Effect of Zn as soil addition and foliar application on yield and protein content of wheat in alkaline soil

Sabir Gul Khattak1, Peter J. Dominy2 and Wiqar Ahmad3*
1 Directorate of Soil and Plant Nutrition, Agriculture Research Institute, 24330 Tarnab, Peshawar, KPK, Pakistan.
2 Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK.
3 Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar, AMK Campus, Mardan, Pakistan.

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Abstract: Zinc (Zn) deficiency in plant tissues is a reflection of both genetic and soil-related factors and is the most widespread problem in cereal crops worldwide, resulting in severe losses in the yield and nutritional quality. Developing cost-effective and quick solutions to Zn deficiency is, therefore, highly important. An experiment was conducted during 2009 – 2010, to assess the effects of various modes and concentrations of applied Zn on wheat yield and nutritional quality grown in alkaline soils.

Both soil addition and foliar spray of ZnSO₄ significantly increased grain yield, 1000 grain weight and grain protein content, while its effect on biological yield and grain protein composition was not significant. Foliar spray of 0.5 % and 1.0 % ZnSO₄ increased grain yield by 10 and 18.8 %, respectively while its soil application at the rate of 5, 10 and 15 kg ha⁻¹ increased grain yield by 18, 32 and 41%, respectively over the control. The treatments receiving ZnSO₄ as 5 kg ha⁻¹ soil + 1.0 % foliar, 15 kg ha⁻¹ soil + 1.0 % foliar and 5 kg ha⁻¹ soil + 0.5 % foliar application recorded 29.5, 29.0 and 27.5 % higher protein contents, respectively over the control. Comparing the value cost ratios (VCR) for the treatments showing higher grain yield and protein content, the VCR for 5 kg ha⁻¹ ZnSO₄ as soil + 1.0 % ZnSO₄ as foliar (10.23) was three times higher than the VCR for 15 kg ha⁻¹ ZnSO₄ as soil + 1.0 % ZnSO₄ as foliar (3.46), thus confirming the superiority of the former over the latter in terms of effectivenes. The results further revealed that despite presumably sufficient native Zn concentration in the soils under study (1.95 mg kg⁻¹), the crop responded positively to Zn treatment and therefore the Zn level of sufficiency (1 mg kg⁻¹) should be reconsidered in accordance with the nature and type of soils.

Keywords: Alkaline soil, foliar application of Zn, modes of Zn application, soil application of Zn, Zn effect on protein content, Zn effect on yield.

INTRODUCTION

Zinc (Zn) deficiency appears to be the most widespread and frequent micronutrient deficiency problem in crop and pasture plants worldwide, resulting in severe losses in yield and nutritional quality. This is particularly the case in cereal production areas and it is estimated that nearly half the soils on which cereals are grown have levels of available Zn low enough to cause Zn deficiency [50 % of soils in Turkey (Eyupoglu et al., 1994), 30 to 70 % of soils in India (Takkar, 1991), 70 % soils in Pakistan (Rashid et al., 1988), 2 m ha of paddy soils in Bangladesh and 8 m ha in China, Japan and the Philippines (Alloway, 2008; 2009)]. Since cereal grains have inherently low Zn concentrations, growing them on these potentially Zn-deficient soils further decreases the grain Zn concentration. It is therefore not surprising that the well-documented Zn deficiency problem in humans occurs predominantly in the countries/regions such as India, China, Pakistan and Turkey where soils are low in available Zn, and cereals are the major source of calorie intake (Alloway, 2008).

Although Zn is required in small amounts, its availability is critical for several key physiological functions in plants including growth regulation, photosynthesis and sugar formation, seed production, and defense mechanisms against various diseases. Its deficiency adversely affects these functions, thus resulting in lower yield and frequently in poor quality crop products (Tahir et al., 2009). Humans consuming crops grown on Zn deficient soils may suffer Zn deficiency...
Alkaline soils with an apparently sufficient Zn level (>1 mg kg\(^{-1}\)) may show reduced Zn availability to crops due to (a) Zn affinity towards adsorption/fixation on the clay adsorption sites, and (b) the high pH of soil (pH 8.0), which might have helped in the formation of unavailable forms of Zn as hydroxides. Zn has greater affinity for adsorption on clay and also it tends to make unavailable zinc hydroxides due to increased pH (Khattak & Pulford, 1999). In such soils crops have responded positively to Zn application by showing increased protein content in grain as well as soil residual Zn concentration (Khattak et al., 2006). A portion of additional Zn probably helps in satisfying the adsorption sites and some of it might be retained as available Zn to plants in solution form. Besides fixation at adsorption sites and the formation of zinc hydroxide in alkaline conditions, low availability of Zn is also attributed to a number of soil and environmental factors including low soil organic matter, calcareous nature of soil, water logging and arid climate (Morvedt et al., 1991; Tandon, 1995; Cakmak et al., 1998).

Estimates suggest that 25% of the world’s population is at risk of Zn deficiency (Maret & Standstead, 2006). Several approaches have been made to overcome Zn deficiency in humans and these include Zn supplementation, food diversification as well as food fortification (Ahmad et al., 2012). Increasing the Zn content in food crops may be a good strategy to overcome its deficiency in people in developing countries. Many researchers endeavoured to chalk out various strategies of its better supplementation, which included; Zn application to soil in the form of ZnSO\(_4\), Zn chelates, soil and foliar spray with different Zn compounds and seed priming (Singh & Abrol, 1985; Yilmaz et al., 1997; Cakmak et al., 1998; Amar et al., 2000; Khattak et al., 2006; Khan et al., 2008; Maqsood et al., 2009). Realising the importance of Zn in plant and human nutrition and the problems associated with its availability to plants in alkaline soils, the present study was conducted to assess the effect of various modes and levels of Zn application on wheat yield and protein content in order to evolve a cost effective strategy to address Zn deficiency in cereals grown in alkaline soils.

**METHODS AND MATERIALS**

The present experiment was conducted in the greenhouse of the Institute of Biological and Life Sciences (IBLS), University of Glasgow, UK during 2009 – 2010. Wheat variety ‘Siran 2008’ obtained from the Institute of Biotechnology and Genetic Engineering (IBGE), University of Agriculture, Peshawar, Pakistan was used for testing the effect of 0, 5, 10 and 15 kg Zn ha\(^{-1}\) (0.0, 2.5, 5.0 and 7.5 mg Zn kg\(^{-1}\) soil in the form of ZnSO\(_4\)) as soil addition and 0, 0.5 and 1% ZnSO\(_4\) solution (0, 5 and 10 g ZnSO\(_4\) per litre of de-ionised water) as foliar application on wheat yield and protein content in alkaline soil. A total of 12 treatment combinations (Table 1) arranged in RCB design with 4 replications were applied in pots filled with 2 kg sandy loam soil of known physico-chemical characteristics (Table 2).

### Table 1: Details of Zn treatments as soil application and foliar spray applied to wheat crop during experiment

| Serial No. | Treatments  | N:P:O\(_2\):K\(_2\)O (kg ha\(^{-1}\)) | Zn as soil treatment (kg ha\(^{-1}\)) | As foliar spray (ZnSO\(_4\)) (%) |
|------------|-------------|---------------------------------|-------------------------------------|---------------------------------|
| 1          | Zn S0 F0 (Control) | 120:65:110                      | 0.0                                 | 0.0                             |
| 2          | 0S-0.5F      | 120:65:110                      | 0.5                                 | 0.5                             |
| 3          | 0S-1.0F      | 120:65:110                      | 1.0                                 | 1.0                             |
| 4          | 5S-0F        | 120:65:110                      | 5.0                                 | 0.0                             |
| 5          | 5S-0.5F      | 120:65:110                      | 5.0                                 | 0.5                             |
| 6          | 5S-1.0F      | 120:65:110                      | 10.0                                | 1.0                             |
| 7          | 10S-0F       | 120:65:110                      | 10.0                                | 0.0                             |
| 8          | 10S-0.5F     | 120:65:110                      | 10.0                                | 0.5                             |
| 9          | 10S-1.0F     | 120:65:110                      | 10.0                                | 1.0                             |
| 10         | 15S-0F       | 120:65:110                      | 15.0                                | 0.0                             |
| 11         | 15S-0.5F     | 120:65:110                      | 15.0                                | 0.5                             |
| 12         | 15S-1.0F     | 120:65:110                      | 15.0                                | 1.0                             |

S: soil treatment; F: foliar spray
Initially, seed grains were soaked in distilled water on a double layered towel paper pad tray and kept in the growth chamber under 18 – 22 °C, 9 hrs light/15 hrs dark, 60 – 70 % humidity and light 150 µMole m⁻² S⁻¹. After germination, 6 healthy seedlings were transferred to each pot and kept in a glass house under day and night lengths of 16 and 8 hrs, respectively with mercury reflector lighting of 400 watts/base E40.

Fertiliser at recommended levels i.e. N:P₂O₅:K₂O at the rate of 120:65:110 kg ha⁻¹ (60:32.5:55 mg kg⁻¹ soil) was added to each treatment in solution form in two splits. Zn as soil treatment or foliar spray was applied in required amount in the form of ZnSO₄. To assess the yield trend with Zn application at lower doses of NPK, the same layout was repeated with half the recommended NPK levels (N:P₂O₅:K₂O at the rate of 60:32.5:55 kg ha⁻¹). Water was provided through plastic trays beneath the pots to avoid nutrient leaching. At boot stage, two healthy plants were cut at the base for analysis of ion retention by plants. At maturity, spikes were removed for grain yield and 1000 grain weight determination, while the remaining plants were harvested to determine the total biomass yield.

**Soil analysis**

The composite soil sample was subjected to various physical and chemical analyses. Existing standard methods were followed for the determination of soil pH (McClean, 1982) and electrical conductivity (EC) (Rhoades, 1996) using 1:5 soil and water suspension. The texture and organic matter content were determined by Bouyoucous method (Bouyoucous, 1962) and Black procedures (1965), respectively.

The nutrient ions were determined by Induced Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Perkin Elmer Optima 4300 DV). A 10 g soil sample was taken in triplicate in 250 mL Erlenmeyer flask to which 20 mL of ammonium bicarbonate di-ethylene trimine penta acetic acid (AB-DTPA) solution was added. Flasks were shaken on a reciprocating shaker at 180 rpm per min for 15 min. The suspension was filtered through a filter paper No. 42 and 5 mL of the AB-DTPA extract from each sample was transferred into 15 mL falcon tubes to which 0.5 mL concentrated HCl was added and mixed on a rotary shaker for 15 min to drive off CO₂. After this, 4.5 mL of distilled water was added to each tube in order to bring the pH of the sample to be in accordance with the pH of the background electrolyte. The solution in the tubes was then analysed by the ICP (OES).

**Grain protein**

To assess the wheat grain protein content, 0.5 g of wheat flour samples were taken in triplicate to which, 0.25 mL protein buffer was added, mixed by Vortex Mixer, and then centrifuged for 15 min at 13 K rpm. The supernatant was removed for spectroscopic analysis at E-280 nm by ultraviolet spectrophotometer in mg/mL i.e. approximately equal to 1 optical density (OD). The absorbance was multiplied by the dilution factor and the protein percentage was calculated as follow:

\[ \% \text{ protein} = \text{absorbance reading} \times \text{dilution} \times \frac{100}{1000} \]

The effect of Zn on the composition of proteins in wheat grain was studied using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

**RESULTS AND DISCUSSION**

Soil samples were analysed before sowing for various physico-chemical characters. The results (Table 2) revealed that the soil texture was a sandy loam, free from excess salinity and alkaline in reaction (pH > 7.0). The soil was moderate in organic matter content (OM > 2 %), and sufficient in macro (N, P, K, S, Ca and Mg) and micronutrients (Fe, Zn, Mn, Cu and B contents) (Table 2).

| Property                  | Mean values |
|---------------------------|-------------|
| Textural class            | Sandy loam  |
| pH (1:5)                  | 8.52        |
| EC (1:5) (dS m⁻¹)         | 0.398       |
| Organic matter (%)        | 2.34        |
| N (%)                     | 0.041       |
| P (mg kg⁻¹)               | 34.44       |
| K (mg kg⁻¹)               | 235.35      |
| Ca (mg kg⁻¹)              | 197.2       |
| Mg (mg kg⁻¹)              | 33.38       |
| S (mg kg⁻¹)               | 76.6        |
| Zn (mg kg⁻¹)              | 1.95        |
| Cu (mg kg⁻¹)              | 1.79        |
| Mn (mg kg⁻¹)              | 138.8       |
| Fe (mg kg⁻¹)              | 216.4       |
| B (mg kg⁻¹)               | 0.618       |
Application of Zn, both as soil and foliar treatments, significantly (p < 0.05) increased the wheat grain yield and 1000 grain weight while the effect on wheat biological yield was not significant. The treatment that received 5 kg ha\(^{-1}\) ZnSO\(_4\) as soil treatment + 1.0 % ZnSO\(_4\) as foliar spray recorded 55 % higher grain yield while that with 15 kg ha\(^{-1}\) ZnSO\(_4\) as soil treatment + 1.0 % ZnSO\(_4\) as foliar spray recorded 48 % higher grain yield over the control. However, the grain yield in both these treatments was not significantly different (Table 3). These results are in line with those reported by Alloway (2008) who states that Zn deficiency in wheat reduces grain yield. Kausar et al., (2001) have reported that despite a sharp increase in fertiliser use in Pakistan, the corresponding increase in yield was only 15 %, which was ascribed to imbalanced use of fertilisers and micronutrient deficiencies, specially of zinc and boron. Qayyum et al. (1987) have also reported a yield loss of 27.8 % in maize when Zn was omitted from the treatments involving NPK and micronutrients.

A positive correlation (R\(^2\) = 0.39) was observed between the Zn uptake by the plant at boot stage and total grain yield (Figure 1). Takkar and Randhawa (1978) have reported a similar positive correlation between the Zn concentration and grain yield and suggested that a Zn concentration below 30 mg Zn kg\(^{-1}\) plant dry matter would result in yields not attaining their maximum potential for the site, and the value of 20 mg Zn kg\(^{-1}\) is the lower critical concentration. Previous studies (Viets,

![Figure 1: Correlation between Zn uptake by plant at boot stage and total grain yield of wheat](image)

Table 3: Effect of Zn application on grain yield (kg ha\(^{-1}\))

| Serial No. | Treatments     | Biomass yield (kg ha\(^{-1}\)) | Grain yield (kg ha\(^{-1}\)) | 1000 weight grain (g) |
|------------|----------------|--------------------------------|----------------------------|-----------------------|
| 1          | Zn S0 F0 (Control) | 3589.3 ± 041.7                 | 1476 ± 054.4                 | 47.44 ± 0.4           |
| 2          | 0S-0.5F         | 3815.5 ± 193.8                 | 1626 ± 075.1                 | 46.1 ± 0.2            |
| 3          | 0S-1.0F         | 3272.5 ± 260.2                 | 1752 ± 060.9                 | 49.3 ± 0.4            |
| 4          | 5S-0.5F         | 3503.8 ± 279.7                 | 1747 ± 125.7                 | 44.5 ± 0.7            |
| 5          | 5S-1.0F         | 3441.0 ± 082.9                 | 1859 ± 139.2                 | 48.2 ± 0.4            |
| 6          | 5S-1.0F         | 4239.8 ± 324.8                 | 2294 ± 184.2                 | 48.2 ± 0.2            |
| 7          | 10S-0F          | 3697.0 ± 666.3                 | 1947 ± 202.2                 | 48.3 ± 0.3            |
| 8          | 10S-0.5F        | 3575.3 ± 301.6                 | 1819 ± 209.0                 | 45.0 ± 1.0            |
| 9          | 10S-1.0F        | 3894.3 ± 095.5                 | 1861 ± 067.5                 | 45.5 ± 0.6            |
| 10         | 15S-0F          | 4093.3 ± 333.6                 | 2085 ± 180.9                 | 46.5 ± 0.0            |
| 11         | 15S-0.5F        | 3595.5 ± 504.2                 | 1901 ± 033.9                 | 50.7 ± 0.3            |
| 12         | 15S-1.0F        | 3569.0 ± 374.8                 | 2132 ± 137.9                 | 47.8 ± 0.2            |

LSD (p < 0.05) | ns | 401.58 | 0.766

S: soil treatment; F: foliar spray
Figures followed by similar letters are not significantly different at the p < 0.05 level
1966; Rashid & Fox, 1992) have suggested the critical value of 15 mg Zn kg\(^{-1}\) as a general value for the interpretation of grain analyses, and more recent work has shown a value of 10 mg Zn kg\(^{-1}\) (Brennan et al., 1993). The treatment of such deficiencies with Zn fertilisers or foliar sprays can increase the yield and also improve the plant’s resistance to ‘foot rot’ fungus (\textit{F. graminarum}) (Gooding & Davies, 1997).

The grain and biomass yields were plotted (Figure 2) to observe their trends with the increasing levels of Zn application. The line for grain yield in the graph showed a significant response (R\(^2\) = 0.51) to increasing levels of Zn, while the biomass was independent (R\(^2\) = 0.06) of increasing levels of Zn. The biomass yield was not affected by 50\% Zn treatments, while the remaining 50\% increased positively. This is in conformity with the results obtained by Rengel and Graham (1996), who reported that the relative production of root and shoot dry matter at deficient compared to sufficient Zn supply for two wheat genotypes tested had no difference. Khattak et al., (2006) have concluded from their experiment on maize tested with various application levels of Zn that there was no statistically significant difference among the treatments regarding fresh biomass yield; anyhow they observed 8 – 11\% increase in biomass yield in various treatments.

Different modes and levels of Zn demonstrated variable effects on 1000 grain weight, and both increase and decrease in 1000 grain weight was observed with respect to the control due to Zn application (Table 3). The highest 1000 grain weight (50.6 g) was recorded in the treatment receiving 15 kg ZnSO\(_4\) as soil and 0.5\% ZnSO\(_4\) solution as foliar spray. 1000 grain weight was statistically different among the levels of Zn applied as soil addition with the best results shown by 15 kg ZnSO\(_4\) ha\(^{-1}\) (48.3 g), while on average over the levels of soil application, different concentrations of foliar application did not show any significant change in 1000 grain weight. Previous investigations (Khattak et al., 2006) have reported an increase in the grain yield of maize and wheat with Zn addition at the rate of 5 kg ha\(^{-1}\) or 2 times as foliar spray of 0.5\% ZnSO\(_4\), and this treatment showed an economical value greater than all the other concentrations added to maize. Yilmaz (1997) has shown increased wheat grain yield by 260\% and 124\%, with Zn application as soil addition and foliar application, respectively.

The present study reveals that Zn with higher levels of NPK performed well (R\(^2\) = 0.47) and almost an increasing trend in the grain yield was observed. With lower doses of NPK (half NPK levels to that of the recommended dose), application of Zn at lower levels both as soil and foliar spray recorded poor performance as compared to half NPK only treatment (Figure 3). This behaviour of potential as in the case of full dose of NPK. Yet, the R\(^2\) value (0.55) showed that Zn increment, irrespective of its mode of application, improved the overall yield of the crop better in the case of half NPK dose as compared to full NPK dose (Figure 3). Increased phosphorus supply enhances the symptoms of Zn deficiency (Brown, 2008), yet, nitrogen appears to affect the Zn status of
crops by promoting plant growth and by changing the pH of the root environment (Alloway, 2008). In many soils, nitrogen is the chief factor limiting the growth and yield and therefore in the case of full dose of NPK, improvements in yield have been found through positive interactions between nitrogen and Zn fertilisers.

Further analysis of the data using minitab statistical package to find the significance of Zn as soil, foliar, and soil and foliar treatments revealed that Zn as soil treatment had a significant (p = 0.031) effect on the grain yield and nearly significant (p = 0.084) effect of foliar spray on grain yield, but no interaction effect of the two modes. These findings are supported by the findings of Yilmaz et al. (1997) who concluded that soil and foliar Zn increased the grain yield of wheat by 260% and 124%, respectively in Zn deficient calcareous soils. The interaction plot (Figure 4) shows that increasing levels of soil application of Zn has an obvious increasing effect on grain yield.

The value cost ratio (VCR) for 5 kg ha\(^{-1}\) ZnSO\(_4\) as soil treatment + 1.0 % ZnSO\(_4\) as foliar spray (10.23) was three times higher over the VCR for 15 kg ha\(^{-1}\) ZnSO\(_4\) as soil treatment + 1.0 % ZnSO\(_4\) as foliar spray (3.46), confirming the superiority of the former over the latter.

Table 4: Economic analysis of Zn application on grain yield of wheat

| Treatment No. | Grain yield (kg ha\(^{-1}\)) | Yield increase (kg ha\(^{-1}\)) | Gross return (Pak Rs) | ZnSO\(_4\) used (kg ha\(^{-1}\)) | Cost of ZnSO\(_4\) (Pak Rs) | Net return (Pak Rs) | % increase over control | VCR |
|--------------|-----------------------------|-------------------------------|-----------------------|--------------------------------|--------------------------|---------------------|------------------------|-----|
| 1            | 1476                        | 000                           | 000000                | 00                             | 000000                   | 000000              | 00.0                   | 00.0 |
| 2            | 1626                        | 150                           | 03607                 | 05                             | 030000                   | 03007              | 10.2                   | 12.0 |
| 3            | 1752                        | 277                           | 06640                 | 10                             | 060000                   | 06040              | 18.8                   | 11.1 |
| 4            | 1747                        | 271                           | 06510                 | 22                             | 052000                   | 05190              | 18.4                   | 04.9 |
| 5            | 1859                        | 383                           | 09200                 | 27                             | 072000                   | 07580              | 26.0                   | 05.7 |
| 6            | 2294                        | 818                           | 19634                 | 32                             | 172000                   | 17714              | 55.4                   | 10.2 |
| 7            | 1947                        | 471                           | 11314                 | 44                             | 264000                   | 08674              | 32.0                   | 04.3 |
| 8            | 1819                        | 344                           | 08247                 | 49                             | 294000                   | 05307              | 23.3                   | 02.8 |
| 9            | 1861                        | 386                           | 09260                 | 54                             | 324000                   | 06020              | 26.2                   | 02.9 |
| 10           | 2085                        | 610                           | 14638                 | 66                             | 396000                   | 10678              | 41.3                   | 03.7 |
| 11           | 1901                        | 425                           | 10210                 | 71                             | 426000                   | 05950              | 28.8                   | 02.4 |
| 12           | 2132                        | 657                           | 15763                 | 76                             | 456000                   | 11203              | 44.5                   | 03.5 |

Note: Wheat grain rate = Pakistan rupees (Pak Rs) 24 kg\(^{-1}\) and ZnSO\(_4\) = Pakistan rupees (Pak Rs) 60 kg\(^{-1}\)
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Table 5: Effect of Zn application on glutenin fraction of grain protein in wheat

| Serial No. | Treatments | Protein (mg mL⁻¹) | Protein (%) | % increase over control |
|------------|------------|-------------------|-------------|------------------------|
| 1          | Zn S0 F0 (Control) | 20.00           | 2.00        | 0.0                    |
| 2          | 0S-0.5F    | 21.90            | 2.19        | 9.5                    |
| 3          | 0S-1.0F    | 24.50            | 2.45        | 22.5                   |
| 4          | 5S-0F      | 25.05            | 2.51        | 25.5                   |
| 5          | 5S-0.5F    | 25.45            | 2.55        | 27.5                   |
| 6          | 5S-1.0F    | 25.90            | 2.59        | 29.5                   |
| 7          | 10S-0F     | 24.50            | 2.45        | 22.5                   |
| 8          | 10S-0.5F   | 23.45            | 2.35        | 17.5                   |
| 9          | 10S-1.0F   | 23.60            | 2.36        | 18.0                   |
| 10         | 15S-0F     | 23.80            | 2.38        | 19.0                   |
| 11         | 15S-0.5F   | 23.70            | 2.37        | 18.5                   |
| 12         | 15S-1.0F   | 25.75            | 2.58        | 29.0                   |

S: soil treatment; F: foliar spray

In terms of effectiveness (Table 4). Although VCR for 0.5 % ZnSO₄ (VCR:12) and 1.0 % ZnSO₄ (VCR:11) as foliar application was the highest among the list of treatments (Table 4), yet, taking into account the yield per unit area of the treatments, the performance of 0.5 % ZnSO₄ (1626 kg ha⁻¹) and 1.0 % ZnSO₄ (1752 kg ha⁻¹) as foliar application only was not satisfactory as compared to 5 kg ha⁻¹ ZnSO₄ as soil + 1.0 % ZnSO₄ as foliar (2294 kg ha⁻¹) and 15 kg ha⁻¹ ZnSO₄ as soil + 1.0 % ZnSO₄ as foliar (2132 kg ha⁻¹). Thus in a country like Pakistan, which faces food deficit due to increased population, the growers must adopt the technology that could increase their production per unit area with acceptable margin of profit. Thus, this study suggested the best level of Zn as 5 kg ha⁻¹ as soil treatment + 1.0 % ZnSO₄ as foliar application as a better option to improve the grain yield on alkaline soil (Table 4).

Application of Zn, both as soil treatment and foliar spray, to wheat crop in alkaline soil significantly (p < 0.05) increased the protein content in wheat grains over the control (Table 5, Figure 5). The treatments receiving 5 kg ha⁻¹ ZnSO₄ soil application + 1.0 % ZnSO₄ solution as foliar application, 15 kg ha⁻¹ ZnSO₄ as soil application + 1.0 % ZnSO₄ solution as foliar spray and 5 kg ha⁻¹ ZnSO₄ as soil application + 0.5 % ZnSO₄ solution as foliar spray application recorded the highest increase in wheat grain protein content, which were 29.5, 29.0 and 27.5 % higher than the control treatment, respectively. It was thus concluded that protein in wheat grain could be increased either by low level of Zn as soil application + comparatively higher dose of foliar application or higher dose of Zn through soil application along with higher concentration of foliar spray. The bars (Figure 5) clearly indicate an initial gradual increase in protein with low Zn concentration levels, but with medium Zn concentration levels it tends to decrease irrespective of its application method. With higher application of Zn at 15 kg as soil treatment and 1.0 % ZnSO₄ as foliar spray, it shows an increase in the protein content. The regression coefficient indicated an overall increase of 21 % in grain protein content with increasing Zn level applied either as foliar application or as soil application (Figure 5).

In calcareous soils, Zn precipitates in unavailable forms for plants, and its uptake and transition to the shoot is inhibited by high concentrations of bicarbonate (Dogar & Van Haj, 1980). However, Zn deficiency in the plants grown in calcareous soils can be recovered fairly readily.

Figure 5: Effect of various modes of treatment and levels of Zn on percent protein content in wheat grain
by application of inorganic Zn salts such as ZnSO\(_4\) to the soil (Nayyar & Takkar, 1980), and such application from external sources linearly increased the grain yield and protein content of two wheat genotypes (Morsheidi & Farahbaksh, 2010). Szakal (1989) and Fecenko and Lozek (1998) have reported similar findings. Khattak et al., (2006) have reported a maximum increase in protein content in the grains of maize obtained from the treatment supplied with 0.5 % ZnSO\(_4\) as foliar spray at the stage when the crop was only 20 – 25 days old. Several researchers have reported significant seed protein and dry matter yield response to Zn fertilisation (Biswas et al., 1977; Tandon, 1992; Zaidan et al., 2010; Keram, 2014). Zn is required for the synthesis of auxin [a growth regulating compound indole acetic acid (IAA)] (Brown et al., 1993). Tryptophan is the most likely precursor for the biosynthesis of IAA and Zn is required for the synthesis of tryptophan (Marschner, 1995). This includes an observed increase in the tryptophan content in rice grains after Zn fertilisation of plants growing on calcareous soil (Singh, 1991).

No major differences were observed in protein profiles of the samples isolated from different Zn treatments (Figure 6) despite the increase or decrease in protein content in various treatments. Alloway (2008) reported that in general the amount of protein in the grains of Zn deficient plants is greatly reduced, but the protein composition remains almost unchanged. In Zn deficient bean leaves, the concentration of free amino acids was 6.5 times greater than in the control but these decreased, and the protein content increased after the administration of Zn for 48 or 72 hours. The mechanism by which Zn deficiency affects protein synthesis is considered to be due to a reduction in RNA and the deformation and reduction of ribosome. The importance of Zn in protein synthesis suggests that a relatively high Zn concentration is required by meristematic tissues where cell division as well as the synthesis of nucleic acid and protein is actively taking place (Brown et al., 1993).

CONCLUSION

This study concludes that alkaline soils are Zn deficient in terms of availability to plants despite the apparent Zn levels in the sufficient range (> 1.0 mg kg\(^{-1}\)). Therefore, this range should be revised in accordance with the nature and type of soils. Foliar application of Zn up to 1.0 % ZnSO\(_4\) solution and 5 kg ha\(^{-1}\) as soil addition increased the yield, while regardless of the method, Zn application increased the protein content in grains. Thus, for higher yield and better quality Zn should be added by farmers cultivating alkaline soils in Pakistan to the extent of 5 kg ha\(^{-1}\) ZnSO\(_4\) as soil addition and 1.0 % ZnSO\(_4\) solution as foliar application.

![Figure 6: Effect of various modes of treatment and levels of Zn on composition of protein in wheat grain](image)

Legends: Coomassie-blue stained SDS-PAGE gel of wheat grain proteins isolated from the 12 Zn treatments. Molecular weight standards are shown on the left.
Lane 1, S0F0: L2, S0F1: L3, S0F2: L4, S1F0: L5, S1F1: L6, S1F2: L7, S2F0: L8, S2F1: L9, S2F2: L10, S3F0: L11, S3F1: L12, S3F2
S = soil Zn application at 0, 1 (5 kg ha\(^{-1}\)), 2 (10 kg ha\(^{-1}\)), 3 (15 kg ha\(^{-1}\))
F = foliar ZnSO\(_4\) application at 0, 1 (0.5 %), and 2 (1 %).
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