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GENETIC DIVERGENCE AND EXTENT OF ASSOCIATION AMONG INDICATORS LINKED TO ZINC CONTENTS AND YIELD IN TROPICAL MAIZE

M. ASLAM^{1*}, S.H. KHAN¹, A. BESHIR³, M. HUSSAIN², and M.A. ASLAM¹

¹Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

²Pakistan Agricultural Research Council, Islamabad, Pakistan

³South Asia Regional Office, CIMMYT, Nepal

*Corresponding author's email: aslampbg@gmail.com

Email addresses of co-authors: shahamgopang@gmail.com, A.ISSA@cgiar.org, sehmimh@yahoo.com, ahmadmalik1152@gmail.com

SUMMARY

Zinc deficiency prevails in one-third of the world's population, which is mainly due to the intake of cereals grown on zinc-deficient soils. Biofortification is a top priority among different solutions to this condition and is an agricultural intervention suitable for farmers and consumers due to accessibility and cost-effectiveness. Maize (*Zea mays* L.) is a short-duration and high-yielding crop that can grow in two seasons per annum and is the most suitable cereal for biofortification. The present study investigated 150 genetically diverse maize inbred lines for grain-zinc contents, yield, and yield-contributing traits, employing an augmented field design. Significant genotypic diversity was prominent for the studied traits. The first four components of the principal component analysis (PCA) depicted an Eigen value of more than one, which explained 73.5% of the total variability. Among inbred lines, E102, E38, E92, E52, E91, E1, E93, E54, E28, E25, E72, E30, and E90 showed higher grain-zinc contents and average grain yields per plant in the PCA-based study. The grain-zinc contents were less than the desired level (33 mg kg⁻¹) notified by HarvestPlus. Creating significant genetic diversity and exploiting its existing level for heterotic performance is a prerequisite to achieve the desired level of grain-zinc contents and yield. Correlation studies revealed a significant positive association of average grain yield per plant with the ear length, the number of grains per row, the number of rows per cob, and 1000-grain weight. Selection of inbred lines based on the standards of positive association with grain yield per plant could improve total yield.

Keywords: Maize, augmented design, biofortification, correlation coefficients, genetic variability, PCA

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Key findings: Improving grain-zinc contents can succeed by selecting early maturing genotypes with less anthesis-silking interval (ASI). Lesser days to silking and days to tasseling cause early mobilization of available zinc from tassel to cob; hence, more zinc is available for grain under long grain-filling periods. High-kernel zinc contents improve grain weight due to enhanced carbohydrate supply for grain filling. Indirect selection to develop zinc biofortified maize genotypes should require high mean values for plant height, total grain weight, ear length, and grain yield and lower mean values for days to silking and days to tasseling.

INTRODUCTION

Zinc is part of many biochemical processes in plants, such as photosynthesis, respiration, and nitrogen metabolism; therefore, it is essential for the growth and development of healthy plants. Additionally, zinc is necessary for cell division and the regulation of gene expression; it is vital in synthesizing proteins, hormones, and enzymes (Hara *et al.*, 2017). Zinc is essential in human health and well-being, including nutrient metabolism. In the body, it is crucial for cell growth and development, immune system functioning, wound healing, breakdown of carbohydrates and proteins, and many more. Zinc contributes to healthy skin, hair, nails, vision, taste, smell, fertility, and reproduction. It is critical for favorable progress and development during pregnancy, childhood, and adolescence (Roohani *et al.*, 2013). Zinc deficiency can lead to many health problems, including poor immunity, stunted growth, impaired wound healing, and skin-related problems (Prasad, 2013).

The 'Green Revolution' ensured food security for an ever-increasing world population. In this achievement, yield, biotic, and abiotic stresses were the prime focus, but compromised nutrition security. Such compromise brought malnutrition to a significant portion of the world's population, especially in developing and low-income countries. At least one out of every three children experienced stunted growth in South Asia (Zaman *et al.*, 2018). This undernourishment leads to impaired cognitive development and susceptibility to chronic diseases. These health issues further deteriorate the communities with associated problems. The COVID-19 pandemic also made

it imperative to focus on health and nutrition (Yousfi *et al.*, 2020). Curbing malnutrition led to several recommended interventions, such as school feeding programs, nutrition education, food fortification, diet diversification, supplementation, nutraceuticals, and biofortification. Among all these, biofortification revealed the best and most practical. Biofortification is an agricultural intervention suitable for farmers and consumers due to accessibility and cost-effectiveness. As cereals are the staple food extensively taken as an energy source for most regions worldwide, therefore, cereal biofortification is the most advantageous. Different biofortified crop varieties have undergone release for general cultivation through conventional plant breeding, i.e., quality protein maize (QPM-maize), pro-vitamin A (PVA) maize, and Zincol in the case of wheat (Garg *et al.*, 2018). Zinc biofortification of staple grain crops also has long-term benefits, as it can provide sustained access to adequate levels of zinc for generations (Buturi *et al.*, 2021).

The zinc biofortification of maize offers a sustainable, low-cost approach to improve zinc status in humans without necessary supplements. It provides a way for farmers to increase their crop yield and income. Therefore, zinc biofortification in maize is an urgent agricultural strategy for improving zinc status in low-income countries and helping to reduce zinc deficiency (Maqbool and Beshir, 2019). Screening of germplasm for diversity is a preliminary step in developing zinc-rich maize, and this diversity helps to create novel gene combinations in hybridization programs. The continued use of different statistical and biochemical approaches helps to identify the desirable genes in germplasm. In this regard, the study materialized to screen out zinc-rich

inbred lines from the available genetically diverse germplasm, based on morphological and physiological markers linked with target traits, to serve as selection criteria in zinc biofortification and investigation of association level of targeted traits with other standards.

MATERIALS AND METHODS

The genetic material consisted of 150 maize inbred lines collected from various sources, including the Plant Genetic Resource Institute, Islamabad (with diverse origins), the Maize and Millet Research Institutes of Pakistan, and the Maize Breeding and Genetics Laboratory, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.

Inbred lines' sowing followed an augmented field design (Federer and Raghavarao, 1975). Replication on check genotypes ensued instead of on test genotypes. Planting inbred lines occurred on 5-m long beds with a standard planting geometry, i.e., 75 cm row-to-row and 25 cm plant-to-plant distances. Sowing continued on five blocks, each containing 30 test inbred lines and four exotic biofortified hybrids as the check entry. Each block contained 34 entries. Assessment of genotypic diversity focused on plant height, ear height, days to tasseling, days to silking, anthesis silking interval, ear length (cm), the number of rows per cob, the number of grains per row, average grain yield per plant (g), 1000-grain weight (g), and grain-zinc contents (mg/kg). Principal component analysis (PCA) aided in reducing dimensions of multivariate data into the first few components responsible for maximum variation to visualize diversity and select best-performing inbred lines.

Statistical analysis

The R-software ran an analysis of variance (ANOVA) and principal component analysis (PCA) biplot. Performing descriptive statistics assessed trends of data. The correlation coefficient for studied traits of maize inbred lines also used the R- software.

Zinc quantification

At physiological maturity, five ears per entry incurred harvesting with the husk and drying up to 14% moisture level according to the protocol devised by HarvestPlus. Representative grain samples, drawn and ground into fine powder, used 0.5 g fine powder for biochemical analysis. For grain-zinc contents, nitro-perchloric acid digestion mixture (HNO₃:HClO₄, 2:1) mixed to the sample for digestion underwent storing overnight for preliminary digestion until completion of vigorous reaction. The Kjeldahl apparatus had heating cycles of 150 °C for 1 hour and 235 °C for a half hour until all fumes disappeared. The digested samples sustained dilution to make a final volume of 50 ml. After dilution, samples analyzed for grain-zinc contents used the atomic absorption spectrophotometer running the zinc lamp for emitting a wavelength (213.9 nm). The absorbance revealed zinc concentration in the sample. Employing this protocol by Zarcinas *et al.* (1987) included a few modifications. Detection of zinc contents used the following formula:

$$\text{Zn contents (mg/kg)} = \text{Curve reading} \times \text{dilution factor}$$

Soil Zn analysis

Collecting soil samples randomly with the help of an Auger transpired in five locations at a 0–30 cm depth. Collected samples attained mixing thoroughly, with a 0.5 kg final sample drawn and tested in the soil testing laboratory at the Ayub Agriculture Research Institute (AARI), Faisalabad, for determining the soil zinc profile.

RESULTS

Augmented analysis of variance for screening experiment

Screening the soil for zinc deficiency ensued (0.9 ppm). The analysis of variance for

Table 1. Analysis of variance (ANOVA) for the augmented field design consisting of 154 maize genotypes, including 150 studied genotypes along with 4 commercial checks. The analysis of data for agronomic, yield-contributing, and grain-zinc content-related traits.

SOV	Df	PH	EH	DT	DS	ASI	EL	NGPR	NRPC	GY	TGW	Zn
Block un adj.	4	1329.31**	651.38**	240.08**	243.57**	5.84**	36.17**	22.3**	15**	2413.68**	9460.8**	22.77**
Gen/trt.adj.	153	891.74**	287.49**	40.79**	37.55**	1.51**	6.49*	46.46**	12.8**	662.2**	2662.8**	19.05**
Check	3	1145.56**	331.99**	121.67**	128.85**	0.33 ^{ns}	10.1*	30.18**	95.67**	1650**	12683**	272.65**
Check + check vs. aug.	150	886.66**	286.6**	39.17**	35.73**	1.53**	6.41*	46.78**	11.15**	642.4**	2462.4**	13.98**
Gen/trt. Unadj.	153	920.4**	301.43**	46.979**	43.82**	1.62**	7.37**	46.98**	13.11**	723.5**	2888.1**	19.64**
Block.adj	4	131.2	61.32	3.2	3.675	1.45**	2.23	2.2	3.5	68.8	846.6	0.29
Augmented	149	759.4**	283.89**	45.35**	42.12**	1.65**	6.652**	43.25**	11.25**	650.6**	2669.3**	11.92**
Check vs. augmented	1	24239.1**	2822.62**	65.96**	42.76**	1.80**	106.69**	653.06**	42.215**	8802.5**	6096.4**	410.49**
Residuals	12	37.9	18.48	5.33	4.14	0.25	1.91	4.93	2.17	49.1	646.1	0.71

PH: plant height (cm), EH: ear height (cm), DT: days to tasseling, DS: days to silking, ASI: anthesis silking interval, EL: Ear length (cm), NGPR: number of grains per row, NRPC: number of rows per cob, TGW: thousand grain weight (g), GY: average grain yield per plant (g), Zn: grain-zinc contents (mg/kg), ns: non-significant, *: significant at 5%, **: significant at 1%.

Table 2. Analysis for the Standard Errors of Means for all studied agronomic, yield-contributing, and zinc content-related traits along with their comparisons of the adjusted means.

Standard error of difference	DT	DS	ASI	PH	EH	EL	NGPR	NRPC	GY	TGW	Zn
Between check means (SEd1)	1.46	1.28	0.31	3.90	2.71	0.87	1.40	0.93	4.43	16.07	0.53
Between any two means of test genotypes of the same block (SEd2)	3.26	2.87	0.71	8.70	6.07	1.95	3.14	2.08	9.90	35.94	1.19
Between any two entries of the different block (SEd3)	3.65	3.21	0.80	9.73	6.79	2.18	3.51	2.32	11.1	40.18	1.33
Between means of test and check genotypes (SEd4)	2.73	2.4	0.61	7.28	5.08	1.63	2.62	1.74	8.28	30.07	0.99

Table 3. Analysis of the descriptive statistics including mean, standard deviation, minimum, maximum, standard error, and coefficient of variability for the agronomic, yield-contributing, and zinc content-related traits in indigenous maize germplasm.

Trait	PH	EH	DT	DS	ASI	EL	NGPR	NRPC	GY	TGW	Zn
Mean	140.24	65.46	55.22	58.45	3.22	10.54	19.73	13	55.28	255.07	14.6
S.D.	29.87	18.01	6.97	6.82	1.49	2.46	6.66	3.6	25.49	52.34	3.68
Min.	35.15	13	41.1	43.7	0.4	5.47	6.15	6	23.64	155.8	6.02
Max.	195.87	100.92	72.35	76.95	6.65	19.92	39.15	27	151.41	395.05	29.06
S.E.	2.41	1.45	0.56	0.55	0.12	0.2	0.54	0.29	2.05	4.22	0.3
C.V.%	4.29	6.45	4.17	3.47	15.71	12.85	10.95	11.21	12.21	9.89	5.6

PH: plant height (cm), EH: ear height (cm), DT: days to tasseling, DS: days to silking, ASI: anthesis silking interval, EL: Ear length (cm), NGPR: number of grains per row, NRPC: number of rows per cob, GY: average grain yield per plant (g), TGW: thousand grain weight (g), Zn: grain-zinc content (mg/kg).

augmented field design (Table 1) showed that all genotypes (inbred lines and exotic checks) were significantly variable for studied traits. Blocking (adjusted) was non-significantly different for studied traits, whereas blocking (unadjusted) proved significantly different and was found efficient. Checks, augmented, check vs. augmented, and check + check vs. augmented showed significant variability except for anthesis-silking interval (ASI) in checks, which showed considerable variability. It could be from a robust blocking effect whereby the variability caused by the external conditions or systematic variations among blocks is substantial, causing the differences between the blocks to overshadow the studied treatment effects. Different standard errors calculation (Table 2) compared differences between check means, any two means of test genotypes in the same block, any two entries of the different blocks, and means of test and check genotypes (SEd1, SEd2, SEd3, and SEd4 respectively), providing critical comparisons and variation patterns in data. Descriptive statistics (Table 3) revealed the mean, standard deviation, range, and coefficient of variations, which provided information about trends in data. Maximum values for grain yield per plant and grain-zinc contents in a screened material were 151.41 g/plant and 29.06 mg/kg, while minimum values were 23.64 g/plant and 6.02 mg/kg, with mean values of 55.28 g/plant and 14.6 mg/kg, respectively.

Principal component analysis

Two separate PCAs proceeded, one for all traits (Figure 1) and the second for grain-zinc contents and average grain yield per plant (Figure 2). The overall PCA depicted 73.5% variability (PC1 = 28.4%, PC2 = 22.7%, PC3 = 12.6%, and PC4 = 9.8%) explained by the first four components having an Eigenvalue more than one (Table 4). Meanwhile, PCA for grain-zinc contents and average grain yield per plant explained 100% variability (PC1 = 65.9% and PC2 = 34.1%) in the first two components. Yield and yield-contributing traits explained significant variations in the first principal component. Reproductive and vegetative phase traits contributed remarkable variations in the

second and third principal components, respectively. Inversely, grain-zinc contents (Zn) and ear height (EH) contributed notable variations in PC4 (Table 5). The best and poor-performing inbred lines appeared by spotting the inbred lines on the PCA biplot according to their trait vector. Best-performing inbred lines with higher recorded values showed plots on the positive axis of the trait vector, whereas inbred lines with lower recorded values were on the negative axis (Figures 1 and 2). PCA biplot based on grain-zinc contents and average grain yield per plant aided in selecting suitable parents for future hybridization programs. Inbred lines spotted on the positive axis of the trait vector were applicable for selection as lines, and those on the negative axis were suitable as testers. Thus, inbred lines shortlisted as female parents (lines) were E102, E38, E92, E52, E91, E1, E93, E54, E28, E25, E72, E30, and E90, and as male parents (tester) were E77, E71, and E100. Only groups of three least-performing inbred lines served as testers. The recorded values for grain-zinc contents and average grain yield per plant of identified lines are available in Table 6.

Correlation analysis among studied parameters

The average grain yield per plant showed a maximum significant positive association with the number of rows per cob, followed by 1000-grain weight, ear length, the number of grains per row, and grain-zinc contents. Traits, i.e., days to silking and days to tasseling, exhibited a non-significant correlation with the average grain yield per plant. Grain-zinc contents (Zn) had a considerable negative link with days to silking and days to tasseling (DT), and plant height (PH), ear height (EH), ear length (EL), and 1000-grain weight (TGW), revealed notable positive associations with grain-zinc contents (Zn). Days to tasseling (DT) showed a strong positive correlation with days to silking (DS). Plant height indicated a sturdy positive relationship with ear height (EH). Ear length (EL) showed a positive significant correlation with the number of grains per row, the number of rows per cob (NRPC), and 1000-grain weight (TGW). Plant height (PH) and ear height (EH)

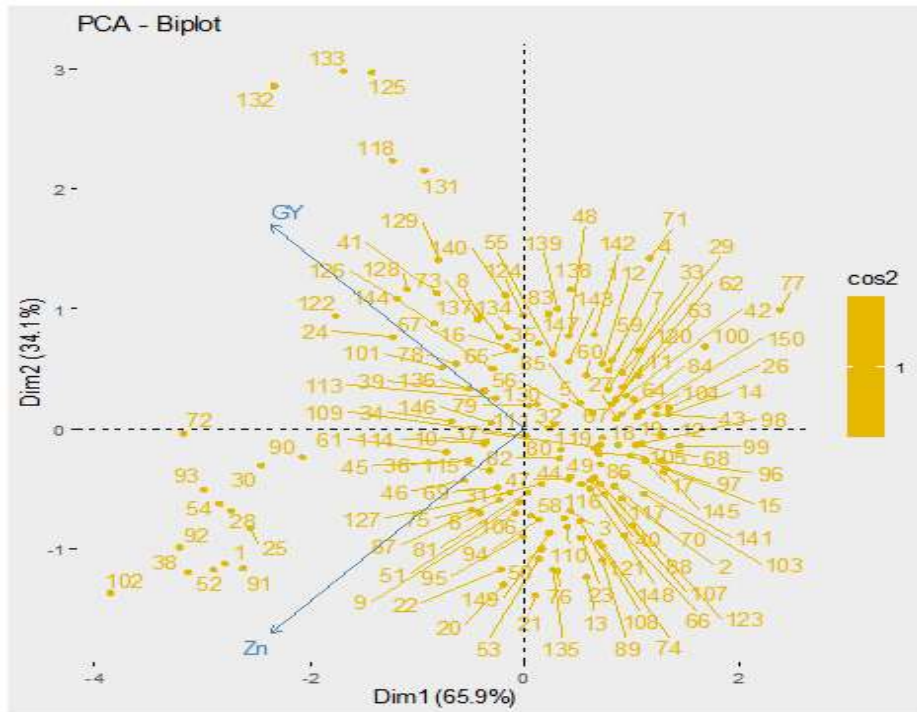


Figure 1. Principal Component Analysis for the two most important traits, i.e., grain yield (GY) and grain-zinc contents (Zn), analyzing all studied mungbean genotypes.

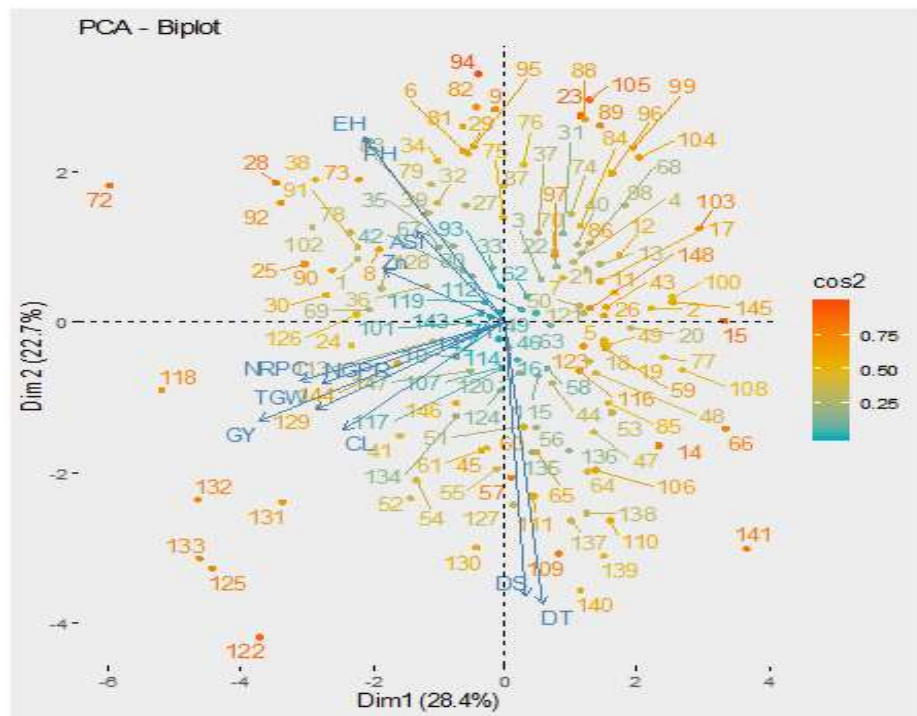


Figure 2. Principal Component Analysis for all studied traits as compared with all studied genotypes to find the best suitable combination of traits with genotypes.

Table 4. Eigen vector values for the calculated principal component analysis along with the proportion of the variation explained by each of the Eigen value vector.

Principal component	1	2	3	4	5	6	7	8	9	10	11
Eigenvalues	3.13	2.50	1.38	1.07	0.72	0.70	0.61	0.54	0.21	0.14	0.00
Explained variation	28.40	22.70	12.60	9.80	6.60	6.30	5.50	4.90	1.90	1.30	0.00
Cumulative variation	28.40	51.20	63.80	73.50	80.10	86.40	92.00	96.80	98.70	100.00	100.00

Table 5. Principal component based on contribution of traits – contribution of each of the traits to each of the four main principal components of the conducted principal component analysis.

Variables	PH	EH	DT	DS	ASI	EL	NGPR	NRPC	GY	TGW	Zn
PC1	-0.27	-0.27	0.07	0.04	-0.17	-0.32	-0.36	-0.40	-0.48	-0.37	-0.24
PC2	0.35	0.36	-0.54	-0.53	0.18	-0.21	-0.12	-0.11	-0.19	-0.17	0.10
PC3	0.49	0.47	0.39	0.43	0.13	-0.27	0.06	0.01	-0.13	-0.15	-0.26
PC4	0.16	0.20	0.16	0.03	-0.71	0.00	-0.11	-0.10	-0.03	-0.05	0.62

Table 6. Inbred lines selected as parents based on various calculations, particularly for the zinc contents and grain yield. A total of 13 lines and 3 testers were selected as parents for the breeding experiments.

Lines	E102	E38	E92	E52	E91	E1	E93	E54	E28	E25	E72	E30	E90	Testers	E77	E71	E100
Zn (mg/Kg)	27.5	25.2	24.9	24.6	23.9	23.7	23.2	23.1	22.5	22.4	22.02	21.4	20.4	Zn	6.02	7.9	8.9
GY (g)	99.6	84.8	94.6	80.8	80.6	90.8	53.6	69.8	97.8	91.8	115.3	76.8	87.6	GY	32.3	62.3	35.6

Table 7. Correlation coefficients calculated for all studied traits of indigenous maize germplasm, including the agronomic, yield-contributing, and zinc content-related parameters.

Trait	PH	EH	DT	DS	ASI	EL	NGPR	NRPC	GY	TGW	Zn
PH	1										
EH	0.85**	1									
DT	-0.25**	-0.26**	1								
DS	-0.22**	-0.22**	0.98**	1							
ASI	0.23 ^{ns}	0.23**	-0.28**	-0.09 ^{ns}	1						
EL	-0.05 ^{ns}	0.00 ^{ns}	0.08 ^{ns}	0.09 ^{ns}	0.06 ^{ns}	1					
NGPR	0.21**	0.17**	0.06 ^{ns}	0.09 ^{ns}	0.13 ^{ns}	0.34**	1				
NRPC	0.2**	0.19**	0.03 ^{ns}	0.06 ^{ns}	0.14 ^{ns}	0.27**	0.37**	1			
GY	0.14 ^{ns}	0.15 ^{ns}	0.07 ^{ns}	0.11 ^{ns}	0.16 ^{ns}	0.55**	0.52**	0.67**	1		
TGW	0.11 ^{ns}	0.06 ^{ns}	0.06 ^{ns}	0.08 ^{ns}	0.10 ^{ns}	0.38**	0.31**	0.41**	0.61**	1	
Zn	0.16**	0.21**	-0.17**	-0.19**	-0.08 ^{ns}	0.21**	0.12 ^{ns}	0.15 ^{ns}	0.31**	0.19**	1

Residual effect = 0.27

PH: plant height (cm), EH: ear height (cm), DT: days to tasseling, DS: days to silking, ASI: anthesis silking interval, EL: Ear length (cm), NGPR: number of grains per row, NRPC: number of rows per cob, TGW: thousand grain weight (g), GY: average grain yield per plant (g), Zn: grain-zinc contents (mg/kg), ns: non-significant, *: significant at 5%, **: significant at 1%.

displayed a negative substantial linkage with days to silking (DS) and days to tasseling (DT) (Table 7). Yield sustained influences from several factors. Considering the overall association of 100% among the studied parameters, the percentage of associations underwent division per their correlation coefficients. Based on correlation coefficients among studied traits, the NRPC showed association at 20.36%, TGW - 18.54%, EL - 16.72%, NGPR - 15.81%, Zn - 9.42%, ASI - 4.86%, EH - 4.56%, PH - 4.26%, DS - 3.34%, and DT - 2.13% with GY. Percent relatedness of Zn with PH, EH, EL, NGPR, NRPC, GY, and TGW was 8.94%, 11.73%, 11.73%, 6.70%, 8.38%, 17.32%, and 10.61%, respectively. The association with reproductive phase indicators was significantly negative with DT (9.50%), DS (10.61%), and ASI (4.47%).

DISCUSSION

Soil zinc analysis showed a deficiency of zinc (0.90 ppm), which provided an opportunity for reliable screening of inbred lines. Darai *et al.* (2020) categorized soils with 0.5–1 ppm zinc as low-zinc soils. Zinc-efficient genotypes perform better in Zn-deficient soils. These zinc-efficient genotypes are potential candidate lines for parents in hybridization programs for grain-zinc biofortification (Karim *et al.*, 2012; Mazhar *et al.*, 2021; Khan *et al.*, 2023). The existence of significant genetic variability is essential for improving traits. The nature of variability directs breeders to sketch out breeding programs. The maize germplasm in the current study showed significant variability for studied traits. Blocking (unadjusted), checks, augmented entries, check vs. augmented, and checks + check vs. augmented were also noteworthy. Blocks (adjusted) were non-significant variables for all studied traits except ASI. It indicated homogeneity within experimental blocks. Similar findings regarding an augmented field design came from Saba *et al.* (2017) while working on common beans. Nzuve *et al.* (2014) and Maqbool *et al.* (2019) reported significant variability in evaluated maize germplasm for studied traits.

Diversity for average grain yield per plant ranged from 23.6 to 151.4 g. Grain-zinc contents ranged from 6.02 to 29.06 mg/kg, showing sufficient genetic diversity for improving grain yield and grain-zinc contents. Agrawal *et al.* (2012) and Goswami *et al.* (2014), working with different genetic materials, reported similar ranges of 7 to 29.8 mg/kg and 10.6 to 21.3 mg/kg, respectively. Guo *et al.* (2020), working on two double haploid populations (DH₁ and DH₂) and drought tolerant maize of Africa (DTMA), found kernel zinc ranging from 16.86 to 37.45 mg/kg (DH₁), 18.38 to 37.93 mg/kg (DH₂), and 18.35 to 39.53 mg/kg (DTMA), with averages of 24.59, 25.59, and 27.11 mg/kg, respectively. The genetic diversity of grain-zinc contents revealed by genotypes may have a basis in the hyper-accumulation of zinc due to the zinc efficiency of cultivars, production of organic ligands, and chelators that bind zinc from the soil, making it more mobile in the plant, and due to the variable activity of heavy metal transporters. The available genetic diversity for all studied traits appears in summary statistics. The standard deviation (SD) reflects the spread out of the sample data from the trait mean. The SD calculated for grain-zinc contents in this study was 3.68. Guo *et al.* (2020) found SD 3.41, 4.01, and 3.5 for kernel zinc contents using drought-tolerant maize of Africa (DTMA) and double haploids (DH₁ and DH₂), respectively, which indicated that DTMA has lesser influences from genotype by environment interaction than both DH populations in expressing kernel zinc contents.

PCA is a dimension reduction multivariate analysis extensively used in research. It boosts selection efficacy because all traits undergo computing and presentation simultaneously. Principal components correspond to the number of traits studied, contributing variability in decreasing order from PC1 until the last component (Aslam *et al.*, 2017). In this study, out of the 11 principal components, four components contributed to maximum variability, thus disregarding subsequent components, which need no demonstration. PCA explains the most decisive traits responsible for genotypic differences. Associated traits bore groupings in one

principal component, reducing total components (Johnson and Wichern, 2002). Following the criteria of Kovacic (1994), principal components with an Eigenvector value of one were valid for further study, with the remaining not considered. Two PCAs transpired, one for all traits and the second for the average grain yield per plant and grain-zinc contents, representing 73.5% variability (PC1 = 28.4%, PC2 = 22.7%, PC3 = 12.6%, and PC4 = 9.8%), and 100% variability (PC1 = 65.9% and PC2 = 34.1%) of the total variability of the data, respectively. The best and poor-performing inbred lines showed plotting on the PCA biplot according to their trait vector.

Best performing inbred lines with higher recorded values reached plotting on the positive axis of the trait vector, while inbred lines with lower recorded values are on the negative axis. Among the evaluated materials, inbred lines E102, E38, E92, E52, E91, E1, E93, E54, E28, E25, E72, E30, and E90, were potential candidate lines for grain-zinc contents and average yield per plant, and E77, E71, and E100 were poor performing, which could serve as male parents (tester). Aslam *et al.* (2017) used PCA to select lentil genotypes efficiently against salinity. Maqbool *et al.* (2015) employed PCA for screening chickpea germplasm under multiple environments. Different scientists used PCA for screening treatments in various crops like chickpeas (Aslam *et al.*, 2014), maize (Mustafa *et al.*, 2015), wheat (Ashraf *et al.*, 2015), cotton (Latif *et al.*, 2015), and common bean (Saba *et al.*, 2017).

Correlation between traits is due to pleiotropic effects of a gene or due to tight linkages of genes of the same haplotype, which helps in simultaneously improving different traits beneficial for selecting indirect traits that are laborious or expensive to screen directly. For indirect selection, the high heritability of traits and positive association with commercial traits is a prerequisite (Saba *et al.*, 2017). Traits with high positive correlation and high heritability prove better for developing selection criteria. The significant association between the average grain yield per plant with the number of rows per cob, 1000-grain

weight, ear length, and the number of grains per row indicates influences of these traits on the average grain yield per plant, therefore denoting yield contributors. Further, it dictates choosing inbred lines with higher mean values for yield-contributing traits. Similar findings came from Ahsan *et al.* (2013), Ali *et al.* (2014), and Maqbool *et al.* (2019). They reported that the number of grains per row influenced the yield potential in maize. No negative correlation between the average grain yields per plant emerged with all the studied traits, indicating that positive association is helpful in simultaneously improving yield and other traits by selecting both loci at a time.

These associations underwent further dissection to understand the nature of correlations. NRPC and NGPR reflected total grains per ear; their significant positive correlation with GY indicates the increase in grain yield correlated with the rise in the total number of grains/ear. The total number of grains per ear increased by selecting long-ear genotypes with decreased ASI, with more silking florets produced in long ears. The EL showed a significant positive correlation with GY, as more silking florets occurred in long ears. TGW showed a significantly positive association with GY, where the means increase in kernel weight increases GY. TGW, in turn, rises with an increase in the grain-filling period. The Zn had a significant and positive association with GY because grain-zinc contents are essential for seedling vigor, thus improving subsequent crop stages and GY. Welch (1999) reported that kernel zinc reservoirs are vital for healthy seedling growth, which applying any zinc cannot substitute.

The study results indicate that a negative link of Zn with reproductive phases that causes an increase in grain-zinc contents is successful through selecting early maturing genotypes with less ASI. Maximum amounts of zinc are necessary for developing pollens than any other part of the plant. Therefore, it is crucial in pollen development and viability (Sharma *et al.*, 1987). It may imply that few DS and DT lead to early remobilization of available zinc from tassel to cob; thus, more zinc is available for grain under long grain-filling periods. The positive association of Zn

with PH and EH indicates that vegetative growth increases with increase in grain-zinc contents. This correlation explains zinc-deficiency symptoms in plants as due to Zn deficiency, auxin production is less, which is necessary for cell elongation; therefore, a decrease in intermodal distance under zinc deficiency causes a reduction in plant height and placement of ear at a lower node (Brown *et al.*, 1993). The positive correlation of Zn and TGW may refer to the role of zinc in carbohydrate metabolism. Liu *et al.* (2020) reported that an increase in zinc supply increases the carbohydrate (source) supply for grain filling (sink), resulting in a rise in TGW. The increasing kernel zinc contents enhances TGW in maize (Ziaeyan and Rajaie, 2012). It implies that for developing zinc biofortified maize genotypes, an indirect selection of genotypes having high mean values for PH, TGW, EL, and GY and a lower mean value for DS and DT could be effective.

CONCLUSIONS

The prevalence of genetic diversity acts as raw material for improving desired traits. Significant genetic diversity for average grain yield per plant and grain-zinc contents prevails in the studied maize germplasm of Pakistan, which needs further scrutiny to enhance the stagnant yield potential of maize with an improved nutrient status and is crucial for food and nutrient security. The identified diversity is beneficial for future breeding programs. Correlation coefficients showed that the average grain yield per plant revealed a positive association with yield-contributing traits and grain-zinc contents. Selecting these linked traits enables simultaneous improvement of yield and quality traits.

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REFERENCES

- Agrawal PK, Jaiswal SK, Prasanna BM, Hossain F, Saha S, Guleria SK, Gupta HS (2012). Genetic variability and stability for kernel iron and zinc concentration in maize (*Zea mays* L.) genotypes. *Indian J. Genet. Plant Breed.* 1(72): 421-428.
- Ali Q, Ahsan M, Tahir MH, Basra SM (2014). Gene action and correlation studies for various grain and its contributing traits in maize (*Zea mays* L.). *Bothalia.* 44(2): 80-91.
- Aslam M, Ahmad K, Maqbool MA, Bano S, Zaman QU, Talha GM (2014). Assessment of adaptability in genetically diverse chickpea genotypes (*Cicer arietinum* L.) based on different physio-morphological standards under ascochyta blight inoculation. *Int. J. Adv. Res.* 2(2): 245-255.
- Aslam M, Maqbool MA, Zaman QU, Shahid M, Akhtar MA, Rana AS (2017). Comparison of different tolerance indices and PCA biplot analysis for assessment of salinity tolerance in lentil (*Lens culinaris*) genotypes. *Int. J. Agric. Biol.* 19(3).
- Brown PH, Cakmak I, Zhang Q (1993). Form and function of zinc plants. In: Zinc in Soils and Plants. Springer, Dordrecht.
- Buturi CV, Mauro RP, Fogliano V, Leonardi C, Giuffrida F (2021). Mineral biofortification of vegetables as a tool to improve human diet. *Foods* 10(2): 223.
- Darai R, Sarker A, Pandey MP, Dhakal KH, Kumar S, Sah RP (2020). Genetic variability and genotype × environment interactions effect on grain iron (Fe) and zinc (Zn) concentration in lentils and their characterization under Terai environments of Nepal. *Adv. Nutr. Food Sci.* 5(1): 1-2.
- Federer WT, Raghavarao D (1975). On augmented designs. *Biometrics* pp. 29-35.
- Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, Arora P (2018). Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Front. Nutr.* 12. <https://doi.org/10.3389/fnut.2018.00012>.
- Goswami R, Kumar J, Hossain F, Thirunavukkarasu N, Manjaiah KM, Agrawal PK, Reddy SS, Guleria SK, Gupta HS (2014). Analyses of the genetic variability for kernel-iron and kernel-zinc in maize genotypes. In: 12th Asian Maize Conference and Expert Consultation on Maize for Food, Feed and Nutritional Security, *Book of Abstracts*.

- Guo R, Dhliwayo T, Mageto EK, Palacios-Rojas N, Lee M, Yu D, Ruan Y, Zhang A, San Vicente F, Olsen M, Crossa J (2020). Genomic prediction of kernel zinc concentration in multiple maize populations using genotyping-by-sequencing and repeat amplification sequencing markers. *Front. Plant Sci.* 11: 534.
- Hara T, Takeda TA, Takagishi T, Fukue K, Kambe T, Fukada T (2017). Physiological roles of zinc transporters: Molecular and genetic importance in zinc homeostasis. *J. Physiol Sci.* 67(2): 283-301.
- Johnson RA, Wichern DW (2002). Applied Multivariate Statistical Analysis. Upper Saddle River, NJ: Prentice hall.
- Karim MR, Zhang YQ, Tian D, Chen FJ, Zhang FS, Zou CQ (2012). Genotypic differences in zinc efficiency of Chinese maize evaluated in a pot experiment. *J. Sci. Food Agric.* 92(12): 2552-2559.
- Khan SH, Aslam M, Bibi A, Khan HZ (2023). GGE biplot analysis for zinc quality and yield stability of exotic maize hybrids. *SABRAO J. Breed. Genet.* 55(1): 268-278. <http://doi.org/10.54910/sabrao2023.55.1.25>.
- Kovacic Z (1994). Multivariate Analysis. Faculty of Economics. University of Belgrade. Serbian.
- Latif A, Bilal M, Hussain SB, Ahmad F (2015). Estimation of genetic divergence, association, direct and indirect effects of yield with other attributes in cotton (*Gossypium hirsutum* L.) using biplot correlation and path coefficient analysis. *Trop. Plant Res.* 2(2): 120-126.
- Liu DY, Zhang W, Liu YM, Chen XP, Zou CQ (2020). Soil application of zinc fertilizer increases maize yield by enhancing the kernel number and kernel weight of inferior grains. *Front. Plant Sci.* 28(11): 188.
- Maqbool MA, Aslam M, Ali H, Shah T, Farid B, Zaman QU (2015). Drought tolerance indices based evaluation of chickpea advanced lines under different water treatments. *Res. Crop.* 16(2): 336-344.
- Maqbool MA, Aslam M, Beshir A, Khan MS, Saeed MT (2019). Studying selection criteria and genetic variability for improvement of indigenous maize in Pakistan. *Pak. J. Agric. Sci.* 56(4): 819-827.
- Maqbool MA, Beshir A (2019). Zinc biofortification of maize (*Zea mays* L.): Status and challenges. *Plant Breed.* 138(1): 1-28.
- Mazhar MW, Ali Q, Ishtiaq M, Ghani A, Maqbool M, Hussain T, Mushtaq W (2021). Zinc-aspartate-mediated drought amelioration in maize promises better growth and agronomic parameters than zinc sulfate and L-aspartate. *SABRAO J. Breed. Genet.* 53(2): 290-310.
- Mustafa HS, Farooq J, Bibi T, Mahmood T (2015). Cluster and principle component analyses of maize accessions under normal and water stress conditions. *J. Agri. Sci. Belgrade* 60(1): 33-48.
- Nzuve F, Githiri S, Mukunya DM, Gethi J (2014). Genetic variability and correlation studies of grain yield and related agronomic traits in maize. *J. Agric. Sci.* 6: 166-176.
- Prasad AS (2013). Discovery of human zinc deficiency: Its impact on human health and disease. *Adv. Nutr.* 4(2): 176-190.
- Roohani N, Hurrell R, Kelishadi R, Schulin R (2013). Zinc and its importance for human health: An integrative review. *J. Res. Med. Sci.* 18(2): 144.
- Saba I, Sofi PA, Zeerak NA, Mir RR, Gull M (2017). Using augmented design for evaluation of common bean (*Phaseolus vulgaris* L.) germplasm. *Int. J. Curr. Microbiol. Appl. Sci.* 6(7): 246-254.
- Sharma PN, Chatterjee C, Sharma CP, Agarwala SC (1987). Zinc deficiency and anther development in maize. *Plant Cell Physiol.* 28: 11-18.
- Welch RM (1999). Importance of seed mineral nutrient reserves in crop growth and development. In: Z. Rengel (Ed.). Mineral Nutrition of Crops: Fundamental Mechanisms and Implications. Binghamton: Food Products Press. pp. 205-226.
- Yousfi N, Bragazzi NL, Briki W, Zmijewski P, Chamari K (2020). The COVID-19 pandemic: How to maintain a healthy immune system during the lockdown—a multidisciplinary approach with special focus on athletes. *Biol. Sport* 37(3): 211-216.
- Zaman QU, Aslam Z, Yaseen M, Ihsan MZ, Khaliq A, Fahad S, Bashir S, Ramzani PM, Naeem M (2018). Zinc biofortification in rice: Leveraging agriculture to moderate hidden hunger in developing countries. *Arch. Agron. Soil Sci.* 64(2): 147-161.
- Zarcinas BA, Cartwright B, Spouncer LR (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis.* 18(1): 131-146.
- Ziaeyan AH, Rajaei M (2012). Combined effect of zinc and boron on yield and nutrients accumulation in corn. *Int. J. Plant Prod.* 3: 35-44.