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Effect of Salicylic Acid Foliar Application on Two Wheat Cultivars Grown under Zinc Stress

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Abstract: The aim of this study was to investigate the effect of foliar application of salicylic acid (SA) on alleviating Zn stress in young wheat plants. Two rigorous pot experiments were conducted with two spring wheat cultivars growing on soil artificially contaminated with Zn. The experimental design included three levels of soil contamination with Zn: 0, 300 and 700 mg kg^{-1}, and three levels of SA concentration: 0, 0.5 and 1 mM. Foliar spray of SA was applied twice at an interval of two weeks. Wheat biomass was harvested two months after plant emergence. Both cultivars showed similar biomass reduction due to Zn phytotoxicity, but differed in the accumulation and distribution of this metal in the plant. The positive effect of SA foliar application was obtained only for one of the two tested cultivars, where a reduction in the Zn translocation from the roots to the aboveground part was observed. As a consequence, the decrease in biomass caused by the toxicity of Zn was limited. A greater positive effect of SA application on wheat biomass was observed at 700 than at 300 mg kg^{-1} Zn in soil. The different responses of the cultivars to the SA was probably related to their different defense mechanisms against Zn stress.

Keywords: *Triticum aestivum*; Zn stress; salicylic acid; foliar spray; alleviation of phytotoxicity

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1. Introduction

Zinc is a metallic element in which both deficiency and excess are harmful to humans, animals and plants. Although in some regions of the world, the deficiency of zinc in the human diet is a significant health problem [1], its excess is harmful to the environment in other regions.

Environmental pollution by heavy metals (HM) is a consequence of industrial development and urbanization. The main sources of excess Zn in soil are mining, metallurgy, the burning of fossil fuels and waste. In addition, agricultural use of fertilizers, pesticides and biosolids carries a serious risk of excess Zn in soils. Zinc contamination of soils is an environmental problem found worldwide. Most Zn is mined in China, where contamination of soils with this metal is common [2]. High soil Zn concentrations are also encountered in European countries such as Spain, France, Italy, Slovenia and Greece [3]. An excess of Zn in soil can also be found in some areas of Poland, although only 1.4% of soils in the country are contaminated with this element [4].

Although Zn is an essential micronutrient for plants, Zn excess is harmful. This element is easily taken up by plants from contaminated soils, and its excessive concentration in the plant disrupts several metabolic and physiological processes and consequently limits yield. Zn stress induces several adverse changes in wheat, such as stunting [5], root damage [6], decrease in chlorophyll content in leaves [7], photosynthetic disorders [8] and variation in enzyme activities [6,7].

Metals can enter the plant symplast in several ways: by simple diffusion, by passive transport through channel proteins, or by active transport through carrier proteins. The last one is the most important because it offers the most control. These carrier proteins are

generally referred to as metal transporters and often have different affinities for different heavy metals. Zn most likely crosses the plasma membrane via members of the ZIP transporter family [9,10].

When plants are exposed to a high Zn concentration, different plant species employ different strategies in response to metal-induced stress. Plants can secrete various organic substances by the roots that bind metal ions in the soil and block their transport into the plants [11] or by modifying the pH of the rhizosphere so that the metals take forms that are unavailable to plants [12]. Plants can also inhibit excessive Zn influx through limiting the relevant transporters at the transcriptional level or employing efflux strategy, which is the release of metals from the cells again [13]. One of the major Zn efflux transporters is HMA4 ([14]). Another way to deal with the excessive entry of Zn into plant cells is by chelating it by various ligands to reduce its undesirable interaction with cellular compounds. The most important Zn chelators are nicotianamine, glutathione and phytochelatines [15]. One more way to deactivate Zn inside a plant cell is to sequester it in safe places, mainly in the vacuoles [16].

Excessive uptake of Zn leads to oxidative stress, as expressed by the production of toxic reactive oxygen species (ROS) in plant tissues. Antioxidant enzymes such as POD, SOD and CAT play a key role in removing ROS. Heavy metal tolerant species usually produce high levels of these antioxidant enzymes [13].

Numerous studies in recent years have shown that salicylic acid (SA), which is an endogenous plant growth regulator, also acts as a signal substance in the induction of the plant specific response to biotic and abiotic stresses [17]. SA can increase wheat tolerance to drought [18], salinity [19], pathogens [20], heat stress [21] and heavy metals [22].

Studies carried out worldwide have confirmed an increase in plant tolerance to HM due to exogenous application of SA. These papers mainly focused on Cd [23,24], Pb [25] and As [26]. However, most of the studies were conducted under hydroponic conditions where SA was applied intra-root along with a nutrient solution. Relatively few of these studies involved wheat, where seeds were soaked in a SA solution and then sown into soil with a high content of HM. According to Gilvanova et al. [27], soaking of seeds in 0.05 mM SA solution led to the mitigation of Zn phytotoxicity. They found an increase in the stem and root biomass of wheat, an increase in net photosynthesis and a stabilization of energy balance compared to plants not treated with SA. Moreover, based on the increase in malondialdehyde (MDA) content, the antioxidant systems were found to be improved. A similar result of soaking wheat seeds in SA solution was obtained by Moussa and El-Gamal [28] and Agami and Mohamed [29]. In wheat exposed to Cd stress, treatment of seeds with 0.05 mM SA resulted in the mitigation of the negative effects caused by Cd toxicity and an increase in wheat tolerance to this metal. These authors reported enhanced antioxidant activity, increased chlorophyll content, improved water balance and positive changes in chloroplast and root ultrastructure.

The application of SA along with the nutrient solution through the roots also had a positive result. In the study of Basalah et al. [30], wheat plants under Cd stress, while treated with SA, showed better growth and better parameters of photosynthesis and antioxidant system, compared to plants without SA application.

SA foliar application is much less studied than seed or root application, while in agricultural practice farmers often use foliar sprays. Only Semida et al. [31] found mitigation of the phytotoxic effects of Cd on bean seedlings by spraying SA solution, and Hayat et al. [32] showed that the foliar application of SA and proline had a mitigating effect in chickpea growing under Cd stress. However, the reaction of wheat growing on Zn-contaminated soil to SA foliar spraying was not investigated.

Studies using soil are also necessary because water cultures or nutrient-saturated sand do not reflect the conditions under which plants grow in the field.

The aim of our study was thus to investigate the effect of foliar application of SA on reducing Zn stress for young wheat plants growing in pots filled with soil brought from the field.

2. Materials and Methods

2.1. Pot Experiment

To investigate the effect of foliar application of salicylic acid (SA) on wheat (*Triticum aestivum* L.) exposed to excessive Zn concentration in soil, two pot experiments were conducted from 2017 to 2018. In both experiments, plastic pots were filled with 2.5 kg of the same soil brought from a field located in Jelcz-Laskowice near Wroclaw, Poland. The characteristics of the physical and chemical properties of the soil are shown in Table 1.

Table 1. Properties of soils used in the experiments.

pH KCl	Sand	Silt	Clay	Corg	P [1]	K [1]	Mg [2]	Zn [3]
	%				mg·kg^{-1}			
5.7	73	24	3	0.79	76.7	147	107	34

Sand: 2.00–0.05 mm, silt: 0.5–0.002 mm, clay: <0.002 mm, Corg–organic carbon, [1] Enger-Rhiem, [2] Schachtschabel, [3] aqua regia.

The test plants were two spring wheat cultivars Zura and Lagwa (Malopolska Hodowla Roslin Sp. z o.o., Poland). They were chosen for the study because in recent years they have had the highest productivity in Poland and were often grown by farmers.

Two-factor complete randomized design with 4 replications were used in this study. The experimental design included 3 levels of soil contamination with Zn (Ist factor): 0 (Zn0), 300 (Zn1) and 700 (Zn2) mg kg^{-1} and 3 levels of SA concentration (IInd factor): 0 (SA0), 0.5 (SA1) and 1.0 (SA2) mM. Similar Zn levels and SA concentrations were used by other authors [33,34].

For the experiment, the soil was artificially contaminated with Zn. Zinc was added one week before sowing in the form of an aqueous solution $ZnSO_4 \cdot 7H_2O$ (Chempur Company, Poland) and thoroughly mixed with the entire volume of soil in the pot. At the same time, the following doses of macroelements were used as a background for all experimental treatments: N-0.50, P-0.15 and K-0.50 g/pot. In the period between fertilization and sowing, the soil was incubated at a temperature of 25 °C and a humidity of 60% of the field water capacity. One week after wheat emergence, a plant thinning was made down to 25 seedlings in the pot.

SA sprayings were performed twice using SG11 hand sprayer (Andreas Stihl AG & Co. KG, Waiblingen, Germany). The first spraying was conducted 1 week after emergence and the second spray was conducted 2 weeks after the first spraying. The SA water solution was prepared by dissolving an appropriate amount of SA (Chempur Company, Poland) in 50 mL of methanol and then topping it up with water. Methanol was also added to distilled water, which was applied on control treatments instead of SA.

Wheat was harvested 2 months after emergence (BBCH 20–21). Aerial parts of wheat were cut 5 mm above the ground and rinsed with distilled water. Roots were removed from pots, cleaned of soil, prewashed with tap water, and then rinsed for 2 h in distilled water using a rotary stirrer. Aerial parts and roots were dried for 24 h at 40 °C, carefully weighed and finely ground. Soil samples were taken from each pot at the same time as the roots. They were dried at room temperature, ground in a mortar and passed through a sieve with a diameter of 2 mm.

2.2. Chemical Analyses

Soil texture was evaluated by the aerometric method (PN-R-04033: 1998), pH was established potentiometrically in 1 mol KCl dm^{-3} (ISO10390: 2005), total organic carbon in soil (TOC) was determined by Tiurin method using potassium dichromate (PN-ISO14235: 2003), P and K were determined using the Enger–Riehm method (Polish standards No. PN-R-04023: 1996 and PN-R-04022: 1996 adequately) and Mg by the Schachtschabel method (PN-R-04020: 1994). The total Zn concentration in the soil was determined using aqua regia. After digestion, Zn was determined using the FAAS method (ISO 11466: 1995).

Zn in shoots and roots was determined by the FAAS method, having first dry ashed the material in a muffle furnace and digested it with 20% nitric acid (PN-R-04014: 1991). A standard reference material IPE 952 (International Plant-Analytical Exchange) from Wageningen (Netherlands) was used for quality control purposes.

2.3. Calculation of the Bioaccumulation and Translocation Factor

In order to compare Zn accumulation and distribution in plants between cultivars, bioaccumulation factors of this metal for shoots (1), roots (2) and the Zn translocation factor (3) were calculated according to the formulas provided by Melo et al. [35]:

BF_{shoot} = Zn concentration in shoots (mg kg^{-1})/Zn concentration in soil (mg kg^{-1})

BF_{root} = Zn concentration in roots (mg kg^{-1})/Zn concentration in soil (mg kg^{-1})

TF = Zn concentration in shoots (mg kg^{-1})/Zn concentration in roots (mg kg^{-1})

2.4. Statistical Analyses

Results for plant biomass and Zn concentration in shoots, roots and soil are presented as mean values from both experiments. ANOVA for the two-factor experiment in a complete randomization design was performed using AWAR software developed at the Institute of Soil Science and Plant Cultivation in Pulawy, Poland [36]. Differences between means were evaluated according to Tukey's HSD test ($p < 0.05$). Standard errors were calculated using Statgraphics v 5 software (StatPoint Technologies, Inc., Warrenton, VA, USA).

3. Results

3.1. Biomass Yield

Under natural soil Zn concentration (Zn0) without salicylic acid (SA), the Zura wheat cultivar produced about 10% less shoot biomass compared to the Lagwa cultivar (Figure 1). At soil Zn1 concentration, shoot yield of both cultivars decreased compared to the plants grown on Zn0. The reduction in biomass of Zura was 32% and that of Lagwa was 38%.

Higher soil contamination (Zn2) resulted in a more severe shoot yield depression than on Zn1. Zura and Lagwa cultivars produced 83% and 76% less shoot biomass with respect to Zn0.

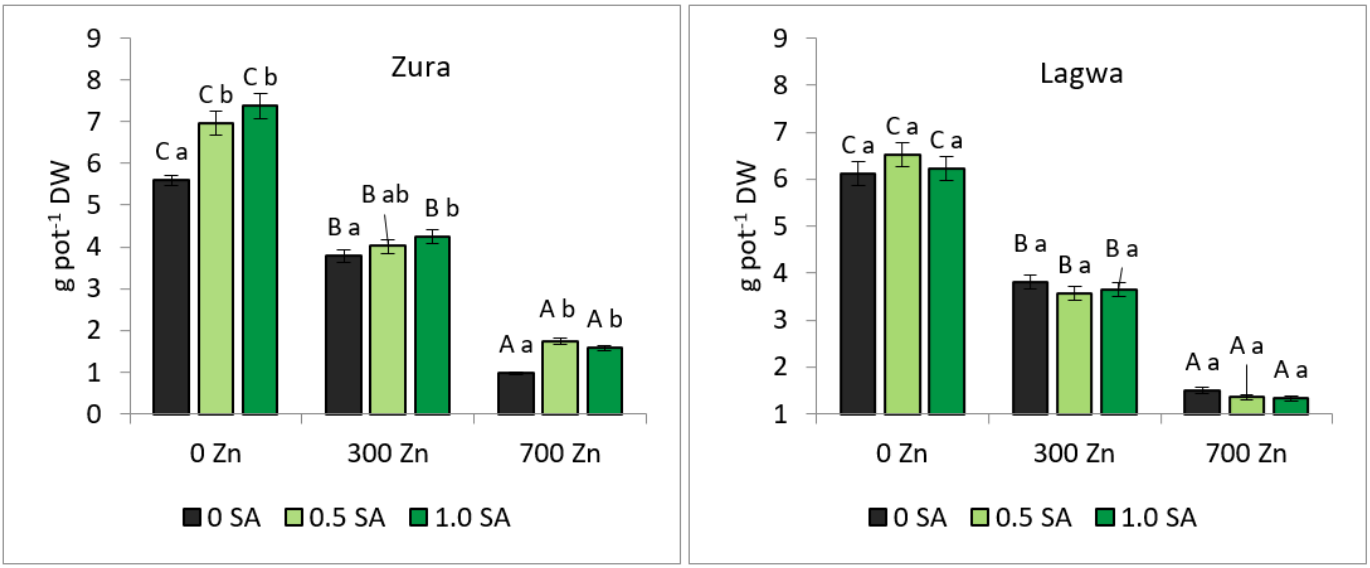

Figure 1. Biomass of wheat shoots–mean over 2 years. Means marked with different capital letters differ significantly between the Zn levels within each level of SA, and means marked with different small letters differ significantly between the SA levels within each level of Zn (according to Tukey's HSD test at $p < 0.05$). Vertical bars represent standard errors (n = 4).

On soil with natural Zn concentration (Zn0), foliar application of SA resulted in a statistically significant increase in Zura shoot biomass. Compared to plants that were not treated with foliar spray (SA0), a 25% and 30% higher shoot yield was obtained for SA1 and SA2 rates, respectively. No positive response to SA was observed in Lagwa.

Zura also responded positively to SA application under Zn soil contamination conditions. At the Zn1 soil contamination level, a statistically significant 12% increase in shoot biomass was observed after spraying wheat with SA2 concentration compared to the SA0 plants. A greater yield-forming effect of SA application was observed on Zn2 contaminated soil than on Zn1. The shoot biomass increase in Zura was similar for SA1 and SA2 and averaged 70% over SA0. Lagwa did not respond with shoot biomass increase to SA application under Zn-contaminated soil conditions.

3.2. Zn Concentration in Plants

Zn concentration in shoots of Zura and Lagwa cultivars grown on soil uncontaminated with this element (Zn0) and without SA, was 49 and 57 mg kg^{-1}, respectively, while in roots it was 116 and 168 mg kg^{-1} (Figures 2 and 3).

Under soil contamination with Zn1 dose, Zura accumulated 24-fold more Zn in its shoots and 21-fold more in its roots compared to plants on Zn0 (Figure 2). In contrast, Lagwa showed a 20-fold increase in Zn concentration in both shoots and roots (Figure 3).

On Zn2-contaminated soil, the wheat accumulated more with this metal than on Zn1. The Zn concentration in the shoots of Zura increased in relation to Zn0 by 60-fold and in the roots by 70-fold (Figure 2). In Lagwa, a 46-fold increase in Zn concentration was observed in both shoots and roots (Figure 3).

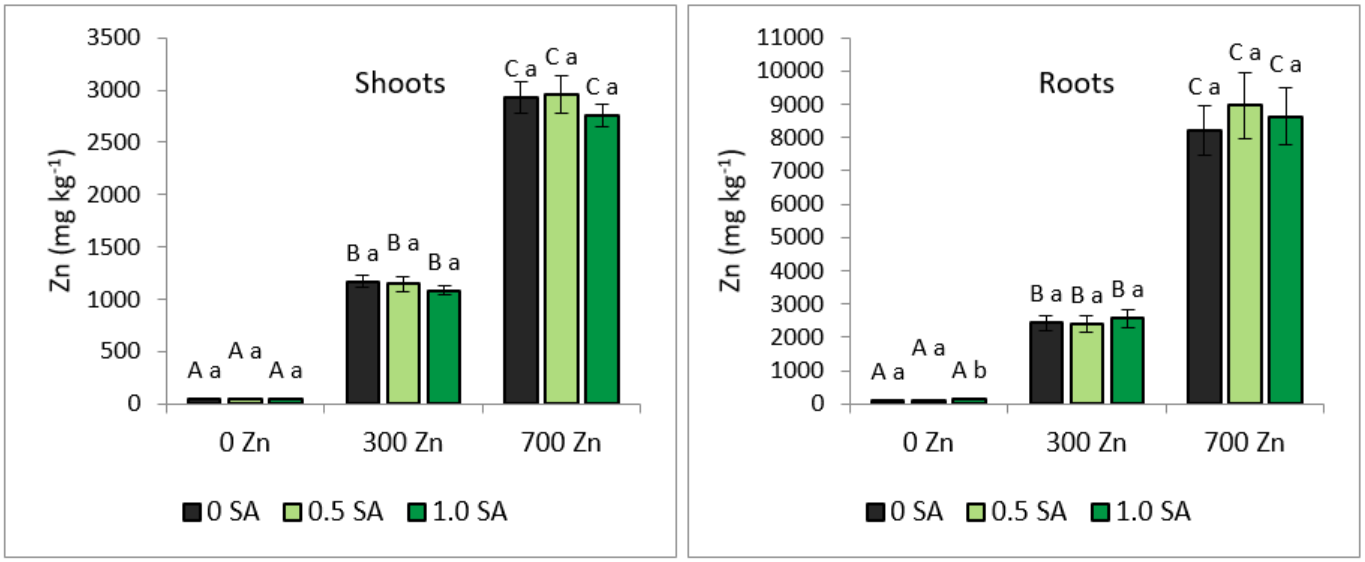

Figure 2. Zn concentration in shoots and roots in Zura cultivar–mean over 2 years. Means marked with different capital letters differ significantly between the Zn levels within each level of SA, and means marked with different small letters differ significantly between the SA levels within each level of Zn (according to Tukey's HSD test at $p < 0.05$). Vertical bars represent standard errors (n = 4).

SA treatment of plants growing on Zn0, as well as on Zn1 and Zn2, did not alter the Zn concentration in the Zura shoots, and a small but significant increase from 116 to 139 mg kg^{-1} was observed in the roots only on Zn0 (Figure 2). Lagwa showed a significant increase in Zn concentration in the shoots and roots of plants grown on Zn2. SA application resulted in a 14% increase in Zn in the shoots, regardless of SA concentration, and a 10% increase in roots under SA2 (Figure 3).

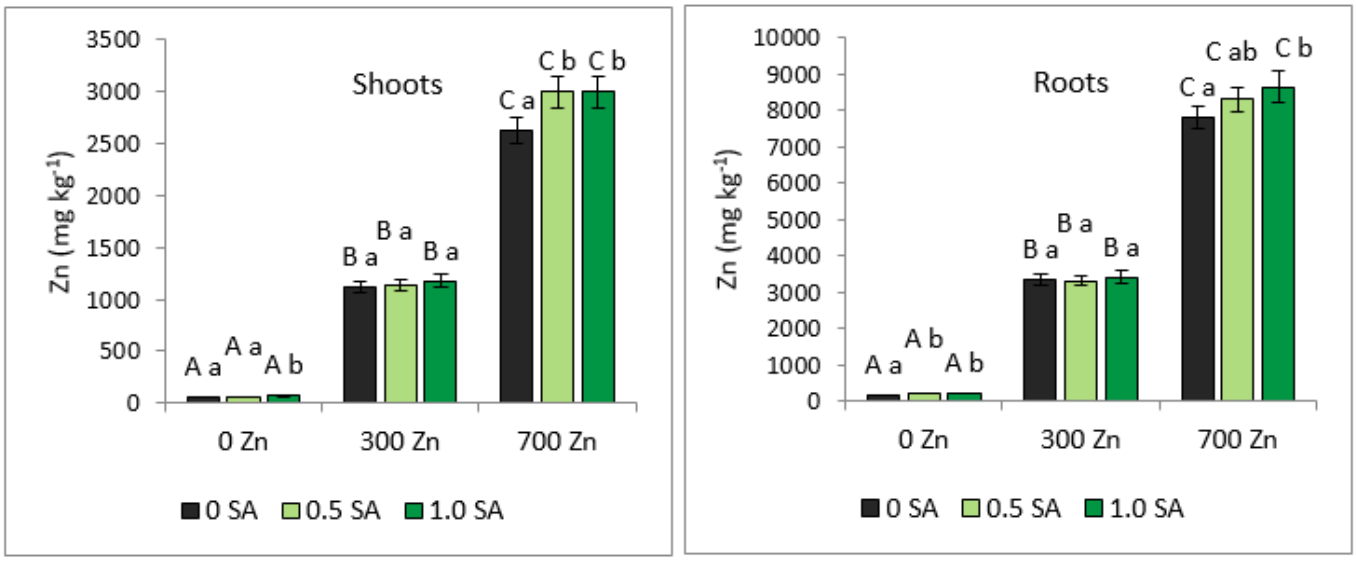

Figure 3. Zn concentration in shoots and roots in Lagwa cultivar–mean over 2 years. Means marked with different capital letters differ significantly between the Zn levels within each level of SA, and means marked with different small letters differ significantly between the SA levels within each level of Zn (according to Tukey's HSD test at $p < 0.05$). Vertical bars represent standard errors (n = 4).

3.3. Zn Concentration in the Soil

Zn concentration in the soil after crop harvest was almost identical for both cultivars (Table 2). The soil with natural concentration of this metal (Zn0) contained, on average from SA treatments, 36–38 mg kg^{-1} Zn depending on the cultivar. The Zn1 dose raised this concentration to 350–362 mg kg^{-1}, while Zn2 to 747–773 mg kg^{-1}, respectively for Zura and Lagwa. According to Polish law, both levels of Zn1 and Zn2 contamination exceed the permissible Zn limit for coarse-textured soil with pH $\leq$ 6.5, which is 300 mg kg^{-1}. There was no significant effect of SA on the Zn concentration in the soil.

Table 2. Zn concentration in soil after harvest in mg kg^{-1} (±SE).

Treatment	Zura			Lagwa		
	0 SA	**0.5 SA**	**1.0 SA**	**0 SA**	**0.5 SA**	**1.0 SA**
0 Zn	34 ± 0.69 A a	37 ± 0.76 A a	37 ± 0.76 A a	36 ± 0.73 A a	38 ± 0.78 A a	39 ± 0.80 A a
300 Zn	342 ± 6.98 B a	356 ± 7.27 B a	353 ± 7.21 B a	358 ± 7.31 B a	363 ± 7.41 B a	366 ± 7.47 B a
700 Zn	700 ± 14.3 C a	754 ± 15.4 C a	786 ± 16.0 C a	807 ± 16.5 C a	750 ± 15.3 C a	761 ± 15.5 C a

Values marked with different capital letters differ significantly between the Zn levels within each level of SA, and values marked with different small letters differ significantly between the SA levels within each level of Zn (according to Tukey's HSD test at $p < 0.05$).

3.4. Zn Accumulation and Distribution in the Plant

3.4.1. Bioaccumulation Factors

On Zn0 and Zn1 without SA, both wheat cultivars accumulated similar amounts of Zn in the shoots as evidenced by similar values of BF_{shoot} (Table 3). In the roots, however, more Zn was accumulated by Zura than by Lagwa, as indicated by BF_{root}.

On Zn2 without SA, Zura accumulated significantly more with this metal than Lagwa. Bioaccumulation factors BF_{shoot} and BF_{root} for Zura were 4.2 and 11.8, while they were 3.3 and 9.7 for Lagwa, respectively. Moreover, Zura responded to increasing soil Zn concentration differently than Lagwa. As soil contamination increased from Zn1 to Zn2 in Zura, there was an increase in bioaccumulation factors BF_{shoot} by 23%, and BF_{root} by 66%. In the Lagwa cultivar, the increase in these factors was minor and amounted to 6% and 4%, respectively.

Table 3. Bioaccumulation factors of Zn (±SE).

Treatment	Zura			Lagwa		
	0 SA	**0.5 SA**	**1.0 SA**	**0 SA**	**0.5 SA**	**1.0 SA**
			BF_{shoot}			
0 Zn	1.4 ± 0.03	1.3 ± 0.03	1.3 ± 0.03	1.6 ± 0.03	1.6 ± 0.04	1.8 ± 0.04
300 Zn	3.4 ± 0.07	3.2 ± 0.07	3.1 ± 0.06	3.1 ± 0.06	3.1 ± 0.06	3.2 ± 0.07
700 Zn	4.2 ± 0.09	3.9 ± 0.08	3.5 ± 0.07	3.3 ± 0.07	4.0 ± 0.08	3.9 ± 0.08
			BF_{root}			
0 Zn	3.4 ± 0.07	3.2 ± 0.07	3.8 ± 0.08	4.6 ± 0.09	5.4 ± 0.011	5.4 ± 0.012
300 Zn	7.1 ± 0.14	6.8 ± 0.15	7.3 ± 0.15	9.3 ± 0.19	9.1 ± 0.019	9.3 ± 0.20
700 Zn	11.8 ± 0.24	11.9 ± 0.25	11.0 ± 0.22	9.7 ± 0.20	11.1 ± 0.23	11.3 ± 0.24

The response of Zura to SA application under Zn-contaminated soil conditions manifested by a decrease in BF_{shoot} compared to plants without SA. In general, the concentration of SA2 was more effective than SA1 in reducing Zn accumulation in wheat shoots. It was especially visible for the plants from Zn2, where due to the SA2 spraying, 16% lower BF_{shoot} was found compared to the SA untreated plants. At the same time, the changes in BF_{root} for Zura amounted to a few percent and were ambiguously targeted.

Lagwa responded differently to the SA application than Zura. On Zn1, after spraying with SA, Lagwa did not change BF_{shoot} and BF_{root} compared to SA0, while on Zn2, there was an increase in Zn accumulation in shoots, as evidenced by a 20% higher BF_{shoot}, on average for both doses of SA. At the same time, there was a 14% and 17% increase in BF_{root} in relation to SA1 and SA2, respectively.

3.4.2. Translocation Factors

The tested wheat cultivars differed not only in the level of Zn accumulation, but also in the distribution of this metal between the aerial parts and the roots. The Zn translocation factor (TF) indicates that the Zura inherently showed an increased Zn transport from roots to shoots compared to Lagwa. This is evidenced by the TF values of Zn0 without SA, which were 0.43 and 0.34 for Zura and Lagwa, respectively (Table 4).

Table 4. Translocation factors of Zn (TF) (±SE).

Treatment	Zura			Lagwa		
	0 SA	**0.5 SA**	**SA2**	**0 SA**	**0.5 SA**	**1.0 SA**
0 Zn	0.43 ± 0.02	0.42 ± 0.02	0.35 ± 0.01	0.34 ± 0.02	0.30 ± 0.02	0.33 ± 0.01
300 Zn	0.47 ± 0.02	0.49 ± 0.03	0.43 ± 0.01	0.33 ± 0.02	0.34 ± 0.01	0.35 ± 0.03
700 Zn	0.36 ± 0.03	0.33 ± 0.01	0.32 ± 0.01	0.34 ± 0.04	0.36 ± 0.03	0.35 ± 0.01

Moreover, as soil contamination increased from Zn1 to Zn2, Zura reduced Zn transport from roots to shoots. In plants on Zn2, the TF translocation rate decreased by 23% compared to plants on Zn1. No such phenomenon was observed in the Lagwa cultivar. The TF for Lagwa remained at the same level regardless of the Zn concentration in the soil.

The SA2 application resulted in a change in Zn distribution in Zura, which was expressed by a reduction in Zn transport from roots to shoots. Depending on the level of soil contamination with Zn0, Zn1 and Zn2, the translocation factor TF decreased by 19%, 9% and 11%, respectively, compared to plants on SA0. At the same time, no changes in TF values were observed in Lagwa due to SA application.

4. Discussion

The two wheat cultivars tested in our experiments, Zura and Lagwa, showed similar sensitivity to excess Zn. Both with a lower level of soil contamination (Zn1) and with more than twice the concentration of this metal in the soil (Zn2), the two tested cultivars

showed a similar reaction in a reduction in biomass. At the same time, differences were found between these cultivars in the amount of Zn accumulated in shoots and roots and its distribution in the plant. These differences were observed especially under conditions of higher soil Zn contamination, where Zura took up significantly more Zn from the soil compared to Lagwa. This was confirmed by higher bioaccumulation factors BF_{shoot} and BF_{root} for this cultivar. Furthermore, for Zura, as soil contamination increased from Zn1 to Zn2, the bioaccumulation factor BF_{root} increased by 66%, while at the same time the translocation factor (TF) decreased by 23%. This indicates an increase in Zn accumulation in the roots and a reduction in Zn transport to the shoots under the condition of increasing Zn concentration in the soil. On the contrary, no such phenomenon was observed in Lagwa. The BF_{root} and TF factors remained practically unchanged, regardless of the level of soil contamination with Zn. On the other hand, Pan et al. [37] found in *Spartina alterniflora* an increase in translocation factor (TF) together with an increase in Zn concentration, suggesting the translocation of this metal from roots to shoots, which is opposite to the Zura wheat. The differences between the studied wheat cultivars in the amount of Zn uptake and its distribution in the plant, with similar biomass reduction due to Zn toxicity, may indicate the different natural defense mechanisms of these cultivars against Zn stress. In general, plants have developed numerous defense mechanisms adapting them to high levels of heavy metals (HM) in the soil. These are based on either excluding the stress factor, that is a limitation of metal uptake from the soil, or uptake of the metal and its detoxification within the plant.

The most likely defense mechanisms that Lagwa activated was to reduce Zn uptake from the soil, as evidenced by the BF_{shoot} and BF_{root} remaining at the same level despite a more than twofold increase in soil Zn concentration. Unlike Lagwa, the Zura cultivar did not benefit from a mechanism that limits Zn uptake from the soil, and probably developed a mechanism for Zn detoxification inside the plant. According to the literature, HM inactivation in the plant involves, among other things, complexation of the ions and their transport to metabolically inactive sites, with the participation of substances such as glutathione (GSH), phytochelatins (PCs) and metallothionein (MT). Metals can thus be transported and retained in the vacuole or cell wall [38–40]. Sousa et al. [41] showed that high levels of HM in the environment, including Zn, are not toxic to *Halimione portulacoides* (L.) because they are immobilized outside of the key metabolic sites. These authors demonstrated that all plant organs of *H. portulacoides* mostly retain metals in the cell wall, while the concentration of metals in the intracellular compartment is much lower. Most metals were found in the cell wall of the root, followed by the stems and leaves. In a study by Pan et al. [37], the cell wall and cytoplasmic supernatant were shown to be important Zn storage sites in *Spatrina alterniflora* growing under Zn stress conditions.

In our experiment, the two wheat cultivars of Zura and Lagwa under Zn stress differed in their responses to foliar SA application. Similarly, in the study of Arshad et al. [42], one of the barley genotypes under Pb excess conditions responded better to foliar SA application than the other. It seems that the different responses of Zura and Lagwa to foliar SA spray were related to the type of cultivar-specific defense mechanism deployed against Zn phytotoxicity. Zura, which developed a Zn detoxification mechanism associated with reduced transport of this metal from roots to shoots and accumulation mainly in roots, responded to exogenous SA application with an increase in shoot biomass. This response was a consequence of a further enhancement of the reduction in Zn translocation from the roots to the aerial parts due to SA application. Hossain et al. [43] reported that the protective role of SA against HM stress involves not only the regulation of ROS and antioxidants, but also the proper distribution of metals in the plant. Sheng et al. [44] found that the addition of 0.5 mM SA to a nutrient solution significantly alleviated the adverse effects of Mn stress in wheat, including by reducing Mn translocation from roots to shoots. In our experiment, in the Zura cultivar endogenous SA is probably involved in the distribution of excessive Zn in the plant, and its application enhances the protective effect against the phytotoxicity of this metal. It should be assumed that in the Lagwa cultivar, endogenous SA does not

play such a big role in the defense mechanism as with the Zura cultivar. Hence, foliar spray of SA was not effective here.

It was found that the SA-induced increases in the Zura biomass was higher in the soil contaminated with Zn2 than the Zn1 dose, with no significant difference in biomass at 0.5 mM and 1 mM SA concentration. However, application of 1 mM SA resulted in greater changes in Zn accumulation and distribution in the plant compared with 0.5 mM. Sahu and Sabat [45] suggest that the level of SA concentration has an impact on the profile of antioxidant enzymes in wheat plants, which may explain the differential effect of different SA concentrations in Zura cultivar. However, Maghsoudi et al. [34] obtained better results by applying 1 mM SA compared to 0.5 mM SA, only at the highest As contamination levels in the soil. They reported lower levels of MDA and H_2O_2, which indicates an increase in the activity of the antioxidant system.

Differences between cultivars in response to SA sprayings were evident not only under Zn stress but also on Zn-uncontaminated soil. In the Zura cultivar, which responded positively to SA under Zn stress conditions, an increase in shoot biomass was observed after SA spraying also on the control treatment without Zn. In contrast, in Lagwa, under both Zn stress conditions and low soil Zn concentration, no biomass increase was observed after SA application. It is likely that in the Zura cultivar, SA plays an important role not only in the defense mechanism against excess Zn but also in other metabolic processes. Hayat et al. [46] found that SA stabilizes the structure and permeability of cell membranes and increases the absorption of nutrients, nitrogen fixation and the activity of nitrate reductase. The literature presents works describing the effects of exogenous SA application on plants growing under non-stressed conditions. Hayat et al. [17] showed positive effects of exogenous SA application on bioproductivity, plant growth, photosynthesis, plant water relations and various enzymatic activities. Moussa and El-Gamal [28] found an increase in root length and biomass as well as a decrease in the level of MDA and H_2O_2 in wheat whose seeds were treated with SA. In contrast, some studies have shown detrimental effects of SA application expressed in a reduction in height and biomass of wheat and basil shoots [44,47].

5. Conclusions

Both tested wheat cultivars growing on Zn-contaminated soil showed lower productivity compared to those growing on soil uncontaminated with this metal. Two months since its emergence, a decrease in shoot biomass reached 38% at a soil Zn concentration of about 350 mg kg^{-1}, and 83% at a concentration of about 750 mg kg^{-1}.

Spraying wheat twice with SA resulted in the alleviation of Zn phytotoxicity, but only for one of the two tested cultivars. This was probably related to the different defense mechanisms of these cultivars against Zn stress. A greater yield-forming effect of SA application occurred at higher soil contamination with Zn and was manifested by a 70% increase in shoot biomass compared to wheat not treated with SA. This increase was attributed to a reduction in Zn translocation from roots to aerial parts that occurred after foliar SA application. The recommended concentration of SA solution for foliar spray of wheat for agricultural practice is 1 mM.

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