Effect of foliar application of Cu, Zn, and Mn on yield and quality indicators of winter wheat grain

Arkadiusz Stepien[1*], and Katarzyna Wojtkowiak[2]

ABSTRACT

Micronutrients are part of many crucial physiological plant processes. The combined application of N and micronutrients helps in obtaining grain yield with beneficial technological and consumer properties. The main micronutrients needed by cereals include Cu, Mn, and Zn. The subject of this study was to determine yield, quality indicators (protein content and composition, gluten content, grain bulk density, Zeleny sedimentation index, and grain hardness), as well as mineral content (Cu, Zn, Mn, Fe) in winter wheat grain (*Triticum aestivum* L.) fertilized by foliar micronutrient application. A field experiment was carried out at the Educational and Experimental Station in Tomaszkowo, Poland. The application of mineral fertilizers (NPK) supplemented with Cu increased Cu content (13.0%) and ω, α/β, and γ (18.7%, 4.9%, and 3.4%, respectively) gliadins in wheat grain. Foliar Zn fertilization combined with NPK increased Cu content (14.9%) as well as high (HMW) and low molecular weight (LMW) glutenins (38.8% and 6.7%, respectively). Zinc fertilization significantly reduced monomeric gliadin content and increased polymeric glutenin content in grain, which contributed in reducing the gliadin:glutenin ratio (0.77). Mineral fertilizers supplemented with Mn increased Fe content in wheat grain (14.3%). It also significantly increased protein (3.8%) and gluten (4.4%) content, Zeleny sedimentation index (12.4%), and grain hardness (18.5%). Foliar Mn fertilization increased the content of ω, α/β, and γ gliadin fractions (19.9%, 9.5%, and 2.1%, respectively), as well as HMW and LMW glutenins (18.9% and 4.5%, respectively). Mineral NPK fertilization, combined with micronutrients (Cu + Zn + Mn), increased Cu and Zn content in grain (22.6% and 17.7%, respectively). The content of ω, α/β, and γ gliadins increased (20.3%, 10.5%, and 12.1%, respectively) as well as HMW glutenins (7.9%).

Key words: Grain quality indicators, micronutrients, protein composition, *Triticum aestivum*, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) yield with favorable quality parameters is determined by both the genotype and environmental conditions, including the weather pattern and agricultural practices that must be properly selected in terms of type and intensity. The modification of the quality characteristics of common wheat grain influenced by basic fertilization has been the subject of numerous scientific studies. Svečnjak et al. (2013) demonstrated that N fertilization increases both grain yield and micronutrient content (14.0% Fe, 9.2% Zn, 19.7% Mn, 13.2% Cu, 15.1% Ni, and 23.0% Cd, respectively) in wheat grain. Similarly, Zhang et al. (2012a) demonstrated that an adequate N supply facilitates Fe, Zn, Mn, and Cu accumulation in wheat grain. Piekarczyk et al. (2011) indicated that both variety and N fertilization are significant agricultural elements with independent effects on crop yield and quality of winter wheat grain. Zecevic et al. (2010), Campillo et al. (2010), Piekarczyk et al. (2011), and Mandic et al. (2015) stated that increasing the N rate improves wheat grain quality, particularly gluten content and the sedimentation index. Johansson et al. (2003) claim that both the varietal factor and mineral fertilizer rates have the greatest impact on protein groups (albumins, globulins, gliadins, high molecular weight [HMW], low molecular weight [LMW]) and glutenins. Labuschagne et al. (2006) concluded that it is the N fertilizer rate and not the time of application that affects the fractional composition of proteins. According to Yue et al. (2007), the glutenin macro polymer content and the N application rate was closely related to the regulatory effect of N on HMW synthesis.

The effects of N fertilization on protein synthesis are well known, but not so the role played by micronutrients in setting both the levels and composition of proteins. Micronutrients are involved in numerous physiological processes that are essential for plants. Copper plays an important role in the metabolism of N compounds. Manganese, along with Zn, has an effect on protein biosynthesis by adjusting the activity of peptidases and controlling protein metabolism (Hänsch and Mendel, 2009). A critical requirement of micronutrients occurs at the stem elongation stage (BBCH 30-39) (BBCH Monograph, 2001) when the plant goes through an intense cell division process. In agricultural practice, the extra foliar feeding plant treatment is more and more often applied; it allows the rapid introduction during the growing season of nutrients that are in short supply in the plants when soil nutrient deficiency occurs or uptake is hindered (Wojtkowiak et al., 2014).

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[1] University of Warmia and Mazury in Olsztyn, Faculty of Environmental Management and Agriculture, pl. Lódzki 3, 10-718 Olsztyn, Poland.

[*] Corresponding author (arkadiusz.stepien@uwm.edu.pl).

[2] University of Warmia and Mazury in Olsztyn, Faculty of Technical Sciences, ul. Heweliusza 10, 10-718 Olsztyn, Poland.

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Albumins and globulins are distributed in all parts of a caryopsis, usually as elements of its structure or catalysts; because they are soluble, they are lost in gluten washout. Gliadins and glutenins are reserve proteins affecting the rheological properties of gluten differently. Gliadins transform it into a viscous liquid and increase its stretchability. Glutenins are responsible for its elasticity and strength (Wieser, 2007). According to Konopka et al. (2007a), the gliadin:glutenin ratio in common wheat should be close to 1:1. Its increase or decrease is a sign of deterioration of the functional properties of the hydrated gluten complex (Johansson et al., 2004). The aim of this study was to determine the yield, content of minerals (Cu, Zn, Mn, and Fe) and proteins, as well as their composition, and selected technological parameters of the winter wheat grain fertilized by foliar micronutrient application.

MATERIALS AND METHODS

Winter wheat (*Triticum aestivum* L.) 'Boomer' was cultivated in the 2011-2012 and 2012-2013 seasons at the Educational and Experimental Station in Tomaszkowo (53°72' N; 20°42' E), Poland. The experiment was carried out by the random block method with three replicates on gray-brown podzolic soil with a granulometric composition of silty clay loam, complex 4, and class IIIb. The soil characteristics were as follows: pH 5.95, 7.90 g C_{org} kg^{-1}, 0.95 g N_{total} kg^{-1}, 64.2 mg available P kg^{-1}, 186.8 mg available K kg^{-1}, 7.6 mg available Mg kg^{-1}, 3.2 mg available Cu kg^{-1}, 1900 mg available Fe kg^{-1}, 7.5 mg available Zn kg^{-1}, and 202 available mg Mn kg^{-1}.

The following micronutrient fertilization variants were tested in the experiment: (1) NPK (mineral fertilizer control): At all sites, N fertilization was applied at 90 kg ha^{-1} divided as in-soil application of 54 kg·ha^{-1} (urea 46%) at the tillering stage (BBCH 22-23) and foliar application of 36 kg N ha^{-1} (10% urea solution) at the stem elongation stage (BBCH 30-31); triple superphosphate (46%) at 30.2 kg P ha^{-1} and potassium salt (56%) at 83.1 kg K ha^{-1} were applied at pre-sowing; (2) NPK+Cu: Mineral fertilization as in the NPK variant + foliar fertilization with 0.2 kg Cu ha^{-1} (1% $CuSO_4$ solution); (3) NPK+Zn: Mineral fertilization as in the NPK variant + foliar fertilization with 0.2 kg Zn ha^{-1} (1% $ZnSO_4$ solution); (4) NPK+Mn: Mineral fertilization as in the NPK variant + foliar fertilization with 0.2 kg Mn ha^{-1} (0.5% $MnSO_4$ solution); and (5) NPK+Cu, Zn, Mn: Mineral fertilization as in the NPK variant + foliar fertilization with 0.2 kg Cu ha^{-1}, 0.2 kg Zn ha^{-1}, and 0.2 kg Mn ha^{-1}.

Copper, Zn, and Mn (individually or combined) were applied to leaves as aqueous solutions at the stem elongation stage (BBCH 30-31).

The plot area was 6.25 m^2 and winter triticale (*Triticosecale* Wittm. ex A. Camus) was grown as a forecrop. Sowing density of winter wheat 'Boomer' was 550 seeds m^{-2} and 285.0 kg ha^{-1}, and the distance between rows was 12 cm. Tillage treatments included plowing immediately after harvesting the forecrop. To cover the post-harvest residues, pre-sowing plowing and harrowing were carried out prior to sowing winter wheat. A combined cultivator and seed drill were used at all sites just before sowing to mix mineral fertilizers and prepare the soil for sowing. Weeds were controlled with herbicides in 2012: florasulam 5 g L^{-1}, aminopyralid 10 g L^{-1}, 2,4-dichlorophenoxyacetic acid 180 g L^{-1} (10 m^3 ha^{-1}; Mustang Forte 195 SE, Dow AgroSciences, Warsaw, Poland); fenoxaprop-P-ethyl 69.0 g L^{-1} and mefenpyr 75 g L^{-1} (12 m^3 ha^{-1}; Puma Universal 069 WG, Bayer Crop Science, Leverkusen, Germany); and in 2013: iodosulfuron-methyl-sodium 2 g L^{-1}, mesosulfuron-methyl 10 g L^{-1} (4.5 m^3 ha^{-1}; Atlantis 12 OD Bayer Crop Science) + iodosulfuron-methyl-sodium 25 g L^{-1}, amidosulfuron 100 g L^{-1} (1.5 m^3 ha^{-1}; Sekator 125 OD, Bayer Crop Science) in the spring after wheat vegetation resumed (BBCH 21-29). No protection against pests and diseases was applied.

The protein and wet gluten contents, bulk density, Zeleny index, and grain hardness were determined with an NIR System Infratec 1241 Analyzer (FOSS, Hillerod, Denmark), which takes transmission measurements of near-infrared waves (570-1050 nm).

To determine micronutrients, the grain was mineralized in a mixture of acids, HNO_3 and $HClO_4$ (4:1), and the content of Cu, Zn, Mn, and Fe was determined in the soil extract by the atomic absorption spectroscopy (AAS) method with a spectrophotometer (Z-8200, Hitachi, Japan).

The quantitative and qualitative characteristics of proteins were determined by the reversed-phase high-performance liquid chromatography (RP-HPLC) technique as developed by Konopka et al. (2007a). This was performed with a chromatograph (1050 series, Hewlett Packard, Palo Alto, California, USA). Detection of protein fractions was performed at a wavelength of 210 nm, and their identification consisted in analysing the spectra and retention times of reference proteins (specific classification was based on wheat grain protein retention time). Protein content was expressed in mAU s^{-1} (milli-absorbance units per second).

The results were processed with STATISTICA 10.0 software (StatSoft, Tulsa, Oklahoma, USA). Statistical calculations were performed with one-way ANOVA. Besides the basic parameters, standard deviation, standard error, and statistically homogeneous groups were determined by Tukey's test at $\alpha = 0.05$. Coefficients of linear correlation (Pearson's r) were calculated.

Monitoring temperature and precipitation continued during the experiment (Table 1). Monthly temperature distribution patterns during the winter wheat growing seasons (2011-2012 and 2012-2013) were similar and did not deviate from the multi-annual mean. Precipitation was higher in September of the first year (2011) by 21.8 mm and 10.6 mm in relation to the multi-annual values. In October and November 2011, it was lower by 39.0 mm and 31.1 mm, respectively, in relation to the following year's experiment and by 13.1 mm and 30.7 mm, respectively, in relation to the recorded multi-annual precipitation. It should be noted that the mean total multi-annual precipitation was exceeded by more than 2-fold at the start of the vegetation stage (April 2012). Total precipitation was similar at the beginning of the

Table 1. Weather conditions in 2011-2012 and multi-annual mean for 1981-2010.

Year	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May	June	July	Aug	Sept-Aug
						Temperature (ºC)							Mean
2011-2012	14.1	8.3	3.1	2.3	-1.7	-7.5	3.0	7.8	13.4	15.0	19.0	17.7	7.9
2012-2013	13.5	7.4	4.9	-3.5	-4.6	-1.1	-3.5	5.9	14.8	17.5	18.0	17.4	7.2
1981-2010	12.8	8.0	2.9	-0.9	-2.4	-1.7	1.8	7.7	13.5	16.1	18.7	17.9	7.9
						Rainfall (mm)							Total
2011-2012	67.5	29.5	14.1	25.8	61.8	27.7	24.1	73.1	51.7	103.2	121.0	45.1	644.6
2012-2013	45.7	68.5	45.2	11.8	44.1	22.6	18.1	28.5	54.5	61.2	121.9	37.6	559.7
1981-2010	56.9	42.6	44.8	38.2	36.4	24.2	32.9	33.3	58.5	80.4	74.2	59.4	581.8

heading stage (May) in each year of the experiment. In June 2012 (end of heading), precipitation exceeded precipitation in 2013 by 40%. In July, precipitation in each year of the experiment was similar, but exceeded the multi-annual precipitation (39% on the average).

RESULTS AND DISCUSSION

Significantly more Fe (11.1%) and Mn (12.9%) were recorded in the first year, and more Cu (40.9%) and Zn (9.9%) in the second year in winter wheat grain. Mineral fertilization with NPK combined with micronutrients (Cu, Zn, and Mn), applied individually or combined, changed the mineral content in the grain of winter wheat 'Boomer' (Table 2).

In our study, the winter wheat grain means for micronutrients were 2.33 mg Cu kg^{-1}, 53.1 mg Fe kg^{-1}, 29.7 mg Zn kg^{-1}, and 33.8 mg Mn kg^{-1} (Table 2). Szira et al. (2014) found comparable contents of Cu and Mn, and less Fe and Zn in 24 varieties of wheat cultivated under various site conditions. As compared to our studies, Koców (2009) found a higher Cu content, and a lower Fe, Zn, and Mn content in the grain of wheat fertilized with micronutrient fertilizer Plonvit Z (INTERMAG, Olkusz, Poland). Grain Cu content increased under the influence of extra fertilization with a combination of Cu and Zn and micronutrients (NPK+Cu, Zn, Mn). In relation to the only mineral (NPK) fertilization variant, Cu content increased by 22.6% (NPK+Cu, Zn, Mn), 14.9% (NPK+Zn), and 13.0% (NPK+Cu).

Grain Fe content increased (14.3%) only after spraying with a urea solution along with a $MnSO_4 \times 5H_2O$ solution at the stem elongation stage (BBCH 30-32).

The combined application of NPK with Cu, Zn, and Mn decreased Fe content by 11.1% (Figure 1). These results are different from those obtained by Wang et al. (2015) where the foliar application of Zn resulted in a significant increase in the Fe concentration. According to Pahlavan-Rad and Pessarakli (2009), foliar application of Zn increased the concentration of Zn and Fe in the grain by 99% and 8%, respectively, while the application of Mn increased by 7% the Mn concentration in the grain. In the study by Narwal et al. (2012), foliar application of Zn, Fe, and Mn increased the content of those minerals in the grain of 14 varieties of winter wheat. According to Kutman et al. (2011), enriching N fertilization with Zn in the form of foliar fertilizers increased Zn and Fe content in the grain.

Table 2. Micronutrient content in winter wheat grain with foliar micronutrient application and mean for fertilization methods.

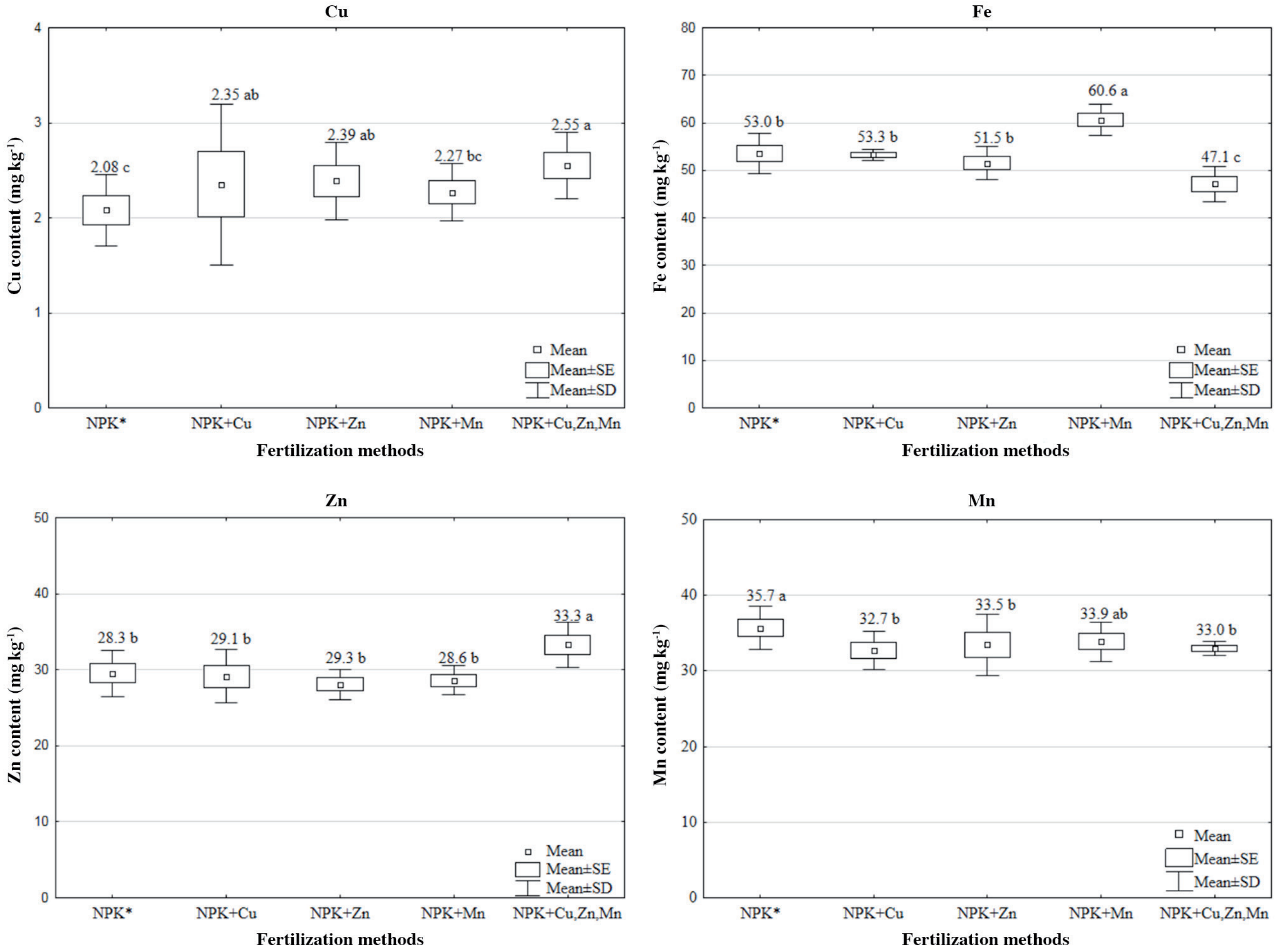
Year		Cu	Fe	Zn	Mn
		mg kg^{-1}			
2012	x	1.93b	55.9a	28.4b	35.8a
	SD	0.263	4.589	3.924	1.937
2013	x	2.72a	50.5b	31.1a	31.7b
	SD	3.700	8.150	2.608	2.265

x: Mean, SD: standard deviation.
Means with the same letter are not significantly different according to Tukey's test ($\alpha < 0.05$).

In our study, the only factor contributing to the significant increase of 17.7% in Zn content was the application of combined micronutrients. According to Zhang et al. (2012b), foliar application of only 0.4% $ZnSO_4$ $7H_2O$ increased Zn content in the grain by 58%; Peck et al. (2008) recorded twice the concentration of that element in the grain. This was not confirmed in our study. All fertilizer variants under analysis reduced the content of Mn in the grain as compared to mineral fertilization without micronutrients (Figure 1).

According to El-Ghamry et al. (2009), foliar micronutrient application leads to an increase in wheat yield and the macro- and micronutrient content in its grain. However, the application of micronutrients in agricultural practice does not always increase its content in the plant (Wojtkowiak and Stepien, 2015). Any deviation in the correct ratio of the elements may lead to a disturbance in absorption and transport, which are attributed to the antagonism among elements. Foliar fertilizers used as aqueous solutions (sulfates) must be absorbed by the leaf tissue before they can be assimilated. Before being metabolized by a plant, a nutrient applied on the leaf surface must pass through a waxy layer, a cuticle, the cell wall, and the cell membrane. All of these elements hinder permeation of nutrients into leaf cells. The beneficial action of micronutrients (Mn, Cu, and Zn) is an effect of, among other things, plant metabolism stimulation, which results in intensified nutrient uptake by the plant's root system (Cakmak, 2008; Hänsch and Mendel, 2009). Weather conditions in the growing periods of winter wheat 'Boomer' cultivated in eastern Poland significantly affected grain yield and the analyzed qualitative characteristics (Table 3). The highest value of the qualitative indicators under study was exhibited by the grain in 2013. This could have been influenced by different thermal conditions in the winter of 2012-2013, which encouraged the transition to the vernalization stage combined with the optimum distribution of precipitation. Zecevic et al. (2014) confirmed the effect of weather conditions in the growing

Figure 1. Content of micronutrients in winter wheat grain provided with foliar application of micronutrients, average for years of investigation.



NPK*: Mineral fertilizers; Cu, Zn, Mn: micronutrients; SE: standard error; SD: standard deviation.
Means with the same letter are not significantly different according to Tukey's test ($\alpha < 0.05$).

periods on the analyzed parameters of the quality of wheat grain (proteins, gluten, and Zeleny index). After analyzing the technological indicators for the grain in the second year of the experiment, increases were found in protein content (8.9%), gluten content (29.0%), Zeleny sedimentation index (34.7%), bulk density (9.3%), and grain hardness (28.6%). According to Malakouti (2008) and Wang et al. (2015), micronutrients and the interactions between them have a positive effect on the physiological processes of plants, which is reflected in improved grain yield and quality. This was not confirmed by our study, and the experimental factors had no effect on the differentiation of grain yield (6.83 to 7.32 t ha^{-1}). According to Bameri et al. (2012), the absence of effects of the combined application of Zn, Fe, and Mn on an increase in wheat yield may be the result of antagonisms between Fe, Zn, and Mn.

In the abovementioned studies, the total content of proteins (10.4% to 11.0%) and gluten (22.6% to 23.7%) in the wheat grain was not significantly different regardless of the applied micronutrients.

The Zeleny sedimentation index, which allows the quantitative and qualitative determination of gluten in flour, ranged from 24.1 to 28.0 cm^3. Grain bulk density ranged from 74.8 to 75.4 kg hL^{-1}, and the hardness index ranged from 29.8 to 36.5. The extra application of spraying with Mn, along with urea, at the stem elongation stage contributed to an increase in the content of the parameters under analysis (total protein content of proteins by 3.8%, gluten content of 4.4%, Zeleny sedimentation index of 12.4%, and grain hardness of 18.5%). According to Hänsch and Mendel (2009), Mn activation was seen in enzymes of N metabolism (glutamine synthetase and arginase), which may have increased the grain quality parameters discussed here.

Zhang et al. (2012a) state that foliar Zn application has a slight effect on protein concentration and gluten characteristics Shewry et al. (2013) found that the amount of gluten in the wheat grain ranges from 24.1% to 61.2%, and the sedimentation index ranges from 13.5 to 50 mL. Warechowska et al. (2013) point out a mean Zeleny sedimentation index value of 31.4 cm^3 for popular wheat varieties cultivated in Europe.

Johansson et al. (2003) indicate that environmental conditions, including the years of the study, modify the amount and size distribution of mono- and polymeric proteins in the wheat grain. According to Konopka et al. (2007b), the grain obtained under water deficit conditions contain less HMW and LMW glutenins, as well as albumins, globulins, and γ gliadins. In our study, despite similar weather conditions in the years of the experiment (except for lower precipitation in April and June 2013), higher protein fraction contents were obtained in 2013 (Tables 4 and 5).

Many studies have shown a relationship between N fertilization and the deposition of storage proteins in the grain (Johansson et al., 2003; Labuschagne et al., 2006; Yue et al., 2007; Fuertes-Mendizábal et al., 2010; Chope et al., 2014); on the other hand, there is no information about the effects of extra micronutrient fertilization on fractional protein composition. Our study demonstrated that albumins and globulins (catalytic and structural proteins) accounted for 20.7% to 23.2% of total proteins.

Supplementing basic mineral fertilization with Mn and a combination of micronutrients (Cu, Zn, Mn) increased the content of albumins and globulins by 3.8% and 4.2%, respectively (Table 4). Gluten proteins (gliadins and glutenins) were the predominant protein fraction (76.8% to 79.3%). Mineral fertilization combined with foliar spraying with Cu or Mn, or a combination of micronutrients (Cu, Zn, Mn), contributed to increasing the accumulation of gliadins by 5.3%, 7.3%, and 11.8%, respectively. Extra fertilization with Zn and Mn increased the proportion of glutenin proteins by 15.5% and 8.4%, respectively, in the wheat grain. Similarly, Peck et al. (2008) studied the applied micronutrients and found a clear effect of Zn fertilization on the decrease in the content of gliadins and glutenins. A possible mechanism of the effect of Zn and Mn on protein is the interaction with cysteine and methionine residues in protein and the formation of disulfide linkages during polymerization.

Table 3. Grain yield, protein content, grain density, gluten content, Zeleny index, and hardness of winter wheat grain with foliar micronutrient application.

Fertilization methods		Grain yield	Protein content	Density	Gluten content	Zeleny index	Hardness
		t ha^{-1}	%	kg hL^{-1}	%		
NPK	x	7.11a	10.6b	75.3a	22.7bc	24.9b	30.8b
	SD	1.326	0.367	3.531	0.234	3.67	5.144
NPK+Cu	x	6.83a	10.6ab	74.8b	22.6bc	24.8b	32.8b
	SD	1.774	0.204	3.684	0.190	2.43	0.828
NPK+Zn	x	7.06a	10.4b	74.9ab	22.3c	24.1b	30.3b
	SD	1.230	0.794	3.750	0.999	4.93	5.640
NPK+Mn	x	7.32a	11.0a	75.4a	23.7a	28.0a	36.5a
	SD	1.557	0.478	3.744	0.449	4.46	7.814
NPK+Cu, Zn, Mn	x	7.25a	10.6b	74.9ab	23.0b	24.9b	29.8b
	SD	1.530	0.792	3.560	0.634	5.54	5.598
			Annual means				
2012	x	5.85b	10.17b	71.73b	22.55b	21.59b	28.03b
	SD	0.513	0.429	0.358	0.731	1.94	3.413
2013	x	8.38a	11.08a	78.40a	29.09a	29.09a	36.06a
	SD	0.560	0.264	0.259	0.676	2.04	4.447

NPK: Mineral fertilizers; Cu, Zn, Mn: micronutrients; x: mean; SD: standard deviation.
Means with the same letter are not significantly different according to Tukey's test ($\alpha < 0.05$).

Table 4. Protein content and composition (peak area) of winter wheat grain with foliar micronutrient application.

Fertilization methods		Albumins and globulins	Σ Gliadins	Σ Glutenins	Gliadin: glutenin
		mAU s^{-1}			
NPK	x	17 012b	30 770d	32 047c	0.96b
	SD	10.37	68.42	217.07	0.007
NPK+Cu	x	16 512c	32 393c	30 910d	1.05a
	SD	23.66	153.04	164.57	0.002
NPK+Zn	x	16 407c	28 521e	37 009a	0.77c
	SD	134.88	220.89	445.50	0.027
NPK+Mn	x	17 664a	33 026b	34 752b	0.95b
	SD	99.60	219.41	244.82	0.006
NPK+Cu, Zn, Mn	x	17 725a	34 396a	32 058c	1.07a
	SD	132.24	216.25	197.02	0.007
		Annual means			
2012	x	16 167b	26 623b	31 647b	0.84b
	SD	581.00	209.80	307.30	0.118
2013	x	17 961a	37 015a	35 063a	1.06a
	SD	532.00	191.80	280.60	0.107

NPK: Mineral fertilizers; Cu, Zn, Mn: micronutrients; x: mean, SD: standard deviation.
Means with the same letter are not significantly different according to Tukey's test ($\alpha < 0.05$).

Table 5. Protein fractions of gliadins and glutenins in winter wheat grain with foliar micronutrient application.

			Gliadins		Glutenins	
Fertilization methods		ω	α/β	γ	HMW	LMW
		mAU s^{-1}				
NPK	x	2101b	16 601c	12 067d	8772d	23 275b
	SD	31.25	71.76	8.69	39.39	205.66
NPK+Cu	x	2493a	17 421b	12 479b	8778d	22 132c
	SD	24.51	103.24	25.36	60.52	214.61
NPK+Zn	x	1937c	15 685d	10 899e	12 173a	24 836a
	SD	13.97	220.98	31.04	17.35	428.73
NPK+Mn	x	2519a	18 181a	12 325c	10 430b	24 322a
	SD	28.01	132.80	64.60	20.32	216.09
NPK+Cu, Zn, Mn	x	2527a	18 341a	13 527a	9466c	22 592bc
	SD	13.03	244.77	53.79	53.60	231.07
		Annual means				
2012	x	1951b	15 161b	9513b	8502b	23 145b
	SD	25.70	104.30	87.30	40.00	108.60
2013	x	2679a	19 331a	15 005a	11 346a	23 717a
	SD	23.70	94.70	37.10	37.00	143.40

NPK: Mineral fertilizers; Cu, Zn, Mn: micronutrients; x: mean; SD: standard deviation; HMW: high molecular weight; LMW: low molecular weight. Means with the same letter are not significantly different according to Tukey's test ($\alpha < 0.05$).

As a result of the combination of micronutrients applied with mineral fertilization, the gliadin:glutenin ratio ranged from 0.77 to 1.07, and fertilizer variants contributed to their significant differentiation. Supplementing basic fertilization (NPK) with Zn decreased the number of gliadins (28 521) and the highest accumulation of glutenins (37 009), which resulted in a significant decrease in the gliadin:glutenin ratio (0.77). According to Konopka et al. (2007a), the gliadin:glutenin ratio in bread wheat grain should be close to 1:1. An increase or decrease in the relationship between the fractions indicates the deterioration of functional characteristics of their hydrated gluten complex.

Foliar micronutrient fertilization combined with mineral fertilization significantly differentiated the content of monomeric gliadins and polymeric glutenins as compared to mineral fertilization (Table 5). Foliar fertilization with Cu (NPK+Cu), Mn (NPK+Mn), and combined micronutrients (NPK+Cu, Zn, Mn) increased the content of ω gliadins (18.7%, 19.9%, and 20.3%, respectively), α/β gliadins (4.9%, 9.5%, and 10.5%, respectively), and γ gliadins (3.4%, 2.1%, and 12.1%, respectively). Extra Zn fertilization (NPK+Zn) decreased the content of ω, α/β, and γ gliadins by 7.8%, 5.5%, and 9.7%, respectively. The level of LMW glutenin subunits in the wheat grain was higher than HMW glutenin subunits. Mineral fertilization (NPK) along with Zn, Mn, and combined micronutrients (NPK+Cu, Zn, Mn) increased the content of HMW glutenins by 38.8%, 18.9%, and 7.9%, respectively. The content of LMW glutenin subunits increased by 6.7% and 4.5% following the supplementation of basic fertilization with Zn and Mn. Extra fertilization with Cu (NPK+Cu) and combined micronutrients (NPK+Cu, Zn, Mn) decreased the content of LMW glutenins by 4.9% and 2.9%, respectively. Luo et al. (2001) conclude that the content of HMW and LMW fractions is genetically conditioned while Chope et al. (2014) indicate the predominant role of N in forming relationships. According to Fuertes-Mendizábal et al. (2010), an increase in the content of HMW glutenins allows the formation of disulfide bonds, which leads to an increase in the degree of polymerization for protein aggregates and, as a consequence, improves the quality of bakery products.

Svečnjak et al. (2013) point out that grain yield is correlated with the content of basic micronutrients. In our study, a correlation analysis (Table 6) demonstrated a positive relationship between Cu content in the grain and grain yield (r = 0.777), protein content (r = 0.546), bulk density (r = 0.805), Zeleny sedimentation index (r = 0.653), and grain hardness (r = 0.442). The content of Mn in the grain was negatively correlated with grain yield and technological indicators for the grain under assessment. The content of Fe in the grain was positively correlated with the gliadin:glutenin ratio (r = 0.530) and negatively correlated with grain yield (r = -0.399), bulk density (r = -0.472), total glutenins (r = -0.493), and HMW glutenins (r = -0.471). Oury et al. (2006) and Zhao et al. (2009) confirm the negative relationship between Fe content and grain yield. However,

Table 6. Correlations between micronutrient content in grain and grain yield and yield quality of winter wheat with foliar micronutrient application.

Specification		Cu	Mn	Fe	Zn
Grain yield		0.777	-0.659	-0.399	0.419
Protein content		0.546	-0.605	ns	ns
Density		0.805	-0.742	-0.472	0.422
Gluten content		ns	-0.384	ns	ns
Zeleny index		0.653	-0.654	ns	ns
Hardness		0.442	-0.574	ns	ns
Gliadins		ns	ns	ns	0.413
Glutenins		ns	ns	-0.493	0.395
Gliadin:glutenin		ns	ns	0.530	ns
Gliadins	ω	ns	ns	ns	ns
	α/β	ns	ns	ns	ns
	γ	ns	ns	ns	0.499
Glutenins	HMW	ns	ns	-0.471	0.389
	LMW	ns	ns	ns	ns

ns: Nonsignificant differences ($\alpha < 0.05$), HMW: high molecular weight, LMW: low molecular weight.

the strength of these correlations depends on environmental conditions (White and Broadley, 2009). In our study, a positive correlation occurred between grain Zn content and grain yield (r = 0.419), grain bulk density (r = 0.422), total gliadins (r = 0.413), γ gliadins (r = 0.499), total glutenins (r = 0.395), and HMW glutenins (r = 0.389). Oury et al. (2006) and Zhao et al. (2009) confirmed the negative relationship between Zn content and grain yield.

CONCLUSIONS

In conclusion, a statistical analysis confirmed the effect of the years of the experiment on grain yield and all analyzed quality parameters for the grain of winter wheat 'Boomer'. Mineral fertilization supplemented with Cu, Zn applied individually or combined (Cu, Zn, Mn) increased Cu content in winter wheat grain. Manganese fertilization increased Fe content; a combination of micronutrients (Cu, Zn, Mn) increased grain Zn content. Extra Mn fertilization significantly increased protein and gluten content, Zeleny sedimentation index, and grain hardness. Under Zn fertilization, there was a significant reduction of monomeric gliadin content and an increase of polymeric glutenin content in the grain, which reduced the gliadin:glutenin ratio. As a result of foliar fertilization with Cu, Mn, and a combination of micronutrients, the content of ω, α/β, and γ gliadins increased. Fertilization with Cu increased the content of low molecular weight glutenins, and fertilization with Zn and Mn increased high molecular weight and low molecular weight glutenin fractions. Applying a combination of micronutrients increased the content of high molecular weight glutenins.

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