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IRON-ZINC BASED GENETIC DIVERSITY ASSESSMENT IN MAIZE (*ZEA MAYS* L.) GENOTYPES

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SUMMARY

Hidden hunger is one of the most important challenges of the current era, and genetic biofortification is the most feasible, cheapest, and sustainable way to provide a balanced diet to the community. Given the value of biofortification in food grains, the relevant study sought to screen maize inbred lines for kernel Fe and Zn contents and estimate their bioavailability using molar ratios. One hundred maize inbred lines planted during spring 2018 in soil contained optimal levels of Fe and Zn. Maize genotypes evaluation comprised plant height, days to tasseling, silking, maturity, cob length, number of rows per cob, grains per row, grains per cob, 100-grain weight, grain yield per plant, grain Fe, Zn, and phytic acid contents. Significant differences emerged for all the studied traits. The results of the correlation study indicated that grain Fe and Zn contents had a positive genetic link with each other while a non-significant negative association with phytic acid and grain yield. A substantial positive correlation of grain yield occurred with rows per cob, grains per row, and grains per cob. Cluster and principal component analyses ran through, with PA/Fe and PA/Zn molar ratios calculated to estimate the mineral bioavailability. Based on the genetic variability for grain yield, Fe, Zn, and PA contents, four clusters resulted, and the first two PCs had an eigenvalue of more than one and depicted 76.91% of the total variance. Genotypes M-11, M-41, M-45, M-56, M-60, M-61, M-66, M-80, M-96, and M-98 showed high Fe and Zn contents with low molar ratios and are potential to benefit further breeding programs to develop biofortified maize hybrids.

Key words: Biofortification, principal component analysis, iron, zinc, phytic acid

Key findings: An occurrence of high genetic variability in maize inbred lines for Fe-Zn contents indicated that genotypes having high concentrations of these minerals with good bioavailability could serve in developing biofortified maize hybrids. A positive correlation between Fe and Zn contents recommends the possibility of increasing both the micronutrients simultaneously.

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INTRODUCTION

Maize is not a staple of Pakistan; however, it ranks as the second most important cereal crop after wheat (GOP, 2022). Pakistan is currently self-sufficient in maize production for domestic needs. More than 60% of grain production presently serves as poultry feed (Khan *et al.,* 2020). Hence, Pakistan is an indirect consumer of maize. The poultry industry is one of the most valuable sectors of the Pakistani economy. Poultry contributes 35% in total meat production in Pakistan and is the cheapest source to meet the demand and supply gap of meat protein. Feed is the most expensive part, i.e., 60%–70% of total expenses, while cereals and oilseed meals are major constituents of feed and the source of macro and micronutrients. (Asghar *et al.,* 2018; Mallick *et al*., 2020; Guevarra *et al*., 2022; Ali and Alshugeairy, 2023). In plant sources, required micronutrients are in minute quantities but are essential for metabolic processes (Yousaf *et al.,* 2018).

About two billion individuals suffer from micronutrient deficiency causing mental abnormalities, poor health and development, low productivity, and even death. Its effects are more drastic in the first 1,000 days of a child’s growth, from conception to two years (Victora *et al.,* 2008). Iron and zinc deficiencies are the most prevalent and life-threatening micronutrient deficits worldwide. Iron (Fe) deficiency causes anemia, leading to maternal and prenatal mortality and neurodegenerative diseases (Gozzelino and Arosio, 2016). In Pakistan, reports have revealed that more than 51% of women of reproductive age and 28.6% of children younger than five are anemic (Ali *et al.,* 2020). Zinc (Zn) deficiency causes hypogonadism, anorexia, depression, and cognitive dysfunction and reduces immunity (Prasad, 2008). About 47% of pregnant women and 18.6% of children under five years are zinc deficient in Pakistan (Asghar *et al.,* 2018).

Combating Fe and Zn deficiencies can result in diets with high and bioavailable Fe and Zn contents. Fortification has a nearly century-long record of success and safety, proven effective for preventing specific diseases, including birth flaws (Tulchinsky, 2010). Successful biofortification by conventional means depends solely on the available genetic diversity in the primary, secondary, and tertiary gene pools (Garg *et al*., 2018). High genetic variability in maize grains for Fe (19.31–50.64 ppm) and Zn (12.60–37.18 ppm) contents have had reports from many scientists that ensure the success of a breeding program to develop micronutrients-enriched maize genotypes (Sharma *et al.,* 2020; Fernandez *et al*., 2023; Mukhlif *et al*., 2023). A significant positive correlation between Fe and Zn indicates that micronutrient improvement can occur together (Akinwale and Adewopo, 2016). With the increasing prevalence of micronutrient deficiencies, this valuable study aimed to screen maize genotypes for high grain Fe and Zn and low PA contents.

MATERIAL AND METHODS

Experimental materials comprised 100 maize inbred lines collected from the Maize and Millets Research Institute, Yousafwala, Sahiwal, Pakistan, and planted in research areas of the Department of Plant Breeding and Genetics, College of Agriculture, University of Sargodha, Pakistan. Before sowing, soil analysis ensued to check Fe and Zn micronutrient concentrations. The soil analysis report disclosed the Fe and Zn concentrations in the soil at 5.04 and 0.98, respectively, then considered adequate (Estefan *et al.,* 2013). During the spring of 2018, seeds of maize inbred lines coded as M-1 to M-100 and sown in 20" × 14" sized polythene bags contained 20 kg soil mixture per bag. Five seeds per genotype per bag were planted in three repeats using a completely randomized design. Retention of three plants per bag continued after germination. Compliance with standard agronomic practices like proper sowing time, application of fertilizers, hoeing, irrigations, and plant protection measures transpired throughout the experiment. Plants selfing of each genotype ensued until their harvesting separately at physiological maturity.

Plant parameters

At proper stage, data recording of five plants per genotype per replication ran for plant height in cm (at maturity), days to tasseling (from date of sowing to date of tassel emergence), days to silking (from date of sowing to date of silk emergence), days to maturity (from date of sowing to date of physiological maturity), cob length (cm), number of rows per cob, grains per row, grains per cob, 100-grains weight (g), grain yield per plant (g), grain Fe content (mg/kg), grain Zn content (mg/kg), and grain phytic acid content (mg/100g). Data collection of these traits happened after harvest.

Estimation of grain Fe and Zn contents (mg/Kg)

Kernel Fe and Zn contents (ppm) calculation used the wet digestion method given by Estefan *et al.* (2013). Digesting oven-dried grounded seed sample of one g weight proceeded with 15 ml of HNO3-HCLO4 (2:1) as digestion mixture. After adding the acid mixture and leaving the specimens overnight, digestion followed on a hotplate at 350 °C for 1-2 h until a colorless solution appeared. The increase in the volume to 15 ml required using distilled water. Filtered samples underwent analysis using atomic absorption spectrophotometer (Shimadzu AA-6300, Japan) according to standards.

$$\text{Fe, Zn (ppm)} = \text{Fe, Zn (ppm from calibration curve)} \times \text{Dilution factor}$$

The prepared standards employed commercially available aqueous (1000 ppm) stock solutions (Certipur®) of Fe and Zn and purified de-ionized water.

Phytic acid content (mg/100g)

The Modified Holt method (Harland and Morris, 1995) helped determine phytic acid estimation. Ground seed sample of one g attained digesting with 20 ml of HNO_3 by continuous shaking, with 1 ml filtrate separated and 0.4 ml distilled water and 1 ml ferric ammonium sulfate (21.6%) added to the filtrate. Samples placed in a boiling water bath for 20 min received 5 ml of isoamyl alcohol after cooling. Adding 0.1 ml of ammonia solution to the specimens continued with centrifuging at 3000 rpm for 10 min. Alcoholic layer separation followed, with the color intensity read at 465 nm on a spectrophotometer with standards against amyl alcohol blank. The phytic acid calculation resulted from a calibration curve of benchmarks made from sodium phytate (P-8810, Sigma-Aldrich, St. Louis, USA).

Statistical analysis

Data recorded from different plant traits underwent analysis of variance (ANOVA) and correlation coefficients estimation using Statistix 8.1. Cluster and principal component analyses performed XLSTAT (Microsoft Excel data analysis add-on).

RESULTS AND DISCUSSION

Analysis of variance

Analysis of variance (ANOVA) estimates extended for plant height (PH), days to tasseling (DT), days to silking (DS), days to maturity (DM), cob length (CL), rows per cob (RC), grains per row (GR), grains per cob (GC), grain yield per plant (GY), 100-grain weight (100GW), iron (Fe), zinc (Zn), and phytic acid (PA) to observe the variation among maize genotypes. The results revealed significant differences ($P \leq 0.05$) among maize genotypes for all the studied traits. Mean squares, genotypic and phenotypic variances, and broad

Table 1. Mean squares, genotypic and phenotypic variances, and broad sense heritability among the maize genotypes for the studied traits.

Plant Trait	MS	EMS	σ^2 G	σ^2 P	h^2 $_{(BS)}$ %
DF	99	200			
Plant height	536.07**	46.63	163.14	209.78	77.77
Days to tasseling	15.16**	7.42	2.58	10.00	25.81
Days to silking	15.76**	7.93	2.61	10.54	24.76
Days to maturity	25.54**	6.97	6.19	13.16	47.04
Cob length	8.09**	1.16	2.31	3.47	66.6
No. of rows per cob	8.59**	3.07	1.84	4.91	37.48
No. of grains per row	24.95**	3.46	7.16	10.63	67.40
No. of grains per cob	2704.2**	575.62	709.53	1285.15	55.21
Grain yield per plant	138.55**	29.79	36.25	66.05	54.89
100-grain weight	4.42**	1.29	1.04	2.33	44.73
Iron content	87.94**	10.11	25.94	36.05	71.96
Zinc content	429.08**	17.03	137.35	154.38	88.97
Phytic acid content	196375**	9798	62192.33	71990.33	86.39

Note: MS = Mean squares of genotypes; EMS = Error mean squarer; σ^2G = Genotypic variance; σ^2P = Phenotypic variance; h^2 $_{(BS)}$ = Broad sense heritability.

sense heritability of the studied plant traits are in Table 1. Observations on plant height, cob length, grains per row, grains per cob, grain yield per plant, iron, zinc, and phytic acid contents showed high broad sense heritabilities (55% to 89%), suggesting that genetic improvement in these traits can be possible through simple selection while qualities having moderate $h^2_{(BS)}$ like days to maturity, grains per cob, grain yield per plant, and 100-grain weight demand more attention and skill during selection to improve them genetically. Study findings are in line with Abrha *et al.* (2013), Kumar *et al.* (2015), and Bhiusal *et al.* (2017). They also observed high genetic variability among maize genotypes for Fe and Zn contents (Bänziger and Long, 2000; Gregorio, 2002; Brkic *et al.,* 2004; Oikeh *et al.,* 2004; Chakraborti *et al.*, 2011). Cichy *et al.* (2005), Simic *et al.* (2009), and Lung'aho *et al.* (2011) also reported that Fe and Zn contents could gain enhancements in maize grains through biofortification with high heritabilities.

Correlation studies of plant traits for screening of maize genotypes

The correlation coefficients of studied plant traits appear in Figure 1. Correlation coefficients indicated that plant height had a highly significant positive association with grain yield-related traits like cob length, number of rows per cob, number of grains per cob, and 100-grain weight, whereas having a significant negative correlation with PA. The remaining traits showed a non-significant relationship with plant height. Days to tasseling have a strong positive correlation with days to silking and days to maturity while having a weak association with the remaining traits, either positive or negative directions, especially Fe, Zn, and PA contents. Days to silking have a significantly positive relationship only with days to maturity and a non-significantly positive correlation with 100-GW and PA but a negative association with all other traits. On days to maturity, non-significantly positive or negative associations occurred with all studied plant traits. Cob length exhibited a significantly positive correlation with RC, GR, GC, and GY though notably negative with PA. Cob length also showed a weak positive association with Fe and Zn contents. The number of rows per cob displayed a strong positive association with GR, GC, and GY and a poor Zn content, yet a non-significantly negative correlation with 100 GW, Fe, and PA. The number of grains per row had a strong positive correlation with SC and GY but a negative with 100 GW, Fe, Zn, and PA. Grain yield per plant and 100-grain weight had negative links with Fe, Zn, and PA. Iron and zinc contents in grain showed a significant

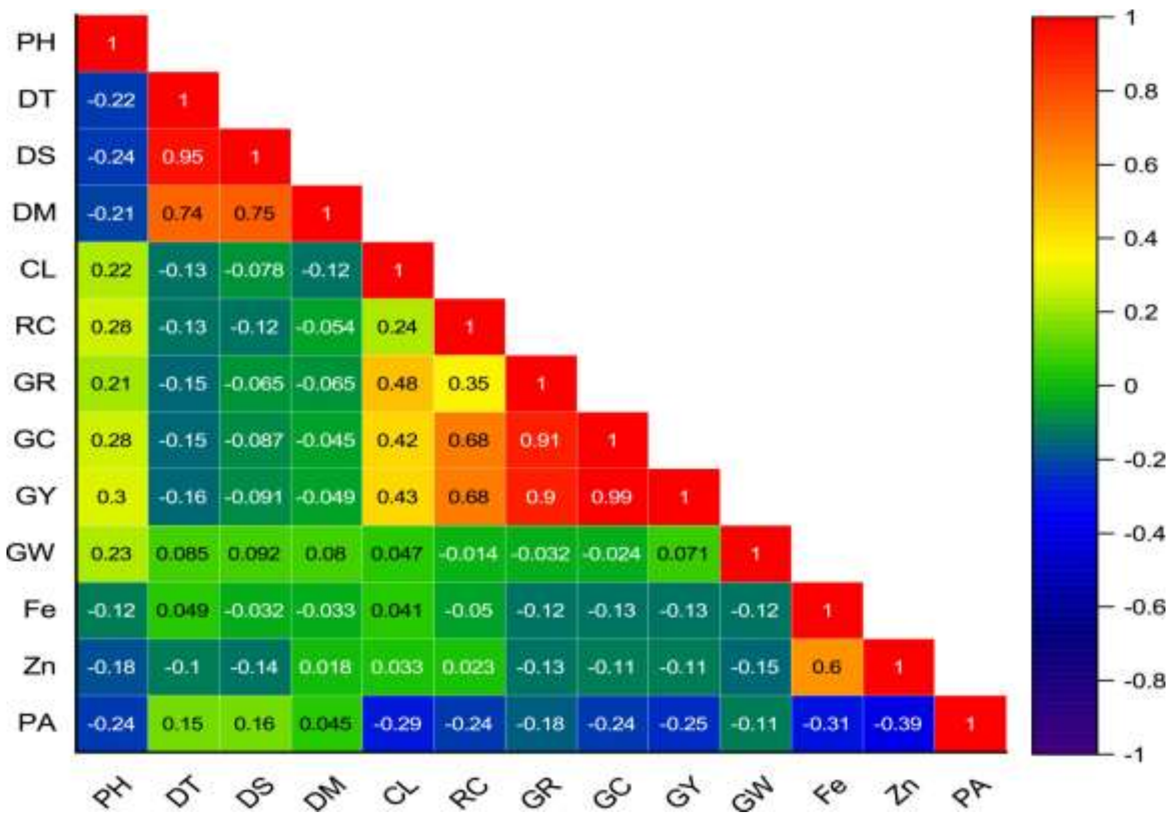

Figure 1. Correlation coefficients among the morphological and quality traits in maize genotypes.
* = Significant ($P < 0.05$); ** = Highly Significant ($P < 0.01$)

Note: PH=Plant height; DT=Days to tasseling; DS=Days to silking; DM=Days to maturity; CL=Cob length; RC=No. of rows per cob; GR=No. of grains per row; GC=No. of grains per cob; GY=Grain yield; GW=100-Grain weight; Fe=Iron contents; Zn=Zinc contents; PA=Phytic Acid contents.

positive correlation with each other and had a negative relationship with PA.

The correlation coefficients study indicated that Fe and Zn contents in all maize genotypes provided a strong positive association with one another, and improving both traits can be done through hybridization followed by selection. Otherwise, phytic acid content related negatively with all the studied plant traits. Results align with the studies of Gregorio (2002), Cakmak *et al.* (2010), and Dragicevic *et al.* (2013). Chukwudi *et al.* (2022) and Govindaraj *et al*. (2022) reported a positive association between Fe and Zn content; however, they found a negative correlation among Fe-Zn and yield-related traits in maize (Akinwale and Adewopo, 2016; Guo *et al.,* 2020; Bojtor *et al.,* 2021).

Cluster analysis

This segment engaged four important plant traits, i.e., grain yield per plant, iron, zinc, and phytic acid contents, to screen 100 maize inbred lines. Cluster analysis assists in checking the genetic diversity in maize genotypes based on these traits. The scrutiny classified inbred lines into four clusters based on their genetic differences, as presented in Figures 2 and 3. Cluster-1 comprised 31 genotypes, which indicated that all inbred lines within a group were genetically similar and showed similar behavior for yield-contributed and quality traits. Similarly, Cluster-2 displayed 21 genotypes, Cluster-3 exhibited 22 genotypes, and Cluster-4 had 26 genotypes (Table 2). Inbred lines of Cluster-1 provided

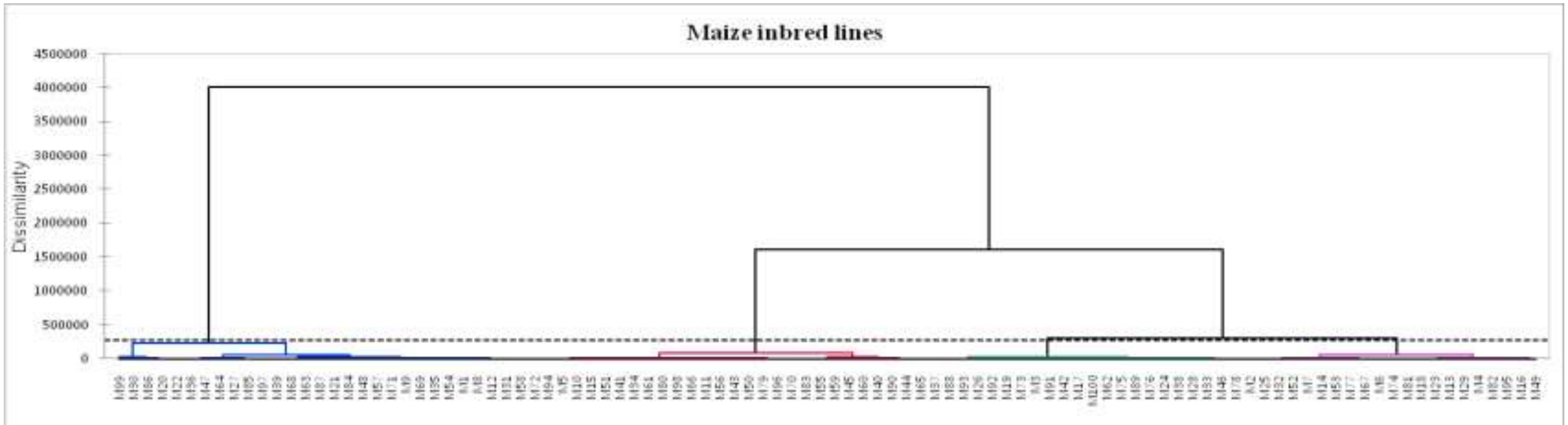

Figure 2. Dendrogram of 100 maize inbred lines obtained through cluster analysis.

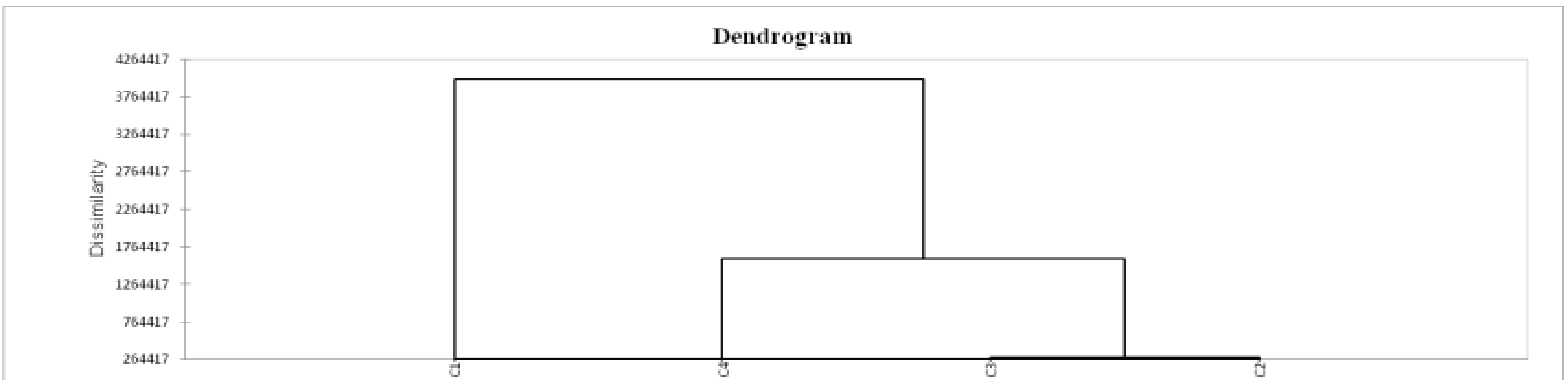

Figure 3. Class distribution of dendrogram making four clusters.

Table 2. Grouping of 100 maize inbred lines into four clusters based on studied plant traits.

Cluster 1	M1: M8: M9: M12: M20: M21: M22: M27: M30: M31: M35: M36: M39: M47: M48: M54: M57: M58: M63: M64: M68: M69: M71: M72: M84: M85: M86: M87: M94: M97: M99
Cluster 2	M2: M4: M6: M7: M13: M14: M16: M18: M23: M25: M29: M32: M49: M52: M53: M67: M74: M77: M81: M82: M95
Cluster 3	M3: M17: M19: M24: M26: M28: M33: M37: M38: M42: M46: M62: M73: M75: M76: M78: M88: M89: M91: M92: M93: M100
Cluster 4	M5: M10: M11: M15: M34: M40: M41: M43: M44: M45: M50: M51: M55: M56: M59: M60: M61: M65: M66: M70: M79: M80: M83: M90: M96: M98

Table 3. Mean values of studied plant traits for all four clusters.

Trait	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Grain Yield	9.44	12.35	10.49	12.55
Fe Conc.	23.81	25.45	24.56	28.50
Zn Conc.	35.95	39.01	38.14	49.51
PA Conc.	1601.64	1202.37	1369.30	972.58

Table 4. Principal component analysis of the studied traits in maize genotypes.

	PC1	PC2	PC3	PC4
Eigenvalue	1.88	1.20	0.54	0.39
Variability (%)	46.95	29.97	13.42	9.67
Cumulative %	46.95	76.91	90.33	100

Table 5. Eigenvectors of the studied trait of maize genotypes.

	PC1	PC2	PC3	PC4
GY	-0.035	0.836	0.540	0.091
Fe	0.607	-0.203	0.457	-0.618
Zn	0.633	-0.133	0.120	0.753
PA	-0.480	-0.492	0.697	0.205

the lowest mean values of Fe, Zn contents, and grain yield per plant, whereas the highest mean value of PA. Cluster-2 exhibited almost average values of all the studied traits, and Cluster-4 gave the highest mean values of Fe, Zn, and GY, with the lowest mean value of PA (Table. 3). The results of cluster analysis implied that inbred lines present in Cluster-1 and Cluster-4 had broad genetic diversity for different studied plant traits and might benefit maize hybridization to develop micronutrients biofortified maize hybrids. Scientists like Liu *et al.* (2006), Subramanian and Subbaraman (2010), Shrestha (2016), and Vivodik *et al.* (2021) also used cluster analysis in maize and grouped the maize genotypes into homogenous subsets.

Principal component analysis

PCA computation ran through 100 maize inbred lines to explain the total variance in the data set and the relationship of response variables. Four PCs extracted were according to the most important traits, i.e., GY, Fe, Zn, and PA. The eigenvalues, total, and cumulative variances are in Table 4. PC1 and PC2 showed eigenvalues of more than one and contributed significantly to the total variability among genotypes. PC1 contributed 46.95% in the total genetic variance mainly associated with Fe and Zn, with factor loadings of 0.607 and 0.633, respectively. PC2 contributed 29.96% to the total variation, with the grain yield per plant (GY) as the major contributing trait, with factor loadings of 0.836 (Table 5). The results of the presented study agree with the findings of Okporie (2008), Shrestha (2016), and Yousaf *et al.* (2018), who also stated that PCs with more than one eigenvalue had the potential use to identify the genetic variation of maize genotypes.

Estimation of iron and zinc bioavailability in maize genotypes

The PA/Fe and PA/Zn molar ratios estimated iron and zinc bioavailability in the food. As a rule, the lower the proportions, the more will be the mineral bioavailability (Gemede, 2020). Thus, PA/Fe and PA/Zn molar ratios in grains of maize genotypes' calculations ranged from 15.72 to 87.25 for Fe and 14.11 to 133.18 for Zn contents (Figure 4). The results revealed that maize inbred lines coded as M-11, M-41, M-43, M-44, M-45, M-55, M-56, M-60, M-61, M-66, M-80, M-81, M-83, M-96, and M-98 exhibited low PA/Fe molar ratio, ranging from 15.71 to 29.93. Similarly, M-11, M-16, M-34, M-41, M-44, M-45, M-55, M-56, M-60, M-61, M-66, M-70, M-80, M-83, M-96, and M-98 showed minimum PA/Zn molar ratio, ranging from 14.11 to 19.79, indicating high bioavailability of Fe and Zn contents in the grains of these genotypes. Therefore, these inbred lines could be useful in developing F_1 hybrids with more Fe and Zn bioavailability in maize kernels than the parents. Negative relationship of molar ratios with bioavailability came from past studies in maize and rice (Ma *et al.,* 2005; Queiroz *et al.,* 2011; Gemede, 2020; Khampuang *et al.,* 2021; Wang *et al.,* 2021).

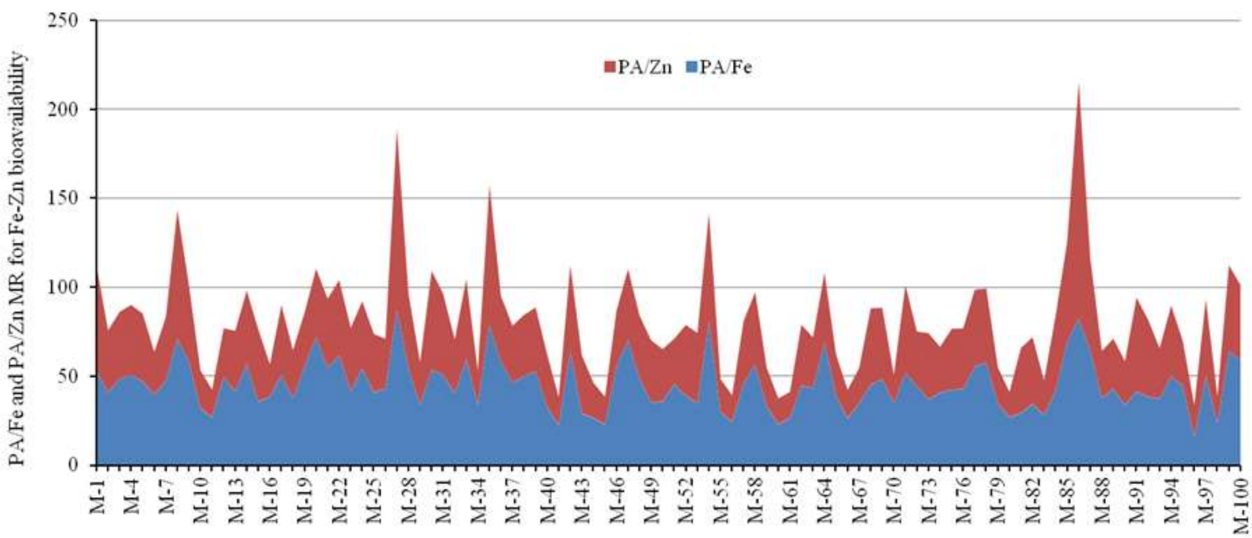

Figure 4. PA/Fe and PA/Zn molar ratios for Fe-Zn bioavailability in maize genotypes.

CONCLUSIONS

Based on the results, the conclusion indicates a possibility to enhance Fe and Zn contents in maize grain through hybridization that will help reduce micronutrient malnutrition in humans through direct consumption of maize while reducing the cost of phytase enzyme in poultry feed. In this study, maize inbred lines M-11, M-41, M-45, M-56, M-60, M-61, M-66, M-80, M-96, and M-98 emerged having high Fe and Zn contents with low molar ratios and have the potential to benefit developing biofortified maize hybrids. However, a negative but non-significant association of Fe-Zn contents with grain yield per plant suggests a slight compromise in grain yield of micronutrients biofortified maize hybrids.

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