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Wheat genotype responses to phosphorus and zinc: Differential growth, mycorrhizal interactions, and zinc accumulation

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ABSTRACT

Agronomic methods for increasing wheat's grain zinc (Zn) content have been found to be an effective means of preventing human zinc deficiency. To assess the response of three wheat genotypes to varying quantities of phosphate (P) and zinc (Zn) for grain yield and Zn accumulation, a pot study was conducted. Using a completely randomized design (CRD) factorial combination, two Zn levels (0, 5 kg ha^{-1}) and three P levels (0, 90, 120 kg ha^{-1}) were applied. The study revealed that wheat genotypes responded differently to varied doses of P and Zn. In order to considerably increase yield (grain and straw) and related features (number of spikelets per spike, spike length, number of grains per spike, and 1000-grain weight), the combination of 5 kg Zn and 90 kg P ha^{-1} was used. The most effective combination for all wheat genotypes was found to be 5 kg Zn + 90 kg P ha^{-1}, which significantly increased grain yield (23 %) and related attributes including number of spikelets per spike (11 %), spike length (10 %), number of grains per spike (17%) and 1000-grain weight (10 %) as compared to control. In general, all wheat genotypes showed decreased mycorrhizal root infection (205-290 %) and grain zinc uptake (22-45 %) when exposed to a higher dose of P (120 kg ha^{-1}) and vice versa. The genotype TD-1 had the higher agronomic Zn efficiency (139 % and 235 %) at 5 and 90 kg Zn and P ha^{-1} as compared to other genotypes NIA-WR1 and Chakwal-86 respectively. Similarly, it was proved that applying 5 kg of Zn ha^{-1} along with P level of 90 kg ha^{-1} was the best combination to maximize the potential yield of Chakwal-86. Among the genotypes, NIA-WR1 accumulated the higher quantity of Zn (51 % and 40 %) in its grains at 5 kg Zn along with 90 kg P ha^{-1} compared to the genotypes TD-1 and Chakwal-86 respectively. The same genotype, NIA-WR1 accumulated the maximum Zn (44.26 µg g^{-1}) in its grains even without the application of exogenous Zn. This indicates that it is Zn-efficient and capable of thriving in Zn-deficient soils. It can utilize the Zn present in the native soil to achieve a high zinc content in its grains. However, further research is needed before general recommendations can be made for wheat growers.

1. Introduction

Micronutrient malnutrition, particularly zinc deficiency, is a serious global health issue, affecting around 17% of the world's population, mostly in developing countries. Zinc is an essential micronutrient that plays a crucial role in the growth and development of plants, humans, and animals [1]. Approximately one quarter of the world's population is facing zinc deficiency . In Pakistan, around half of the population suffers from micronutrient deficiencies, and over 40% of women and 20.6% of children are zinc deficient [2]. Consequences of zinc deficiency include stunted growth, increased susceptibility to infections, complications during pregnancy, and impaired cognitive development [3, 32]. The World Health Organization (WHO) states that zinc deficiency is a public health issue in over 70% of nations, with young children and pregnant women being the most affected [4]. Wheat is a staple food in Pakistan, but typically contains low zinc content. Wheat grown in alkaline, calcareous soils common in semiarid regions of Pakistan is highly susceptible to zinc deficiency, leading to low grain yield and zinc concentration [5]. Zinc plays a critical role in various plant functions, including tryptophan production, membrane integrity, pollen tube formation, enzyme activation, and protein synthesis [6]. Over 70% of soils in Pakistan are deficient in zinc, and lack of awareness among growers about the benefits of zinc fertilizers is a major challenge [7]. The zinc concentration in wheat grains often remains around 25–30 $\mu g\ g^{-1}$ dry weight. However, for zinc biofortified wheat, to enhance human zinc intake and health, a zinc concentration of over 50 $\mu g\ g^{-1}$ dry weight in wheat grains is desired [8]. Application of zinc salts like $ZnPO_4$ and $ZnSO_4$ through soil, foliar spray, or seed priming can significantly improve wheat yield and grain zinc content [9]. Saha et al. [10] indicated that excess phosphorus can inhibit zinc uptake in plant roots, while zinc can decrease the transfer of phosphorus from roots to shoots. This interaction can lead to reduced nutrient availability for plants, negatively impacting their growth and yield. Previous research has shown that colonization by arbuscular mycorrhizal fungi (AMF) can contribute up to 24.3% of the total above-ground Zn in wheat and 12.7% in barley when inoculated with *Rhizophagus irregularis* under varying soil Zn concentrations [11]. However, increased P application negatively affects Zn uptake across different crops, including maize and soybean, primarily due to reduced AMF colonization. Specifically, AMF colonization accounted for 79-89% of the negative effects observed on Zn uptake when P levels were elevated [38]. Certain wheat genotypes have developed efficient mechanisms for zinc translocation to shoots, reducing zinc antagonism with other nutrients like phosphorus, making them zinc-efficient [12]. The increasing costs of fertilizers and lack of awareness pose significant challenges for wheat growers in using zinc fertilizers for wheat cultivation. There is an urgent need to identify wheat genotypes that can effectively extract indigenous Zn from the soil for optimal growth and accumulate higher levels of Zn in grains. This is crucial for addressing Zn malnutrition in humans. Therefore, the current study aims to explore Zn-efficient genotypes and determine the optimal combination of soil-applied zinc and phosphorus to achieve maximum wheat yield with increased grain zinc content.

2. Materials and Methods

The pot study was conducted to assess the performance of three wheat genotypes, namely NIA-WR1, TD-1, and Chakwal-86, in terms of mycorrhizal root infection, zinc accumulation, and grain yield. The study was conducted in plastic pots filled with 5 kg of soil within a greenhouse at the Nuclear Institute of Agriculture (NIA) located in Tandojam. Six treatments were employed, including two levels of zinc (Z1 = no additional zinc, and Z2 = zinc @ 5 kg ha^{-1}) and three levels of phosphorus (P0 = no external phosphorus, P1 = phosphorus @ 90 kg ha^{-1}, and P2 = phosphorus @ 120 kg ha^{-1}), following a completely randomized design (CRD) in factorial combination with three replications. Seeds of the selected varieties were planted in the pots, with five plants maintained in each. Phosphatic fertilizer (DAP) was applied at sowing, while nitrogen fertilizer was applied in stages at sowing, tillering, and booting. The plants were irrigated with canal water at 50 % of field capacity, and manual weeding was carried out to manage weeds. The physico-chemical properties of the soil used in the study were determined before filling the pots (Table. 1). Upon maturity, the plants were harvested, and data on plant growth, yield, and related attributes were collected.

2.1 Soil Analysis

Soil analysis involved collecting surface soil (0-15 cm) from the NIA experimental farm. The soil was mixed, air-dried, and sieved through a 2 mm sieve. The soil texture was determined using the Bouyoucus hydrometer method [13] by calculating the proportions of sand, silt, and clay and comparing them using the USDA textural class triangle. Total nitrogen was determined using the Kjeldahl digestion method with sulfuric acid, as reported by Mulvaney [14]. Other nutrients (phosphorus, potassium, and zinc) were extracted using the AB-DTPA extraction method. The concentration of extractable phosphorus was determined using a spectrophotometer (U-2900UV/VIS, Hitachi, Japan) at a wavelength of 882 nm. Potassium concentration was determined using a flame photometer following the procedure of Soltanpour and Schwab [15], and zinc content was measured using an atomic absorption spectrophotometer (Analytical Jena AAS-NOVA-400, Germany) equipped with a flame ionization detector, following the method described by Rashid [16].

2.2 Plant Tissue Analysis

Plant tissue and grain samples were dried in an oven at 70 degrees Celsius for 72 hours. After grinding, the samples were digested in a di-acid mixture (HNO_3 and $HClO_4$) following the procedure by Jones and Case [17]. The digested samples were then analyzed for phosphorus concentration using the vanadate-molybdate yellow color method with a spectrophotometer [18] at a wavelength of 470 nm. Zinc concentration in the plant material was estimated using an atomic absorption spectrophotometer (Analytical Jena AAS-NOVA-400, Germany) equipped with a flame ionization detector, as outlined by Rashid [16]. Agronomic zinc efficiency (ZNUE) was evaluated using the formula provided by Fageria [19].

$$\text{Agronomic ZNUE} = \frac{\text{Grain yield of Zn fertilized plots} - \text{Grain yield of Zn unfertilized plots}}{\text{Quantity of Zn applied}}$$

The grain Zn accumulation was calculated using the following formula

$$\text{Zn accumulation } (\mu g \text{ plot}^{-1}) = \text{Grain Zn concentration} (\mu g \text{ g}^{-1}) \times \text{Grain dry weight (g plot}^{-1})$$

The categorization of wheat genotypes as zinc-efficient and zinc-responsive was assessed based on yield parameters and ZnUE, as reported by Rawal (39).

2.3 Arbuscular Mycorrhizal Colonization Assay

In harvested roots, arbuscular mycorrhizal colonization was assessed using the "slide" method outlined by Giovannetti and Mosse [20]. The roots were rinsed with deionized water (dH2O) to remove soil particles. Approximately 3 cm-long root tips were excised and preserved in ethanol for further processing. Initially, the root tips were immersed in 10% (w/v) KOH and incubated at 65 °C in a water bath for an hour. Subsequently, the KOH solution was discarded, and the samples were treated with 10% HCl (v/v) at 65 °C for 15 minutes. After removing the HCl solution, the root tips were then treated with a 0.05% (w/v) trypan blue solution at 65 °C for 25 minutes. The processed root samples were stored in lactic acid until microscopic evaluation of mycorrhizal colonization. Ten pieces of root tips were examined under the microscope for each sample, focusing on a standard section of the root system. The presence or absence of colonization was recorded for each of the 10 pieces, providing a score ranging from 0 (no indication of infection) to 10 (highly infected) for each sample.

2.4 Statistical Analysis

Data collected for growth, yield parameters, and zinc relationships were analysed using ANOVA with the MSTAT-C computer software [21]. Treatment means were compared using the least significant difference (LSD) method at $P < 0.05$. Correlations among various parameters and graphical representation of data were conducted using Microsoft Excel (Redmond, WA, USA).

3. Results And Discussion

The experimental soil had a silty clay loam texture and was non-saline, but deficient in nutrients including nitrogen (N), P and Zn, and organic matter, while having sufficient potassium (K) as depicted in Table. 1. The study was conducted in a subtropical climate, where the crop experienced mean monthly temperatures ranging from 15 to 30 °C, relative humidity between 43% and 56%, and mean monthly sunshine hours ranging from 8.52 to 10.05 (Table. 2). Differential responses in growth, yield, and nutrient accumulation were observed with varying applications of phosphorus (P) and zinc (Zn). Zinc application did not significantly affect the number of tillers per plant (Table. 3).

Table 1

Soil Analyses of Experimental Site

Characteristics	Unit	Values
EC (1:2.5)	dS m^{-1}	0.82
pH (1:2.5)	-	7.6
Organic matter	%	0.75
Kjeldahl N	%	0.051
Extractable P	µg g^{-1}	5.01
Exchangeable K	µg g^{-1}	148
Extractable Zn	µg g^{-1}	0.58
Textural class	-	Silty clay loam

However, it did improve yield and yield parameters such as plant height, spikelets per plant, spike length, thousand grain weight, grains per spike, biological yield, and grain yield in zinc-responsive genotypes. The tested wheat genotypes (NIA-WR1, TD-1, and Chakwal-86) exhibited varying responses in growth, yield, and nutrient uptake to different levels of soil-applied phosphorus (P) and zinc (Zn), as reported by Singh *et al.* [22]. Previous studies [23] have shown that using seeds with high zinc content (50 mg kg^{-1}) can improve seedling emergence, grain yield, and grain zinc concentration. The differential response of genotypes to Zn application is influenced by their genetic makeup and physiological traits, which can be affected by environmental conditions [24-25]. Therefore, screening genotypes for high Zn use efficiency is crucial for sustainable agriculture.

Growth and yield responses to soil-applied Zn and P: Application of Zn at 5 kg ha^{-1} along with 90 kg P ha^{-1} was recorded as the most suitable combination, resulting in a significant increase in yield-contributing traits (Table. 4) including number of spikelets per spike (16.35), spike length (9.57 cm), number of grains per spike (24.04), thousand grain weight (43.02 g), biological yield (27.52 g pot^{-1}) and grain yield (10.92 g pot^{-1}) (Tables. 4 and 5). However, the application of zinc either in the absence of phosphorus (P0) or with a high dose of phosphorus (120 kg ha^{-1}) did not play a significant role in improving the growth and yield traits of the wheat genotypes studied. According to Bharti et al. [26] there was no significant effect of zinc application combined with phosphorus on the number of tillers per plant, indicating that zinc application may not stimulate tillering in certain genotypes, possibly due to reduced enzyme activity. Aboyeji *et al.* [27] reported that applying zinc (5 kg ha^{-1}) with a higher dose of phosphorus (120 kg ha^{-1}) did not significantly affect growth, yield traits, and nutrient accumulation in groundnut. Their findings suggested a decrease in groundnut yield traits when zinc was applied with phosphorus at 120 kg ha^{-1}, possibly due to elevated phosphorus concentration leading to increased zinc accumulation in plant shoots [28-29].

Table 2

The meteorological data of the experimental site during the course of study

Month	Temperature (°C)			Total Rainfall (mm)	Mean Relative Humidity (%)	Mean Sunshine (hrs/day)
	Min	Max	Mean			
Nov	14.8	30.8	22.8	0	52	8.73
Dec	10.3	25.9	18.0	0	56	8.63
Jan	7.0	23.4	15.1	0	52	8.80
Feb	10.0	26.5	18.2	0	50	8.52
Mar	15.6	32.0	23.8	0	49	8.95
April	21.1	39.2	30.0	3	43	10.05

Table 3

Analysis of variance (anova) for growth and yield traits of wheat genotypes

Trait	Treatment Interaction	sum of square	Mean square	Probability Value
Biological yield	Treatment	169.294	33.8587	0.0000**
	Variety	142.365	71.1824	0.0000**
	Treatment*Variety	38.279	3.8279	0.4724 ns
Grain yield	Treatment	26.2441	5.2848	0.0000**
	Variety	31.8246	15.9123	0.0000**
	Treatment*Variety	26.1727	2.6173	0.0000**
Plant height	Treatment	297.553	59.5107	0.0000**
	Variety	116.175	58.0875	0.0000**
	Treatment*Variety	112.258	11.2258	0.0072**
Spikelets per spike	Treatment	17.0022	3.4004	0.0001**
	Variety	29.4614	14.7307	0.0000**
	Treatment*Variety	7.0750	0.7075	0.1665 ns
Spike length	Treatment	4.6931	0.93863	0.0037**
	Variety	1.2284	0.61418	0.0725ns
	Treatment*Variety	3.8803	0.38803	0.0999ns
Thousand grain weight	Treatment	114.285	22.8571	0.0063**
	Variety	11.908	5.9541	0.3690ns
	Treatment*Variety	24.733	2.4733	0.9234ns
Grains per spike	Treatment	147.433	29.487	0.0000**
	Variety	420.467	210.234	0.0000**
	Treatment*Variety	122.243	12.224	0.0030**
Tillers per plant	Treatment	1.96695	0.39339	0.0731ns
	Variety	0.07934	0.03967	0.7993ns
	Treatment*Variety	0.90751	0.09075	0.8669ns

**= Significant at 0.05 level   ns= non-significant at ≤ 0.05 level

The wheat genotype Chakwal-86 had the maximum plant height at 76.22 cm, followed by NIA-WR1 at 74.37 cm, while TD-1 had the minimum plant height at 72.63 cm. Chakwal-86 remained the best performer, with significantly high numbers of spikelets per spike (16.68), spike length (9.41 cm), and grains per spike (26.08). However, the genotypes did not differ significantly in thousand grain weight. Similarly, Chakwal-86 attained significantly high biological yield at 27.50 g pot^{-1} and grain yield at 11.07 g pot^{-1} compared to the other genotypes tested (Table 5).

In the current study, genotype Chakwal-86 demonstrated significantly higher growth and yield traits when 5 kg ha^{-1} of zinc was applied alongside the recommended phosphorus dose, compared to other tested genotypes. Variability among genotypes can be attributed to mechanisms involved in nutrient (zinc and phosphorus) uptake, such as the secretion of organic acids and phytosiderophores, as well as the translocation and internal utilization of micronutrients [30], influencing grain micronutrient concentration and yield [24].

Table 4

Effect of Various Zn and P levels on growth and yield parameters of wheat

Trait	Genotype	TREATMENTS						Varietal Mean
		P0Z1	P1Z1	P2Z1	P0Z2	P1Z2	P2Z2	
Plant Height (cm)	NIA-WR1	70.78 b	76.44 a	75.44 a	69.33 b	77.33 a	76.89 a	74.37 B
	TD-1	69.22 b	77.22 a	70.11 b	70.56 b	77.44 a	71.22 b	72.63 C
	Chakwal-86	75.11 a	77.89 a	74.89 a	75.22 a	77.78 a	76.44 a	76.22 A
	Treatment Mean	71.70 C	77.19 A	73.48 BC	71.70 C	77.52 A	74.85 B	
Number of Spikelets/Spike	NIA-WR1	13.89 g	14.78 e-g	15.00 e-g	15.00 e-g	16.33 b-d	15.44 d-f	15.07 B
	TD-1	14.78 e-g	15.00 e-g	15.22 d-f	14.55 fg	15.89 c-e	15.44 d-f	15.15 B
	Chakwal-86	15.55 d-f	16.89 a-c	17.78 a	15.78 c-e	16.83 a-c	17.22 ab	16.68 A
	Treatment Mean	14.74 D	15.56 BC	16.00 AB	15.11 CD	16.35 A	16.04 AB	
Spike Length (cm)	NIA-WR1	8.34 f	9.36 a-d	9.33 a-d	8.61 d-f	9.36 a-d	9.28 a-e	9.05 B
	TD-1	8.56 ef	9.33 a-d	8.78 c-f	9.42 a-c	9.89 a	9.78 ab	9.29 AB
	Chakwal-86	9.17 a-e	9.78 ab	9.72 ab	9.22 a-e	9.45 a-c	9.11 b-e	9.41 A
	Treatment Mean	8.69 C	9.49 AB	9.28 AB	9.09 BC	9.57 A	9.39 AB	
Number of grains per spike	NIA-WR1	18.44 ef	19.97 ef	17.55 f	19.33 ef	23.67 cd	27.00 ab	20.99 B
	TD-1	19.00 ef	20.22 ef	21.11 de	18.67 ef	19.89 ef	20.38 ef	19.88 B
	Chakwal-86	24.33 bc	23.78 cd	25.56 a-c	27.22 ab	28.55 a	28.22 a	26.28 A
	Treatment Mean	20.59 B	21.32 B	21.41 B	21.74 B	24.04 A	25.20 A	

Means sharing the same letter are statistically non-significant at probability level of ≤ 0.05.

Increasing phosphorus (P) levels reduced the zinc (Zn) concentration in the grains of all three wheat varieties (NIA-WR1, TD-1, and Chakwal-86) compared to their respective controls where no P was applied. Among the three varieties, the maximum grain zinc concentration was 65.63 µg g^{-1} in NIA-

WR1 at a P level of 90 kg ha^{-1} where 5 kg ha^{-1} of zinc was applied. This was followed by 62.80 µg g^{-1} in the same genotype at a P level of 120 kg ha^{-1} with 5 kg Zn ha^{-1}. In contrast, the minimum grain zinc concentration was 22.18 µg g^{-1} in genotype TD-1 at a P level of 120 kg ha^{-1} where no zinc was applied. Notably, NIA-WR1 extracted the maximum zinc (44.26 µg g^{-1}) in its grains without the application of exogenous zinc. This indicates that NIA-WR1 is a zinc-efficient genotype that can utilize the native soil zinc effectively for high grain zinc accumulation. Hacisalihoglu et al. [31] proposed that higher activities of antioxidants (SOD and CA) may contribute to efficient cytoplasmic zinc utilization in genotypes deemed zinc-efficient. Genotype NIA-WR1 extracted the maximum zinc from a deficient zinc treatment, consistent with findings by Imtiaz et al. [12] that zinc-efficient genotypes possess mechanisms for efficient zinc translocation to shoots and mitigate antagonism with other nutrients like phosphorus, lacking in zinc-inefficient wheat genotypes. Zinc application at 5 kg ha^{-1} resulted in a reduction of phosphorus (P) concentration in the wheat grains of all genotypes tested compared to the treatments without zinc application (Fig. 2). The genotype NIA-WR1 had a significantly high phosphorus concentration (0.69%) at a P level of 120 kg ha^{-1} where no zinc was applied, while the minimum phosphorus concentration was noted in genotype Chakwal-86 at a zinc level of 5 kg ha^{-1} with no phosphorus application.

Table 5

Effect of various Zn and P levels on yield parameters of wheat

Trait	Genotype	TREATMENTS						Varietal Mean
		P0Z1	P1Z1	P2Z1	P0Z2	P1Z2	P2Z2	
Thousand Grain Weight (g)	NIA-WR1	37.13 c	40.25 a-c	40.70 a-c	40.30 a-c	42.03 a	42.50 a	40.49 A
	TD-1	40.56 a-c	41.47 a	40.63 a-c	41.50 a	44.03 a	41.60 a	41.63 A
	Chakwal-86	37.27 bc	41.70 a	41.13 ab	40.50 a-c	43.00 a	42.37 a	40.99 A
	Treatment Mean	38.32 B	41.14 A	40.82 A	40.77 A	43.02 A	42.16 A	
Grain Yield (g per pot)	NIA-WR1	8.09 g	10.26 d	9.98 de	8.62 fg	10.29 d	10.13 d	9.56 B
	TD-1	8.76 fg	9.13 ef	7.12 h	10.30 d	10.33 d	10.43 cd	9.35 B
	Chakwal-86	9.78 de	11.76 ab	11.51 ab	10.03 d	12.14 a	11.22 bc	11.07 A
	Treatment Mean	8.88 D	10.38 B	9.53 C	9.65 C	10.92 A	10.59 AB	
Biological Yield (g per pot)	NIA-WR1	21.80 g	25.39 c-f	23.49 e-g	23.52 e-g	26.07 c-e	24.55 d-g	24.14 B
	TD-1	21.37 g	24.07 d-g	23.61 d-g	23.72 d-g	26.84 b-d	24.32 d-g	23.99 B
	Chakwal-86	22.65 fg	30.84 a	28.65 a-c	26.58 b-e	29.64 ab	26.66 b-e	27.50 A
	Treatment Mean	21.94 D	26.77 AB	25.25 BC	24.61 C	27.52 A	25.18 BC	
Harvest Index (%)	NIA-WR1	37.16 bc	40.45 a-c	42.52 a-c	36.82 c	39.54 a-c	41.54 a-c	39.67 A
	TD-1	41.13 a-c	38.11 bc	30.42 d	44.27 a	38.71 a-c	43.11 ab	39.29 A
	Chakwal-86	43.24 ab	38.16 a-c	40.20 a-c	37.80 bc	41.19 a-c	42.08 a-c	40.44 A
	Treatment Mean	40.51 AB	38.91 AB	37.71 B	39.63 AB	39.81 AB	42.24 A	

Means sharing the same letter are statistically non-significant at probability level of ≤ 0.05.

The genotype NIA-WR1 accumulated the maximum zinc (675.15 µg pot^{-1}) in its grains at a zinc level of 5 kg ha^{-1} along with a phosphorus level of 90 kg ha^{-1} (Fig. 3) compared to the other genotypes under study. In contrast, the genotype TD-1 showed the maximum agronomic zinc efficiency (63.67 mg mg^{-1} Zn) at a phosphorus level of 90 kg ha^{-1} compared to the other genotypes tested (Fig. 4). All wheat genotypes responded positively to an increase in grain zinc concentration when a zinc dose of 5 kg ha^{-1} was applied. However, grain zinc concentration decreased when zinc was applied in conjunction with a high dose of phosphorus (120 kg ha^{-1}). Previous studies [23] have indicated that phosphorus application negatively affects grain and shoot zinc concentration in cereal crops due to complex interactions between the two nutrients. Zinc application also reduced grain phosphorus concentration, likely due to antagonistic effects and the formation of zinc-phosphorus complexes [37]. This observation supports the negative correlation between grain phosphorus and zinc concentration found in this study. Zinc application reduced the mycorrhizal root infection of the wheat genotypes under study compared to the treatments where no Zn was applied (Fig. 5). Similarly, an overdose of P (120 kg ha^{-1}) drastically reduced mycorrhizal root infection. The wheat genotypes NIA-WR1 and TD-1 exhibited the maximum root infection percentages, at 85-86% and 82-83% respectively, at P levels of 0 and 90 kg ha^{-1} without Zn application. In contrast, the genotype Chakwal-86 expressed the least mycorrhizal root infection, 15% only, at a P level of 120 kg ha^{-1} along with Zn applied at 5 kg ha^{-1}. Interestingly, high mycorrhizal root infection was exhibited by the Zn-efficient genotype NIA-WR1 compared to other genotypes, and the infection was reduced drastically with application of P.

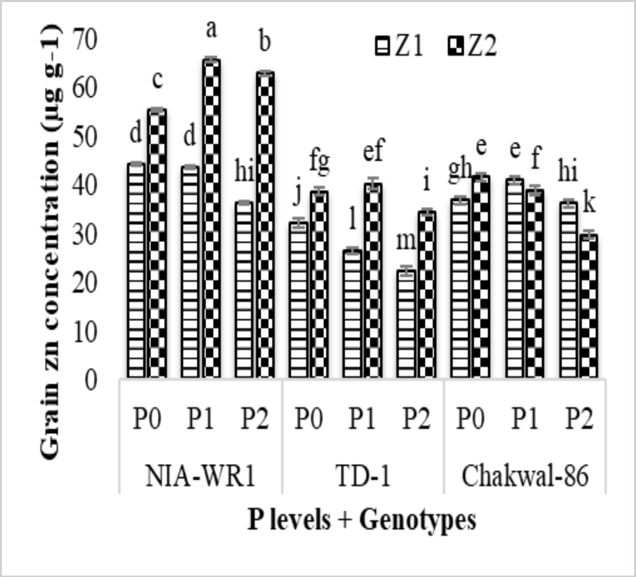

Fig. 1. Grain Zn Concentration (µg g-1) of Wheat Genotypes at Different P and Zn Levels

Z_1= No zinc, Z_2=Zn @ 5 kg ha^{-1}, P_0= without P, P_1= P_2O_5 @ 90 kg ha^{-1}, P_2= P_2O_5 @ 120 kg ha^{-1}.

Mycorrhizal association with plants has a supportive role for P acquisition [33], and it also plays a role in facilitating Zn transport in the soil-mycorrhizae-plant continuum [34]. Coccina et al. [34] reported that high AMF root colonization resulted in enhanced Zn concentration in grains of a commercial organic wheat crop. It did not escalate the P uptake, but the Zn uptake was enhanced through AMF [35].

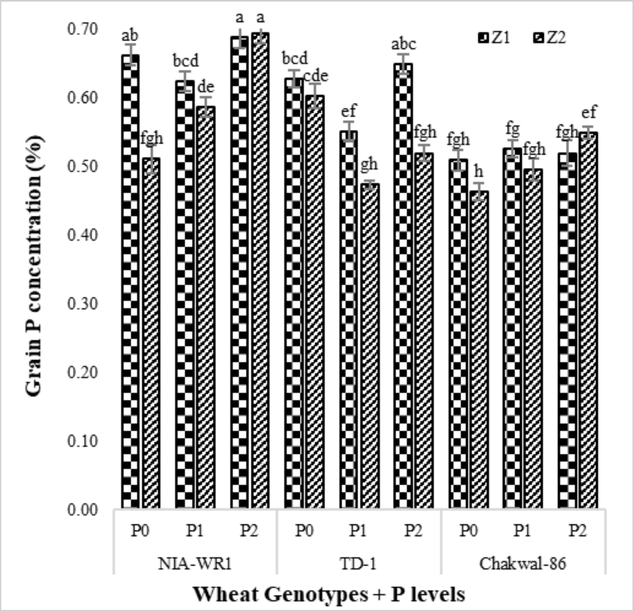

Fig. 2. Grain P Concentration (%) of Wheat Genotypes at Different P and Zn Levels

Z_1= No zinc, Z_2=Zn @ 5 kg ha^{-1}, P_0= without P, P_1= P_2O_5 @ 90 kg ha^{-1}, P_2= P_2O_5 @ 120 kg ha^{-1}.

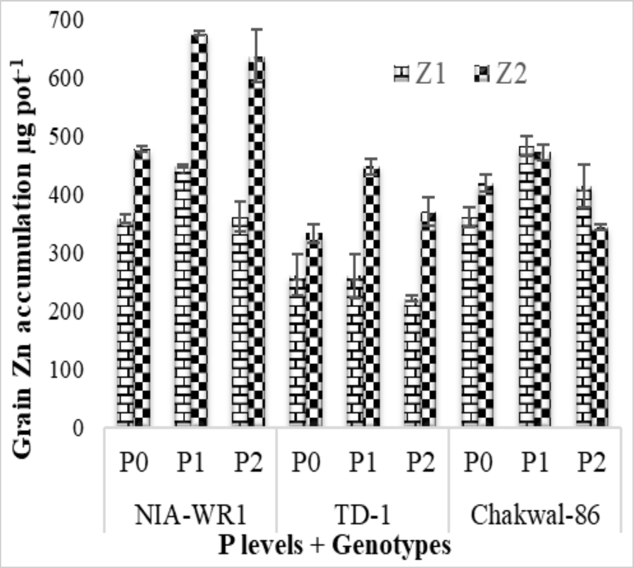

Fig. 3. Grain Zn Accumulation (µg pot-1) of Wheat Genotypes at Different P and Zn Levels

Z_1= No zinc, Z_2=Zn @ 5 kg ha^{-1}, P_0= without P, P_1= P_2O_5 @ 90 kg ha^{-1}, P_2= P_2O_5 @ 120 kg ha^{-1}

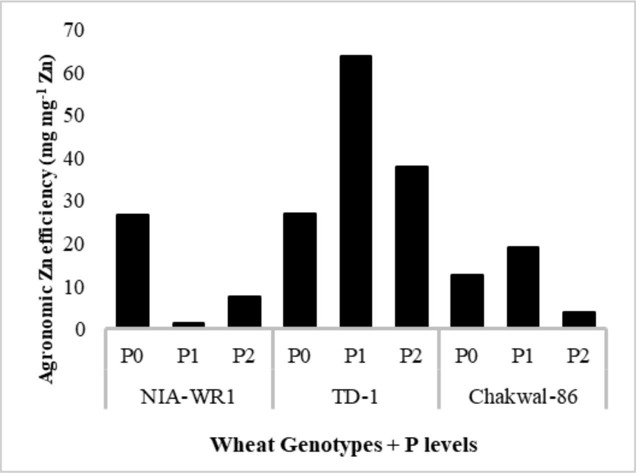

Fig. 4. Agronomic Zn Efficiency (mg mg^{-1} ZN) of Different Wheat Genotypes

Z_1= No zinc, Z_2=Zn @ 5 kg ha^{-1}, P_0= without P, P_1= P_2O_5 @ 90 kg ha^{-1}, P_2= P_2O_5 @ 120 kg ha^{-1}.

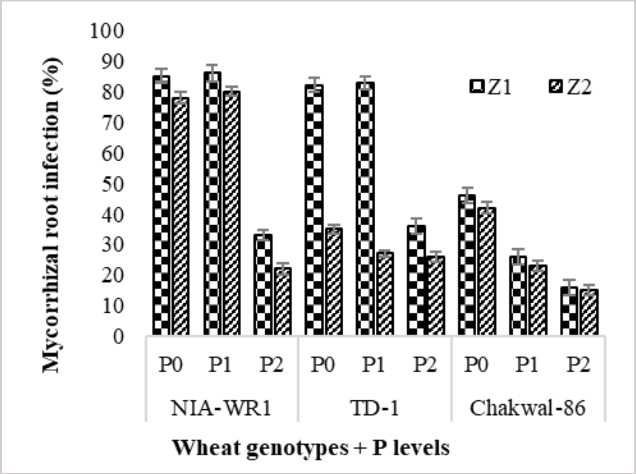

Fig. 5. Arbuscular Mycorrhizal Root Infection (%) of Wheat Genotypes at Different P and Zn Levels

For achieving better AMF colonization and symbiotic benefits, half of the recommended P is suggested to be applied. This is due to the fact that increasing soil-applied P causes a reduction in mycorrhizal colonization, and P doses 50% higher than recommended may diminish the benefits of mycorrhizae [36].

The reduced colonization by AMF and Zn uptake in treatments with excessive phosphorus may also be attributed to the plants shifting to an AMF-independent pathway for nutrients uptake, altered root physiology and microbial dynamics, as well as nutrients imbalances [39, 40].

4. Conclusion

Zinc, as a quality element, improved wheat growth, yield, and grain Zn concentration. The genotype NIA-WR-1 proved to be Zn-efficient, as it extracted more Zn from the treatment where no exogenous Zn was applied. The application of 5 kg Zn ha^{-1} along with 90 kg P ha^{-1} was found to be the best-suited combination to achieve high yields in the Zn-inefficient but responsive genotype Chakwal-86. In contrast, an overdose of P (120 kg ha^{-1}) not only reduced arbuscular mycorrhizal colonization and grain Zn concentration, but also had a negative effect on overall wheat growth and yield.

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