

Seed priming with zinc improves field performance of maize hybrids grown on calcareous chernozem

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Highlights

- Seed priming with Zn resulted in an average increase of maize grain yield by about 18% compared to control, and by about 8.4% compared to water priming.
- Zn-priming promoted plant growth and increased final plant height of three maize hybrids.
- Overall experiment plant growth parameters were correlated with grain yield components and grain yield.
- Overall effect of seed priming on grain Zn concentration was significant, but it was increased by Zn-priming in two hybrids.
- Using the seeds with elevated Zn content can improve overall field performance of maize grown on calcareous chernozem.

Abstract

Delivery of micronutrients to plants through seed priming improves seedling vigour and increases crops yields. Two-year field trial was conducted in Pančevo, Serbia, with aim to study the effect of seed priming with zinc (Zn) on field performance of three maize hybrids on calcareous chernozem deficient in plant available Zn. Seed priming treatments were: control (without priming), water priming and priming with 4 mM zinc sulphate water solution. Seed priming had significant effect on early plant growth, plant height, yield components, grain yield and grain Zn concentration. Zn-priming promoted plant growth and increased final plant height. Across two growing seasons with contrasting precipitation

and three tested maize hybrids, Zn-priming resulted in an average increase of grain yield by about 18% compared to control, and by about 8.4% compared to water priming. A significant relationship between plant growth parameters, grain yield components and grain yield was detected. Grain Zn concentration was increased by Zn-priming in two hybrids in the season with less precipitation and in one hybrid in the second season. The results imply that using the seeds with elevated Zn content can improve overall field performance of maize grown on calcareous chernozem.

Introduction

Zinc (Zn) is an essential microelement for higher plants and plays an important role in plant growth, gene expression, structures of enzymes, photosynthesis, pollen development, sugar transformation, protein synthesis, membrane permeability, signal transduction and auxin metabolism (for review see Hacısalihogly, 2020). Soils with low plant-available Zn and Zn deficiency in crops are reported in many countries, and around 50% of cereal production area worldwide has soils with low plant available Zn (Cakmak, 2009). Availability of Zn for plants is affected by high soil pH, high bicarbonates and drought (Alloway, 2008), whilst high application of phosphorus fertilizers lowers grain Zn concentration in wheat and maize (Nikolic *et al.*, 2016; Zhao *et al.*, 2020). Zn can be applied to crops through soil and foliar fertilizers or by seed treatments. Foliar Zn application may have limited effectiveness (Fageria *et al.*, 2009), including differential response of cultivars to applied form of Zn as shown for barley (Moshfeghi *et al.*, 2019), whilst in some soils, Zn can be fixed and consequently not utilized by the crop (Rengel, 2015).

Using seeds with elevated micronutrient content can improve seedling vigour and increase crops yields (Welch, 1986). Nutrient seed priming is a pre-sowing treatment, efficient and low cost technique used to increase nutrients content in seed. There are advantages to this approach, since each seed is exposed to the nutrient which is available in early growth stages, and it is less costly due to less required amounts. Primed Zn is translocated

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from seeds to shoots during germination and seedling development as shown in maize (Imran *et al.*, 2015) and rice (Prom-u-thai *et al.*, 2012). Maize seed reserves are exhausted at the 3-4 leaf stage (Cooper and MacDonald, 1970); therefore, seed priming with Zn, in addition to seed hydration provides substantial Zn reserve that plants can utilize at early growth stage. Moreover, seed priming with Zn alone or with Zn and Mn can also help plants to cope with stressful conditions such as salt stress and low root zone temperature, by promoting early plants growth in comparison to water priming (Imran *et al.*, 2013; Imran *et al.*, 2018). In addition, important role of Zn fertilization for early plants growth was shown for maize by increased net photosynthetic rate in leaf development stage (Liu *et al.*, 2016), and also alleviated drought stress in wheat plants by Zn-mediated increase in photosynthesis pigment and active oxygen scavenging substances, and reduction in lipid peroxidation (Ma *et al.*, 2017). Zn-priming promotes seed germination and seedling vigour in rice and wheat (Rehman *et al.*, 2015, Harris *et al.*, 2008), also germination and field emergence of maize (Foti *et al.*, 2008). Furthermore, Zn-priming was more efficient than Zn-foliar treatment in increase of yield of wheat grown on Zn-deficient calcareous soil (Yilmaz *et al.*, 1997) and equally efficient as Zn addition to soil in maize (Harris *et al.*, 2007). Esper Neto *et al.* (2020) recently shown that seed priming with nano-scale ZnO improves maize seedling growth. Positive effect of Zn-priming on grain yield and grain nutritional quality was also shown in common bean (Tabesh *et al.*, 2020). Hassan *et al.* (2019) also showed that wheat seed priming with 0.01 M zinc sulphate water solution increased straw and grain Zn level, and the same treatment improved the stand establishment, seedling growth, chlorophyll content, morphological and yield parameters, grain yield and seed Zn concentrations in mungbean (Haider *et al.*, 2020a, 2020b).

Plant species differ in their sensitivity to Zn deficiency, and maize is more susceptible than other crops such as rye or oats (Broadley *et al.*, 2012; Mattiello *et al.*, 2015), and it is the first crop with reported Zn deficiency symptoms (Maze, 1914). Maize is the top ranked cereal globally, and it is the main crop in terms of cultivated area in Serbia, providing a substantial amount of food supply of 29 kg/capita/year (FAOSTAT, 2018; <http://www.fao.org/faostat/en/#data>). In Serbia, high pH and often calcareous soils prevail in the one-third of arable land located in the flatland of the Vojvodina Province (Manojlović and Singh, 2012) where soils with low plant-available Zn were recorded (Nikolic *et al.*, 2016). Yet, little attention is paid to application of Zn in maize production, and there is a general lack of research on this topic, including seed priming techniques, and grain micronutrients level such as Zn. Therefore, the aim of this study was to examine the effect of Zn-priming on plants growth, yield, yield components, and grain Zn and protein concentration of maize hybrids grown on calcareous chernozem.

Table 1. Growing season weather conditions for 2015 and 2016.

Month	Main air temperature (°C)			Precipitation (mm)		
	2015	2016	Ten-year mean (2005-2014)	2015	2016	Ten-year mean (2005-2014)
April	11.9	14.1	13.7	25.0	67.8	42.0
May	18.5	16.7	18.3	88.2	93.4	87.6
June	23.3	21.6	22.2	20.1	160.6	83.9
July	27.5	22.5	24.1	4.8	103.2	51.7
August	25.5	20.6	23.5	69.1	14.2	53.8
September	20.9	17.8	18.6	86.4	39.8	53.2
Mean/Sum	21.3	18.9	20.1	293.6	479.0	378.2

Materials and methods

Experimental site

Two-year field trial was conducted under rainfed conditions at experimental site of Institute Tamiš, Pančevo, Serbia in 2015 and 2016. The annual on-site precipitation sum is around 650 mm and precipitation distribution differs remarkably over seasons, but in Serbia, maize crop is irrigated mostly in double-cropping system. In the first growing season, summer was very warm and dry, and precipitation sum was much lower than in the second maize growing season, also lower in comparison to mean value for ten-year period (Table 1). The chernozem soil had following properties: pH 8.1, organic matter 3.1%, CaCO₃ 11.1%, total N 0.21%, AL-extractable P₂O₅ and K₂O were 20.0 mg and 25.8 mg per 100 g of soil, respectively. Concentrations of DTPA-extractable Zn in soil were 0.53 mg kg⁻¹ and 0.57 mg kg⁻¹ in the first and the second season, respectively. Mineral fertilizers were applied as NPK and urea, with N 170 kg ha⁻¹, P₂O₅ 60 kg ha⁻¹ and K₂O 60 kg ha⁻¹.

Plant material and priming treatments

Seeds of three maize hybrids of different maturity classes NS 6030 (FAO 600), NS 4023 (FAO 400), and NS 3022 (FAO 360) were provided by the Maize Department of Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia. Seed priming comprised of the following treatments: priming with water, priming with 4 mM zinc sulphate (ZnSO₄·7H₂O) aqueous solution, and non-primed seeds were control. Seeds of all hybrids were primed as described by Imran *et al.* (2013). During 24 h treatments, seeds were soaked in distilled water or zinc sulphate solution and placed in the dark. Afterwards, Zn-primed seeds were rinsed thoroughly with distilled water for one minute to remove the priming solution. All treated seeds were air dried close to original weight. Ninety seeds for each hybrid per plot were subjected to priming treatments. Seed Zn concentration and content were determined before (control) and after Zn-priming treatment (Table 2).

Experimental design, treatments and sampling

The experiment was laid out as randomized complete block design with four replications. Nine treatments were factorial combination of seed priming (control, water priming and Zn-priming) and three hybrids and were laid on thirty-six plots. The size of each plot consisted of four 5 m long rows, with a 75×22 cm sowing pattern. The sowing was performed in the third decade of April in both growing seasons. To obtain plants dry biomass, five random uniform plants were harvested six weeks after emergence, at phenological stage of nine or ten unfolded leaves (BBCH 19), and shoot biomass was oven dried at 80°C at a constant temperature. Plant

height was determined at harvest, using five plants per each plot. Maize was harvested at the fully ripe stage and plants from border rows were harvested separately and were not used for further measurements. Grain yield was calculated at 14% of moisture content. Number of grains per ear and 1000 grain weight were determined, and samples for determination of grain protein and Zn concentration were taken.

Analytical methods. Zn concentration was measured in seeds and harvested grains, and protein concentration was measured in grains. To obtain grain Zn concentration samples were air dried and milled, and 0.5 g of dry samples were microwave digested with 10 ml of HNO_3 + 2 mL H_2O_2 (Milestone ETHOS UP, Milestone Inc., Shelton, USA). Grain Zn concentration was determined by inductively coupled plasma optical emission spectroscopy (Varian Vista-PRO CCD Simultaneous ICP-OES, Varian, Inc.). Protein concentration in grain was determined by near-infrared spectroscopy (NIR Analyzer, INSTALAB 600, Dickey John, USA).

Statistical analyses

Obtained data were processed by ANOVA. The fixed effects of growing season and hybrid on maize shoot dry weight, final plants height, yield components, grain yield and grain Zn and protein concentration were analysed. Random effects were trial blocks and seed priming. ANOVA procedure and multiple comparison Tukey's test ($P < 0.05$) were adopted. Statistical analyses was performed using SPSS 15.0 package (IBM Corporation, Armonk, New York, USA) for Windows Evaluation version.

Results

Zn-priming remarkably increased Zn concentration and content within the seeds of all examined hybrids (Table 2). Seed Zn content in Zn-primed seeds was 8 to 10-fold higher than in control.

Significant three-way interactions between growing season, hybrid and seed priming were detected for grain yield and 1000-grain weight (Table 3). There was a significant interaction between hybrid and seed priming for shoot dry weight and grain yield across two growing seasons (Table 3); differences between means of three hybrids with three seed priming treatments were significant (Figure 1), also when two-way ANOVA was carried out for individual seasons (results not shown). In hybrid NS 6030 relative shoot weight differences between Zn-priming and control (28%) was greater than in NS 4023 (21%) and NS 3022 (8%) (Figure 1). For grain yield, relative differences between Zn-priming and control was greater in hybrid NS 3022 (21%) than in NS 6030 (19%) and NS 4023 (12%). Significant interactions between growing season and seed priming were detected for shoot dry weight, final plant height, grain yield and grain Zn concentration. There was a significant interaction between growing season and hybrid for final plant height, grain yield, 1000-grain weight and grain protein concentration. Grain Zn concentration and 1000-grain weight were significantly affected by growing season, hybrid and seed priming. When one-way ANOVA was carried out for individual hybrids and growing seasons, showing the effects of seed priming on examined parameters, it was detected that Zn-priming enhanced early plants growth of all tested hybrids; shoot dry weight measured six weeks

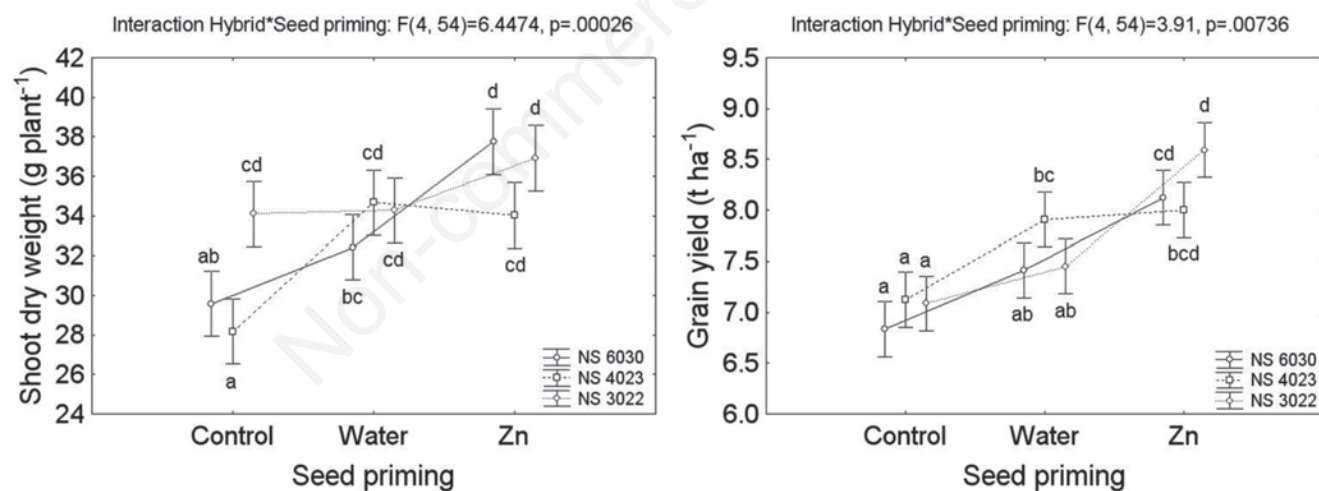


Figure 1. Effect of hybrid and seed priming interaction on shoot dry weight and grain yield of three maize hybrids. Mean values marked with the same letter are not significantly different at $P < 0.05$. Vertical bars denote 95% confidence intervals.

Table 2. Zn concentration and content in seeds of maize hybrids before and after Zn-priming.

Treatment	NS 6030		Hybrids NS 4023		NS 3022	
	Seed Zn concentration ($\mu\text{g g}^{-1}$)	Seed Zn content ($\mu\text{g seed}^{-1}$)	Seed Zn concentration ($\mu\text{g g}^{-1}$)	Seed Zn content ($\mu\text{g seed}^{-1}$)	Seed Zn concentration ($\mu\text{g g}^{-1}$)	Seed Zn content ($\mu\text{g seed}^{-1}$)
Control	24	10	36	12	36	12
Zn-priming	298	116	334	116	258	95

after emergence was significantly increased by Zn-priming in comparison to control overall experiment, except in hybrid NS 3022 in the second growing season, and relative increase ranged from 4 to 30% (Figure 2). In contrast, positive significant effect of water priming on shoot dry weight was recorded only in two hybrids in the first growing season. Final plants height was significantly increased by Zn-priming in comparison to control overall experiment, whilst differences between two priming treatments were significant in the second season (Figure 2). This was supported by significant growing season and seed priming interaction across hybrids (Table 3); relative increase of shoot dry weight and final plants height by water priming in comparison to control in two consecutive seasons was 16.8 and 2.7%, and 3.4 and 2.9%, respectively, whilst increase by Zn-priming was consistent in two seasons (results not displayed).

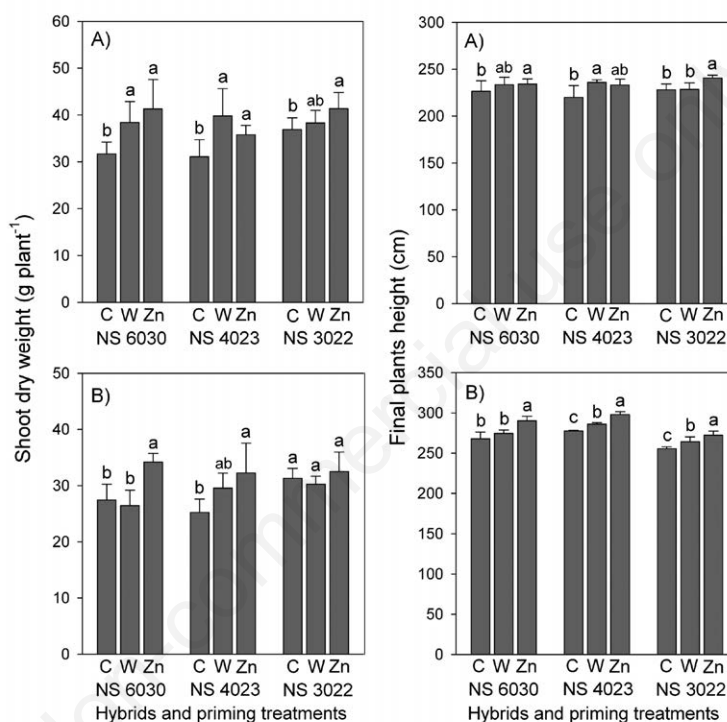


Figure 2. The effect of water priming (W) and Zn-priming (Zn) on shoot dry weight measured six weeks after emergence, and final plant height of three maize hybrids in A) 2015 and B) 2016; C denotes control. Values are means of four replications and the bars represent standard deviation. Mean values for each hybrid and each growing season marked with the same letter horizontally are not significantly different at $P < 0.05$ (after Tukey's test following a 3-way ANOVA).

Table 3. ANOVA results of the effects of growing season, seed priming and hybrid on plants growth, grain yield, yield components, grain Zn and protein concentration.

Source of variation	Shoot dry weight	Final plant height	Grain yield	Grain number per ear	1000 grain weight	Grain protein concentration	Grain Zn concentration
Growing season (GS)	<0.001	<0.001	<0.001	<0.001	<0.001	0.14	<0.001
Seed priming (SP)	<0.001	<0.001	<0.001	<0.001	<0.001	0.50	0.0483
Hybrid (H)	<0.001	<0.001	0.051	<0.001	<0.001	<0.001	<0.001
GS × SP	0.0019	0.0337	<0.001	0.07	0.10	0.30	<0.001
GS × H	0.63	<0.001	<0.001	0.07	<0.001	<0.001	0.09
SP × H	<0.001	0.50	<0.0073	0.54	0.42	0.16	0.57
GS × SP × H	0.1139	0.14	0.0016	0.63	0.0093	0.98	0.06

(Table 5), whilst grain protein concentration ranged from 8 to 9.4% overall experiment and it was not significantly affected by priming treatments in each tested hybrid (results not shown).

Grain yield was significantly correlated with shoot dry weight, final plant height, number of grain per ear and 1000-grain weight (Table 6). Significant correlation was also detected between plants growth parameters and 1000-grain weight, whilst grain Zn concentration was correlated with final plant height.

Discussion

Zn-priming increased the seed Zn content of all tested hybrids,

which was 8 to 10-fold higher than in control seeds (Table 2). Similar increases were reported in earlier studies after Zn-priming of barley (Ajouri *et al.*, 2004) and maize (Harris *et al.*, 2007; Imran *et al.*, 2013).

Although significant interaction of hybrid and seed priming for shoot dry weight indicated the greatest response of NS 6030 to Zn-priming (Figure 1), early plants growth as well as final plants height were significantly promoted in other hybrids in both seasons (Figure 2). Based on growing season by seed priming significant interaction seems that positive effect of water priming on maize growth was more pronounced under conditions with lack of precipitation. Similar results for maize seed Zn-priming and water priming were reported by Harris *et al.* (2007), whilst water priming did not enhance maize growth in a greenhouse experiments by

Table 4. Effect of priming treatments on grain number per ear, 1000-grain weight and grain yield of maize hybrids in 2015 and 2016.

Priming treatments	Grain number per ear			1000-grain weight (g)			Grain yield (t ha ⁻¹)		
	NS 6030	Hybrids NS 4023	NS 3022	NS 6030	Hybrids NS 4023	NS 3022	NS 6030	Hybrids NS 4023	NS 3022
2015									
Control	557 ^b	616 ^a	608 ^b	275 ^a	201 ^b	201 ^b	5.32 ^b	6.23 ^a	6.63 ^b
Water	546 ^b	597 ^a	605 ^b	252 ^a	200 ^b	198 ^b	5.80 ^b	6.04 ^a	6.78 ^b
Zn	590 ^a	657 ^a	641 ^a	272 ^a	218 ^a	233 ^a	6.55 ^a	6.14 ^a	8.17 ^a
2016									
Control	651 ^b	686 ^b	700 ^b	410 ^b	371 ^b	352 ^a	8.36 ^c	8.02 ^b	7.54 ^b
Water	648 ^b	730 ^{ab}	794 ^a	434 ^a	387 ^{ab}	324 ^b	9.02 ^b	9.78 ^a	8.13 ^b
Zn	695 ^a	754 ^a	807 ^a	438 ^a	407 ^a	362 ^a	9.70 ^a	9.86 ^a	9.02 ^a

Values are means of four replicates. ^{a-c}Mean values marked with the same letter are not significantly different at P<0.05 (after Tukey's test following a one-way ANOVA).

Table 5. Effect of priming treatments on grain Zn concentration of maize hybrids in 2015 and 2016.

Priming treatments	Grain Zn concentration (mg kg ⁻¹)		
	NS 6030	Hybrids NS 4023	NS 3022
2015			
Control	21.1 ^b	24.9 ^a	22.0 ^b
Water	22.5 ^b	23.5 ^a	21.1 ^b
Zn	25.2 ^a	24.0 ^a	23.5 ^a
2016			
Control	30.4 ^a	23.4 ^a	21.9 ^b
Water	33.5 ^a	25.1 ^a	23.6 ^a
Zn	29.9 ^a	26.5 ^a	24.3 ^a

Values are means of four replicates. ^{a-b}Mean values marked with the same letter are not significantly different at P<0.05 (after Tukey's test following a one-way ANOVA).

Table 6. Simple correlations between variables evaluated for three maize hybrids grown with seed priming treatments across two growing seasons (n=36).

	Grain yield	Shoot dry weight	Final plant height	Number of grains per ear	1000-grain weight	Grain Zn concentration
Shoot dry weight	0.644***					
Final plant height	0.698***	0.385*				
Number of grains per ear	0.523***	0.378*	ns			
1000-grain weight	0.706***	0.478***	0.473***	0.867***		
Grain Zn concentration	ns	ns	0.430***	-0.374*	ns	
Grain protein concentration	ns	ns	ns	ns	ns	ns

*Significant at P<0.05 level; ***significant at P<0.001 level; ns, not significant.

Subedi and Ma (2005). In contrast, water and both zinc sulphate and nano-Zn-priming did not increase plant height and total dry biomass of forage maize grown on soil with high plant available Zn (Sharifi *et al.*, 2016). Therefore, additional effect of Zn on plants growth in present study, can also be associated with concentration of plant-available Zn in experimental soil that was very close to widely accepted critical deficiency limits of 0.5 mg kg⁻¹ (Cakmak *et al.*, 1999).

Present field study clearly demonstrated that Zn-priming significantly increased grain yield in all tested hybrids (Table 4), resulting in relative increase of 18% over control across hybrids and two contrasting seasons (Figure 3). Differential response of hybrids to priming treatments and the greatest yield increase by Zn-priming in NS 3022 across two seasons (Figure 1) might be associated with hybrids maturity class and their response to drought stress, and requires further research. Furthermore, positive significant effect of Zn-priming was more pronounced than the effect of water priming on plants growth and yield components (Figure 2, Table 4) which were positively correlated with grain yield (Table 6). Prevailing positive effect of Zn-priming over water priming is consistent with findings by Harris *et al.* (2007). In addition, water priming has not been efficient for an increase of maize grain yield in temperate-humid conditions (Subedi and Ma, 2005). In study by Mohsin *et al.* (2014), grain yield was also positively associated with plant height, 1000-grain weight and ear growth parameters of maize under various Zn applications, including Zn-priming.

Grain Zn level of all tested hybrids over treatments ranged from 21.1 to 33.5 mg kg⁻¹ (Table 5). This is consistent with study by Brkić *et al.* (2004) who evaluated large number of maize genotypes in similar agro-ecology. Although priming treatments significantly affected grain Zn concentration overall experiment, significant increase by Zn-priming in both seasons was recorded in two hybrids, whilst grain protein concentration remained unaffected, and there was no correlation between these grain quality parameters (Table 5). On the other hand, Mohsin *et al.* (2014) reported significant increase of grain Zn concentration simultaneously with decrease of protein concentration in two maize hybrids by seed priming with 1% or 2% Zn water solution. Harris *et al.* (2007) also

obtained remarkable increase of maize grain Zn level by water and Zn priming, along with very low grain yields. In our study, increase of grain Zn level by Zn-priming can be attributed to enhanced plant growth, thus increased nutrients uptake, including Zn. Grain yield is seriously considered in breeding programs for improvement of Zn level in cereals (Garcia-Oliveira *et al.*, 2018; Mageto *et al.*, 2020). Absence of negative correlation between grain yield and grain Zn level in present study indicates that grain Zn level can be maintained with increase of grain yield in modern maize hybrids. Recent studies also confirm that grain Zn level is not inversely related to wheat grain yield (Chen *et al.*, 2017; Khokhar *et al.*, 2018).

Conclusions

In summary, the study has demonstrated that Zn-priming increased grain yield of tested maize hybrids grown on calcareous Chernozem potentially deficient in plant available Zn, during two growing seasons with contrasting precipitation regime. Water priming had beneficial effect on grain yield in the season with favourable weather conditions. It appears that Zn-priming promoted plants growth, which led to increase of grain yield components, and in some cases of grain Zn concentration. Therefore, Zn delivery to crops through seeds can be used as effective technique to improve grain yield of maize on soils with limited Zn available to plants.

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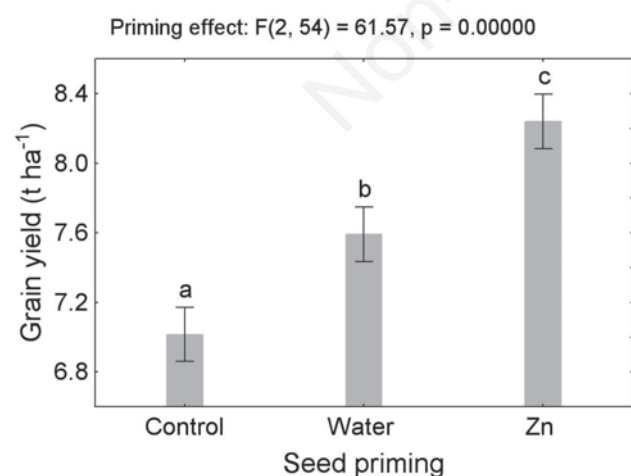


Figure 3. Effect of seed priming treatments on grain yield across two growing seasons and three maize hybrids. Mean values marked with the same letter are not significantly different at $P < 0.05$ (after Tukey's test following a 3-way ANOVA). Vertical bars denote 95% confidence intervals.

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