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Characterizing wheat genotypes for zinc efficiency based on high grain yield and zinc uptake

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Abstract

Given the substantial loss of crop productivity in zinc (Zn) deficient soils, the development of cultivars with improved absorption and utilization capabilities is required to sustain the productivity of low input agricultural systems. In this regard, field experiments were conducted over two years to test ten wheat genotypes for Zn efficiency at two Zn levels (0 and 5 kg Zn ha^{-1}). The data analysis revealed that the effects of years and years' interactions with genotypes and Zn levels were non-significant. However, genotypes and Zn levels significantly (P < 0.05) affected grain yield, Zn uptake and Zn efficiency indicators. Zn stress factor (ZnSF) varied between 3.80 to 13.39%, signifying the differential sensitivity of wheat genotypes to Zn deficiency. For low, medium, and high-performance rankings at each Zn level, each parameter was given an index score of 1, 2, and 3, respectively. Additionally, the genotypes were divided into five groups according to their total zinc uptake and grain yield at low zinc. With a total index score of 26, SDT-V11 was classified as HGY-HZn (High grain yield-high Zn uptake) genotype. With corresponding total index scores of 25, 23, and 22, the three genotypes—SDT-V8, AST-V2, and NIA-AS-14-1—were assigned to the HGY-MZn (High grain yield-medium Zn uptake) group. Zincol-2016, with the least cumulative index score of 18, was categorized as medium grain yield-low Zn uptake (MGY-LZn) cultivar. This kind of classification will help future breeding efforts to increase the efficiency of nutrient utilization.

Keywords: Classification, Indexing, Wheat genotypes, Zn acquisition, Zn deficiency

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Introduction

Wheat, the staple grain of Pakistan, was grown on 9.6 million hectares, with production of about 31.4 million tons in year 2023-24 (Iqbal et al., 2018; Abbas & Shafique, 2019; Government of Pakistan, 2024). It contributes to 72% of Pakistanis' daily caloric intake (Mehmood et al., 2020; Alamgeer et al., 2022). With the start of green revolution, high-yielding crop cultivars were introduced, which were more than doubled agricultural yields and decreased starvation while also accelerating the depletion of soil macro- and micronutrient reserves. This led to widespread nutrient deficiencies in crop plants across the globe (Shewry et al., 2016). Zinc (Zn) insufficiency has become one of the most significant micronutrient deficiencies impacting filed crops on alkaline soils, which make up at least 30% of all arable land worldwide (Cakmak and Kutman, 2018). Over 50% of the world's soil used to cultivate cereals now have persistent zinc deficiency problems. Because Zn absorption and accumulation are severely restricted in such conditions, cereal crops are unable to realize their full output potential (Cakmak, 2008).

Zinc is a crucial micronutrient involved in numerous plant biochemical activities including enzyme activation, chlorophyll formation, auxin synthesis, and pollen development (Begum et al., 2016; Liaquat et al., 2023). According to Cakmak (2008), zinc deficiency is a serious problem for almost all key crops, including sugarcane, oilseeds, cereals, fodders, and fiber crops. Additionally, zinc is an essential element for humans, and the World Health Organization considers a zinc deficiency to be a key contributing factor to disease (Narváez-Caicedo et al., 2018). Zinc deficiency in crop plants can be alleviated with the application of Zn containing fertilizers to the soil. However, fertilization is not always effective in correcting a zinc shortage in the alkaline-calcareous soils of Pakistan due to soil Zn fixation (Zhao et al., 2018). Chelated Zn fertilizers can avert the soil fixation of Zn, but relatively higher cost hinders their adoption by the resource-poor farmers. On the other hand, foliar fertilization with 0.2-0.5% zinc sulphate heptahydrate can effectively be applied to combat Zn deficiency (Cakmak & Kutman, 2018). Nevertheless, this approach is time-consuming, and it might not work as well in cases of severe zinc shortage or if it is not used promptly (Pandey et al., 2013). The expense of applying foliar zinc fertilizer accounts for 90% or more of the whole cost (Ram et al., 2016).

Soil Zn deficit problem can be sustainably addressed by developing Zn-efficient crop cultivars that thrive in low-Zn soils with minimal fertilizer use, while simultaneously safeguarding the environment. For this purpose, categorization of crop genotypes according to their performance in terms of growth and production in nutrient-deficient environments is fundamental element of every breeding endeavor (Raza et al., 2023). Gerloff (1977); Jhanji et al. (2013) established a classification scheme that divided genotypes into four groups according to responsiveness and efficiency. Since this grouping was done solely on the basis of population means, a little departure from population mean may categorize the genotype as efficient or inefficient. Therefore, this classification might not be more helpful for large-scale germplasm screening (Aziz et al., 2011). Using a metroglyph technique, Bilal et al. (2018) divided cultivars into nine groups. By dividing efficiency and responsiveness into three categories i.e. low, medium, and high. This classification approach places a sizable percentage of cultivars in the medium group. The identification of Zn efficient wheat genotypes is of utmost importance for maximizing yield and tackling Zn malnutrition. Rasheed et al. (2020) asserted that increased zinc concentration in grain is not guaranteed by zinc efficiency. Therefore, in wheat genotypes grown on Zn-deficient soils, Zn application must be ensured to optimize grain yield and improve grain Zn contents. To assist breeders in incorporating zinc efficiency traits into high-yield wheat varieties and to address malnutrition, this study aimed to identify zinc-efficient wheat genotypes that exhibit higher yields and increased zinc uptake in zinc-deficient conditions.

Materials and Methods

Experimental site, design and treatment plan

Field experiments were conducted over two consecutive Rabi seasons (2020-21 and 2021-22) at the Nuclear Institute of Agriculture in Tandojam. The experimental soil had a texture of silty clay loam, an alkaline pH_e of 7.96, and was non-saline (EC_e = 2.20 dS m^{-1}). The soil had 0.73% organic matter, 0.035% total nitrogen, and 5.51, 178 and 0.36 mg kg^{-1} AB-DTPA extractable phosphorus, potassium and zinc, respectively. Ten wheat genotypes viz., NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, SDT-V11, NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-4, NIA-AS-14-5 and Zincol-2016 were grown under standard management practices. The study employed a split-plot design with zinc treatments as main plots and genotypes in sub-plots, replicated three times. Each 5 m² experimental unit, planted with a single genotype, consisted of ten 2.5 m rows spaced 0.2 m apart. Zinc treatments included no added zinc and 5 kg Zn ha^{-1}, applied by broadcasting during the first irrigation (25–30 days after sowing). One-third of the recommended nitrogen dosage (120 kg ha^{-1}) was administered at the time of sowing, while the remaining two-thirds was evenly distributed and applied during the tillering and booting phases of crop. The entire amount of P (90 kg P_2O_5 ha^{-1}) and K (60 kg K_2O ha^{-1}) were applied at sowing time.

Plant sampling and analysis

After the crops were harvested at maturity, the grain and straw from each sub-plot were manually threshed and weighed to calculate their yields (kg ha^{-1}). Random samples of both grain and straw were taken from each replication, then dried in an oven at 70 °C until they reached a stable weight, followed by fine grinding using a Wiley mill. The finely ground samples of grain and straw underwent digestion with a 5:1 mixture of nitric acid and perchloric acid, following the method outlined by Jones and Case (1990). The digested grain and straw samples were then analyzed for zinc concentration using an atomic absorption spectrophotometer.

Zn efficiency related parameters

Various Zn efficiency related parameters viz., zinc stress factor (ZnSF), Zn harvest index (ZnHI), Zn physiological efficiency index (ZnPEI), and biological efficiency index (ZnBEI) were calculated using the following formulae of Irfan et al. (2017):

$$\text{Grain (or straw) Zn uptake (g ha}^{-1}) = \frac{\text{Grain (or straw) yield (kg ha}^{-1}) \times \text{Grain (or straw) Zn concentartion (mg kg}^{-1})}{1000}$$

$$\text{Total Zn uptake (g ha}^{-1}) = \text{Grain Zn uptake (g ha}^{-1}) + \text{straw Zn uptake (g ha}^{-1})$$

$$\text{ZnSF (\%)} = \frac{\text{Grain yield at high Zn (kg ha}^{-1}) - \text{Grain yield at low Zn (kg ha}^{-1})}{\text{Grain yield at high Zn (kg ha}^{-1})}$$

$$\text{ZnHI (\%)} = \frac{\text{Zn uptake in grain (g ha}^{-1})}{\text{Total Zn uptake (g ha}^{-1})} \times 100$$

$$\text{ZnPEI (kg g}^{-1}) = \frac{\text{Grain yield (kg ha}^{-1})}{\text{Total Zn uptake (g ha}^{-1})}$$

$$\text{ZnBEI (kg g}^{-1}) = \frac{\text{Grain + straw yield (kg ha}^{-1})}{\text{Total Zn uptake (g ha}^{-1})}$$

Indexing and classification of wheat genotypes

Wheat genotypes were categorized into three groups: low, medium, and high scoring, based on the index score assigned for each parameter at varying zinc levels. Using the approach described by Bilal et al. (2018), the indexing process incorporated absolute values along with the population mean (μ) and standard deviation (SD) for each genotype and parameter. Each genotype received a score of 1, 2, or 3, with 1 representing low scoring (when the mean was below μ - SD), 2 indicating medium scoring (when the mean was between μ - SD and μ + SD), and 3 denoting high scoring (when the mean was above μ + SD). The total index score for each genotype was calculated by adding individual scores for all parameters. For the ZnSF measure, a reversed scoring system was applied, as genotypes with higher ZnSF values were more susceptible to low zinc stress. By plotting grain yield (kg ha^{-1}) on the x-axis and total zinc uptake (g ha^{-1}) on the y-axis, all genotypes were categorized into nine distinct groups. In this classification, low, medium, and high grain yields are indicated as LGY, MGY, and HGY, while the zinc uptake levels of the tested genotypes are denoted as LZn, MZn, and HZn.

Statistical analysis

The yield data and other zinc-related parameters were analyzed using analysis of variance (ANOVA) with the aid of STATISTIX 10, following the methods outlined by Steel et al. (1997). To assess differences between treatment means, Tukey's Honestly Significant Difference (HSD) test was employed, applying a significance level of $P \leq 0.05$.

Results

The data pertaining to yield and various Zn-related parameters were subjected to a three-way ANOVA, including year, Zn treatment, wheat genotypes and their interactions in the model (Table 1). An additional 2-way ANOVA was performed on the mean data of two years as the effect of the year was non-significant.

Grain yield, straw yield and Zn stress factor

The statistical analysis found that both zinc levels and genotypic variability had a significant impact on grain yield ($P < 0.01$). However, there was no significant interaction between zinc levels and genotypes. When zinc levels were low, the grain yield varied considerably among different genotypes, ranging from 4275 kg ha^{-1} (NIA-AS-14-4) to 5755 kg ha^{-1} (SDT-V8), with an average yield of 5117 kg ha^{-1}. The grain yield of genotypes with 5 kg Zn ha^{-1} varied from 4483 (NIA-AS-14-4) to 6417 kg ha^{-1} (AST-V2) with a mean value of 5576 kg ha^{-1}. The study found that zinc deficiency hindered plant growth in the soil, as demonstrated by the lower grain yields in plots that did not receive zinc supplementation. Averaged across Zn levels, the genotypes SDT-V8, AST-V2, and NIA-AS-14-1 produced the highest yields and NIA-AS-14-4 the lowest. On average, the grain yield increased by 9% with Zn application but the genotypes responded differently to the applied Zn viz., the increase was only 4.2% in SDT-V11 but 15.6% in NIA-AS-14-3. Higher grain yield of genotype AST-V2 and SDT-V8, regardless of zinc levels, is a notable finding and it can be attributed to higher grain and/or total Zn absorption and other Zn efficiency indices.

The straw yield of test genotypes varied from 5575 to 6692 kg ha^{-1} with a mean value of 6057 kg ha^{-1} at low Zn level (Table 2). With the application of 5 kg Zn ha^{-1}, it ranged from 5733 to 6967 with an average value of 6435 kg ha^{-1}. The genotypes AST-V2 and NIA-AS-14-1 produced the highest and NIA-AS-14-5 produced the lowest straw yield across both Zn level. Averaged across the genotypes, application of 5 kg Zn ha^{-1} resulted in 6.2% increase in straw yield.

The relative reduction in grain yield caused by Zn deficiency stress varied significantly across wheat genotypes, as revealed by ZnSF (Table 1). The tested genotypes' ZnSF ranged from 3.80 to 13.39% (Table 2). The genotypes SDT-V11 and NIA-AS-14-4 recorded the lowest reduction in grain yield due to Zn deficiency (ZnSF = 3.80 and 4.58%), while NIA-MB-2, AST-V2 and NIA-AS-14-3 observed more than 10% reduction in grain yield. Zinc stress factor further aids in identifying Zn responsive and non-responsive genotypes. It also shows the genotype's relative ability to yield grain when Zn is added. The genotypes SDT-V11 and NIA-AS-14-4 were the low responders, while NIA-MB-2, AST-V2 and NIA-AS-14-3 were the high responders to Zn applications.

Grain and straw Zn concentration

Grain Zn concentration was significantly affected by Zn levels, genotypes and their interaction (Table 1). Zn concentration in grains of various genotypes ranged from 20.84 to 37.18 mg kg^{-1} at low Zn and from 34.91 to 55.30 mg kg^{-1} at high Zn level (Table 3). Averaged across both Zn levels, the genotypes Zincol-2016 and NIA-AS-14-3 depicted the lowest and the highest grain Zn concentration, respectively. On an average, Zn concentration in grain increased from 30.85 mg kg^{-1} at low Zn to 39.83 mg kg^{-1} at high Zn level, depicting 29% increase in Zn concentration with the application of Zn fertilizer @ 5 kg ha^{-1}. However, the response of the genotypes varied significantly to the applied Zn as the grain Zn concentration increased by 78.3% for Zincol-2016 and only 5.3% for NIA-MN-1. Straw Zn concentration increased from 27.15 mg kg^{-1} in low Zn treatment to 36.14 mg kg^{-1} in high Zn, amounting to 33.1% increase over control (Table 3). Among various genotypes, SDT-V11, NIA-AS-14-1 and NIA-AS-14-3 recorded the highest Zn concentration in straw across both Zn levels. Generally, it ranged between 18.32 (Zincol-2016) to 33.33 (SDT-V11) in low Zn treatments and from 29.87 (SDT-V8) to 46.63 (NIA-AS-14-3) at high Zn level.

Grain and straw Zn uptake

On an average, grain Zn uptake increased by 41.1% (from 158 to 223 g ha^{-1}) with Zn application (Table 4). The Zn uptake differed significantly among the genotypes. NIA-AS-14-1 and NIA-AS-14-3 accumulated the highest, while Zincol-2016, NIA-AS-14-4 and NIA-MB-2 accumulated the lowest amount of Zn in grains across both Zn levels. At low Zn level, Zincol-2016 and SDT-V8 recorded the lowest (94 g ha^{-1}) and the highest grain Zn uptake (191 g ha^{-1}), respectively. At high Zn level, NIA-AS-14-4 recorded the lowest (159 g ha^{-1}) while NIA-AS-14-3 the highest grain Zn uptake (316 g ha^{-1}). Likewise, the straw Zn uptake was significantly affected by Zn levels, genotypes and their interaction (Table 1). Application of 5 kg Zn ha^{-1} resulted in 41.1% increase in straw Zn uptake over control/low Zn level. Genotypes SDT-V11, NIA-AS-14-1 and NIA-AS-14-3 showed the highest average straw Zn uptake across both Zn levels, while SDT-V8 and Zincol-2016 recorded the lowest values (Table 4). Straw Zn uptake varied between 116 to 186 g ha^{-1} at low Zn level, while it ranged between 176 to 291 g ha^{-1} at high Zn level. Significant (P < 0.05) interaction between Zn × G showed that genotypes behaved differently for total Zn uptake at both Zn levels. On average for all genotypes, total Zn uptake significantly increased from 321 g ha^{-1} under Zn deficiency to 453 g ha^{-1} in Zn-treated plots. When Zn was not applied, the total Zn uptake varied substantially from 210 to 368 g ha^{-1} and it varied between 373 to 607 g ha^{-1} in case Zn application (Table 4).

Zinc efficiency relations

The ZnHI shows the ability of a genotype to translocate fraction of total Zn uptake (grain + straw) into grain. Zinc application @ 5 kg ha^{-1} could not significantly affect ZnHI, indicating that the tested genotypes behaved similarly in terms of ZnHI at both Zn levels (ZnHI < 50%) (Table 5). However, genotypes differed considerably for ZnHI at both Zn levels. It varied from 42.39% for NIA-AS-14-4 to 55.63% for SDT-V8 at low Zn level. At high Zn level, ZnHI ranged from 42.92 to 56.65%. On average for both Zn levels, genotypes NIA-AS-14-4 and SDT-V8 exhibited the lowest and the highest ZnHI, respectively. The ZnPEI and ZnBEI illustrate how effectively a crop can utilize Zn to produce grain or above ground biomass for every unit of Zn deposited in the above ground portions. The Zn level, genotype, and genotype × Zn level interaction all had a significant impact on the Zn physiological efficiency index (ZnPEI). Significant differences in ZnPEI between wheat genotypes at each Zn level were found by analysis of variance (Table 1). Zincol-2016 was the most efficient in converting the accumulated Zn to grain production at control, while NIA-AS-14-3 was the least efficient among all genotypes (Table 6). In Zn fertilized plots, genotypes SDT-V8 and NIA-AS-14-3 attained the highest and the lowest ZnPEI values, respectively. A perusal of data revealed that ZnPEI was significantly higher (16.25 kg g^{-1}) at low Zn level where total Zn uptake was lower than at adequate Zn supplies (12.52 kg g^{-1}). This indicates that an inverse effect of Zn application on ZnPEI. Like ZnPEI, ZnBEI was influenced by zinc level, genotype and genotype × Zn level interaction (Table 2). ZnBEI was almost two-fold higher than ZnPEI at both Zn levels. The ZnBEI dropped by 24.1% upon the application of 5 kg Zn ha^{-1}. When Zn (5 kg ha^{-1}) was supplied, the extent of variability between wheat genotypes for ZnBEI was lower (19.77 - 30.68 kg g^{-1}) than when Zn was not added (30 - 52.43 kg g^{-1}). Averaged across both Zn levels, the highest and the lowest value of ZnBEI was observed by Zincol-2016 and NIA-AS-14-3, respectively (Table 5).

Table 1 Summary of three-way and two-way analysis of variance (ANOVA) for various parameters

Parameters	Calculated F – values for various sources of variations						
	Year (Y) df = 1	Zn Level (Z) df = 1	Genotype (G) df = 9	Y×Z df = 1	Y×G df = 9	Z×G df = 9	Y×Z×G df = 9
Grain yield	1.1	40	26.73	0.33	0.47	0.74	0.22
Straw yield	2.03	8.45	3.28	1.97	1.13	0.59	0.35
Grain Zn concentration	0.1	164.13	17.38	0.02	0.78	7.41	0.18
Straw Zn concentration	0.06	205.12	18.94	0.04	1.31	5.73	0.39
Grain Zn uptake	1.27	323.27	43.4	0.05	1.1	11.48	0.33
Straw Zn uptake	2.03	284.31	12.52	1.36	1.37	5.74	0.97
Total Zn uptake	2.87	527.18	36.47	0.45	0.92	13.21	0.45
Zn stress factor	2.59	-	7.14	-	2.08	-	-
Zn harvest index	0.08	0.06	9.73	1.27	0.99	0.81	0.46
ZnPEI	0.65	189.45	12.23	2.09	0.66	8.25	0.85
ZnBEI	0.49	338.18	32.21	0.2	1.84	15.62	0.75

Tabulated F: F(1,78) = 3.96; F(9,78) = 2.00; F(1,38) = 4.10; F(9,38) = 2.14; ZnPER and ZnBER represent zinc physiological and biological efficiency indexes, respectively.

Table 2 Grain and straw yield, and zinc stress factors of wheat genotypes as affected by zinc application (data are average of two years)

Genotypes	Grain yield (kg ha^{-1})		Straw yield (kg ha^{-1})		Zinc stress factor (%)
	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}	
Zincol-2016	4550 ± 95 (2)	5000 ± 173 (2)	6350 ± 38 (2)	6633 ± 60 (2)	8.22 ± 0.76 (2)
NIA-MN-1	4817 ± 233 (2)	5133 ± 246 (2)	6267 ± 377 (2)	6867 ± 309 (3)	5.57 ± 1.34 (2)
NIA-MB-2	4500 ± 132 (1)	5167 ± 183 (2)	5850 ± 260 (2)	6600 ± 328 (2)	12.74 ± 1.57 (1)
AST-V2	5698 ± 184 (2)	6417 ± 292 (3)	6683 ± 66 (3)	6967 ± 300 (3)	11.10 ± 1.13 (2)
SDT-V8	5755 ± 73 (3)	6200 ± 202 (3)	5928 ± 374 (2)	5950 ± 257 (1)	7.05 ± 1.22 (2)
SDT-V11	5750 ± 104 (3)	5992 ± 33 (2)	5583 ± 164 (1)	6342 ± 220 (2)	3.80 ± 0.50 (3)
NIA-AS-14-1	5742 ± 58 (3)	6100 ± 236 (2)	5575 ± 218 (1)	6317 ± 373 (2)	5.64 ± 1.04 (2)
NIA-AS-14-3	4945 ± 185 (2)	5717 ± 242 (2)	5913 ± 266 (2)	6262 ± 224 (2)	13.39 ± 1.21 (1)
NIA-AS-14-4	4275 ± 14 (1)	4483 ± 44 (1)	6692 ± 166 (3)	6683 ± 220 (2)	4.58 ± 0.92 (2)
NIA-AS-14-5	5133 ± 164 (2)	5550 ± 180 (2)	5727 ± 312 (2)	5733 ± 497 (1)	7.18 ± 1.51 (2)
Mean	**5117 B**	**5576 A**	**6057 B**	**6435 A**	**7.93**
$HSD_{0.05}$					
Zn Level (Z)	**147.17**		**250.32**		**-**
Genotype (G)	**546.31**		**929.18**		**6.03**
Z×G	**NS**		**NS**		**-**

Data showed in the columns represent mean values ± standard error of mean. Index scores are presented in parenthesis. $HSD_{0.05}$ stands for honestly significant difference at 5% probability level

Table 3 Grain and straw zinc concentration of wheat genotypes as affected by zinc application (data are average of two years)

Genotypes	Grain Zn concentration (mg kg^{-1})		Straw Zn concentration (mg kg^{-1})	
	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}
Zincol-2016	20.84 ± 0.63 (1)	37.16 ± 1.55 (2)	18.32 ± 0.86 (1)	33.11 ± 0.53 (2)
NIA-MN-1	33.14 ± 1.52 (2)	34.91 ± 2.68 (2)	26.75 ± 0.67 (2)	31.41 ± 1.78 (2)
NIA-MB-2	28.35 ± 0.98 (2)	35.14 ± 0.48 (2)	26.74 ± 1.63 (2)	31.96 ± 1.54 (2)
AST-V2	30.93 ± 1.60 (2)	40.80 ± 2.57 (2)	26.57 ± 0.75 (2)	33.50 ± 2.15 (2)
SDT-V8	33.26 ± 1.61 (2)	37.24 ± 1.77 (2)	26.08 ± 1.51 (2)	29.87 ± 0.90 (1)
SDT-V11	31.78 ± 0.52 (2)	35.91 ± 0.23 (2)	33.33 ± 2.60 (3)	38.87 ± 1.22 (2)
NIA-AS-14-1	29.22 ± 0.66 (2)	46.27 ± 2.43 (3)	28.95 ± 0.54 (2)	43.07 ± 1.49 (3)
NIA-AS-14-3	37.18 ± 1.72 (3)	55.30 ± 2.68 (3)	30.79 ± 1.54 (2)	46.63 ± 1.17 (3)
NIA-AS-14-4	29.52 ± 1.53 (2)	35.70 ± 0.47 (2)	25.76 ± 1.22 (2)	32.12 ± 0.21 (2)
NIA-AS-14-5	34.26 ± 1.64 (2)	39.92 ± 1.95 (2)	28.26 ± 1.15 (2)	40.90 ± 1.76 (2)
Mean	**30.85 B**	**39.83 A**	**27.15 B**	**36.14 A**
$HSD_{0.05}$				
Zn Level (Z)	**1.56**		**1.23**	
Genotype (G)	**5.59**		**4.58**	
Z×G	**8.95**		**7.33**	

Data showed in the columns represent mean values ± standard error of mean. Index scores are presented in parenthesis. $HSD_{0.05}$ stands for honestly significant difference at 5% probability level.

Classification and indexing of wheat genotypes

For each parameter, wheat genotypes were classified into categories of low, medium, and high efficiency based on the criteria of Bilal et al. (2018). Categorization based on index scores was applied to several key parameters, i.e. yield, Zn concentration, Zn uptake, ZnSF, ZnPEI, and ZnBER at both zinc levels (Tables 6 and 7). Most of the genotypes were classified as medium in terms of their characteristics. Based on grain yield, two genotype were categorized as low (< 4533 kg ha^{-1}), five as medium (4533 – 5700 kg ha^{-1}), and three as high (> 5700 kg ha^{-1}) at low Zn level (Table 6). At high Zn, one genotype was categorized as low (< 4956 kg ha^{-1}), seven as medium (4956-6195 kg ha^{-1}), and two as high (> 6195 kg ha^{-1}) (Table 7). The results of our study indicated that, at lower zinc levels, the genotypes were categorized into five distinct groups according to their grain yield and overall zinc absorption (see Fig. 1). This type of classification would facilitate the identification of wheat genotypes capable of thriving in zinc-deficient soils and assist in selecting parent plants for hybridization aimed at developing zinc-efficient varieties. With corresponding total index scores of 25, 23, and 22, the three genotypes—SDT-V8, AST-V2, and NIA-AS-14-1—were assigned to the HGY-MZn (High grain yield-medium Zn uptake) group. With a total index score of 26, genotype SDT-V11 was assigned to the HGY-HZn group. This genotype can be used for soils with a variety of Zn concentrations and was effective in both acquiring and using zinc for grain yield in low Zn availability conditions. The LGY-MZn group comprised NIA-MB-2 and NIA-AS-14-4. Genotypes. NIA-AS-14-3, NIA-AS-14-5 and NIA-MN-1 were the members of MGY-MZn group. Zincol-2016 fell into the medium grain yield-low zinc group (MGY-LZn). Zincol-2016 also attained the lowest index score of 18.

Table 4 Grain, straw and total zinc uptake of wheat genotypes as affected by zinc application (data are average of two years)

	Grain Zn uptake (g ha^{-1})		Straw Zn uptake (g ha^{-1})		Total Zn uptake (g ha^{-1})	
Genotypes	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}
Zincol-2016	94 ± 3.39 (1)	185 ± 5.42 (2)	116 ± 7.64 (1)	218 ± 5.48 (2)	210 ± 13.21 (1)	403 ± 10.97 (2)
NIA-MN-1	159 ± 5.55 (2)	178 ± 7.86 (2)	167 ± 13.59 (2)	215 ± 11.50 (2)	326 ± 13.79 (2)	393 ± 20.87 (2)
NIA-MB-2	127 ± 1.08 (2)	181 ± 1.97 (2)	156 ± 2.27f (2)	210 ± 1.71 (2)	283 ± 6.62 (2)	391 ± 2.85 (2)
AST-V2	176 ± 6.64 (2)	261 ± 10.41 (2)	178 ± 4.32 (2)	232 ± 9.06 (2)	353 ± 11.06 (2)	493 ± 13.28 (2)
SDT-V8	191 ± 4.72 (3)	231 ± 17.08 (2)	153 ± 1.42f (2)	176 ± 5.65 (1)	344 ± 5.85 (2)	408 ± 22.61 (2)
SDT-V11	182 ± 6.15 (2)	214 ± 2.10 (2)	186 ± 14.53 (3)	245 ± 3.79 (2)	368 ± 22.09 (3)	460 ± 5.05 (2)
NIA-AS-14-1	167 ± 4.58 (2)	282 ± 12.66 (3)	161 ± 3.68 (2)	271 ± 11.94 (3)	328 ± 5.33 (2)	552 ± 6.00 (3)
NIA-AS-14-3	183 ± 11.94 (2)	316 ± 16.04 (3)	181 ± 11.86 (2)	291 ± 10.68 (3)	364 ± 22.35 (2)	607 ± 9.97 (3)
NIA-AS-14-4	126 ± 8.59 (2)	159 ± 1.62 (1)	173 ± 5.73 (2)	214 ± 11.14 (2)	299 ± 2.63 (2)	373 ± 10.93 (1)
NIA-AS-14-5	175 ± 4.27 (2)	221 ± 8.40 (2)	161 ± 7.53 (2)	230 ± 12.51 (2)	337 ± 10.12 (2)	451 ± 20.72 (2)
Mean	**158 B**	**223 A**	**163 B**	**230 A**	**321 B**	**453 A**
$HSD_{0.05}$						
Zn Level (Z)	**7.56**		**7.24**		**11.43**	
Genotype (G)	**28.07**		**26.88**		**41.82**	
Z×G	**44.91**		**43.02**		**66.55**	

Data showed in the columns represent mean values ± standard error of mean. Index scores are presented in parenthesis. $HSD_{0.05}$ stands for honestly significant difference at 5% probability level.

Table 5 Zinc harvest index (ZnHI), zinc physiological efficiency (ZnPEI) and biological efficiency ratio (ZnBEI) of wheat genotypes as affected by zinc application (data are average of two years)

	ZnHI (%)		ZnPEI (kg g^{-1})		ZnBEI (kg g^{-1})		Total index score	
Genotypes	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}	0 kg Zn ha^{-1}	5 kg Zn ha^{-1}
Zincol-2016	45.12 ± 1.37 (1)	45.95 ± 1.52 (2)	21.86 ± 1.12 (3)	12.41 ± 0.13 (2)	52.43 ± 2.80 (3)	28.98 ± 0.34 (2)	18	20
NIA-MN-1	48.78 ± 1.13 (2)	45.36 ± 0.91 (2)	14.97 ± 0.85 (2)	13.16 ± 1.03 (2)	34.26 ± 0.68 (2)	30.68 ± 1.21 (3)	23	22
NIA-MB-2	45.09 ± 0.77 (1)	46.25 ± 0.27 (2)	15.93 ± 0.43 (2)	13.23 ± 0.38 (2)	36.74 ± 1.70 (2)	30.16 ± 0.64 (2)	19	20
AST-V2	49.89 ± 0.31 (2)	52.89 ± 1.83 (2)	16.21 ± 0.74 (2)	13.03 ± 0.47 (2)	35.20 ± 1.10 (2)	27.21 ± 1.19 (2)	23	22
SDT-V8	55.63 ± 0.88 (3)	56.65 ± 1.75 (3)	16.75 ± 0.50 (2)	15.31 ± 0.46 (3)	33.99 ± 1.31 (2)	29.92 ± 1.01 (2)	25	20
SDT-V11	49.84 ± 1.48 (2)	46.59 ± 0.37 (2)	15.86 ± 0.58 (2)	13.10 ± 0.09 (2)	31.18 ± 1.61 (2)	26.91 ± 0.46 (2)	26	20
NIA-AS-14-1	51.21 ± 2.07 (2)	51.03 ± 2.48 (2)	17.55 ± 0.42 (2)	11.06 ± 0.54 (2)	34.61 ± 0.41 (2)	22.50 ± 0.05 (1)	22	24
NIA-AS-14-3	50.66 ± 4.09 (2)	52.03 ± 2.36 (2)	13.63 ± 0.33 (1)	9.42 ± 0.24 (1)	30.00 ± 1.39 (1)	19.77 ± 0.32 (1)	20	23
NIA-AS-14-4	42.39 ± 2.73 (1)	42.92 ± 1.27 (1)	14.38 ± 0.14 (2)	12.07 ± 0.46 (2)	36.86 ± 0.23 (2)	30.01 ± 0.73 (2)	21	16
NIA-AS-14-5	52.17 ± 0.96 (2)	49.14 ± 1.01 (2)	15.37 ± 0.91 (2)	12.44 ± 0.78 (2)	32.32 ± 0.83 (2)	25.17 ± 0.90 (2)	22	19
Mean	**49.08**	**48.88**	**16.25 A**	**12.52 B**	**35.76 A**	**27.13 B**		
$HSD_{0.05}$								
Zn Level (Z)	**NS**		**0.54**		**0.93**			
Genotype (G)	**5.80**		**1.97**		**3.42**			
Z×G	**NS**		**3.14**		**5.44**			

Data showed in the columns represent mean values ± standard error of mean. Index scores are presented in parenthesis. The overall index score is calculated by adding the values shown in parentheses for each parameter of the respective genotype at each zinc level, as detailed in Tables 3-6. $HSD_{0.05}$ stands for honestly significant difference at 5% probability level.

Table 6 Index scoring of wheat genotypes for various parameters at low Zn (0 kg ha^{-1}) level in soil. The genotypes are classified as low if their mean is < μ - SD, medium if mean is μ - SD to μ + SD, and high if the mean is > μ + SD (Bilal et al., 2018)

Parameters	Low (score 1)	Medium (score 2)	High (score 3)
Grain yield	**< 4533**	**4533-5700**	**> 5700**
(kg ha^{-1})	NIA-MB-2, NIA-AS-14-4	Zincol-2016, NIA-MN-1, AST-V2, NIA-AS-14-3, NIA-AS-14-5	SDT-V8, SDT-V11, NIA-AS-14-1
Straw yield	**< 5639**	**5639-6475**	**> 6475**
(kg ha^{-1})	SDT-V11, NIA-AS-14-1	Zincol-2016, NIA-MN-1, NIA-MB-2, SDT-V8, NIA-AS-14-3, NIA-AS-14-5	AST-V2, NIA-AS-14-4
Grain Zn concentration	**< 26.45**	**26.45-35.25**	**> 35.25**
(mg kg^{-1})	Zincol-2016	NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, SDT-V11, NIA-AS-14-1, NIA-AS-14-4, NIA-AS-14-5	NIA-AS-14-3
Straw Zn concentration	**< 23.24**	**23.24-31.06**	**> 31.06**
(mg kg^{-1})	Zincol-2016	Zincol-2016, NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-4, NIA-AS-14-5	SDT-V11
Grain Zn uptake	**< 126**	**126-190**	**> 190**
(g ha^{-1})	Zincol-2016	NIA-MN-1, NIA-MB-2, AST-V2, SDT-V11, NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-4, NIA-AS-14-5	SDT-V8
Straw Zn uptake	**< 143**	**143-183**	**> 183**
(g ha^{-1})	Zincol-2016	NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-4, NIA-AS-14-5	SDT-V11
Total Zn uptake	**< 274**	**274-368**	**> 368**
(g ha^{-1})	Zincol-2016	NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-4, NIA-AS-14-5	SDT-V11
Zn stress factor (%)[†]	**> 11.32**	**4.54-11.32**	**< 4.54**
	NIA-MB-2, NIA-AS-14-3	Zincol-2016, NIA-MN-1, AST-V2, SDT-V8, NIA-AS-14-1, NIA-AS-14-4, NIA-AS-14-5	SDT-V11
Zn harvest index (%)	**< 45.17**	**45.17-52.98**	**> 52.98**
	Zincol-2016, NIA-MB-2, NIA-AS-14-4,	NIA-MN-1, AST-V2, SDT-V11, NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-5	SDT-V8
Zn physiological efficiency	**< 13.98**	**13.98-18.53**	**> 18.53**
index (kg g^{-1})	NIA-AS-14-3	NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, SDT-V11, NIA-AS-14-1, NIA-AS-14-4, NIA-AS-14-5	Zincol-2016
Zn biological efficiency index	**< 29.50**	**29.50-42.02**	**> 42.02**
(kg g^{-1})	NIA-AS-14-3	NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, SDT-V11, NIA-AS-14-1, NIA-AS-14-4, NIA-AS-14-5	Zincol-2016

[†] For zinc stress factor, the genotypes are scored as low if their mean is > μ+SD, medium if mean is μ-SD to μ+SD, and high if the mean is < μ-SD.

Table 7 Index scoring of wheat genotypes for various parameters at high Zn (5 kg ha^{-1}) level in soil. The genotypes are classified as low if their mean is < μ - SD, medium if mean is μ - SD to μ + SD, and high if the mean is > μ + SD (Bilal et al., 2018)

Parameters	Low (score 1)	Medium (score 2)	High (score 3)
Grain yield (kg ha^{-1})	**< 4956** NIA-AS-14-4	**4956-6195** Zincol-2016, NIA-MN-1, NIA-MB-2, SDT-V11, NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-5	**> 6195** AST-V2, SDT-V8
Straw yield (kg ha^{-1})	**< 6044** SDT-V8, NIA-AS-14-5	**6044-6827** Zincol-2016, NIA-MB-2, SDT-V11, NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-4	**> 6827** NIA-MN-1, AST-V2
Grain Zn concentration (mg kg^{-1})	**< 33.39** -	**33.39-46.28** Zincol-2016, NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, SDT-V11, NIA-AS-14-4, NIA-AS-14-5	**> 46.28** NIA-AS-14-1, NIA-AS-14-3
Straw Zn concentration (mg kg^{-1})	**< 30.37** SDT-V8	**30.37-41.92** Zincol-2016, NIA-MN-1, NIA-MB-2, AST-V2, SDT-V11, NIA-AS-14-4, NIA-AS-14-5	**> 41.92** NIA-AS-14-1, NIA-AS-14-3
Grain Zn uptake (g ha^{-1})	**< 172** NIA-AS-14-4	**172-273** Zincol-2016, NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, SDT-V11, NIA-AS-14-5	**> 273** NIA-AS-14-1, NIA-AS-14-3
Straw Zn uptake (g ha^{-1})	**< 198** SDT-V8	**198-263** Zincol-2016, NIA-MN-1, NIA-MB-2, AST-V2, SDT-V11, NIA-AS-14-4, NIA-AS-14-5	**> 263** NIA-AS-14-1, NIA-AS-14-3
Total Zn uptake (g ha^{-1})	**< 376** NIA-AS-14-4	**376-530** Zincol-2016, NIA-MN-1, NIA-MB-2, AST-V2, SDT-V8, SDT-V11, NIA-AS-14-5	**> 530** NIA-AS-14-1, NIA-AS-14-3
Zn harvest index (%)	**< 44.67** NIA-AS-14-4	**44.67-53.09** Zincol-2016, NIA-MN-1, NIA-MB-2, AST-V2, SDT-V11, NIA-AS-14-1, NIA-AS-14-3, NIA-AS-14-5	**> 53.09** SDT-V8
Zn physiological efficiency index (kg g^{-1})	**< 10.98** NIA-AS-14-3	**10.98-14.06** Zincol-2016, NIA-MN-1, NIA-MB-2, AST-V2, SDT-V11, NIA-AS-14-1, NIA-AS-14-4, NIA-AS-14-5	**> 14.06** SDT-V8
Zn biological efficiency index (kg g^{-1})	**< 23.47** NIA-AS-14-1, NIA-AS-14-3	**23.47-30.79** Zincol-2016, NIA-MN-1, AST-V2, SDT-V8, SDT-V11, NIA-AS-14-4, NIA-AS-14-5	**> 30.79** NIA-MB-2

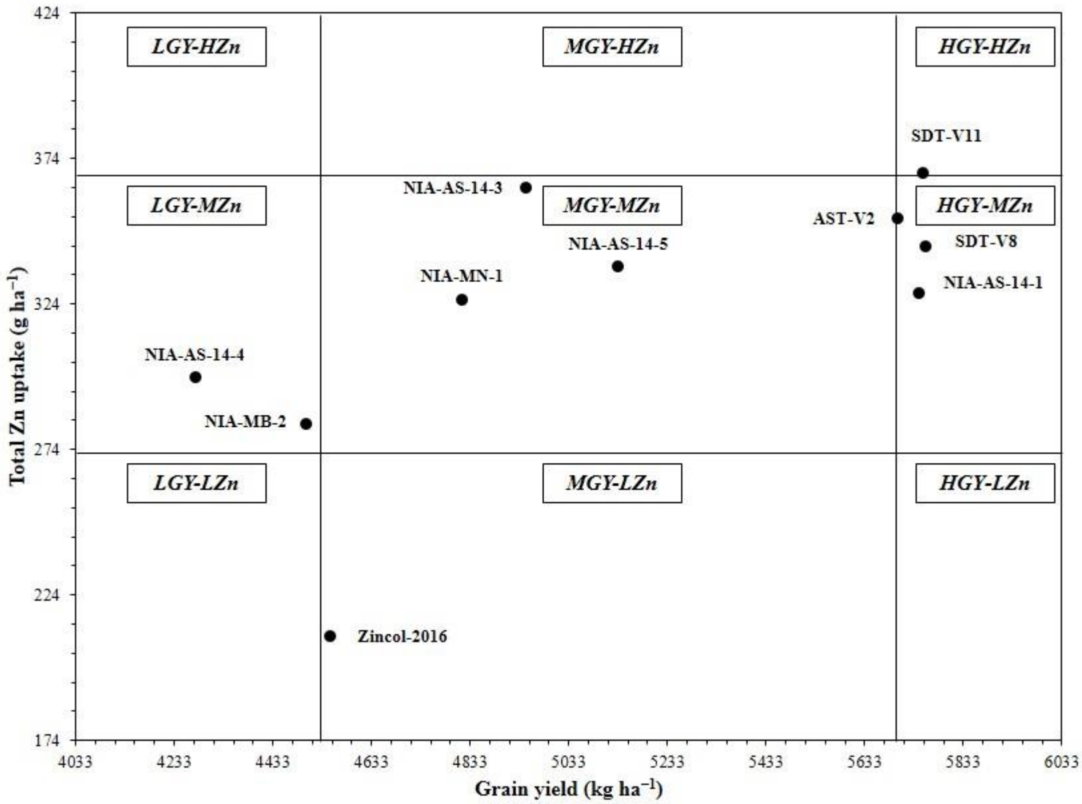

Fig. 1 Classification of ten wheat genotypes based on their grain yield and total Zn uptake at deficient Zn level according to Bilal et al. (2018). In this classification, LGY, MGY, and HGY indicate low, medium, and high grain yields, respectively, while LZn, MZn, and HZn represent low, medium, and high zinc absorption by the genotypes evaluated.

Discussion

The primary goal of our study was to evaluate and pinpoint wheat genotypes that thrive in soils with different zinc (Zn) levels and make efficient use of added Zn. The effectiveness of either native or added Zn is typically measured by the amount of biomass or grain yield generated per unit of Zn fertilizer applied to the crops (Rawal et al., 2022). The wheat genotypes under investigation exhibited marked differences in their yield and Zn-related characteristics. Similarly, for all yield and Zn-related indicators (except Zn harvest index), the effects of Zn treatments were also significant. The interaction effects of genotype × Zn level was significant for the majority of the examined parameters, with the exception of grain yield, straw yield, and ZnHI. It is important to highlight that all the parameters examined showed a significant increase with higher levels of soil zinc, except for ZnHI, ZnPEI, and ZnBEI. This indicates that the experimental soil was low to medium in available zinc, making it appropriate for screening purposes, as the growing medium should be lacking in the nutrient being studied. Soils having < 0.5 ppm of DTPA-extractable zinc are generally regarded as potentially deficient in zinc and may benefit from the application of zinc fertilizers (Chatterjee et al., 2018).

In our investigation, the grain yield of the examined genotypes corresponded with the overall uptake of zinc at both low and high soil zinc levels. The increase in grain yield linked to greater zinc absorption was attributed to the positive impact of zinc on wheat productivity and its function as a catalyst or enhancer in various metabolic and physiological activities (Khan et al., 2023). Additionally, zinc activates the enzymes that assist in the production of proteins and carbohydrates. Moreover, studies have shown that zinc supplementation leads to enhanced growth characteristics in wheat, such as increased chlorophyll content, greater leaf area index, taller plants, and a more robust root system (Kanwal et al., 2020). Kumar et al. (2022); Singh et al. (2019) have reported that the application of zinc leads to enhanced wheat grain yields. They observed a strong and statistically significant positive correlation between grain yield and total zinc uptake ($r > 0.50$, $P < 0.01$) at both zinc treatment levels, suggesting that genotypes that absorb greater amounts of zinc from the soil are likely to achieve higher grain yields.

The current study found no signs of a yield dilution effect on the concentration of zinc in the grain. In contrast, a modest positive correlation ($r = 0.22$; $P = 0.25$) was observed between the zinc concentration in grain and the yield of the grain. These findings are consistent with the research conducted by Khokhar et al. (2018); Velu et al. (2019); Rehman (2019). Contrarily, Gomez-Coronado et al. (2016) observed a significant negative relationship between the gain yield and grain Zn concentration due to yield dilution effect. The other possible reason might be the rain-fed Mediterranean conditions where limited water availability may have hindered Zn uptake and translocation. Similarly, in highly acidic soils, wheat grain yield had a highly significant negative relationship with grain Zn (Pant et al., 2020). Nonetheless, the impact of yield dilution on the concentration of zinc in grains can be counteracted by enhancing the movement of zinc from unproductive plant areas to the grain, as indicated by the zinc harvest index (Wang et al., 2018; Liu et al., 2019). As noted earlier, increasing zinc accumulation in grains and facilitating the transfer of zinc from leaves to grains was accomplished by the overexpression of the ferritin gene (Liu et al., 2016) in genetically engineered wheat varieties, and the nicotianamine synthase 2 gene (Singh et al., 2017). This led to a concurrent rise in both grain weight and zinc content.

The current research demonstrated a substantial and positive relationship between grain yield and the uptake of zinc in grains ($r > 0.65$, $P < 0.01$), supporting this claim. The results of our study showed that < 50% of total Zn was accumulated in grain which suggests that sufficient room is available for the breeders to increase the ZnHI. Hence, selection strategies to increase wheat Zn utilization efficiency in alkaline calcareous soils should focus on enhancing Zn harvest index which will help in increasing grain yield without affecting the grain Zn concentration. Additionally, a significant correlation was found between grain yield and ZnHI ($r > 0.60$, $P < 0.001$), indicating that the genotypes AST-V2 and SDT-V8, which have a greater capacity to move accumulated zinc to the grains, achieved higher grain yields at both zinc levels. Furthermore, it is recommended to choose genotypes with elevated ZnHI for further studies on the root and shoot characteristics that facilitate the translocation of zinc from source to sink (grains) (Wissuwa et al., 2008).

The ZnPEI and ZnBEI metrics illustrate how well a crop can utilize zinc (Zn) and assess its capability to generate grain yield or biomass based on the amount of Zn absorbed from the soil. Our analysis of these two-efficiency metrics revealed that there were no consistent relationships among grain yield, ZnPEI, and ZnBEI. This suggests that most wheat genotypes exhibit similar internal Zn utilization efficiency, which restricts the potential for using these parameters to select an ideal Zn-efficient genotype (Irfan et al., 2017). Both ZnPEI and ZnBEI showed significant variability across different genotypes, both under control conditions and with sufficient zinc treatments. These indices tended to decline as zinc supply increased (Table 5). Because ZnPEI and ZnBEI are influenced by the amount of zinc absorbed or the zinc available in the soil, genotypes that exhibited high ZnPEI and ZnBEI under control conditions demonstrated lower values at adequate zinc levels, leading to higher zinc concentrations in these genotypes. Our findings also indicate that ZnHI could be used as a partial criterion for evaluating genotypes. Furthermore, these results suggest that the wheat genotype classified under HGY-HZn (SDT-V11) was particularly effective in acquiring and utilizing zinc from the soil to enhance grain yield, even in conditions of zinc deficiency (Fig. 1). Genotypes that demonstrate enhanced abilities to acquire or utilize zinc should be considered for cultivation in soils with varying zinc levels (Singh et al., 2020). The exceptional performance of SDT-V11 was also validated by its highest index score of 26 (see Table 6). Meanwhile, the genotypes NIA-AS-14-1, SDT-V8, and AST-V2 showed moderate zinc acquisition but were effective in producing grain yield, suggesting their superior utilization efficiency.

Conclusion

The wheat genotypes analyzed in this research exhibited notable differences in grain yield, zinc uptake, and zinc efficiency metrics. This genetic variation could be advantageous for developing improved genotypes with enhanced zinc efficiency. All things considered, our research points to the fact that genotypes viz., SDT-V11, NIA-AS-14-1, SDT-V8 and AST-V2 with great potential for grain production and/or strong ability to collect zinc from soil, and high zinc translocation towards the grain can thrive well on Zn deficient soils. In addition, classifying and indexing wheat genotypes is an effective method for grouping the available germplasm based on zinc efficiency.

Conflict of interest: The authors declare that they have no conflict of interest.

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