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Zn KERNEL CONTENT-BASED BIODIVERSITY ANALYSIS STUDIES FOR BIOFORTIFICATION IN INDIGENOUS MAIZE (*ZEA MAYS* L.) GERMPLASM

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Abstract

Zn is an essential trace mineral for all forms of life due to its essential role in expression of genes, development of cells and replication. The World Health Organization recommends a daily intake of 6.7 to 15 mg of zinc for humans. Diversity determination among the inbred lines is essential for the heterosis breeding. Genetic biofortification of staple crops like maize presents a sustainable strategy to address malnutrition. The present study was conducted to estimate genetic diversity for Zn content in 50 inbred lines and evaluate their performance by using the correlation and principal component analysis in randomized complete block design with three replications. Zinc content in the grains was quantified by using Atomic Absorption Spectrophotometry technique and six inbred lines 468 Zai, 738 MMRI 32, 749 4B-9, 739 4B-3, 737-339 and 476 Zai was found with higher Zn concentrations. A positive correlation was observed between zinc content and most of the observed traits, except for plant height (PH) and kernel yield (KY), which exhibited a negative association. PCA study revealed that the first three principal components had eigenvalue more than one indicated broad genetic base and accounted for 65.3% of the total variation. The identification of high-level genetic diversity could be used for maize improvement and inbred lines with higher zinc contents are valuable for breeders aiming to develop biofortified maize.

Key words: Maize, Zn, Genetic variability, Biofortification, Correlation, Principal component analysis, Biplot.

Introduction

Maize (*Zea mays* L.) among cereal crops ranked third after wheat and rice worldwide. It generates billions of dollars annually worldwide (Mabberley, 2008). In Pakistan maize contributes 3 percent value in agriculture and 0.7 percent to GDP (Govt. of Pakistan, 2023). It is a nutritious crop and serves as a vital source of food for humans, forage for animals and raw material for industries. Due to its importance, it has earned the title of queen of cereals. Maize kernel contains 10% protein, 72% starches, 4.8% oil, and 1.7% ashes (Tahir *et al*., 2009). More than 200 million population globally used corn as staple crops, and it provides 15% protein and 20% calories of the world (Nuss & Tanumihardjo, 2010).

Zn is an essential trace mineral for all forms of life due to its essential role in expression of genes, development of cells and replication. Over 300 enzymes and 1000 transcription factors rely on Zn for their proper functioning (Prasad, 2012). Zn is an essential micronutrient that plays a key role in the various cellular processes, including protein synthesis nucleic acid metabolism (DNA synthesis) and gene transcription (Maxfeld *et al*., 2023). Zn regulates intracellular signaling pathways in innate and adaptive immune cells, influencing immune responses such as antibody production, inflammatory signaling, and lymphocyte differentiation (Stiles *et al*., 2024). Consequently, it is crucial for the proper functioning of the immune system.

Malnutrition one of the foremost socio-economic problems faced by developing countries like Pakistan. It is estimated that 60–70% of the population in Asia and Sub-Saharan Africa may be at risk of insufficient zinc intake (Prasad, 2006). One third of the population of the world in developing countries undergoes in acute Zn deficiency (Takkar & Shukla, 2015). According to another study two billion people are Zn deficient around the world (Prasad, 2012). The susceptible populations which include babies, young children, pregnant and lactating women due to their greater Zn demands, as they are at critical stages of growth and physiological needs (Black *et al*., 2008). About 82% of pregnant women in the world are at the risk of insufficient Zn intake to meet the necessities of pregnancy (Shukla *et al.,* 2016). Ensuring the necessary amounts of Zn in diet should be a major strategy to overcome child illness, increase growth and decrease the mortality rate in developing countries. Deficiency of Zn is major cause of nutrient disorders in humans and in children its effects are more common (Boonchuay *et al.,* 2013). The human body cannot produce zinc and requires Zn daily because there is no long-term storage of Zn in body. The recommended daily intake of zinc is 10 mg for men and 8 mg for women (Schoofs *et al*., 2024).

Addressing zinc deficiency requires a comprehensive approach. Although a varied and nutritionally enriched diet is an effective remedy, this is often unattainable for impoverished populations in developing countries (Enserink, 2008). Many people in the world which are Zn deficient use maize as staple food and directly depends on it. Therefore, a sustainable and practical solution lies in the production of staple foods, particularly maize, that are enriched with essential micronutrients like zinc. This can be achieved through advanced agricultural techniques such as genetic biofortification of staple crops. It is considered an ideal approach because it benefits the poor community, especially in the rural areas, who have limited access to commercially fortified foods. Maize constitutes a significant portion of the diet and accounts for 50% of total zinc intake in rural areas (Oikeh *et al.*, 2003). The nutritional quality of maize varies, and its low zinc content significantly contributes to hidden hunger. To tackle this issue, genetic biofortification stands out as a particularly effective strategy.

Genetic diversity is the base for any crop improvement program. Investigation of the genetic variability of parental combinations is a crucial step for effective breeding. Relying solely on single-character evaluation through statistical methods can lead to incomplete or occasionally inaccurate interpretations. Therefore, it is essential to analyze morphological and biochemical traits together. The focus of this study is to evaluate and confirm the maize inbred lines that have higher Zn content which can be further used for the development of biofortified maize hybrids.

Material and Methods

Total fifty inbred lines of maize crop collected from various research institutions in Pakistan were used in current experiment shown in (Table 1). The experiment was carried out at the research area of University of Agriculture Faisalabad, utilizing RCBD with three replications. A spacing of 6 inches between plants and 2.5 feet between rows was maintained. All essential precautions were taken to protect the plants from biotic and abiotic stresses.

Data recorded and estimation of Zn kernel content: In each replication, five plants were randomly chosen and tagged for observation. At full maturity the cobs from tagged plants were harvested and data for the Zn, morphological and yield traits such as ear diameter (ED), cob length (CL), number of kernel rows per cob (NKRC), number of grains per cob (NGC), plant height (PH), hundred kernel weight (HKW), cob diameter (CD), kernel yield (KY), zinc (Zn), and ear height (EH) were recorded. Harvested ears with husks were dried in the shade to reduce kernel moisture content to 14% post-harvest. Representative grain samples were taken and ground into a fine powder using a Cyclotech. To determine kernel zinc (Zn) concentration, a digestion process using a 7:3 diacid mixture (HNO3: HClO4) was carried out following the atomic absorption spectrometry (AAS) method (Zarcinas *et al.*, 1987). The Kjeldahl Apparatus was used to digest grain samples. The Zn concentration in the prepared samples was measured with an atomic absorption spectrophotometer, according to (Anon., 1990). The instrumental handling conditions for Zn are detailed in (Table 2).

Table 1. List of inbred lines of maize used in experiment.

S. No.	Inbred lines	S. No.	Inbred lines	S. No.	Inbred lines
1.	374 Zai	18.	354 Zai	35.	455 Zai
2.	370 Zai	19.	352 Zai	36.	747 MMRI 24
3.	375 Zai	20.	347 Zai	37.	749 4B-9
4.	388 Zai	21.	342 Zai	38.	750 210
5.	379 Zai	22.	340 Zai	39.	748 4
6.	382 Zai	23.	344 Zai	40.	751 HSRZ
7.	367 Zai	24.	343 Zai	41.	752 231
8.	377 Zai	25.	462 Zai	42.	753 4B-15
9.	366 Zai	26.	358 Zai	43.	754 4B-6
10.	371 Zai	27.	364 Zai	44.	755 223
11.	376 Zai	28.	363 Zai	45.	736 HBR-4
12.	364 Zai	29.	460 Zai	46.	737 339
13.	381 Zai	30.	467 Zai	47.	738 MMRI-32
14.	359 Zai	31.	456 Zai	48.	739 4D-3
15.	358 Zai	32.	470 Zai	49.	740 MMRI-21
16.	363 Zai	33.	469 Zai	50.	741 MMRI-26
17.	348 Zai	34.	468 Zai		

Table 2. The experimental conditions utilized in Zn investigation by AAS.

Parameters	Set values
Burner head	Standard type
Lamp current (mA)	10.0
Flame	Air-C_2H_2
Burner height (mm)	7.5
Slit width (nm)	1.3
Fuel gas pressure (Flow rate) (kpa)	6
Wavelength (nm)	213.9
Oxidant gas pressure (Flow rate) (kpa)	160

Statistical analysis

The recorded data from each replication for the recorded traits was average. The analysis of variance was calculated by using Statistix 8.1 (Steel *et al.*, 1997). The correlation among the recorded traits was obtained by using mean values R package "psych." The principal component analysis was performed by using approach given by Iqbal *et al.*, (2015).

Results

Analysis of variance: Collected data from the recorded morphological, yield and biochemical traits were subjected to analysis of variance and significant differences were observed for all the recorded traits except hundred kernel weight which showed non-significant differences in present experiment (Table 3). The highest variations were recorded for the traits number of grains per cob (67.64), followed by kernel yield (48.77) and ear height (22.5), at same time the traits zinc content (5.99), plant height (9.13) and the number of kernel rows per cob (9.85) was observed with lowest variations.

Estimation of zinc in inbred lines: The zinc content in maize inbred lines was measured using atomic absorption techniques. According to the average zinc concentrations it was found that the inbred lines 468 Zai, 738 MMRI 32, 749 4B-9, 739 4B-3, 737-339, and 476 Zai exhibited higher levels of zinc content (Fig. 1).

Table 3. Analysis of variance based mean squares of recorded traits.

Traits	ED	CL	NKRC	NGC	PH	HKW	CD	KY	Zn	EH
SS	2837.28	294.25	434.16	524686	29365.4	1399.99	324.69	219532	1320.49	12688.1
MS	59.11	6.13021	9.045	10930.9	611.779	29.1664	6.76438	4573.58	27.5102	264.335
F	1.66*	1.54**	7.31**	1.62*	4.27**	1.25	3.06**	3.09**	21.23**	1.73*
CV	18.29	13.93	9.85	67.64	9.13	21.93	10.2	48.77	5.99	22.5

****:** Significant at 1% level and ***:** Significant at 5% level. ED= Ear diameter (cm), CL= Cob length (cm), NKRC= Number of kernel rows per cob, NGC= Number of grains per cob, PH= Plant height (cm), HKW= Hundred kernel weight (g), CD= Cob diameter (cm), KY= Kernel yield (g), Zn= zinc (ppm), and EH= Ear height (cm)

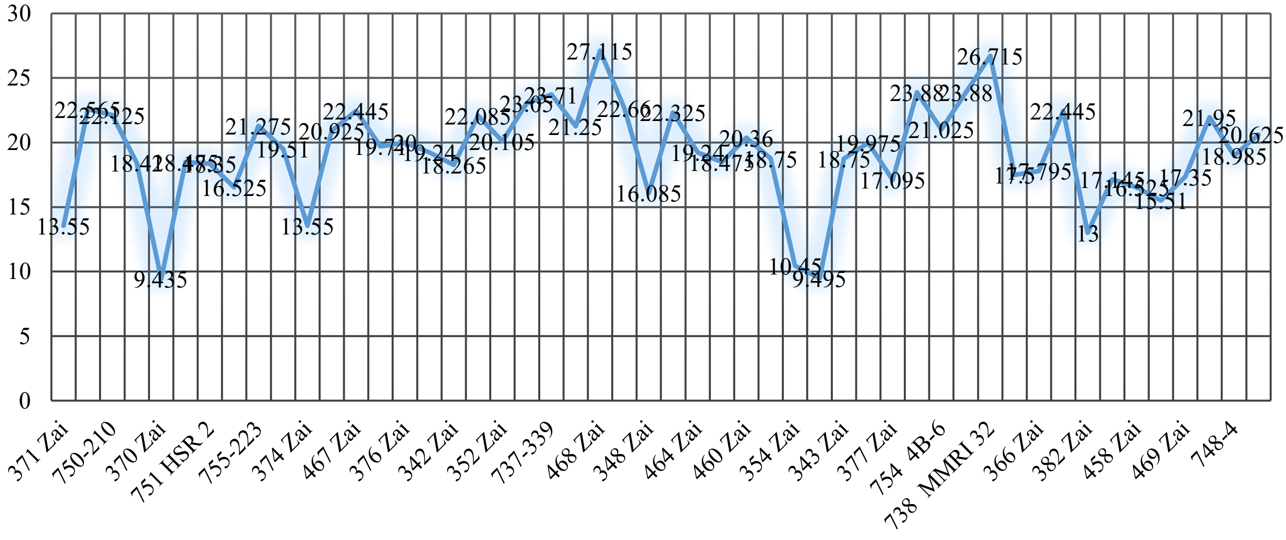

Fig. 1. Concentrations of Zn kernel content in inbred lines of maize.

Correlation analysis: The results presented in (Fig. 2) indicate that the Zn content exhibited a positive correlation with traits HKW, NKRC, NGC, CL, ED, and EH while negative correlation was shown with the traits PH, CD, and KY. Interestingly, PH showed a negative association with Zn but displayed positive associations with all the other variables, including a highly significant positive association with CD, EH, and HKW. The cob diameter showed negative correlation with Zn and postive with all remaining traits. The number of grains per cob, hundred kernel weight and ear height showed positive association with all the recorded traits. The number of kernel rows per cob obserevd in negative association with KY, but a positive association with all other traits. Cob length had a negative correlation with KY and a positive correlation with other characters. Ear diameter was found to have a positive association with all selected traits except KY with negative association. Kernel yield exhibited a positive association with EH, NGC, CD, PH, and HKW, but a negative association with Zn, ED, CL, and NKRC.

Principal component analysis: Principal component analysis was conducted using quality, yield and morphological traits. Tables 4 and 5 presents the eigenvalues, percentage variance, cumulative percentage variance, and factor loadings for the various studied characters. The scree plot in Figure 3 revealed that the eigenvalues of three principal components (PCs) exceeded more than one. These three PCs explain the variation 65.3% contained in the data among the inbred lines, as shown in Table 4. PC1 contributed 34% to the total divergence of the study population. Notably, PC1 was primarily influenced by the following traits NGC (0.456), CD (0.443), ED (0.404), NKRC (0.362), CL (0.342), PH (0.313), EH (0.255), HKW (0.094), KY (0.092), and Zn (0.039). PC2 contributed 19.5% of the total variability of the studied traits among the inbred lines. The main factors contributing to PC2 were Zn (0.363), NKRC (0.266), CL (0.244), NGC (0.232), and ED (0.157), while HKW (-0.068), CD (-0.160), EH (-0.367), PH (-0.459), and KY (-0.531) had negative weights. PC3 contributed 11.8% to the total variability of the recorded traits among the inbred lines. The main contributing factors to PC3 were Zn (0.618), EH (0.543), PH (0.283), HKW (0.057), and CL (0.162), while ED (-0.107), CD (-0.171), NKRC (-0.188), NGC (-0.214), and KY (-0.298) had negative contributions to this component.

The PCA biplot reveals that the inbred lines 736 HBR-4, 374 Zai, 354 Zai, and 464 Zai exhibit a wide range of characteristics. All the observed variables show a positive correlation in the 1st principal component. In the 2nd component, there is a negative association observed for the traits HKW, PH, EH, KY, and CD (Fig. 4). The inbred lines in PC2 and PC3 contributed more for Zn content. The 1st principal component is influenced by the inbred lines 464 Zai, 468 Zai, 374 Zai, 455 Zai and 359 Zai while 2nd principal component influenced by the inbred lines 752-231, 463 Zai, 354 Zai, 736 HBR-4 and 462 Zai. Third principal component influenced by the inbred lines 382 Zai, 467 Zai, 738 MMRI 32, 463 Zai and 371 Zai. PCA biplot shows relationship among the variables and genotypes (Fig. 3). The longer arrows of Zn, PH, KY, CD, NGC and NKRC indicate the stronger influence of these traits on the principal components.

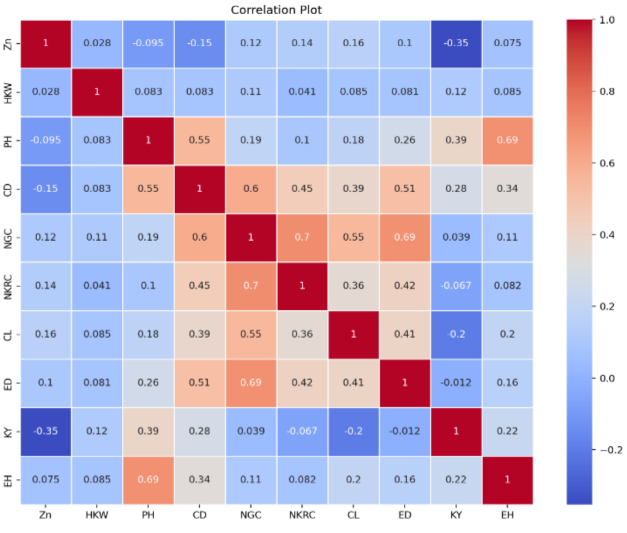

Fig. 2. Correlation analysis of Zn content and other morphological traits in maize.
ED= Ear diameter (cm), CL= Cob length (cm), NKRC= Number of kernel rows per cob, NGC= Number of grains per cob, PH= Plant height (cm), HKW= Hundred kernel weight (g), CD= Cob diameter (cm), KY= Kernel yield (g), Zn= Zinc (ppm), and EH= Ear height (cm).

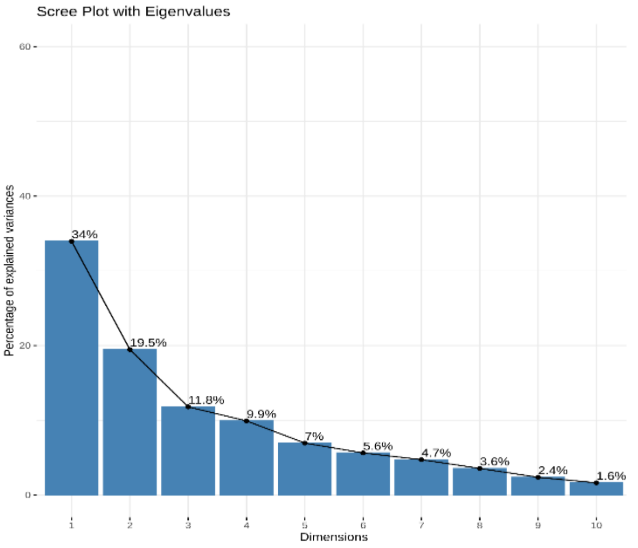

Fig. 3. Scree plot for principal component analysis of maize inbred lines.

Discussion

ANOVA of maize inbred lines is critical tool for addressing the genetic variability and identifying potential for breeding improvements. It aids in the identification of significant variations for targeted traits which is essential for selecting the superior accession for the hybridization programs. The present study resulted in nonsignificant differences for hundred kernel weight which are in line with the results of Rahman *et al.*, (2012). Significant differences were observed for the remaining traits, indicating that selecting lines based on these parameters would be beneficial for improving these traits. The results of the present experiment agree with the findings of (Menkir, 2008; Al-Naggar *et al.*, 2022).

The Zinc content inmmaize kernels was determined by using the technique Atomic Absorption Spectroscopy technique and the genotypes was identifie d with higher Zn content in present eThese results are inline with the findings of (Cömertpay *et al.*, 2016; Langyan *et al.*, 2022) in maize crop. These genotypes would be helpful in breeding programs for the development of Zn enriched maize genotupes.

Correlation analysis for maize yield traits involves examining the association among various morphological and yield traits. It helps the researchers in identifying the most influential yield traits for breeding programs to enhace the productivity of crops. Ogunniyan & Olakojo, (2014) observed positive associations between kernel yield and plant height of miaze crop similar to our experiment. Positive association of kernel yield with PH, EH, HKW, CD and CL was oberevd by Keerthana *et al.*, (2022) in maize crop which are in agreement to our experiment. Reults based on our experiment suggestts that by focusing on these traits kernel tield could be enhanced. Negative association of Zn with kernel yield in rice crops was obserevd by Tripathy *et al.*, (2020) similar to our experiment suggesting that enhancing zinc levels may adversely affect the kernel yield. The positive correlation between Zn and most yield traits in this experiment indicates that enhancing these traits could lead to an increase in Zn concentration in maize kernels.

Principal Component Analysis (PCA) is highly reliable and objective evaluation and is widely utilized method for evaluating and comprehensively assessing germplasm resources in various crops (Hu *et al.*, 2024). In present study ten different traits was evaluated in 50 maize imbred lines and three components showed the eigen value more than one and contributed 65.3% to the total variation. The inbred lines contributed contributed positively for Zn content in first three components. First three components showed the eigrn values more than one and explain 65.3% variation. Ali *et al.*, (2015) also resulted three componetns with with eigen values more than one for maize crop similar to our experiment. The PCA biplot analysis displays the variables with the longer length of vectors indicates the contribution of these traits in variation (Sultana, 2019). The biplot analysis revealed that the traits Zn, PH, KY, CD, NGC, EH and NKRC indicates the stronger influence of these traits in differentiation of inbred lines. Contrary to this the traits CL, ID and HKW with short arrows were the least descriminator. These reults of present experiment are in agreement with the findings of (Belalia *et al.*, 2019). The traits with longer vectors are the most important for the evaluation of inbred lines in maize crop while traits with short vectors are leat important for the evaulation. The PCA based groupimg of maize inbred lines of present study are in line with the findigns of (Mijangos-Cortes *et al.*, 2007; Jaric *et al.*, 2010) who identified that plant height, ear height and kernel yiled are the most distinguishing traits for the characteristing the maize crop. Aslam *et al.*, (2024) also identified Zn content as the major discriminating traits for the characterization of maize germplasm similar to our results. the identification of high level genetic diversity could be used for maize improvement and inbred lines with higher zinc contents are valuable for breeders aiming to develop biofortified maize.

Table 4. Pricnciple component analysis for Zn and other morphological traits of maize inbred lines.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Zn	0.039	0.363	0.618	-0.082	0.523	-0.141	-0.331	-0.265	0.008	0.046
HKW	0.094	-0.068	0.057	-0.972	-0.095	0.045	0.156	-0.048	-0.012	0.0137
PH	0.313	-0.459	0.283	0.116	-0.021	-0.033	0.128	-0.197	-0.71	-0.189
CD	0.443	-0.160	-0.171	0.091	-0.058	0.003	0.054	-0.731	0.425	0.134
NGC	0.456	0.232	-0.214	-0.037	0.127	-0.013	-0.116	0.165	0.099	-0.789
NKRC	0.362	0.266	-0.188	0.0352	0.371	0.614	0.230	0.141	-0.214	0.355
CL	0.342	0.244	0.162	0.009	-0.647	0.153	-0.537	0.104	-0.108	0.205
ED	0.404	0.157	-0.107	0.011	0.044	-0.738	0.230	0.276	-0.058	0.345
KY	0.092	-0.531	-0.298	-0.118	0.369	-0.039	-0.629	0.196	0.020	0.180
EH	0.255	-0.367	0.543	0.097	-0.008	0.169	0.205	0.424	0.493	0.004

ED= Ear diameter (cm), CL= Cob length (cm), NKRC= Number of kernel rows per cob, NGC= Number of grains per cob, PH= Plant height (cm), HKW= Hundred kernel weight (g), CD= Cob diameter (cm), KY= Kernel yield (g), Zn= zinc (ppm), and EH= Ear height (cm)

Table 5. Summary of principal component analysis in maize inbred lines.

Importance of components	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Eigen value	3.371	1.928	1.190	0.989	0.683	0.581	0.466	0.355	0.237	0.195
Standard deviation	1.842	1.395	1.086	0.995	0.833	0.750	0.687	0.596	0.486	0.404
Proportion of variance	0.339	0.194	0.117	0.099	0.069	0.056	0.047	0.035	0.023	0.016
Cumulative proportion	0.339	0.534	0.652	0.751	0.820	0.877	0.924	0.959	0.983	1.000

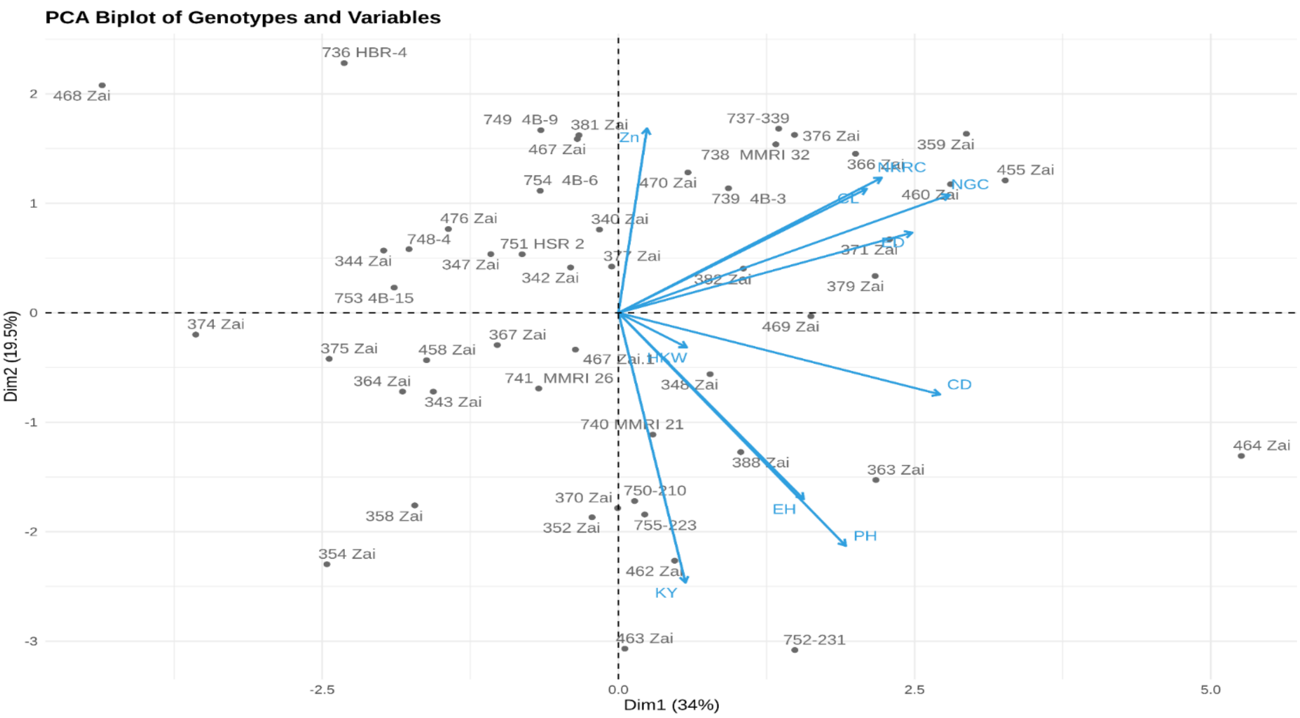

Fig. 4. PCA Biplot of morphological and biochemical traits of maize inbred lines
ED= Ear diameter (cm), CL= Cob length (cm), NKRC= Number of kernel rows per cob, NGC= Number of grains per cob, PH= Plant height (cm), HKW= Hundred kernel weight (g), CD= Cob diameter (cm), KY= Kernel yield (g), Zn= zinc (ppm), and EH= Ear height (cm)

Conclusion

The average zinc concentrations showed that the inbred lines 468 Zai, 738 MMRI 32, 749 4B-9, 739 4B-3, 737-339, and 476 Zai had significantly higher levels of zinc. These specific inbred lines, known for their superior zinc concentrations and improved bioavailability, have potential for use in breeding programs that aim to develop maize hybrids with enhanced zinc content. By including these high-zinc lines, it is possible to produce maize varieties that can better address nutritional deficiencies and contribute to improved public health outcomes.

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