Zinc Absorption through Leaves and Subsequent Translocation to the Grains of Bread Wheat after Foliar Spray

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Abstract: Agronomic biofortification could possibly be a promising strategy to overcome zinc (Zn) deficiency in wheat; however, the cultivar’s response to foliar applications is enigmatic when it comes to the relative efficiency of Zn absorption and accumulation. To decipher that enigmatic response, this study was designed with the objectives (i) to track the amount of Zn absorbed through leaves after foliar application, (ii) to calculate the amount of the absorbed Zn actually translocated and stored in the grains, and (iii) to calculate the relative efficiency of the high yielding cultivars in terms of their Zn absorption and translocation. The results reveal that 0.90% of the zinc sprayed was absorbed through leaves, and 43% of the absorbed Zn was translocated to the grains. The cultivars significantly varied for their Zn absorption (0.71–1.07%) and subsequent translocation of the absorbed Zn (23–66%). Foliar zinc treatment also improved growth attributes such as leaf area, height, spikelet per spike, number of grains per spike, grain yield, leaf and grain Zn content, and grain protein content. These findings suggest a need for cautious parent selection in devising the breeding strategies intended for biofortification.

Keywords: translocation; foliar spray; grain Zn content; yield; protein content; Zn deficiency; biofortification

1. Introduction

Zinc (Zn) deficiency in the diet is a major issue globally due to the low uptake in plants, the soil being deficient, Zn becoming unavailable, and low absorption and intake by human beings [1,2]. Zinc has been identified as an essential micronutrient for humans, plants, and animals [3]. Zn deficiency in plants can lead to disturbance of the metabolism, growth, and development of plants, as the enzymes are directly affected. It plays a key role in many physiological processes such as metabolic activities, chlorophyll and protein synthesis, and strengthening the immune response in animals and humans [4]. Zinc is required for the normal development of around 10% of all proteins in the human body [5,6].

Zinc, being an inevitable requirement of biological systems, plays regulatory, structural, and functional roles in the enzymes essential for metabolic activities. It acts as a catalytic or co-catalytic component in human biological systems and has a structural role in protein biosynthesis and nucleotide metabolism [7]. There are numerous zinc-regulated enzymes in plants such as carbonic aldolase, anhydratase and fructose 1, 6-bisphosphatase.

The deficiency of zinc occupies the 17th position among the twenty significant factors that commonly cause death in humans (WHO, 2009). About one third of the world’s population is zinc-deficient, particularly children under the age of five who require zinc-containing proteins for growth and development [8]. In Pakistan, about 18.6% of children
below five years and about 22.1% of women of reproductive age were zinc deficient in the National Nutrition Survey conducted in 2018 [9].

Wheat products are the primary source of zinc in underdeveloped countries [10]. Wheat is one of the world’s most commonly produced cereals, which accounts for 50 percent of the daily calorie intake in many developing countries [11]. About 13.4 billion hectares of land are used globally for cultivation of wheat [12]. In Pakistan, wheat is a staple food and has got over 43% area of the country under production [13]. In wheat grains, zinc can be accumulated via two sources: (1) zinc is continually taken from the soil by roots and directly translocated into grain; (2) zinc is deposited in vegetative tissues (leaves and stems) and subsequently remobilized into grain, as graphically explained in Figure 1. The proportional contributions of these two sources to Zn accumulation in grain rely on a variety of plant and soil parameters, such as micronutrient and water availability during growth of plants compared to other chemical fertilizers. Zn-EDTA (Na$_2$Zn-EDTA), which has shown a superior effect on the productivity and beneficial effects on the quality of wheat grains [16] and grain zinc concentration with zero environmental footprint. They have shown a superior effect on the productivity and growth of plants compared to other chemical fertilizers. Zn-EDTA (Na$_2$Zn-EDTA), which contains about 12% Zn, is the most common source of zinc in a chelated form [2].

Zinc absorption by various methods such as soil, foliar, or seed treatments, focuses on increasing zinc accumulation in plants [17]. These treatments not only raise the quality of the produce but also the grain yield. The best technique for applying Zn was found to be soil application of zinc.

Biofortification is a process of enhancing the bioavailable nutrient contents in grains and pulses of crops. If zinc concentrations in staple food could be increased, it would have beneficial effect on the health of people living in rural areas that otherwise may not be able to purchase either fortified or naturally zinc-enriched food. Two possible biofortification strategies are genetic and agronomic. In genetic biofortification, plant breeding is involved in the development of new cultivars capable of accumulating higher zinc contents in grains. In agronomic biofortification, fertilizers are used to enrich grain zinc contents [5,14]. Therefore, the process of biofortification is an outstanding technique to mitigate micronutrient deficiency [15].

Zinc sulfates and synthesized zinc amino acid chelates are common sources of zinc fertilizers. Recent studies have shown that zinc-amino acid chelate foliar spray imparts beneficial effects on the quality of wheat grains [16] and grain zinc concentration with zero environmental footprint. They have shown a superior effect on the productivity and growth of plants compared to other chemical fertilizers. Zn-EDTA (Na$_2$Zn-EDTA), which contains about 12% Zn, is the most common source of zinc in a chelated form [2].

Zinc absorption by various methods such as soil, foliar, or seed treatments, focuses on increasing zinc accumulation in plants [17]. These treatments not only raise the quality of the produce but also the grain yield. The best technique for applying Zn was found to be soil application of zinc.

![Schematic diagram of zinc treatments to wheat crop.](image-url)

Figure 1. Schematic diagram of zinc treatments to wheat crop.
of the produce but also the grain yield. The best technique for applying Zn was found to be the foliar application, as it is directly absorbed through the leaf surface, transported to the phloem, and then translocated to the developing grains. This improves the yield components by increasing the activity of photosynthetic enzymes. Zinc fertilizers applied via soil can cause significant zinc losses due to Zn being unavailable and leaching from the root zone. However, this approach has been widely practiced because it requires no additional labor, equipment, or distribution infrastructure. Foliar fertilization, on the other hand, prevents fertilizer losses induced by leaching, and fertilizer is also quickly accessible to the plant.

The high nutrient ratio in leaves is also produced by Zn foliar fertilization compared to soil application [18]. Its foliar spray in combination with herbicides, fungicides and insecticides can also provide better results [19]. The appropriate timing of zinc foliar application in bread wheat is equally important to enhance the growth, yield, reproduction, and zinc accumulation in the grains. Significant improvement in vegetative as well as reproductive growth of wheat was observed by foliar application on almost all growth stages of wheat as reported in the literature. Different studies related to the timing of foliar zinc application are presented in Figure 2.

Numerous studies in literature have been reporting different methods of application of Zn, time of Zn application, Zn molecular form for its application, and genes affecting Zn transport and accumulation [4,9,11,16,19]. However, information on the amount of zinc applied, absorbed, and accumulated in wheat grains is still not very well unearthed. In this experiment, a deliberate effort was employed to better understand (a) how much Zn is absorbed through leaves after foliar application of 6 mM zinc sulphate and amino acid

![Figure 2. Timing of zinc foliar application on wheat reported in the literature. (G = germination, S = seedling, T = tillering, SE = stem elongation, B = Booting) [10,14,16,20–35].](image-url)
chelates, (b) how much of the absorbed zinc is further translocated to the grains, and (c) to what extent do cultivars differ from each other in their Zn absorption.

2. Materials and Methods

2.1. Experimental Design and Layout

Field experiments were conducted in the Seed Centre, University of Punjab, Lahore. The experiment was conducted during 2020–2021. Seed sowing was performed at the recommended rate of 150 kg ha\(^{-1}\) with hand drilling in rows in their respective sub-plots. Certified seeds of wheat cultivars were collected from the Federal Seed Certification and Registration Department, Lahore. Five wheat cultivars including Pakistan-2013, Faisalabad-2008, Johar-2016, Chakwal-50, and Gandum-1 were used in the experiment. Before sowing, the seeds were checked for germination rate. The physical and chemical properties of soil are listed in the Table 1.

<table>
<thead>
<tr>
<th>Texture</th>
<th>pH</th>
<th>E.C (mS cm(^{-1}))</th>
<th>SOM (%)</th>
<th>N (mg kg(^{-1}))</th>
<th>P</th>
<th>K</th>
<th>Zn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loam</td>
<td>7.0</td>
<td>0.8</td>
<td>0.79</td>
<td>50</td>
<td>4.9</td>
<td>112</td>
<td>0.68</td>
<td></td>
</tr>
</tbody>
</table>

E.C = Electrical conductivity; SOM = Soil 1.

All other agronomic methods, such as fertilizer applications and the level of irrigations, were kept the same except for zinc application. The experiment was laid out in a randomized complete block design. The experimental area was 364 ft\(^2\), which was further divided into two blocks, and each block was divided into 20 subplots with a one-foot non-experimental area between each subplot. There were four replications in each block. Fertilizers to these plots were added at the recommended rate of 100:85:60 kg hec\(^{-1}\) (NPK). Nitrogen was given as a split dose, i.e., half the dose at the time of sowing and half the dose at the time of active tillering. The monthly weather conditions in Lahore during 2020–2021 are presented in Table 2.

<table>
<thead>
<tr>
<th>Months</th>
<th>Temperature (°C)</th>
<th>Rainfall (mm)</th>
<th>Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max</td>
<td>Min</td>
<td>Average</td>
<td>Max</td>
</tr>
<tr>
<td>November</td>
<td>25.3</td>
<td>12.1</td>
<td>18.8</td>
</tr>
<tr>
<td>December</td>
<td>19.3</td>
<td>7.7</td>
<td>13.7</td>
</tr>
<tr>
<td>January</td>
<td>17.0</td>
<td>6.6</td>
<td>12.4</td>
</tr>
<tr>
<td>February</td>
<td>24.2</td>
<td>11.4</td>
<td>18.2</td>
</tr>
<tr>
<td>March</td>
<td>29.6</td>
<td>17.3</td>
<td>23.8</td>
</tr>
<tr>
<td>April</td>
<td>34.0</td>
<td>20.0</td>
<td>26.0</td>
</tr>
</tbody>
</table>

Source: Pakistan Meteorological Department.

2.2. Zinc Treatments and Morphological Data

During the experiment, the foliar zinc application method was investigated using two zinc sources. Foliar application of 6 mM of zinc sulphate and zinc amino acid chelates was given during the early growth phase (tillering stage) and late growth phase (booting stage) of wheat to all cultivars. In the very late afternoon, foliar application of both zinc sources was carried out to prevent potential leaf damage caused by salts on sunny days and at high temperatures during the day. Data were collected for different parameters during the vegetative and reproductive phases. Plant height was measured from the soil level to the tip of the plant including awns. Leaf area was measure by taking leaf length and width multiplied by the correction factor 0.75. Five representative spikes were collected from each plot, counted, and the average was reported as spikelet per spike. These spikes
were threshed individually and grains from each spike were counted and the average was reported as the number of grains per spike.

2.3. Chemical Analysis

2.3.1. Leaf and Grain Analysis

Zinc analysis of leaf and grain samples from each strand was evaluated using the spectrophotometric zinc analysis method [33]. After the calculation of leaf Zn content and grain Zn content, the translocation factor was estimated by dividing the grain Zn content to leaf Zn content multiplied by 100, and was written in percentage amount translocated.

2.3.2. Protocol for Zinc Analysis

The leaf samples were first dried in a convection oven at 70 °C for 8 h. However, the grains were air dried at room temperature for a night under a ceiling fan, to make sure that the samples are dry enough for grinding. The samples were crushed and 1 g of ground powder was added to a flask containing 8 mL of HNO$_3$ and HClO$_4$ (1:1 ratio) for acidic digestion. The samples were then placed in the dark until a clear solution was obtained. The solution was filtered and the volume was raised to 100 mL. The FAAS (model: GBCSAVAAT AA Australia) was then used for zinc metal determination.

2.3.3. Protocol for Grain Protein Analysis

Two grams of grains were crushed with PVP (polyvinyl polypyrrolidone) and 4 mL of 0.1 M phosphate buffer solution was added in the ratio of 1:2 (plant material: buffer solution). The slurry obtained was centrifuged at 4 °C for 10 min at 8000 rpm. The protein content of the supernatant was then calculated. In 1 mL biuret reagent, 0.1 mL supernatant was added. Parallel control was used, and was run in parallel. The optical density was then measured using a spectrophotometer at a wavelength of 545 nm. The total protein content was determined using the bovine serum albumin (BSA) standard protein curve using the following formula:

Protein content (mg g$^{-1}$ fresh weight) = Curve value × total extract/extract used × Weight of tissue × 1000.

2.4. Statistical Analysis

All the data were statistically analyzed using SPSS software version 20. The descriptive statistics were also conducted and results are presented as mean ± standard deviation and the least significant difference test was used to compare mean values at the 5% significance level. The normal distribution of residuals was found satisfactory to proceed for two-way ANOVA. The treatments, cultivars, and their interactions were studied. The cultivars’ means were subjected to comparative analysis (Duncan multiple range test) to rank cultivar means and their relative differences. Correlation analysis was carried out by linear regression programs to study the interaction between different wheat parameters.

3. Results

3.1. Amount of Foliar Zn Applied and Absorbed in Leaves

Two zinc forms, i.e., ZnSO$_4$·7H$_2$O and Zn amino acid chelates, were sprayed at 6 mM concentrations. The total amount of Zn sprayed after two foliar applications was calculated as 1.2 g (for both applications, i.e., at the tillering and booting stages) as shown in Table 3. As a result of the ZnSO$_4$·7H$_2$O foliar spray, the average amount of the Zn absorbed through leaves was estimated as 10.83 mg kg$^{-1}$. The maximum amount of Zn absorbed through leaves was 12.85 mg kg$^{-1}$, which is 1.07% of the total amount sprayed and was found in cultivar Pakistan-13, while the minimum amount absorbed was 9.40 mg kg$^{-1}$, which is 0.78% of the total amount sprayed and was found in Chakwal-50 (Table 3). Overall, on average, 0.90% of the total amount sprayed on wheat leaves was absorbed and accumulated (Table 3).
Table 3. Wheat cultivars ranked as per their zinc absorption through leaves and grains via foliar treatment of zinc sources.

<table>
<thead>
<tr>
<th>Zinc Treatment</th>
<th>Cultivars</th>
<th>Amount Given (g)</th>
<th>Leaf Zn (mg kg(^{-1}))</th>
<th>% Absorbed</th>
<th>Grain Zn (mg kg(^{-1}))</th>
<th>% Translocated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foliar application</td>
<td>Faisalabad-08</td>
<td>1.2</td>
<td>8.47 *</td>
<td>0.71%</td>
<td>5.57 *</td>
<td>66%</td>
</tr>
<tr>
<td>(zinc sulphate)</td>
<td>Gandum-1</td>
<td>1.2</td>
<td>10.72 *</td>
<td>0.89%</td>
<td>6.21 *</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>Chakwal-50</td>
<td>1.2</td>
<td>9.40 *</td>
<td>0.78%</td>
<td>3.80 *</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>Pakistan-13</td>
<td>1.2</td>
<td>12.85 *</td>
<td>1.07%</td>
<td>4.00 *</td>
<td>31%</td>
</tr>
<tr>
<td></td>
<td>Johar-16</td>
<td>1.2</td>
<td>12.69 *</td>
<td>1.06%</td>
<td>2.91 *</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>10.83</td>
<td>0.90%</td>
<td>4.50</td>
<td>43%</td>
</tr>
<tr>
<td>Foliar application</td>
<td>Faisalabad-08</td>
<td>1.2</td>
<td>12.73 *</td>
<td>1.06%</td>
<td>2.71 *</td>
<td>21%</td>
</tr>
<tr>
<td>(zinc amino acid chelates)</td>
<td>Gandum-1</td>
<td>1.2</td>
<td>6.87 *</td>
<td>0.57%</td>
<td>2.85 *</td>
<td>41%</td>
</tr>
<tr>
<td></td>
<td>Chakwal-50</td>
<td>1.2</td>
<td>4.87 *</td>
<td>0.41%</td>
<td>1.28 *</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td>Pakistan-13</td>
<td>1.2</td>
<td>9.33 *</td>
<td>0.78%</td>
<td>1.51 ^ns</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>Johar-16</td>
<td>1.2</td>
<td>5.39 *</td>
<td>0.45%</td>
<td>2.29 *</td>
<td>42%</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>7.84</td>
<td>0.65%</td>
<td>2.12</td>
<td>29.2%</td>
</tr>
</tbody>
</table>

* Values differ significantly from each other at \( p \leq 0.05; ^{\text{ns}} \) Non-significant

When Zn amino acid chelates were sprayed, the maximum amount of Zn absorbed in leaves was 12.73 mg kg\(^{-1}\), and was observed in the leaves of the cultivar Faisalabad-08. The minimum amount of Zn was absorbed in the leaves of Chakwal-50, and was estimated as 4.87 mg kg\(^{-1}\). The overall average for all the cultivars in study was 7.84 mg kg\(^{-1}\), as a result of Zn amino acid chelates foliar application (Table 3).

3.2. Amount of Zn Translocated into Grains and Cultivars Efficiency

The second objective of the study was to estimate how much of the absorbed Zn was actually accumulated in grains and was available for human consumption. The findings of the study suggest that the grain Zn content as a result of foliar treatment of zinc sulphate and amino acid chelated zinc exhibited significant differences among the two Zn sources in terms of zinc translocation and ultimately its accumulation in grains. Overall, 43% of the Zn absorbed in leaves was actually translocated to the grains as a result of the ZnSO\(_4\)·7H\(_2\)O foliar spray (Table 3). The maximum amount of the Zn translocated was 5.57 mg kg\(^{-1}\), which is 66% of the amount absorbed in leaves (Table 3). However, the minimum amount of Zn absorbed was 2.91 mg kg\(^{-1}\), which is 23% of the total Zn absorbed in leaves. In the case of foliar spray of Zn amino acid chelates, overall, 29.2% of the Zn absorbed in leaves was translocated to grains. The maximum amount translocated was 2.29 mg kg\(^{-1}\), which is 42% of the total amount absorbed in leaves.

Cultivars also varied significantly in terms of their relative efficiency for grain Zn accumulation (Table 3). The cultivars Faisalabad-08 and Gandum-1 exhibited maximum percentage translocation in grains (66% and 58%, respectively) through foliar application of zinc sulphate compared to other cultivars. The cultivars Johar-16 and Gandum-1 exhibited maximum percentage translocation in grains (42% and 41%, respectively) through foliar application of amino acid chelated zinc compared to other cultivars. Interestingly, Faisalabad-08 ranked fourth among the five cultivars studied, in terms of its Zn absorption in leaves (8.47); however, it ranked first for its efficacy to translocate the absorbed Zn into the grains. Similarly, Johar-16, which was second-best in terms of its efficiency for absorbing Zn in leaves, could translocate only 2.91 mg kg\(^{-1}\), which is only 23% of the total amount absorbed, exhibiting the poorest efficacy for translocation of the absorbed Zn. These findings suggest that the cultivars significantly differ in their efficacy of Zn absorption in leaves and also its subsequent translocation to the grains.
3.3. Vegetative and Reproductive Parameters

The probabilities of F for vegetative and reproductive parameters of wheat with respect to cultivar, treatment, and treatment × cultivar are shown in Table 4. Overall, the cultivars studied in the experiment exhibited significant interactions for all the traits except grain yield. This might be attributed to a higher coefficient of variation among different replications in the experiment for this particular trait. Partial yield loss as a result of bird attacks on the tree side of the experiment could be another reason for grain yield being non-significant. The frequency distribution of ten traits’ phenotype data based on two replicates is presented in Figure 3. All the parameters observed in the study followed normal distribution. The correlation among the different parameters is also shown in Figure 4.

Table 4. Probability of F for vegetative and reproductive parameters of wheat regarding cultivar, treatment, and cultivar × treatment.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Cultivar</th>
<th>Treatment</th>
<th>Cultivar × Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm)²</td>
<td>0.02 *</td>
<td>0.00 **</td>
<td>0.00 **</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>0.00 **</td>
<td>0.00 **</td>
<td>0.00 **</td>
</tr>
<tr>
<td>Grain filling duration</td>
<td>0.00 **</td>
<td>0.93</td>
<td>0.00 **</td>
</tr>
<tr>
<td>Spike length (cm)</td>
<td>0.00 **</td>
<td>0.00 **</td>
<td>0.00 **</td>
</tr>
<tr>
<td>Number of spikelets spike⁻¹</td>
<td>0.00 **</td>
<td>0.01 *</td>
<td>0.00 **</td>
</tr>
<tr>
<td>Number of grains spike⁻¹</td>
<td>0.35</td>
<td>0.00 **</td>
<td>0.00 **</td>
</tr>
<tr>
<td>Leaf zinc content (mg kg⁻¹)</td>
<td>0.40</td>
<td>0.00 **</td>
<td>0.00 **</td>
</tr>
<tr>
<td>Grain zinc content (mg L⁻¹)</td>
<td>0.44</td>
<td>0.00 **</td>
<td>0.00 **</td>
</tr>
<tr>
<td>Grain protein content (%)</td>
<td>0.02 *</td>
<td>0.00 **</td>
<td>0.00 **</td>
</tr>
<tr>
<td>Grain yield (g)</td>
<td>0.06</td>
<td>0.00 **</td>
<td>0.17</td>
</tr>
</tbody>
</table>

* Significant ** Highly significant at p ≤ 0.05.

Figure 3. Frequency distributions of the residuals of various parameters studied.
3.3.1. Leaf Area, Plant Height, and Grain Filling Duration

Leaf area significantly increased in wheat cultivars with foliar zinc treatment compared to no zinc treatment (control) as shown in Table 5. Foliar treatment of zinc amino acid chelates showed a 25% increase in leaf area (55.31 cm²) closely followed by zinc sulphate foliar spray, i.e., a 24% increase (54.08 cm²) compared to the control (41.53 cm²). Due to increased leaf area, the surface area for photosynthesis is increased and contributes to yield components of wheat. The comparison of mean values for plant height in Table 5 shows that healthier and taller plants (116.62 cm) were observed with foliar ZnSO₄·7H₂O application, which is a 6% increase and was statistically different compared to the control (106.48 cm). A non-significant difference was observed in grain filling duration with zinc treatments (Table 5).

Table 5. Mean values and standard deviation for different parameters for the control, foliar ZnSO₄·7H₂O, and foliar zinc amino acid chelate treatments.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Control</th>
<th>Foliar ZnSO₄</th>
<th>Foliar ZnAACh *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm)</td>
<td>41.53 b ± 1.92</td>
<td>54.08 a ± 1.50</td>
<td>55.31 a ± 1.61</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>106.48 c ± 2.77</td>
<td>116.62 a ± 2.27</td>
<td>112.85 b ± 1.84</td>
</tr>
<tr>
<td>Grain filling duration</td>
<td>33.10 a ± 1.19</td>
<td>33.00 a ± 1.03</td>
<td>33.10 a ± 1.01</td>
</tr>
<tr>
<td>Spike length (cm)</td>
<td>17.34 b ± 0.81</td>
<td>18.45 a ± 0.85</td>
<td>18.76 a ± 0.76</td>
</tr>
<tr>
<td>Number of spikelets spike⁻¹</td>
<td>19.89 b ± 0.92</td>
<td>21.26 a ± 0.96</td>
<td>20.76 ab ± 0.94</td>
</tr>
<tr>
<td>Number of grains spike⁻¹</td>
<td>40.40 c ± 1.39</td>
<td>51.90 a ± 1.80</td>
<td>48.36 b ± 1.58</td>
</tr>
<tr>
<td>Leaf zinc content (mg kg⁻¹)</td>
<td>2.23 c ± 0.11</td>
<td>10.70 a ± 0.42</td>
<td>7.83 b ± 0.33</td>
</tr>
<tr>
<td>Grain zinc content (mg L⁻¹)</td>
<td>1.27 c ± 0.32</td>
<td>4.50 a ± 1.34</td>
<td>2.13 b ± 0.71</td>
</tr>
<tr>
<td>Grain protein content (%)</td>
<td>15.04 b ± 1.05</td>
<td>18.65 a ± 1.35</td>
<td>15.52 b ± 1.49</td>
</tr>
<tr>
<td>Grain yield (g)</td>
<td>182.01 b ± 18.73</td>
<td>237.63 a ± 24.08</td>
<td>223.48 a ± 19.97</td>
</tr>
</tbody>
</table>

* Foliar zinc amino acids chelates. Different letters denote significant differences among the means.

3.3.2. Spike Length, Number of Spikelets Spike⁻¹, and Number of Grains Spike⁻¹

The spike length values obtained with different zinc treatments were competitive and non-significant from each other; however, they were significantly different from the control, as shown in Table 5. The maximum number of spikelets spike⁻¹ was observed with foliar application of ZnSO₄·7H₂O (21.26), which is a 6% increase compared to the control, and a minimum number of spikelets spike⁻¹ was observed with the control (19.89).
The maximum number of grains spike\(^{-1}\) (51.90) was achieved with foliar application of ZnSO\(_4\)·7H\(_2\)O, which is a 22% increase, whereas the minimum was achieved with the control (40.40), as shown in Table 5.

3.3.3. Leaf and Grain Zinc Content

The maximum zinc content in leaves was found with foliar application of ZnSO\(_4\)·7H\(_2\)O (10.70 mg kg\(^{-1}\)), and the minimum was found in the control (2.23 mg kg\(^{-1}\)), as shown in Table 5. Grain zinc content also displayed a significant increase with foliar zinc treatments. The maximum zinc content in grain (4.50 mg L\(^{-1}\)) was found with the foliar treatment of ZnSO\(_4\)·7H\(_2\)O, and the minimum value (1.27 mg L\(^{-1}\) and 1.83 mg L\(^{-1}\)) was recorded in the control. One reason for this significant increase with zinc foliar application is due to the direct absorption of zinc to the leaf via foliar application, which is then readily translocated to the grains. Consequently, this treatment increases the grain zinc concentration.

3.3.4. Grain Protein Content

The maximum grain protein content (18.65%) was found with foliar treatment of zinc sulphate, whereas the minimum (15.04%) was found in the control (Table 5). Zinc deficiency in staple crops causes RNA destruction via blocking the activity of RNA polymerase and thus protein synthesis in grains. Hence, provision of zinc via any biofortification method leads to an increase in protein content in wheat grains.

3.3.5. Grain Yield

The maximum grain yield (237.63 g) was recorded with the foliar treatment of ZnSO\(_4\)·7H\(_2\)O followed by a slight decrease in yield with the foliar treatment of zinc amino acid chelates. The minimum value (182.01 g) was recorded in the control as shown in Table 5. Foliar application of zinc sources significantly increased grain yield as compared to the control. Linear regression analysis of leaf zinc with grain zinc, grain protein, and grain yield and that of grain zinc with grain protein and grain yield showing different variations are shown in Figure 5.

![Figure 5. Linear regression analysis of leaf and grain zinc content.](image-url)
Our findings on different cultivars reacting to a specific foliar doses of both zinc sources allowed us to determine the most effective zinc treatment for increasing zinc accumulation and translocation efficiency into the leaves and grains, as well as provide new insight into boosting the effectiveness of Zn fertilizers, thus helping to relieve the health concerns caused by Zn deficiency.

4. Discussion

Zinc plays a key role not only in plant development and growth, but also helps plants to increase the quality of the harvest. Zinc biofortification strategies, and especially foliar Zn application, can help farmers to harvest higher grain yields with enhanced Zn content. It will subsequently help to satisfy the hidden hunger or Zn deficiency in humans. In this context, it is extremely important to understand how Zn is absorbed in various tissues, especially leaves, from where it is then remobilized and allocated to grains. The genetic makeup of the cultivars could possibly enable certain plant to absorb more Zn content than others. Similarly, the timing of zinc application to wheat plants also plays a role in increasing zinc accumulation in leaves and grains. In this study, the foliar zinc application and its subsequent absorption and translocation in the grains was studied in the top five most cultivated cultivars in Pakistan.

On the basis of zinc absorption efficiency via foliar application, cultivars can be classified as zinc-efficient or zinc-inefficient. The zinc-efficient cultivars exhibit better efficiency for zinc absorption including, but not limited to, a better genetic ability to absorb and translocate zinc from the leaf to the grain. In this experiment, we found that cultivars responded differently to zinc treatments, Zn absorption in leaves and its subsequent translocation to the grains. For example, the maximum Zn absorbed in the leaves was 12.85 mg kg\(^{-1}\) and the minimum was 8.47 mg kg\(^{-1}\) when zinc sulphate was foliarly applied. Overall, the average for the Zn absorbed through leaves was 10.83 mg kg\(^{-1}\), which is 0.90% of the total amount of the zinc administered to the plants. However, when zincamino acid chelates were sprayed, the average dropped to 0.65%. This clearly suggests that the cultivars vary significantly for their efficacy of Zn absorption and the source applied. The differences seen in different genotypes for zinc absorption can also be attributed to the different mechanisms in the rhizosphere and within a plant system. Other possible reasons could be protection against super oxide radicals in the soil, improved uptake of zinc by the plant, and differential utilization and its translocation [34,36]. Other reasons for these differences in cultivars could be due to soil and environmental conditions where the cultivars were grown [35].

The greater zinc efficiency in some cultivars might be the result of a different genetic makeup, which can result in enhanced Zn uptake, utilization, and translocation [37]. Kobayashi [38] reported that nicotinamide efflux transporter genes (ENA1 and ENA2) played a critical role in zinc uptake and transport in plants, determining the plant’s translocation efficiency. They reported that effective translocation of zinc and iron in cereal grains were aided by increased levels of DMA (de-oxymugeinic acid) and NA (nicotinamide).

The results of our observations on a significant increase in leaf area are in accordance with the study of Khan and Abdoli [39,40], where leaf area showed an increase with foliar treatment of zinc sulphate. Aziz [41] reported that zinc application triggers growth and development in wheat, resulting in taller wheat plants. Similar findings of a significant increase in plant height were reported by Ramzan [13].

A significant increase in the number of spikelet per spike might be due to the fact that Zn acts as a cofactor in numerous enzymatic pathways, resulting in enhanced fertility and better nutrient uptake in plants. Our results are in line with the findings of Esfandiari [42]. The non-significant change in grain fill duration found in this study shows the robust genetic composition for photoperiod and vernalization in facultative wheat cultivars grown in Pakistan. Arafat [43] also reported no change in grain filling duration as a result of zinc foliar spray, as grain fill duration depends upon the exposure of the crop to sunlight (photoperiod) and temperature, which were experienced by all the plots uniformly. Our
significant results regarding the number of grains per spike were in correlation with the discoveries of Aslam and Zeidan [44,45], who also found an increase in the number of grains per spike with foliar zinc treatment.

Other studies [46] also reported increased grain protein content with foliar application of ZnSO$_4$$\cdot$7H$_2$O. The researchers in [16,37] also reported that grain zinc concentration significantly increased via foliar zinc treatment, as was the case in this study. Abdoli [40] found an increase in grain zinc concentration of up to 28% during the first growing season and 89% during the second growing with foliar zinc application. Our results are aligned with the findings of Abdoli [40], who also found an increase of about 83% in grain yield with zinc foliar application at the stemming and grain filling stages.

5. Conclusions

Foliar application of zinc sulphate significantly increased the leaf and grain Zn content in all wheat cultivars in comparison to the control. However, the cultivars differed substantially in their Zn absorption and translocation capabilities. Faisalabad-08 and Gandum-1 treated with a foliar spray of zinc sulphate exhibited the maximum efficacy for percent of translocation (66% and 58%, respectively) to the grains from the leaves. Gandum-1 was found to be a better parent in both zinc sulphate and zinc amino acid chelate foliar sprays and is a better parent for Zn translocation and accumulation in grains. Hence, in can be chosen for a breeding program focusing on biofortification in Pakistan.

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References
2. Sekhon, B. Chelates for micronutrient nutrition among crops. Resonance 2003, 8, 46–53. [CrossRef]
6. Andreini, C.; Banci, L.; Bertini, I.; Rosato, A. Zinc through the three domains of life. J. Proteome Res. 2006, 5, 3173–3178. [CrossRef]


17. Johnson, S.; Lauren, J.; Welch, R.; Duxbury, J. A comparison of the effects of micronutrient seed priming and soil fertilization on the mineral nutrition of chickpea (Cicer arietinum), lentil (Lens culinaris), rice (Oryza sativa) and wheat (Triticum aestivum) in Nepal. Exp. Agric. 2005, 41, 427–448. [CrossRef]

18. Toor, M.D.; Adnan, M.; Shozib, M.; Ahmad, R. Foliar application of Zn: Best way to mitigate drought stress in plants; A review. IJAR 2020, 6, 16–20.


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