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Influence of zinc treatments on grain yield and grain quality of different maize genotypes

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Abstract: Maize production is intensified with a larger amount of mineral fertilisers in the era of meteorological conditions change, which leads to a decrease in the reserves of microelements in the soil. The aim of this study was to determine the influence of zinc application on grain yield, nitrogen and carbon content in grain of three maize genotypes in the period 2016–2018 (factor A). Factor B: cultivars ZP 427, ZP 548 and ZP 687 belonging to different maturation groups. Factor C: Various zinc treatments were applied: T_1 – control; T_2 – 25 kg Zn^{2+}/ha (35 g of $ZnSO_4$ on the experimental plot) was introduced into the soil before sowing; T_3 – seed treatment (0.129 g of $ZnSO_4 \cdot 7 H_2O$) + foliar treatment (2 L/ha liquid fertiliser 7% Zn^{2+}). The average yield for all examined variables was 7.33 t/ha. On average, T_2 (8.08 t/ha) treatment showed a highly significant effect on the yield in relation to T_1 (7.03 t/ha) and on T_3 (7.21 t/ha). On average, the amount of nitrogen determined for all cultivars was the highest in T_3 (1.52%). The highest carbon content was in T_1 (41.78%), which is at the level of significance of $P < 0.01$ more than T_2 (41.46%), while in relation to T_3 (40.99%) there is no significance.

Keywords: *Zea mays* L.; macroelement; micronutrient; agroclimatic condition

Maize (*Zea mays* L.) is one of the most important cultivated plant species with wide application in human and animal nutrition, in the food industry and in the production of biofuels. It is grown in a number of agro-ecological environments in the world (IITA 2009). Forecasts show that by 2025 the production of maize in the world will increase significantly, while the need for products of this plant species by 2050 in developing countries will double (Rosegrant et al. 2008).

In Serbia, maize is the main plant species both in terms of area and productivity. It is grown on more than a million hectares a year (https://gain.fas.usda.gov/Belgrade_Serbia). Serbia is in the fifth place in terms of maize production from 47 CEFTA (Central European Free Trade Agreement) countries and most countries in the EU and the CIS (Commonwealth of Independent States) (http//www.seedev.org/publikacije/). For the last 100 years, maize breeding has been intensively developed in the direction of creating genotypes with increased yield potential. To realise the genetic potential of fertility, maize requires a large amount of macroelements, especially nitrogen (N). However, for high yields of maize, it is

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very important that maize plants are provided with zinc (Zn). Potarzycki and Grzebisz (2009) states that Zn strongly influences maize in the critical phase of grain formation, which affects the final grain yield. This microelement is an activator of enzymes such as RNA polymerase, superoxide, etc. It participates in the structure and synthesis of proteins, metabolism of carbohydrates, fats and nucleic acids, increases resistance to diseases and unfavourable agroclimatic conditions. It affects the biosynthesis of the plant hormone auxin, which stimulates the growth and development of the root system and quality rooting. Increased need for zinc, especially on arid and semi-arid soils was reported (Rurinda et al. 2014). The concentration of zinc in certain types of soil is within wide limits. Sandy soils have the lowest zinc concentration of 30 mg/kg, while in soils rich in organic matter such as chernozem the concentration can be from 120–150 mg/kg. The level of soil zinc is not correlated with the concentration available to plants, because the mobile part of zinc is on average 1% of the total amount. About 50% of the soluble fractions of zinc is the free ionic form of Zn^{2+} which is the most accessible to plants. However, it does not stay free for long, but binds to colloids and precipitates with hydroxides, urea, phosphates, sulfides and other anions (Kabata-Pendias 2004). Almost 50% of the world's land in cereal production has low available Zn which is the cause of low yields and nutritional quality of grain (Welch and Graham 2004). Lack of zinc in the soil can reduce cereal yield by up to 80%. Great attention is thus paid to the study of the role of zinc in tolerance to biotic and abiotic stress.

The aim of this study was to determine the impact of zinc application on yield and grain quality of different maize genotypes for a period of three consecutive years.

MATERIAL AND METHODS

Design of experimental research. Experimental research was conducted during 2016–2018 on a plot in the municipality of Vladimirci, Mačva region, Serbia (44°36'31.8''N, 19°47'4.2''E). According to the mechanical composition, the soil is on the boundary of clay and loam. Soil chemical characteristics: 1.26% organic carbon – determination of humus content – by the method of oxidation of organic matter, pH_{KCl} 5.26, 2.44 mg P/kg determination of ammonium lactate – determination of readily available phosphorus spectrophotometrically and 163.0 mg K/kg (determination of ammonium lactate – determination of readily available potassium using the flame photometry). The area of the experimental plot was 5 607 m^2, and the elementary plot 14 m^2. The plots are arranged according to the plan of divided plots in four repetitions. The precursor in each year of the research was wheat. All agrotechnical measures were applied in optimal deadlines. For plant nutrition the following treatments were used: 160 kg N/ha (224 g N on the experimental plot), respectively 30 kg N/ha (200 kg/ha NPK 15:15:15) in basic cultivation (42 g N on the experimental plot), 90 kg N/ha (196 kg/ha urea 46% N, $CO(NH_2)_2$) in pre-sowing (126 g N on the experimental plot) and 40 kg N/ha (87 kg/ha urea 46% N, $CO(NH_2)_2$) in top-dressing (56 g N on the experimental plot).

Factor A: Meteorological conditions during the vegetation have a significant impact on maize production. This is especially pronounced in the conditions of natural water regime, and in studies of this type (temperature and precipitation) cannot be omitted.

Factor B: Three cultivars, yellow grain of the tooth type, selected at the Maize Institute Zemun Polje, were sown: ZP 427 (FAO 400); ZP 548 (FAO 500) and ZP 687 (FAO 600).

Factor C: Various zinc treatments were applied: T_1 – control; T_2 – before sowing 25 kg Zn^{2+}/ha was introduced into the soil (35 g of $ZnSO_4$ on the experimental plot); T_3 – seed treatment before sowing + foliar treatment with Zn^{2+}. Before sowing, for one elementary plot, 63 maize seeds were used, immersed in a solution with 0.129 g of $ZnSO_4 \cdot 7\,H_2O$ and kept in the dark for 24 h. After that the seeds were washed with water and dried in filter paper (modified according to Johnson et al. (2005)). In the phenophase of 5–7 leaves, 2 L/ha liquid fertiliser with trade name Nutri Zinc Pro (Agrochemical) was applied foliarly. The fertiliser is intended for nutrition of different plant species on different types of soil, foliar and through an irrigation system. Nutri Zinc Pro contains 7% total zinc (3.5% EDTA – ethylenediamine tetra-acetic acid chelating agent, which is capable of binding metal ions, e.g. sodium, calcium, magnesium, zinc and many others).

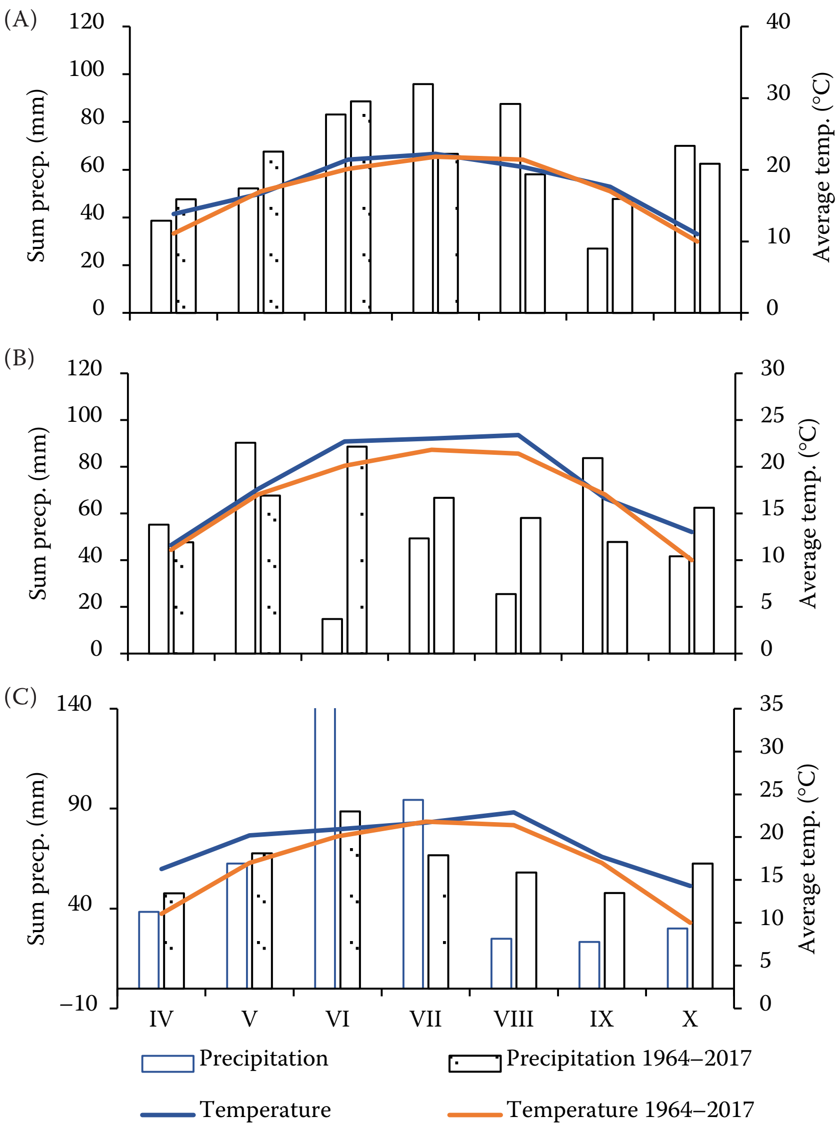
The total content of N and carbon (C) was determined by elemental analysis after dry incineration of the sample on a CHNSO VarioEL III apparatus (Gaithersburg, USA), which is expressed in % (AOAC 2006). The yield was calculated on 14% grain moisture. To evaluate data, descriptive statistics and analysis of variance (ANOVA) in the program DSAASTAT

(Perugia, Italy) were used. Three-way ANOVA was used to test effects of year, genotype, treatment and growing season. All results were calculated at a significance level *LSD* (least significant difference) of 0.01 and 0.05.

RESULTS AND DISCUSSION

Meteorological conditions (air temperature and sum precipitation). Differences between average air temperature and sum precipitation of maize vegetation by years (Figure 1).

During a three-year study, the lowest average temperature during the period of maize vegetation was measured in 2016 (17.5 °C). The average daily average temperatures in 2017 (18.2 °C) and 2018 (19.2 °C) were higher. In the period of development of generative organs (June–July) and grain filling (August–September) in 2017, the calculated average values of the average monthly air temperature were 21.4 °C. In 2018, the calculated temperatures were 0.6 °C lower (20.8 °C). It can be stated that the mean air temperatures were optimal for the development of maize. The amount of precipitation in the examined period was different. The highest sum of precipitation was recorded in 2016, when 454.4 mm fell in the period from April to October, in 2018 it was 426.4 mm. In the same period in 2017, the measured sum precipitation was only 360.3 mm. During the three-year research period, differences were noticed in terms of the distribution of precipitation by months. During the month of May, in the



2016 Average 17.5 °C
Sum 454.4 mm
1964–2017 Average 16.9 °C
Sum 438.9 mm

2017 Average 18.2 °C
Sum 360.3 mm

2018 Average 19.2 °C
Sum 360.3 mm

Figure 1. Average air temperature and sum precipitation in (A) 2016; (B) 2017 and (C) 2018 vegetation period

phases of vegetative development of plants, the sum of precipitation recorded in 2018 was 62.5 mm, while in 2016 it was 52.2 mm and in 2017 it was 90.2 mm. The differences were pronounced in the period June–July, when the lowest precipitation was in 2017 (64.1 mm), then in 2016 (135.4 mm), while in 2018 the highest precipitation was recorded (247.2 mm). Significant differences were recorded also in the grain filling phase (July–August): in 2016, the sum precipitation was 183.4 mm, in 2017 – 74.8 mm and in 2018 – 119.3 mm. In October 2018, the lowest sum precipitation was only 30.0 mm.

Grain yield. Maize is a plant that is grown in the open field and the yield is a very quantitative trait. Environmental factors (A) and treatments (C) affected yield levels at a level of $P < 0.01$ (Table 1). The interaction of genotypes × treatments at the level of $P < 0.05$ determined the differences in the amount of yield. The amount and distribution of precipitation significantly affected the height of maize yields. In the summer period, drought is a regular occurrence, most pronounced in the months of July–August. The highest grain yield in 2016 was 10.93 t/ha. The relatively favourable distribution of precipitation influenced the achievement of high grain yields in the conditions of natural water regime. Compared to 2018 (7.91 t/ha), the increase was 3.02 t/ha (38.18%). In 2017, the water deficit was pronounced in June, July and August, which affected the grain yield to be the lowest, 3.50 t/ha. The differences found were significant at the level of $P <$ 0.01. In Serbia, that time period coincides with the phenological phases of maize flowering, fertilisation and grain pouring. Deficiency of water in the stages of flowering and pollination can lead to changes in silk (later silking, reduces the length of silk) and prevent the development of embryos after pollination. In such unfavourable conditions, the treatment of T_2 (3.86 t/ha) significantly affected the difference in yield (0.33 t/ha) compared to the control (3.53 t/ha). Genotype (B) did not have a statistically significant effect on maize grain yield, although genotype ZP 687 (7.72 t/ha) had the highest grain yield. Treatments with zinc (C) had the effect that on average for all three years the yields were significantly higher compared to the control. The highest yield was in the treatment of soil with zinc (T_2) 8.08 t/ha. Compared to the control, the increase was for 15.34%. Compared to T_3 treatment (7.21 t/ha) there was an increase of 12.25%, which was highly significant.

The obtained results can be explained by the fact that zinc ion was more accessible to plants during T_2 treatment. Similar results were obtained by Abid et al. (2014) that the dry matter yield of fodder maize increased significantly under the influence of N and Zn^{2+} fertilisation. The importance of zinc fertilisation for the early growth of maize plants was shown by Liu et al. (2016) through increasing the rate of photosynthesis in the leaf development phase.

According to Basit et al. (2021) $ZnSO_4$ maize seed priming affected an increase in maize root biomass of 22–48%, shoot biomass 38–69%, in 30-day-old plants, compared to the control. Nciizah et al. (2020) pointed out that the duration of seed priming and the concentration of Zn mean the effect on the percentage of germination and the speed of seed germination.

In order for the zinc ion to be absorbed, it is necessary for the zinc to reach the root surface. Such different results are significantly influenced by the time of application of zinc and the phenophase of plant development. Abdoli et al. (2014) proved that foliar application of Zn^{2+} as $ZnSO_4$ in different phenological phases of development has different effects on the increase of yield components and the concentration of Zn^{2+} in wheat grain. Wang et al. (2017) found that foliar and application of Zn^{2+} over the soil did not significantly affect the yield of maize biomass. In addition to the method of using zinc to increase yields, the amount of zinc is also important. Potarzycki et al. (2010) found that foliar application of Zn^{2+} at a dose of 0.5 and 1 kg Zn^{2+}/ha has a positive effect on maize grain yield, and Jamil et al. (2015) stated that increasing doses of Zn^{2+} (5 and 10 kg Zn^{2+}/ha) increased plant height, tree volume and yield. Potarzycki and Grzebisz (2009) concluded that in a three-year study, yield increased by 18% using foliar treatments ranging from 1.0 to 1.5 kg Zn^{2+}/ha, compared to NPK – only fertilisation treatment. Also, they found that during foliar treatment with 1.0 kg Zn^{2+}/ha, the total N and grain yield significantly increased.

Nitrogen content in maize grain. The chemical composition of maize grains is its most important property. Maize grain, as well as grain of other cereals, contains the following most important chemical components: starch (61–78%), non-starch polysaccharides (about 10%), proteins (6–12%) and fats (3–6%) (Sinha et al. 2011). The nitrogen content in maize grain is very important, because nitrogen is the main element in the composition of crude proteins. The nitrogen content in maize grain and other parts of the plant is different and depends on the amount of macro and micronutrients. Nitrogen in the grain comes from the nitrogen absorbed from the soil during the pouring of the grain and from the nitrogen that is transported from the vegetative organs to the grain. In the conducted research, the average nitrogen content in maize grain was 1.48%.

All examined variables had a highly significant effect on the nitrogen content in the grain (Table 2). Meteorological conditions (A) were the most favourable in 2016, when the highest nitrogen content was determined (1.71%), while in 2018 (1.33%) the nitrogen content was the lowest. In 2017 the nitrogen content was 1.44%. The difference in nitrogen content in maize grain between 2017 and 2018 was not at the level of statistical significance. This difference is assumed to have been affected by a smaller amount of precipitation (–10.9 mm) in August 2018. Genotype (B) and interactions with other variables had a highly significant effect on grain nitrogen content. Cv. ZP 684 had the highest nitrogen content in grain (1.58%). Differences between cultivars are at the level of $P < 0.01$. These differences occur due to different maturation groups, phenotypic characteristics, smaller plants, shorter grain filling time (Ferreira et al. 2012), vegetation length and plant phase in conditions of insufficient water, which confirms the highly significant interaction ratio of AB factors. The use of zinc in treatments (C) significantly $P < 0.01$ affected the nitrogen content in maize grain. The highest nitrogen content in maize grain on average in all cultivars was during T_3 treatment. The average value was 1.52%, which is statistically significantly higher than in the control (1.45%) and T_2 treatment (1.48%).

Carbon content of maize grain. Maize is a high-yielding carbohydrate plant. Maize grain has the most BEM (nitrogen-free extractives), and over 90% of the total amount of BEM is starch, so the chemical composition of maize is a real grain-starch plant. The average starch content in the grain of different maize genotypes is 71% with a variation of 3%. Starch should be the most abundant carbohydrate in the

Table 1. Influence of maize genotypes and applied zinc treatments on yield

Year (A)	Genotype (B)	Treatment (C)			$\bar{x}$ AB	$\bar{x}$ A
		T_1	T_2	T_3		
	427	8.37	11.20	10.60	10.06	
2016	548	11.52	12.42	8.65	10.87	10.93
	687	11.47	12.72	11.37	11.85	
$\bar{x}$ AC		10.45	12.11	10.21		
	427	3.10	3.97	3.41	3.49	
2017	548	3.41	3.61	2.66	3.22	3.50
	687	3.55	4.00	3.63	3.73	
$\bar{x}$ AC		3.53	3.86	3.23		
	427	7.00	10.33	8.58	8.64	
2018	548	6.80	7.61	8.18	7.53	7.91
	687	8.01	6.87	7.83	7.57	
$\bar{x}$ AC		7.27	8.27	8.20	$\bar{x}$ B	
	427	6.16	8.50	7.53	7.40	
$\bar{x}$ BC	548	7.24	7.88	6.50	7.21	
	687	7.68	7.86	7.61	7.72	
$\bar{x}$ C		7.03	8.08	7.21		
Average 2016–2019					7.33	

	A^{**}	B^{ns}	AB^{ns}	C^{**}	AC^{ns}	BC^{*}	ABC^{ns}
F-test	0.00	0.49	0.32	0.00	0.16	0.03	0.09
$LSD_{0.01}$	0.59	1.72	2.97	0.87	1.51	1.51	2.61
$LSD_{0.05}$	0.53	1.22	2.12	0.65	1.12	1.12	1.94

T_1 – control; T_2 – before sowing 25 kg Zn^{2+}/ha was introduced into the soil (35 g of $ZnSO_4$ on the experimental plot); T_3 – seed treatment before sowing + foliar treatment with Zn^{2+}; *LSD* – least significant difference; $^{*}P < 0.05$; $^{**}P < 0.01$; ns – not significant

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Table 2. Nitrogen content in maize grain depending on applied variables (%)

Year (A)	Genotype (B)	Treatment (C) T_1	T_2	T_3	$\bar{x}$ AB	$\bar{x}$ A
2016	427	1.53	1.50	1.79	1.61	1.71
	548	1.69	1.78	1.69	1.72	
	687	1.90	1.76	1.78	1.81	
$\bar{x}$ AC		1.70	1.70	1.68	1.75	
2017	427	1.25	1.31	1.60	1.38	1.44
	548	1.37	1.54	1.24	1.38	
	687	1.60	1.46	1.58	1.55	
$\bar{x}$ AC		1.40	1.40	1.44	1.47	
2018	427	1.06	1.38	1.35	1.20	1.33
	548	1.30	1.37	1.32	1.33	
	687	1.36	1.45	1.33	1.38	
$\bar{x}$ AC		1.24	1.24	1.34	$\bar{x}$ B	
$\bar{x}$ BC	427	1.45	1.48	1.52	1.40	
	548	1.45	1.56	1.41	1.48	
	687	1.62	1.56	1.56	1.58	
$\bar{x}$ C		1.45	1.48	1.52		
Average 2016–2019					1.48	

	A**	B**	AB**	C**	AC**	BC**	ABC**
F-test	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$LSD_{0.01}$	0.05	0.03	0.05	0.03	0.05	0.05	0.09
$LSD_{0.05}$	0.05	0.02	0.03	0.02	0.04	0.04	0.07

T_1 – control; T_2 – before sowing 25 kg Zn^{2+}/ha was introduced into the soil (35 g of $ZnSO_4$ on the experimental plot); T_3 – seed treatment before sowing + foliar treatment with Zn^{2+}; *LSD* – least significant difference; $^{*}P < 0.05$; $^{**}P < 0.01$; ns – not significant

human diet, up to 50%. This is very important for more intensive inclusion of maize in the direct diet of people. Since the starch molecule consists of glucose chains in which carbon is present, it is important to determine the carbon content, because it is the main source of metabolic energy. According to the results of the research in Table 3, the average value of carbon in maize grain was 41.41%. Statistically significant differences were influenced by year (A), treatments (C) and year × treatment interaction (AC) ($P < 0.01$).

The synergistic effect of seed treatment and foliar treatment with Zn has a significant impact. Foliar fertilisation with micronutrients has proven to be necessary, because it enables the application of minerals according to the needs of the plant. Foliar application enables uniformity in the distribution of nutrients. The application of bioactive substances, which can be applied in parallel with foliar fertiliser, also showed good results in affecting plant growth (Ruiz-García and Gómez-Plaza 2013). Studies by Harris et al. (2007) showed that pre-sowing treatment of maize seeds with 1% zinc sulphate solution ($ZnSO_4$) can very easily increase the level of zinc in the seeds. Imran et al. (2017) found under controlled conditions that soybean plants whose seeds were treated with zinc grew as plants to which zinc was provided in the form of a nutrient solution. Rehman and Farooq (2016) found that wheat grain yield can be increased by 33–35% and zinc content in grain up to 25% if wheat seeds are treated with 1.25 and 1.50 kg Zn^{2+}/ha ($ZnSO_4$) before sowing. Esper Neto et al. (2020) found that maize plants whose seeds were treated with zinc oxide nanoparticles before sowing had more intensive germination and greater resistance to stress conditions.

In 2016, the highest carbon content was 43.96%. Compared to 2017 (39.65%), the carbon content was higher by 10.87%, and in 2018 (40.61%) by 9.05%. The differences were significant at the level of $P < 0.01$. The difference of 0.96% between 2017 and 2018 was not statistically significant. Such differences were

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Table 3. Carbon content in maize grain depending on the applied variables (%)

Year (A)	Genotype (B)	Treatment (C)			$\bar{x}$ AB	$\bar{x}$ A
		T_1	T_2	T_3		
	427	44.37	43.52	42.55	43.48	
2016	548	45.24	44.94	43.02	44.40	43.96
	687	45.51	43.87	42.61	44.00	
$\bar{x}$ AC		45.04	44.11	42.73		
	427	40.02	39.82	39.71	39.85	
2017	548	39.97	39.86	39.46	39.76	39.65
	687	39.30	39.36	39.37	39.34	
$\bar{x}$ AC		39.76	39.68	39.51		
	427	40.46	40.42	40.69	40.52	
2018	548	40.46	40.54	40.75	40.58	40.61
	687	40.66	40.79	40.74	40.73	
$\bar{x}$ AC		40.53	40.58	40.73	$\bar{x}$ B	
	427	41.62	41.25	40.98	41.29	
$\bar{x}$ BC	548	41.89	41.78	41.08	41.58	
	687	41.82	41.34	40.91	41.36	
$\bar{x}$ C		41.78	41.46	40.99		
Average 2016–2019					41.41	

	A^{**}	B^{ns}	AB^{ns}	C^{**}	AC^{**}	BC^{ns}	ABC^{ns}
F-test	0.00	0.78	0.80	0.00	0.00	0.91	0.94
$LSD_{0.01}$	1.11	1.31	2.27	0.59	1.02	1.02	1.77
$LSD_{0.05}$	1.01	0.94	1.62	0.44	0.76	0.76	1.32

T_1 – control; T_2 – before sowing 25 kg Zn^{2+}/ha was introduced into the soil (35 g of $ZnSO_4$ on the experimental plot); T_3 – seed treatment before sowing + foliar treatment with Zn^{2+}; *LSD* – least significant difference; $^{*}P < 0.05$; $^{**}P < 0.01$; ns – not significant

probably influenced by higher temperatures and lack of precipitation in 2017 and 2018. The dry period and high temperatures increase the proportion of vitreous endosperm, in which starch granules are smaller and that makes the grain harder. The obtained results are comparable with the results Kljak et al. (2018). The authors determined that the hardness of endosperm is a genetically determined trait, but that climatic traits in the period of maize vegetation, transport and drying after harvest can also affect grain hardness. Treatments (C) had different effects on carbon content. The highest carbon content was found in the T_1 control (41.78%), which was greater than T_2 (41.46%) by 0.78% without statistical significance. In the T_3 treatment (40.99%) the carbon content compared to the control (T_1) was lower by 1.94%, which was at the level of significance $P < 0.01$. In the dry year of 2017, T_3 (1.44%) significantly affected the increased carbon content compared to T_1 and T_2 (1.40%), so it can be said that this treatment mitigated the negative impact of drought.

Based on the obtained results, it can be concluded that the examined factors have a significant influence on the examined variables. The distribution of precipitation and temperature during maize vegetation had a highly significant effect on yield. The most favourable year was 2016 with grain yield (10.93 t/ha) and the most unfavourable was the dry year 2017 with grain yield (3.50 t/ha). Zinc treatment had a significant effect on grain yield, nitrogen and carbon content in grain. The application of Zn to the soil before sowing affected the highest yield (8.08 t/ha). Foliar application of Zn^{2+} affected nitrogen content (1.52%). The carbon content was the highest in the control variant (41.78%). The genotype also showed statistically highly significant variability. The cv. ZP 687 had the highest grain yield (7.72 t/ha) and nitrogen content (1.58%). In unfavourable years for the development and fruiting of maize, the application of Zn^{2+} had a significant impact, and it can be said that the application of Zn^{2+} can reduce unfavourable

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abiotic factors. On average – in the unfavourable year (2017) the yield increased ($T_1 \rightarrow T_2$) by 9%; in 2016 and 2018 by 16% and 14%.

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