr. Genomics, 12(3): 417-38. DOI: 10.1007/s10142-012-0287-y

Rodrigo S., Cuello-Hormigo B., Gomes C., Santamaria O., Costa R., Maria J. Poblaciones M.J. 2015. *Influence of fungicide treatments on disease severity caused by Zymoseptoria tritici and on grain yield and quality parameters of bread-making wheat under Mediterranean conditions.* Eur. J. Plant Pathol., 141: 99-109. DOI: 10.1007/s10658-014-0527-1

Pirgozliev S.R., Edwards S.G., Hare M.C., Jenkinson P. 2002. *Effect of dose rate of azoxystrobin and metconazole on the development of Fusarium head blight and the accumulation of deoxynivalenol (DON) in wheat grain.* Eur. J. Plant. Pathol., 108(5): 469-478.

Schisler D.A., Khan N.I., Boehm M.J., Lipps P.E., Slininger P.J., Zhang S. 2006. *Selection and evaluation of the potential of choline-metabolizing microbial strains to reduce Fusarium head blight.* Biol. Control, 39: 497-506. DOI: 10.1016/ j.biocontrol.2006.08.007

Shewry P.R., Halford N.G. 2002. *Cereal seed storage proteins: structures, properties and role in grain utilization.* J. Exp. Bot., 53(370): 947-958.

Singh H., Macritchie F. 2001. *Application of polymer science to properties of gluten – mini review.* J. Cereal Sci., 33: 231-243. DOI: 10.1006/jcrs.2000.0360

Stępień Ł., Chełkowski J. 2010. *Fusarium head blight of wheat – pathogenic species and their mycotoxins.* World Mycotoxin J., 3(2): 107-119. DOI: http://dx.doi.org/10.3920/ WMJ2009.1193

Wachowska U., Irzykowski W., Jędryczka M., Stasiulewicz-Paluch A.D., Głowacka K. 2013. *Biological control of winter wheat pathogens with the use of antagonistic Sphingomonas bacteria under greenhouse conditions.* Biocontrol Sci. Techn., 23(10): 1110-1122. DOI: 10.1080/ 09583157.2013.812185

Wieser H., Zimmerman G. 2000. *Importance of amounts and proportions of high molecular weight subunits of glutenin for wheat quality.* Eur. Food Res. Technol., 210: 324-330.

Zhang S., Schisler D.A., Boem M.J., Slininger P.J. 2007. *Utilization of chemical inducers of resistance and Cryptococcus flavescens OH 182.9 to reduce Fusarium head blight under greenhouse condition.* Biol. Control, 42: 308-315. DOI: 10.1016/j.biocontrol.2007.05.020