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GGE BIPLOT ANALYSIS FOR ZINC QUALITY AND YIELD STABILITY OF EXOTIC MAIZE HYBRIDS

S.H. KHAN[1], M. ASLAM[1,*], A. BIBI[1], and H.Z. KHAN[2]

[1]Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
[2]Department of Agronomy, University of Agriculture, Faisalabad, Pakistan
Corresponding author's email: aslampbg@gmail.com
Email addresses of co-authors: shahamgopang@gmail.com, ameerbibi@gmail.com, haroon679@hotmail.com

SUMMARY

Zinc deficiency is one of the major causes of malnutrition in the communities due to the consumption of zinc-deficient staple food. With accessibility and affordability, biofortification is an agricultural intervention beneficial for all the stakeholders involved as any actor. A total of 16 exotic zinc biofortified maize hybrids developed at different maize research stations of CIMMYT got introduced and evaluated across three separate locations in major maize growing areas of Punjab and Khyber Pakhtunkhwa (KP), i.e., Faisalabad, Sahiwal, and Nowshera. The decision to introduce exotic zinc-enriched maize germplasm in Pakistan, in collaboration with CIMMYT, Mexico, came from the findings of a related research work—evaluation of diverse indigenous maize germplasm; however, none of the entry qualified for suggested biofortification standard, i.e., 33 mg/kg zinc. Introducing exotic material depends upon its yield stability in the new environment. Therefore, stability analysis is mandatory. Using genotype and genotype into environment (GGE) biplot analysis found the GEI (genotype × environment interaction). Exotic hybrids G16, G4, and G1 performed superior and stable in test environments for studied traits, especially for average grain yield per plant and grain zinc content. These three hybrids gained strong\ recommendations for introduction in Pakistan.

Keywords: Biofortification, stability analysis, GGE biplot, HarvestPlus

Key findings: All tested environments differently influenced NGPR, ASI, and GY forming three mega-environments. Exotic zinc biofortified hybrids G16, G4, and G1 were stable and best performing for studied traits, especially average grain yield per plant and grain zinc content across locations.

Abbreviations: PH: plant height (cm), EH: ear height (cm), DT: days to tasseling, DS: days to silking, ASI: anthesis silking interval, EL: Ear length (cm), NRPC: number of rows per cob, NGPR: number of grains per rows, GY: average grain yield per plant (g), TGW: thousand grain weight (g), Zn: grain zinc content (mg/kg), and FW: Field weight (g). PC= Principal Component, ZmZIP = *Zea mays* Zinc regulated transporter, iron regulated transporter-like protein, ZmNAS5 = *Zea mays* Nicotianamine Synthase, and CIMMYT: International Maize and Wheat Improvement Center.

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INTRODUCTION

In Pakistan, 18.6% of children are zinc deficient, with a higher percentage in rural areas than in urban areas. Likewise, about 22.1% of women of reproductive age are zinc deficient (National Nutritional Survey Pakistan, 2018). Some serious health concerns associated with zinc deficiency are poor neurobehavioral development, delayed growth (growth retardation), impaired immune system (Alloway, 2008), anorexia (reduced appetite), diarrhea, hair fall, weight loss, delayed recovery of wounds, hypertension, taste loss, retarded reproductive system, and lesions on eye and skin (Brown *et al*., 2004). In this situation, there is a need to address the problem of zinc deficiency by any means, whether by supplementation, fortification, or biofortification.

Developing a biofortified staple crop is the best strategy to address the problem, even in low-income parts of the population. This practice will provide the required zinc to masses directly through routine intake. Maize is a very popular grain commercial crop of the region. It occupies the second position as far as grain production is concerned. It contributes 3.2% to value addition in agriculture and 0.7% to the country's GDP (GOP, 2022). Given the two growing seasons per annum, it is available throughout the year as a staple food. It supplies 15% of protein and 30% of caloric requirements for the world population daily (Nyaligwa *et al*., 2017). Maize grain can be ground in making tortillas, bread, cakes, beverages, gruel, and porridges. Grain is also consumed as fried, boiled and parched. Its consumption as food directly and in the form of different by-products make it suitable for biofortification. The suggested target for zinc biofortification is 33 mg/kg in maize (Bouis and Welch, 2010).

The use of various approaches tried to achieve the target of high grain zinc content, i.e., conventional breeding, introduction and stability analysis, marker assisted breeding, quantitative trait loci (QTL) mapping, mutation breeding, and quantitative genetics. High genotypic diversity and transgressive segregants for grain zinc content resulted in hybridizing contrasting parents from F2:F3 populations (Qin *et al*., 2012). Genetic analysis of mapping populations under individual and combined environments showed 15 and 16 QTLs on chromosomes 2, 7, and 9 with iron and zinc co-localized QTLs (Qin *et al*., 2012). A total of 64 QTLs for individual and 67 for multi environment emerged during maize grain minerals (Cu, Mn, Fe, and Zn) accumulation study (Zhang *et al*., 2017). A report said 48 putative genes control zinc accumulation in maize grain. These genes include one gene from CE (cation efflux), one from the ferritin family, and 13 from the ZIP family (zinc-regulated transporter, iron-regulated transporter-like protein). The NRAMP (natural resistance-associated macrophage protein) family includes 16 genes. The YS (yellow stripe) family includes 17 genes for iron and zinc transportation to grain (Sharma and Chauhan, 2008). Identification of QTLs and genes controlling grain zinc accumulation in maize used GWAS (Genome-wide association studies). This technique detects variability in genome and relates it to target trait in variant (Maqbool and Beshir, 2019). Hindu *et al*. (2018) reported 20 SNPs significantly associated with grain zinc accumulation. The identified genes can be cloned for zinc biofortification in maize. Success stories of zinc biofortified maize hybrid developed by CIMMYT includes ICTA HB-18, BIO-MZN01, and ICTA B-15, having additional qualities of high yield and protein, large grain, and early maturity (Maqbool and Beshir, 2019).

Having no evidence of indigenous zinc biofortified maize germplasm present in Pakistan, it becomes necessary to introduce such in Pakistan to address the problem of zinc deficiency on a short-term basis. The best strategy is to introduce zinc-biofortified maize germplasm and evaluate them for their stability under different environments. The best stable introduced germplasm should be used in various breeding programs to develop high-yielding zinc biofortified maize hybrids, followed by seed production locally and then approval from authorities for general cultivation in the country.

Stability reflects consistency in plant characters by plants over multiple environments. Stability analysis is a prerequisite for introducing an exotic material. Developing zinc-enriched, high-yielding, and stable material is necessary to attain zinc-biofortified maize. As quantitative traits are more vulnerable to changes in the environment, therefore, it is crucial to work out genotype by environment interaction (G × E). A significant difference of G × E creates problems in releasing a cultivar for a large area. Some environmental factors are uncontrollable, like climate change, while others are controllable to some extent, like edaphic factors. Environmental factors that affect zinc biofortification are mainly edaphic. GGE biplot analysis computes genotypic effects and genotype × environment interaction by one-way analysis of variance (Yan *et al*., 2000). This study aimed to analyze the stability of grain yield and zinc content of exotic maize hybrids in different environments in Pakistan to address the problem of zinc deficiency.

MATERIALS AND METHODS

Description of study

Sixteen zinc biofortified maize hybrids, introduced from CIMMYT, Mexico (Table 1), and two indigenously cultivated maize hybrids (G-17 and G-18) as yield check, gained evaluation at three different environments during the spring of 2021. G-17 and G-18 are single-cross yellow maize hybrids cultivated in all the maize growing areas of Pakistan as high-yielding maize hybrids. None of the zinc biofortified maize hybrid is available locally as a check. The experiments designed in randomized complete block design had three replications.

The experimental locations consisted of 1. The University of Agriculture, Faisalabad (longitude, 72.3308700°; latitude, 31.9744600°; elevation, 192 masl), 2. Maize and Millets Research Institute (MMRI), Sahiwal (longitude, 73.079109°; latitude, 31.418715°; elevation, 152 masl), and 3. Cereal Crop Research Institute (CCRI), Pirsabak, Nowshera (longitude, 74°; latitude, 32° elevation, 288 masl). These locations are major maize-growing areas in Punjab and Khyber-Pakhtunkhwa. The recorded climatological data included the period of February to June 2021, as presented in Table-2.

Table 1. List of exotic zinc biofortified maize hybrids used in the present investigation along with their codes.

Sr. No.	Hybrid	Code	Sr. No.	Hybrid name	Code	Sr. No.	Hybrid	Code
1	TSCTWCZN-1	G1	7	TSCTWCZN-7	G7	13	TSCTWCZN-13	G13
2	TSCTWCZN-2	G2	8	TSCTWCZN-8	G8	14	TSCTWCZN-14	G14
3	TSCTWCZN-3	G3	9	TSCTWCZN-9	G9	15	TSCTWCZN-15	G15
4	TSCTWCZN-4	G4	10	TSCTWCZN-10	G10	16	TSCTWCZN-16	G16
5	TSCTWCZN-5	G5	11	TSCTWCZN-11	G11	17	FH-949 (LC)	G17
6	TSCTWCZN-6	G6	12	TSCTWCZN-12	G12	18	YH-1898 (LC)	G18

Table- 2. Meteorological conditions subjected to multi-location trial of exotic zinc biofortified hybrids of maize – data contains the parameters of average highest temperature, average lowest temperature, rainfall days, precipitation and humidity for MMRI, UAF, and CCRI.

	MMRI					UAF					CCRI				
Month	Avg High Temp. ℃	Avg low Temp. ℃	Rain fall days	Precipitation (mm)	Humidity %	Avg High Temp. ℃	Avg low Temp. ℃	Rain fall days	Precipitation (mm)	Humidity %	Avg High Temp. ℃	Avg low Temp. ℃	Rain fall days	Precipitation (mm)	Humidity %
Feb	26	12	4	13.89	44	23.8	10.4	5	18	50	20	10	10	105.66	46
Mar	32	17	5	18.05	40	29.5	14.9	7	19	46	25	14	12	112.78	44
Apr	38	24	6	14.04	25	36.4	22.1	9	16	29	32	20	15	94.29	38
May	44	31	5	15.38	18	41.7	28.5	5	12	20	38	26	12	64.52	27
Jun	45	34	6	24.37	22	44.3	32.3	6	12	22	42	30	8	42.12	23

All the standard agronomic and management practices followed the country's prescribed procedures for crop production for all sites (Agriculture Department, Government of the Punjab, 2020). Seedbed preparation began by thrice plowing the soil with the cultivator, followed by the rotavator twice. Basal dose application of 100 kg Di-ammonium phosphate and 50 kg of potassium sulfate was on per acre basis. Planting geometry followed the recommended row length of 5 m, row-to-row space of 0.75 m, and plant-to-plant distance of 0.20 m. For weeds control, spraying of Atrazine + s-metolachlor 720 SC had the dose of 800 ml/acre as pre-emergence weedicide at the time of sowing. Applying three bags of urea were in splits at vegetative stage 5 (V5), vegetative stage 9 (V9), and 15 days before tasseling (Punjab Agriculture Department, 2020). Recording of parameters during the study included the following: plant height (PH; cm), ear height (EH; cm), days to tasseling (DT), days to silking (DS), anthesis silking interval (ASI), ear length (EL; cm), number of rows per cob (NRPC), number of grains per row (NGPR), average grain yield per plant (GY; g), 1000 grain weight (g), grain zinc content (Zn; mg/kg) and field weight per plant (FW; g).

Quantification of grain zinc content

Adopting the protocol devised by Zarcinas *et al*. (1987) helped quantify grain zinc content. Taking representative grain samples, these proceeded to grind into fine powder at 0.5 g. Adding Di-acid digestion mixture Nitric acid HNO3: Perchloric acid HClO4 (2:1 per sample) to each flask occurred, afterward covered with aluminum foils. The samples were left overnight to conduct preliminary digestion and achieve a vigorous reaction. Final digestion proceeded in the Kjeldahl apparatus with heating cycles of 150 °C for one hour and 235 °C for a half hour until all fumes disappeared. Diluting the digested samples ensued to make a final volume of 50 ml. Zinc quantification used the Atomic Absorption Spectrophotometer following the conditions described in AOAC (1990).

Soil zinc status of experimental sites

Soil sample collection from each location employed an auger from five different points, randomly, with a depth of 0–30 cm. The thoroughly mixed collected samples, gathered a 0.5 kg final sample, then sent to the soil testing laboratory at Ayub Agriculture Research Institute, Faisalabad, Pakistan, for the soil zinc profile determination. Results appear in Table 3.

Table3. Soil zinc status of experimental sites

Experimental site	Soil Zn analysis	Remarks
UAF	0.80ppm	deficient
MMRI	0.92ppm	deficient
CCRI	0.96 ppm	deficient

Statistical analysis

Employing GGE biplot analysis (Yan and Kang, 2002) aided the assessment of G × E interaction and highlighted which-won-where, best genotype, and best location information using Gen. stat.16 software.

RESULTS

Visualization of Genotype × Environment interaction and site specific performance of exotic hybrids

Following GGE scatter biplot analysis for all traits, zinc biofortified maize hybrids evaluation occurred across multi-locations to find out which hybrid performed the best and which performed poorly at which location. The first two components (PC_1 and PC_2) of biplot were important, describing the maximum variability for traits. The mega-environments highlighting genotype × environment interaction and biplots dissected into different sectors for identification of site-specific performance. The results are presented in Figure 1.

Identification of representative environment for exotic hybrids

Location-based GGE comparison biplot offers identification of ideal location based on representativeness and discrimination power. The most representative location must be located on average tester coordinate (ATC) abscissa, and the most discriminating location must have the longest vector length. The selection of the best locations for each trait was according to the performance of hybrids. For PH, EH, DS, DT, NRPC, and NGPR, the most conducive environment was MMRI, followed by UAF and CCRI. The best location for ASI was CCRI, followed by MMRI and UAF. UAF emerged as the best location for TGW, GY and Zn, followed by MMRI and CCRI. The best location for FW and EL was UAF, followed by CCRI and MMRI (Figure 2).

Screening of stable exotic hybrids

The genotype-based GGE comparison biplot analysis helped to select the best stable hybrid. Representing the average environment axis (AEA) shows a line which passes from the

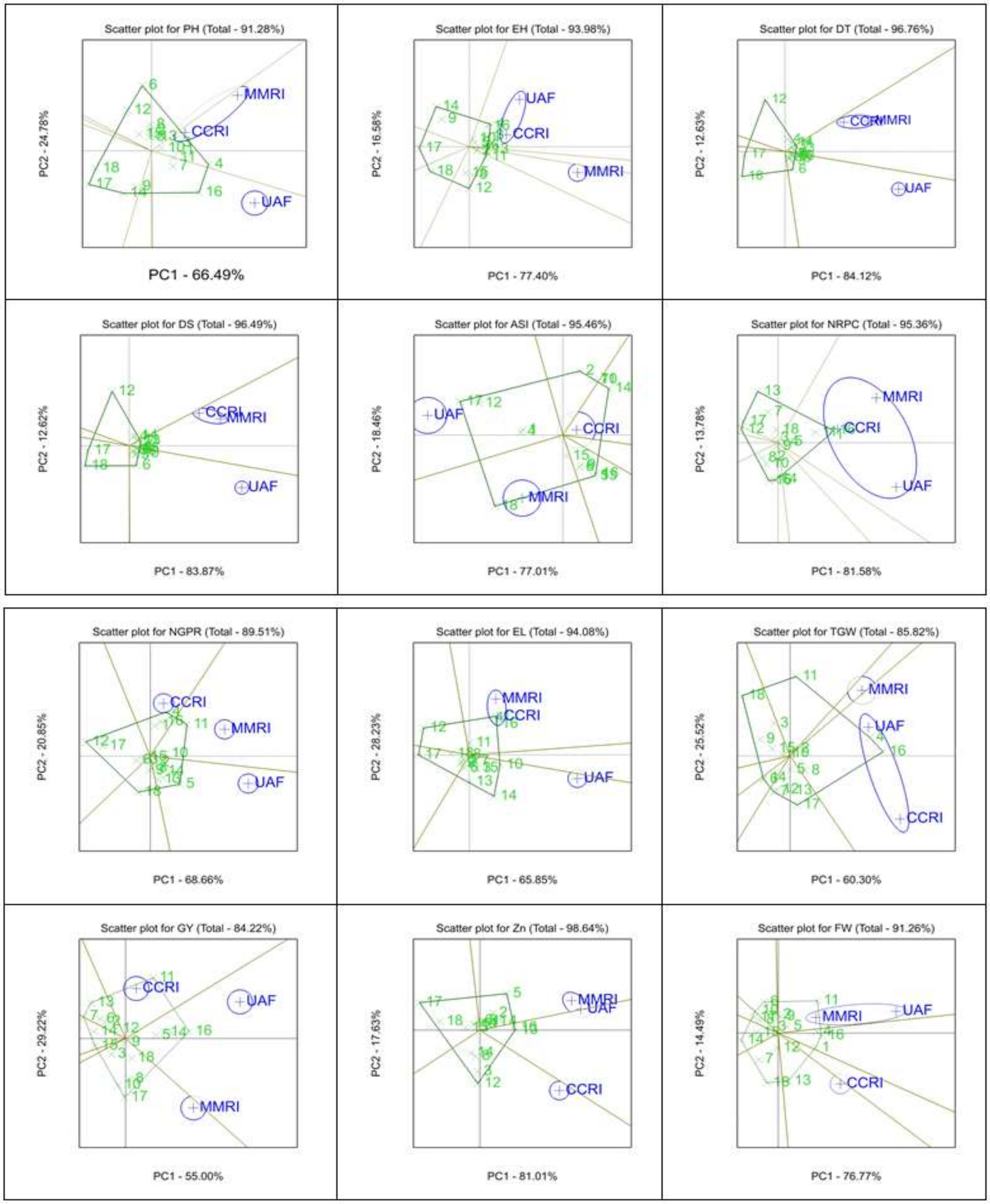

Figure 1. Visualization of which-won-where for the studied traits of exotic Zn biofortified maize hybrids following GGE scatter biplot, where, PH: plant height (cm), EH: ear height (cm), DT: days to tasseling, DS: days to silking, ASI: anthesis silking interval, EL: ear length (cm), NRPC: number of rows per cob, NGPR: number of grains per rows, GY: average grain yield per plant (g), TGW: thousand grain weight (g), Zn: grain zinc content (mg/kg), and FW: field weight (g).

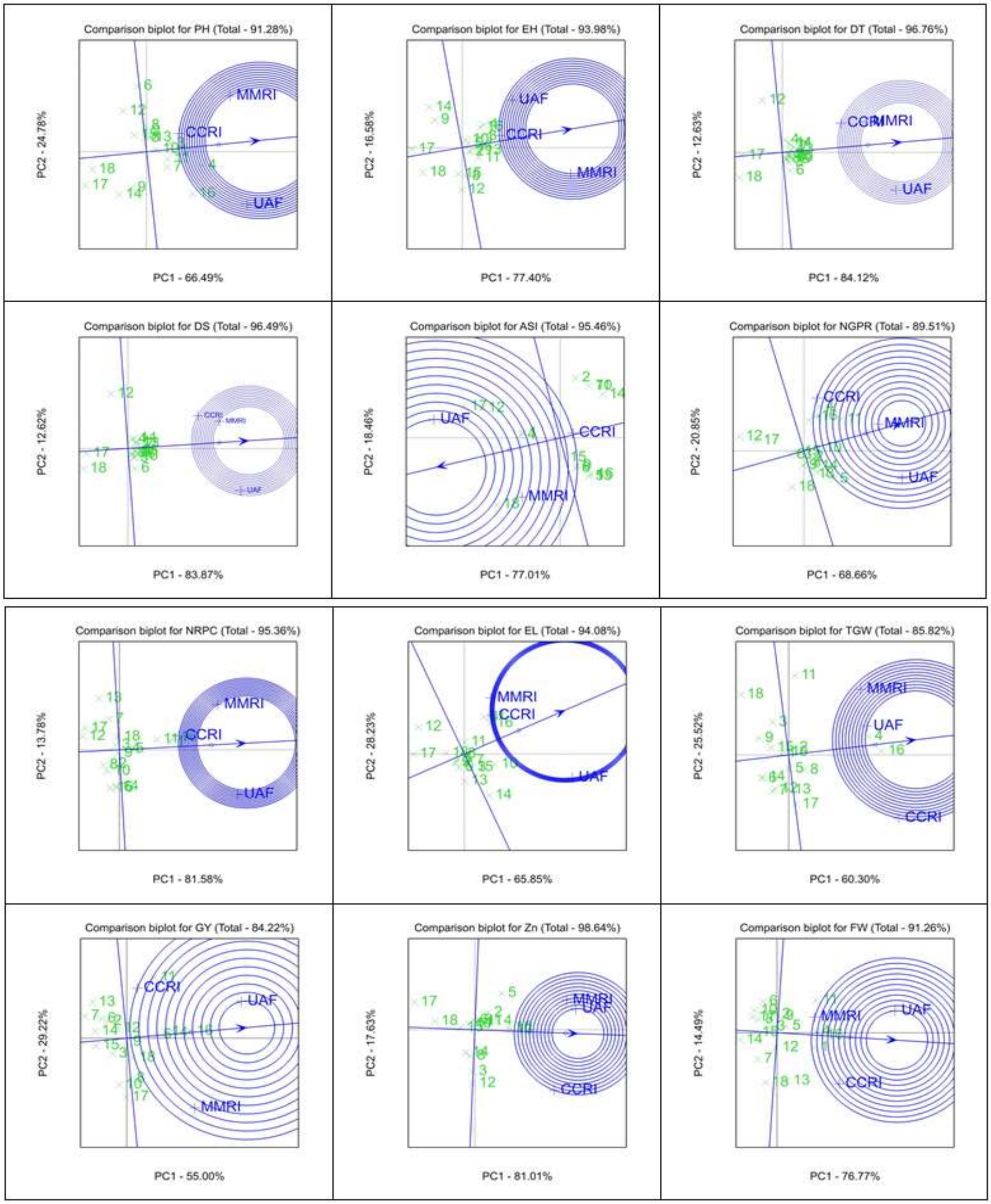

Figure 2. Best location identification for studied traits of exotic Zn biofortified maize hybrids using GGE comparison biplot (environment based), where, PH: plant height (cm), EH: ear height (cm), DT: days to tasseling, DS: days to silking, ASI: anthesis silking interval, EL: ear length (cm), NRPC: number of rows per cob, NGPR: number of grains per rows, GY: average grain yield per plant (g), TGW: thousand grain weight (g), Zn: grain zinc content (mg/kg), and FW: field weight (g).

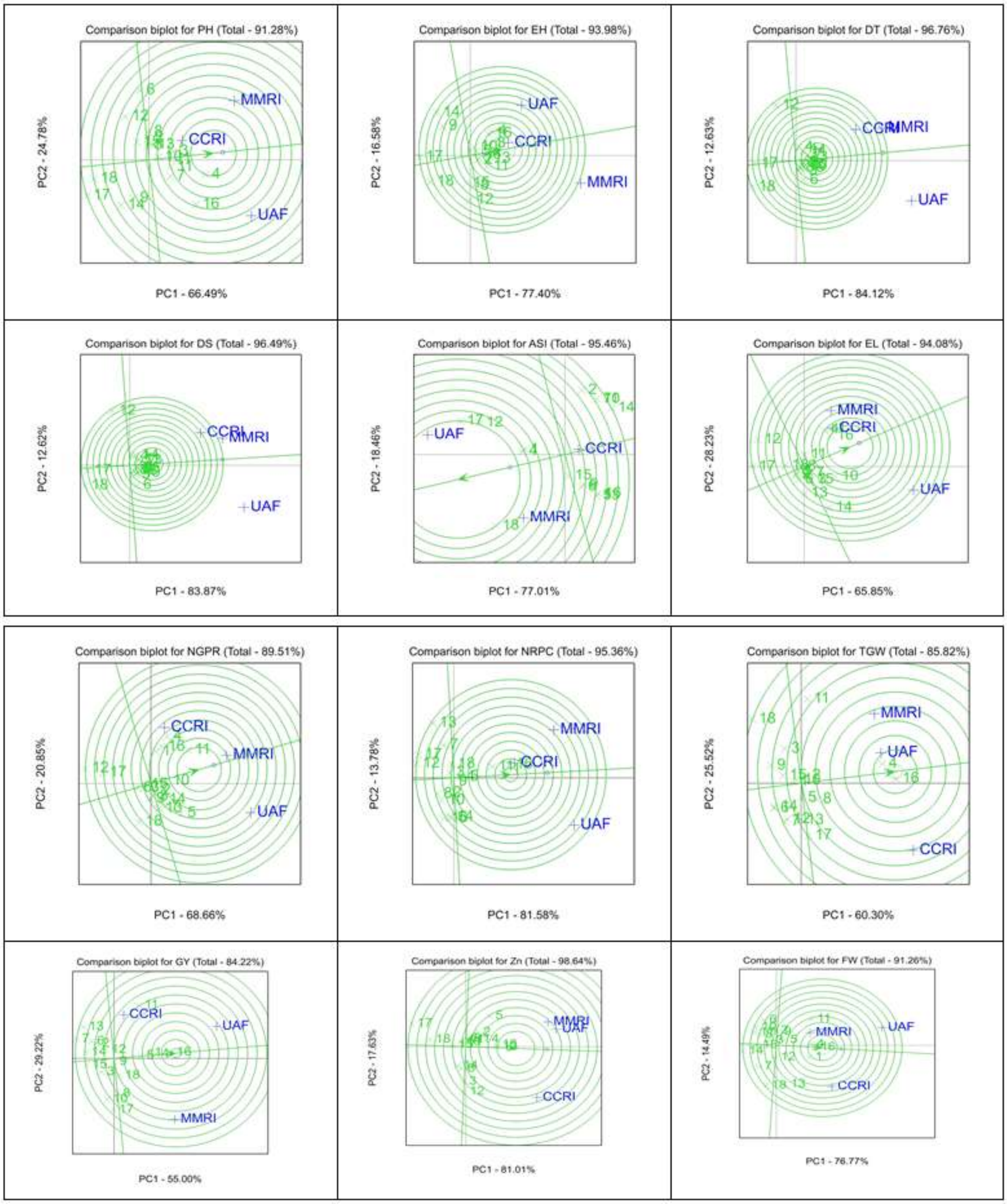

Figure 3. Visualization of best exotic genotypes for studied traits using GGE comparison biplot (genotype based), where, PH: plant height (cm), EH: ear height (cm), DT: days to tasseling, DS: days to silking, ASI: anthesis silking interval, EL: ear length (cm), NRPC: number of rows per cob, NGPR: number of grains per rows, GY: average grain yield per plant (g), TGW: thousand grain weight (g), Zn: grain zinc content (mg/kg), and FW: field weight (g).

circle through the origins in the direction of the abscissa. The average environment ordinate (AOE) is perpendicular to AEA. AOE separates genotypes into two groups based on the mean. Genotypes present on the right side of the AOE have trait performance more than the mean, genotypes on the left side have trait performance less than the mean value, and the genotypes located on the origin have performances equal to the mean. On the AEA axis, in the direction of the arrow, trait performance increases. The AE abscissa helps to identify stable genotypes. Genotypes closer to the AE abscissa are more stable. The best hybrid must be located in the innermost concentric ring or its surrounding. Poor hybrids spread out in the outermost circle. The stable exotic hybrids for studied traits occur in Figure 3.

Mean performance and genotypic comparison

Table 4 presents the mean of 16 exotic and two local hybrids for all studied traits. Grain zinc content ranged from 21.03 to 52.89 mg/kg and the average grain yield ranged from 91.46 to 133 g/plant. Hybrids G6, G4, and G1 performed better than other exotic and indigenous hybrids for GY and Zn traits. All exotic hybrids showed grain zinc content more than the devised criteria of HarvestPlus. Summary statistics highlighted the trends in data. Computation of mean, standard error of mean (SEM), and standard deviation (SD) are in Table 4.

Table 4. Summary statistics and mean comparison.

Hybrid	PH	EH	DT	DS	ASI	EL	NGPR	NRPC	TGW	GY	FW	Zn
1	209.28	115.44	67.89	70.22	2.56	20.80	35.00	21.33	225.57	122.58	243.90	40.46
2	199.44	109.22	67.78	69.67	1.89	15.88	32.89	14.48	231.71	104.98	201.35	43.25
3	211.22	116.61	67.56	69.44	1.89	16.87	31.44	14.98	223.46	107.12	201.87	39.36
4	226.56	117.69	66.67	69.11	2.44	20.23	37.56	19.07	269.33	121.86	242.81	46.43
5	198.11	106.69	68.67	70.56	1.89	15.88	35.33	16.37	228.21	119.56	215.23	47.19
6	196.91	113.58	67.56	69.56	2.00	15.81	30.56	14.99	214.23	99.44	194.16	39.07
7	210.67	109.53	69.89	71.67	1.78	16.90	33.44	14.30	218.68	103.82	197.45	38.54
8	196.67	103.76	66.89	68.89	2.00	16.53	32.56	13.56	235.46	111.87	200.30	38.62
9	189.11	93.13	68.22	70.33	2.11	15.58	31.78	15.00	215.13	111.17	209.73	40.41
10	201.83	107.44	68.00	69.78	1.78	19.14	35.22	13.89	227.31	109.15	193.90	37.65
11	207.11	112.43	68.67	70.44	1.78	17.21	39.56	19.67	236.54	115.99	237.39	41.23
12	186.83	101.56	60.89	63.78	2.89	13.83	23.56	11.67	219.57	109.83	210.43	39.63
13	202.73	115.19	68.78	70.67	1.89	15.70	33.00	13.56	226.17	109.40	216.74	50.21
14	182.31	91.63	68.22	69.89	1.67	17.24	33.33	14.98	214.43	100.55	176.45	37.06
15	190.76	99.28	68.00	70.22	2.22	16.86	31.67	14.22	220.59	102.59	192.30	35.19
16	222.00	116.77	67.00	69.00	2.00	20.47	35.44	22.00	275.34	129.29	246.05	49.98
17	155.83	74.69	52.33	55.00	2.67	12.26	25.78	11.78	229.11	106.70	188.36	21.03
18	161.24	79.44	50.78	53.78	3.00	15.27	30.00	15.33	208.99	112.93	202.04	27.37
Mean	197.14	104.67	65.77	67.89	2.14	16.80	32.67	15.45	228.88	111.05	209.47	39.59
SD	22.89	14.97	6.02	5.66	0.59	2.92	4.96	3.18	22.69	12.23	24.56	7.97
SEM	5.40	3.53	1.42	1.33	0.14	0.69	1.17	0.75	5.35	2.88	5.79	1.88
Min	154.39	72.11	50.00	53.33	1.33	11.81	22.00	11.11	193.83	91.46	174.02	21.03
Max	234.22	123.02	70.78	72.89	3.33	22.31	41.22	22.00	282.22	133.72	251.83	51.89

PH: plant height, EH: ear height, DT: days to tasseling, DS: days to silking, ASI: anthesis silking interval, EL: ear length, NRPC: number of rows per cob, NGPR: number of grains per rows, GY: average grain yield per plant, TGW: thousand grain weight, Zn: grain zinc content, and FW: field weight.

DISCUSSION

The GGE biplot analysis is a powerful statistical tool for the evaluation and partitioning of GEI. Environment/location vector defines the grouping of genotypes in its conducive environment for a particular trait. This grouping primarily depends on PC_1 scores and secondly on PC_2 scores. Blue spherical or round structures represent the mega-environment. Environment performing alike grouped together. Hybrid performing better for a particular trait positioned at a vertex forming a polygon. The polygon gets divided into different sectors by lines originating from the origin. Thus, the sector containing hybrids in a particular environment shows that these hybrids perform better in a respective environment for a trait under consideration. The hybrids without any environment in their sectors are poor performing.

The higher cumulative variation captured by the first two principal components in this study suggested a sufficient explanation of the GEI pattern for studied traits in the environment-centered principal component analysis biplot. GEE biplot accounting for 60% or more variation could be used in the mega-environment (Yan and Kang, 2002).

The scatter plot revealed that all tested environments differently influence for NGPR, ASI, and GY forming three mega-environments indicating the presence of GEI, whereas representation of all other traits were either of the two mega-environments. The comparison GGE biplot featuring location highlights the best location for a trait. The comparison biplot that features genotype ranking highlights best hybrids for trait across given locations. In the presented study, GGE biplots visualize a variable degree of GEI for different traits, necessitating site-specific selection for traits with higher GEI. Maqbool *et al*. (2018) suggested an environment-specific selection for pro-vitamin A biofortified exotic maize hybrids.

Comparison GGE biplot based on environment showed different locations vary suitability for various traits. For characters PH, EH, DS, DT, NRPC, and NGPR, the most conducive environment was MMRI, followed by UAF and CCRI. The best location for ASI, TGW, GY, and Zn was UAF, followed by MMRI and CCRI. Soil zinc analysis reports for multi-locations depicted zinc deficiency at all three locations, with UAF declared as more zinc deficient, followed by MMRI and CCRI. The best location for Zn-deficient study was UAF, followed by MMRI and CCRI. Analysis reports showed zinc-deficiency status for all experimental sites. Darai *et al*. (2020) categorized soils with 0.5–1 ppm zinc as low zinc soils. It makes screening and evaluation of genotypes more reliable because zinc-efficient genotypes have more uptake and remobilize more zinc than in-efficient ones. These zinc-efficient genotypes have potential to become candidate lines for grain zinc biofortification (Karim *et al.,* 2012). It indicates that genotypes growing on more zinc-deficient soils up-regulate zinc transporters more. This result agrees with the finding (Mager *et al.*, 2018) that over-expression of zinc transporter gene family ZmZIP3, ZmZIP4, ZmZIP5, ZmZIP7, and ZmZIP8 occurs in plants when grown in zinc-deficient soil. A reported over-expression of ZmNAS5 was also responsible for producing nicotianamine synthase, which translocates heavy metals to plant parts. The members of Poaceae family release phytosiderophores from roots during zinc deficiency for uptake of zinc by its chelation (Kochian, 1993). For EL, the best location was CCRI, followed by MMRI and UAF. UAF proved the best location for FW, followed by CCRI and MMRI. Theoretically, an ideal genotype shows high mean rank and a consistent performance among all the test environments (Yan and Tinker, 2006; Yan and Kang, 2002). The line passing through the origin and circle of biplot is AEA (average environment axis) and the line perpendicular to it is known as AOE (average ordinate environment). Genotypes present on the right side of AOE are those having a higher mean value with increasing order in the direction of the arrow, while the analysis of stability is from its distance from the circle on the AEA known as the average environment abscissa (Mitrovic *et al.,* 2012, Yan and Kang, 2002). For PH, the proven best hybrids were 4, 1, 11, 3, and 16 and for EH, hybrids 3, 1, 6, 13, 4, and 16 are the best ones. In the case of DT and DS, hybrids 13, 7, 11, and 4 and hybrids 13, 7, 11, 16, and 10revealed as the best, respectively. Similarly, best hybrids found for ASI were 17, 18, 12, 4, and 1. For EL, NGPR, NRPC, TGW, GY, FW, and Zn, hybrids 16, 1, 4, 10, and 11, hybrids 11, 10, 16, 4, and 1, hybrids 1, 16, 11, 5, and 4, hybrids 16, 4, 8, 2, and 10, hybrids 16, 4, 1, 5, and 11, hybrids 16, 4, 1, 11, and 5, and hybrids 13, 16, 4, 2, 5, and 11 came out as the best, respectively. Similar stability models were used by Mitrovic *et al.* (2012) and Maqbool *et al*. (2017).

Exotic biofortified hybrids meet introduction criteria when giving high and stable yields compared with indigenous commercial ones. The selected constant exotic zinc-biofortified crossbreeds perform better for yield and all other studied traits versus the indigenous commercial hybrids. All the exotic hybrids revealed grain zinc content higher than 33 mg/kg, which is the criteria for zinc biofortification in maize. Welch and Graham (2004) set criteria that a yield of biofortified hybrids can be increased or maintained up to acceptable levels by a farming community. Summary statistics revealed trends in data. Standard deviation (SD) highlights how close or far the distribution of a sample is around the mean. The lower value of SD indicates more reliability in the study and less genotype by environmental influence on the trait expression (Guo *et al.,* 2020). Lower values of the standard error of mean (SEM) indicated less fluctuation and more stability of the sample mean of a trait upon repetition. It is helpful for the re-appearance of traits in farmer's fields, as were in research stations.

CONCLUSIONS

Zinc biofortification in maize is a sustainable strategy to nourish zinc-deficient masses in Pakistan. Non-availability of any indigenous zinc-biofortified maize hybrid led to the exotic hybrids' introduction to fill this gap. Yield potential of introduced material must be more or at least at par with superior indigenous hybrids for easy adoption by farmers. Exotic zinc biofortified hybrids G16, G4, and G1 were stable and best performing for studied traits, especially average grain yield per plant and grain zinc content across locations; thus, their imperative recommendation for introduction in Pakistan.

REFERENCES

Agriculture Department Government of Punjab (2020). Publications Crop Production Plans https://dai.agripunjab.gov.pk.

Alloway BJ (2008). Symptoms of zinc deficiency in agricultural and horticultural crops. In: Zinc in soils and crop nutrition; *IZA and IFA*: 59-75. http://www.topsoils.co.nz/wp-content/uploads/2014/09/Zinc-in-Soils-and-Crop-Nutrition-Brian-J.-Alloway.pdf.

AOAC (1990). Official Methods of Analysis. *Association of Official Analytical Chemists*. Arlington. VA. USA.

Bouis HE, Welch RM (2010). Biofortification-a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south*. Crop Sci*. 50:20-32.

Brown K, Rivera J, Bhutta Z, Gibson R, King J, Lonnerdal B (2004). International zinc consultative group: Technical Brief Document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull*. 99-203. https://pubmed.ncbi.nlm.nih.gov/18046856..

Darai R, Sarker A, Pandey M, Dhakal K, Kumar S, Sah R (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: 1-12.

Government of Pakistan, GOP (2022). Economic Survey of Pakistan 2021–2022. Ministry of Food, Agriculture and Livestock, Finance Division, Economic Advisor's Wing, Islamabad.

Guo R, Dhliwayo T, Mageto EK, Palacios-Rojas N, Lee M, Yu D, Ruan Y, Zhang A, San FV, Oleson M, Crossa J, Prasanna BM, Zhang L, Zhang X (2020). Genomic prediction of kernel zinc concentration in multiple maize population using genotyping by sequencing and repeat amplification sequencing markers. *Font. Plant Sci.* 11: 534.

Hindu V, Palacios-Rojas N, Babu R, Suwarno WB, Rashid Z, Usha R, Saykhedkar GR, Nair SK (2018). Identification and validation of genomic regions influencing kernel zinc and iron in maize. *Theo. App. Genet*. 131:1443-1457.

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: 2552-2559.

Kochian LV (1993). Zinc absorption from hydroponic solutions by plant roots. In: Zinc in Soils and Plants. Springer, Dordrecht. (pp. 45-57). https://doi.org/10.1007/978-94-011-0878-2_4.Maqbool MA, Aslam M, Beshir A, Khan MS (2018). Breeding for provitamin A biofortification of maize (*Zea mays* L.). *Plant Breed*. 137(4): 451-69.

Mager S, Schönberger B, Ludewig U (2018). The transcriptome of zinc deficient maize roots and its relationship to DNA methylation loss. *BMC Plant Biol*. 18(1): 1-6. https://doi.org/10.1186/s12870-018-1603-z.

Maqbool MA, Beshir AR (2019). Zinc biofortification of maize (*Zea mays* L.): Status and challenges. *Plant Breed.* 138:1-28.

Mitrovic B, Treski S, Stojakoviã M, Ivanoviã M, Bekavac G (2012). Evaluation of experımental maize hybrids tested in multi-location trials using AMMI and GGE biplot analyses. *Turk. J. Field Crop*. 17(1): 35-40.

National Nutrition Survey (2018). National Nutrition Survey (NNS), Pakistan Nutrition Division, National Institute of Health, Islamabad, Pakistan.

Nyaligwa L, Hussein S, Laing M, Ghebrehiwot H, Amelework BA (2017). Key maize production constraints and farmers' preferred traits in the mid-altitude maize agroecologies of northern Tanzania. *South Afr. J. Plant Soil*. 34(1): 47-53. https://hdl.handle.net/10520/EJC-52e9c8a65.

Qin H, Cai Y, Liu Z, Wang G, Wang J, Guo Y, Wang H (2012). Identification of QTL for zinc and iron concentration in maize kernel and cob. *Euphytica* 187: 345-358.

Sharma A, Chauhan R (2008). Identification of candidate gene-based markers (SNPs and SSRs) in the zinc and iron transporter sequences of maize (*Zea mays* L.). *Current Sci.* 25: 1051-1059.

Welch RM, Graham RD (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *J. Exp. Bot*. 55: 353-364.

Yan W, Hunt LA, Sheng Q, Szlavnics Z (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Sci*. 40(3): 597-605. https://doi.org/10.2135/cropsci2000.403597x.

Yan W, Kang MS (2002). GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. *CRC Press*. https://doi.org/10.1201/9781420040371.

Yan W, Tinker NA (2006). Biplot analysis of multi-environment trial data: Principles and applications. *Can. J. Plant Sci.* 86(3): 623-645. https://doi.org/10.4141/P05-169.

Zarcinas BA, Cartwright B, Spouncer LR (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun. Soil Sci. Plant Anal.* 18(1): 131-146. https://doi.org/10.1080/00103628709367806.

Zhang H, Liu J, Jin T, Huang Y, Chen J, Zhu L, Zhao Y, Guo J (2017). Identification of quantitative trait locus and prediction of candidate genes for grain mineral concentration in maize across multiple environments. *Euphytica* 213: 1-16.