agronomy

MDPI

Article

Genetic Analysis of Zinc, Iron and Provitamin A Content in Tropical Maize (*Zea mays* L.)

Enoobong Udo [1,2,*], Ayodeji Abe [1], Silvestro Meseka [2], Wende Mengesha [2] and Abebe Menkir [2,*]

[1] Department of Crop and Horticultural Sciences, University of Ibadan, PMB 3017, Ibadan 200284, Oyo State, Nigeria
[2] International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan 200284, Oyo State, Nigeria
* Correspondence: e.udo@cgiar.org (E.U.); a.menkir@cgiar.org (A.M.)

Abstract: Breeding maize with high contents of zinc, iron and provitamin A (PVA) could be effective in mitigating micronutrient deficiency in developing countries with a high reliance on maize-based diets. Information on the mode of inheritance of zinc, iron, PVA and grain yield (GY) would facilitate the development of varieties with enhanced contents of these nutrients. Twenty-four yellow to orange maize inbred lines and their 96 F1 hybrids generated using North Carolina Design II, were evaluated alongside four checks for two years at two locations in Nigeria. The effects of environment, hybrid and inbred line were significant for GY and contents of zinc, iron and PVA. The GY, and contents of zinc, iron and PVA of the hybrids ranged from 1.49 to 6.05 t ha^{-1}, 22.51 to 33.33 mg kg^{-1}, 20.04 to 29.65 mg kg^{-1} and 3.55 to 15.28 µg g^{-1}, respectively. Additive gene effects controlled the accumulation of PVA and Fe, whereas both additive and non-additive gene effects controlled the inheritance of Zn and GY. Inbred lines with high general combining ability for GY and single or multiple micronutrients were identified, and could be used to develop hybrids and synthetics that combine high GY with high micronutrient content. Six hybrids combined high GY with high contents of all three micronutrients, and are recommended for further evaluation and possible release. Our study revealed the feasibility of enriching maize with multiple micronutrients without compromising grain yield.

Keywords: additive gene effect; non-additive gene effect; micronutrient deficiency; orange maize

Citation: Udo, E.; Abe, A.; Meseka, S.; Mengesha, W.; Menkir, A. Genetic Analysis of Zinc, Iron and Provitamin A Content in Tropical Maize (*Zea mays* L.). *Agronomy* **2023**, *13*, 266. https://doi.org/10.3390/agronomy13010266

Academic Editor: Ali Raza

Received: 5 December 2022
Revised: 6 January 2023
Accepted: 13 January 2023
Published: 16 January 2023

1. Introduction

Maize is an important crop for global food security and its grains contain about 72% starch, 10% protein, 4.8% fat, 8.5% dietary fibre, 3.0% sugar with no anti-nutrients [1]. However, most maize genotypes are low in content of vitamin A and valuable minerals including Fe and Zn [2,3]. Zn acts as a catalyst for the activation of several enzymes in the body [4]. It is involved in the regulation of gene expression, proper functioning of the immune system, physical growth, reproductive health and neurobehavioral development [5,6]. The role of Fe is manifest in several biological processes such as respiration, energy production, cell division and formation of genetic materials [7]. Vitamin A is necessary for visual health, cellular growth and development and control of gene expression [8]. Symptoms of Zn deficiency include stunted growth in infants, increased oxidation stress, low immunity to infections and slow mental development, whereas Fe deficiency is largely associated with anaemia and fatigue [9]. Vitamin A deficiency has been implicated in blindness and 17% of all deaths in children younger than 5 years in developing countries [10]. Deficiency of micronutrients in the diet leads to 'hidden hunger', necessitating the need for the consumption of foods enriched with adequate content of bioavailable micronutrients. Micronutrient deficiency is widespread in developing countries where there is high reliance on maize-based foods. The low cost of maize production and its growing use in processed products in these countries make it a suitable vehicle for biofortification [11,12]. For example, the

estimated Zn requirement is 1860 μg day^{-1}, therefore, the development of maize varieties with Zn content exceeding 37 mg kg^{-1} would have a significant impact in alleviating Zn deficiency [12]. In addition, on the basis of retention, bioavailability and daily requirements of vitamin A and Fe, coupled with an estimated daily maize consumption of 200 g day^{-1}, increasing the PVA content of maize to 15 μg g^{-1} as set by Harvestplus and Fe content to 40 mg kg^{-1} would positively impact human nutrition in developing countries [13].

The International Institute of Tropical Agriculture with the support of the HarvestPlus challenge program has been involved in developing maize varieties with high levels of Zn and PVA. Previous diversity studies for PVA, Zn and Fe in maize reported the presence of substantial native variation for the three micronutrients in inbred lines [10,14–16], suggesting that breeding maize varieties for enhanced contents of these nutrients is feasible. Maize accumulates provitamin A, through the carotenoid biosynthetic pathway and is later changed to Vitamin A in the human body through activities of certain enzymes [17]. Orange and yellow colour in maize have been associated with higher nutritional value than white, due to the presence of carotenoids, especially zeaxanthin and β-carotene which impart the colour [18,19]. According to [16,20], β-carotene represents about 70% of the PVA content in maize. On the other hand, accumulation of Fe and Zn occurs through uptake from the rhizosphere to the roots, translocated to the shoots, leaves and stems at the vegetative stage and later to the grains during the reproductive stage [15,21]. Micronutrient accumulation in plants is highly influenced by climatic and edaphic factors which may mask the genetic potential of the crop. Nonetheless, high heritability estimates for Zn and Fe accumulation have been reported in maize, highlighting the low presence of environmental effects on phenotypic expression of these traits and the possibility of transferring them to progenies [12,15,22].

Combining ability studies unravel the effects of genes involved in the expression of PVA, Fe and Zn content and facilitate the selection of parents for developing source populations and hybrids with high levels of the three micronutrients. General combining ability (GCA) is the mean performance of an inbred line in a series of hybrid combinations and is associated with additive gene effects while specific combining ability (SCA) is linked to the non-additive genetic effects [23]. Provitamin A in maize, has been the subject of many combining ability studies. While [24] reported that non-additive genetic effects were more important in the inheritance of carotenoids, several other studies have reported the preponderance of additive gene effects conditioning provitamin A content in maize [16,20,25–28]. Similarly, several genetic studies in maize have reported that (GCA was more important than SCA in the regulation of Zn accumulation in maize [15,29,30]. In contrast, [31] found non-additive gene action to be more important than additive gene action for Zn and Fe levels in maize. Considering the contradictory results reported in previous studies, as well as the use of fewer inbred lines for genetic analyses in those studies, there is additional need to unravel the mode of inheritance of Zn, Fe and PVA in tropical maize using more maize inbred lines with diverse genetic backgrounds. Furthermore, most of the genetic studies also focused on one or two micronutrients at a time, whereas deficiencies in Zn, Fe, and PVA have been recorded together in many low-income communities in sub-Saharan Africa. Micronutrients interact during their transportation and functioning in the human body which may increase the bioavailability of each micronutrient [32]. For example, higher PVA levels in cereals could improve the intestinal absorption of Fe from cereals [33]. Therefore, understanding the gene action controlling the three micronutrients is important in identifying parental lines that can be crossed to develop source populations and high-yielding maize hybrids with high levels of PVA and minerals. Increasing PVA, Zn and Fe content should also be achieved without compromising grain yield and other important agronomic traits. The objectives of the present study were therefore to determine (i) the mode of inheritance of Zn, Fe, PVA content as well as grain yield and (ii) the correlations of Zn, Fe and PVA content with grain yield and other agronomic traits in maize.

2. Materials and Methods

2.1. Genetic Materials

Twenty-four (24) yellow to orange endosperm tropical maize inbred lines with low to high levels of Zn and provitamin A (Table S1A) were used in the present study. The inbred lines were crossed using the North Carolina design II [23] at IITA's main research station, Ibadan (7°29′11.99′′ N, 3°54′2.88′′ E, altitude 190 masl). The inbred lines were divided into six groups each containing four inbred lines to generate six sets of crosses. The inbred lines were separated into groups based on similarities in Zn and provitamin A contents that were determined in previous seasons as well as the genetic backgrounds of the lines to maximize productivity in hybrids. Each inbred line in each group was used both as a male and a female parent to generate a total of 96 hybrids (Table S1B).

2.2. Field Evaluation

The 96 hybrids plus two commercial provitamin A biofortified hybrids (Ife Hybrid 3 and Ife Hybrid 4) and two conventional orange maize hybrids (M1124-31 and Oba Super 2) used as checks were arranged in a 25 × 4 alpha lattice design and planted at two testing sites in Nigeria in 2020 and 2021. The testing sites were (Ikenne 3°42′ E, 6°54′ N, altitude 30 masl; Saminaka 8°39′ E, 10°34′ N, altitude 760 masl). The inbred parents were also evaluated in 4 × 6 alpha lattice design in a separate trial alongside the hybrids at two of the testing sites (Ikenne and Saminaka). Each entry in both hybrid and inbred trials was planted in a single 5 m row plot with a spacing of 0.75 m between rows and 0.50 m within rows with two replications. Three seeds were placed in each hill and later thinned to two plants giving a population density of about 50,000 plants ha^{-1}. NPK 15:15:15 fertilizer was applied at the rate of 60 kg N ha^{-1}, 60 kg P ha^{-1}, and 60 kg K ha^{-1} at planting. Urea (46-0-0) fertilizer at 60 kg N ha^{-1} was applied as top dressing at four weeks after planting. Weeds were controlled by application of gramoxone and atrazine as pre-emergence herbicides at the rates of 1.5 L gramoxone and 2.5 L atrazine in 200 L of water per hectare. Manual weeding was subsequently performed to manage the weeds.

2.3. Agronomic Trait Recording

Agronomic data were collected from each plot at the two testing sites in both 2020 and 2021. Days to anthesis (DA) and days to silking (DS) were recorded as the number of days at which 50% of plants in a plot shed pollen and had emerged silks, respectively. Anthesis-silking interval (ASI) was estimated as the difference between DS and DA. Plant height (PH) and ear height (EH) were measured in centimetres as the distance from the base of the plant to the top of the first tassel branch and the node where the upper ear is attached, respectively. Plant aspect (PA) was scored on a scale of 1–5, where 1 represented uniform, clean, vigorous plants with good appearance and 5 represented non-uniform, unhealthy plants with poor appearance. Ear aspect (EA) was scored on a scale of 1–5, where 1 represented clean, well-filled, uniform and large ears while 5 represented incompletely filled ears with pest or disease damage. Harvested ears were shelled and grain moisture content of the grains was measured using a hand-held moisture meter. The grain weight and moisture content were used to compute grain yield adjusted to 15% moisture content. Grain yield was not recorded in the inbred lines trials as most of the plants were weak.

2.4. Minerals Analysis

Five representative plants were self-pollinated in each F1 hybrid for each replication every year. Similarly, uniform plants of each inbred line were self-pollinated in each replication at the two locations for two years. To minimize field contamination, harvested ears were placed into paper bags and carefully shelled by hand in the laboratory. Samples were drawn from the hand-shelled grains and submitted to the Food and Nutritional Sciences Laboratory of IITA for mineral and carotenoid analyses.

A 10 g sample was taken from each plot and milled to fine powder (flour approx., 0.05 mm) using Perten Laboratory Mill 3100 (Perten Instruments). A 7 g sample was then

drawn from each milled sample and loaded in sample cups [34], which were sealed on one end with 4 µm Poly-4 XRF sample film. The sample cups were then loaded into Bruker S2 Ranger for the X-ray fluorescence (XRF) analysis. Sample films were changed after each reading and cups were cleaned to avoid cross-contamination between samples. Measurements were performed twice per sample and mean values were recorded. A 10% sample was also analysed using the inductively coupled plasma-optical emission spectrometry (ICP-OES) to further confirm the accuracy of values obtained from the XRF analysis.

2.5. Carotenoids Analysis

Self-pollinated ears were harvested, dried under ambient temperature with minimal exposure to direct sunlight and shelled separately. Samples of 100 kernels were drawn from each plot in duplicates for carotenoid analysis. Carotenoids were extracted and quantified using High Performance Liquid Chromatography (HPLC) (Water Corporation, Millford, MA, USA) at Food and Nutritional Sciences Laboratory, IITA-Ibadan using a protocol described by [35]. Each 100 kernel-sample was ground to pass a 0.5 mm screen using Perten Laboratory mill (Perten Instruments). A 0.6 g ground sample was transferred to a 50 mL glass centrifuge tube. Then, 6 mL ethanol containing 0.01% butylated hydroxyl toluene was added to the glass tube, which was capped and mixed using a vortex mixer for 15 s. The glass tube was then incubated in 85 °C water bath for 5 min. The glass tubes were removed from the water bath and 500 µL of 80% potassium hydroxide was added to the tubes. Vortexing and incubation were repeated. The samples were moved immediately into an ice bath and 3 mL cold deionized water was added to tube and mixed using a vortex mixer for 15 s. To each sample, 3 mL hexane was added, vortexed and then centrifuged at 1000 rpm for 10 s. Then, 200 µL of internal standard β-Apo 80–carotenal was also added to 10% of the number of samples alongside hexane to validate the efficiency of the extraction process. The hexane fraction was then extracted and transferred into a concentrator tube. Extraction of the hexane fraction was repeated three times by adding 3 mL hexane each time. The hexane extracts from each sample were then dried at 40 °C under nitrogen gas using a concentrator (Organomation Asscociates, Inc., Berlin, MA, USA). The samples were reconstituted in 1 mL of 50:50 methanol/dichloromethane and the tube capped immediately. The extracts were vortexed and transferred into HPLC vials, placed in an autosampler tray and inserted into the HPLC machine. A 50 µL aliquot per sample was injected automatically into the HPLC (Water Corporation, Milford, MA, USA) for analyses of a-carotene, β-carotene (cis and trans-isomers), β-cryptoxanthin, lutein, and zeaxanthin based on calibration of the standard of each carotenoid. The Waters HPLC components, operated with Empower 1 Software, includes a 717 Plus autosampler with temperature control set at 5 °C, a Waters 1525 binary HPLC pump, and a 2996 photodiode array detector for carotenoid quantification. Carotenoids were separated on a 3 µm C30 Carotenoid Column (4.6 × 250 mm) eluted by a mobile phase methanol/water (92:8 v/v) with 10 mM ammonium acetate as solvent A and 100% methyl-tert-butyl-Ether as Solvent B. The gradient was applied for 30 min from 70% solvent A:30% solvent B, to 40% solvent A:60% solvent B. The flow rate was 1.0 mL per minute, the absorbance was measured at 450 nm.

Chromatograms were extracted after analyses and major carotenoids were identified and concentrations calculated using the formula [36]:

$$\mathrm{Cx}\left(\mu\mathrm{g\ g}^{-1}\right) = \frac{\mathrm{Ax}\ \times\ \mathrm{Cs}\left(\mu\mathrm{g\ mL}^{-1}\right) \times \text{total volume of extract (mL)}}{\mathrm{As}\ \times \text{sample weight (g)}}$$

where Cx and Ax were concentration and peak area of carotenoid x, while Cs and As were the concentration and peak area of the standard.

Total carotenoid was calculated as the sum of concentrations of α-carotene, lutein, β-carotene, β-cryptoxanthin, zeaxanthin. Provitamin A was calculated by adding the concentrations of β-carotene, and half concentration each of β-cryptoxanthin and α-carotene [37].

2.6. Data Analysis

Analyses of variance for hybrid and parent trials were carried out using PROC MIXED procedure of SAS 9.4 [38] to generate entry means for micronutrients and agronomic traits for each location-year combination, which is hereafter referred as an environment. Cross-environment analyses were then conducted using adjusted means for block effects.

The test hybrids excluding checks were thereafter subjected to analysis of variance based on North Carolina Design II to estimate combining ability effects for the micronutrients and agronomic traits. In the combined analysis of variance using a mixed model, hybrids, sets, male and female parental lines were considered as fixed effects, while environments, replications within environment and blocks within replications were considered as random effects. The variance components due to hybrids within sets were divided into variance due to female (set), male (set) and female × male (set) interaction and the interaction of each respective variance component with environment was used to compute F tests. The variance due to environment × female × male (sets) was tested using the pooled error variance. The pooled error variance was obtained by dividing error sums of square from all environment combinations with the corresponding sum of error degrees of freedom. The main effects of female (sets) and male (sets) represents the general combining ability (GCA female and GCA male) while female × male (sets) interaction represents specific combining ability (SCA) effects [23].

The relative importance of GCA and SCA (predictability ratio) was calculated as:

$$\frac{2\sigma^2 gca}{2\sigma^2 gca + \sigma^2 sca}$$

where $\sigma^2 gca$ and $\sigma^2 sca$ are variances due to GCA and SCA, respectively.

AGD-R was used to calculate variance components and broad sense heritability for each trait [39]. Simple correlation coefficients between micronutrients and agronomic trait were also calculated from means averaged across environments using PROC CORR of SAS 9.4 [38].

3. Results

3.1. Variation in Zn, Fe and PVA Content

The inbred line and environment had significant effects on Zn, Fe and PVA contents (Table S1). Inbred line × environment interaction had significant effects on β-carotene, PVA, and Fe, but not on Zn. The contribution of inbred line × environment interaction to total variation was much smaller relative to the variability among inbred lines for the micronutrients. Heritability estimates were 0.94 for Zn, 0.80 for Fe, and 0.96 for PVA. Mean micronutrient content of inbred lines averaged across environments ranged from 27.82 to 41.59 mg kg^{-1} for Zn, 19.20 to 31.01 mg kg^{-1} for Fe, and 2.12 to 15.15 μg g^{-1} for PVA (Table 1).

The differences among hybrids and environment were highly significant for the micronutrients and grain yield. Hybrid × environment interaction mean squares were significant for grain yield, PVA and Fe content, but not for Zn content (Table 2). Again, the mean squares for hybrids were much larger than the mean squares for hybrid × environment interaction for all micronutrients including grain yield. The GY, and contents of zinc, iron and PVA of the hybrids ranged from 1.49 to 6.05 t ha^{-1}, 22.51 to 33.33 mg kg^{-1}, 20.04 to 29.65 mg kg^{-1} and 3.55 to 15.28 μg g^{-1}, respectively. The overall mean micronutrient content in hybrids was 28.51 mg kg^{-1} for Zn, 23.61 mg kg^{-1} for Fe, and 8.32 μg g^{-1} for PVA, whereas mean grain yield for hybrids was 4.88 t ha^{-1}. The number of hybrids that accumulated above average concentrations of the micronutrients was 51 for Zn, 35 for Fe and 28 for PVA. Six hybrids combined high grain yield with high contents of Zn, Fe and PVA (Table 3).

Table 1. Mean micronutrients concentrations of 24 parental inbred lines across environments.

Inbred Line	Zn (mg kg^{-1})	PVA (µg g^{-1})	Fe (mg kg^{-1})
1	28.53	10.39	23.42
2	31.10	8.12	23.40
3	30.85	10.07	22.69
4	32.86	8.84	22.73
5	33.36	5.77	23.06
6	32.61	5.85	24.31
7	35.62	8.39	25.00
8	31.45	11.66	19.20
9	36.39	6.56	24.29
10	41.49	5.04	22.44
11	39.86	4.13	31.01
12	36.12	8.33	24.03
13	34.72	5.24	25.21
14	34.75	8.39	24.78
15	41.59	2.12	29.36
16	35.33	9.31	27.89
17	29.61	7.28	27.31
18	31.68	7.99	21.97
19	33.06	10.88	26.96
20	27.82	11.25	24.05
21	37.12	4.88	27.71
22	35.14	10.81	26.16
23	27.65	15.15	20.93
24	28.78	7.41	26.17
Grand mean	33.58	7.72	24.39
LSD ($p < 0.05$)	1.37	0.83	1.46

GM: general mean, LSD: least significant difference, PVA: provitamin.

Table 2. Mean squares from the combined analysis of variance across environments for micronutrients and grain yield of 96 maize hybrids and four commercial checks.

Source	DF	Zn mg kg^{-1}	Fe mg kg^{-1}	Provitamin A (µg g^{-1})	Grain Yield (t ha^{-1})
Env	3	687.98 **	204.94 **	114.49 **	286.98 **
Rep (Env)	4	22.01 **	36.04 **	10.01 **	0.77
Block (Env × Rep)	192	3.39	3.85 **	1.32 **	0.97 **
Hybrid	99	23.58 **	15.89 **	24.95 **	5.08 **
Env × Hybrid	297	3.74	2.87 *	1.25 **	0.87 **
Set	5	133.59 **	33.67 **	57.48 **	5.11 **
Env × Set	15	4.08	4.03 *	1.75 **	1.21 **
Female (Set)	18	19.04 **	16.48 **	40.55 **	5.76 **
Male (Set)	18	19.23 **	30.51 **	43.16 **	7.02 **
Female × Male (Set)	54	8.32 **	4.48 **	2.20 **	2.98 **
Env × Female (Set)	54	5.26 *	3.33 *	1.23 **	0.74
Env × Male (Set)	54	3.37	3.19	1.76 **	0.89 *
Env × Female × Male (Set)	162	2.85	2.04	0.88 *	0.71
Error	220	3.65	2.29	0.68	0.62
CV%		6.70	6.39	10.03	16.16
$\sigma^2_{Additive}$		14.88	9.51	16.06	146.63
$\sigma^2_{Non\text{-}additive}$		4.83	2.13	1.26	92.33
σ^2_{Env}		0.48	0.38	0.18	5.6
Predictability ratio		0.84	0.90	0.96	0.76
Heritability		0.88	0.87	0.92	0.86

*: significant at $p < 0.05$, **: significant at $p < 0.01$, DF: degree of freedom, Env: environment, Rep: replication, CV%: coefficient of variation, $\sigma^2_{Additive}$: additive variance, $\sigma^2_{Non\text{-}additive}$: non-additive variance σ^2_{Env}: environmental variance.

Table 3. Mean values for micronutrients content and grain yield for 96 hybrids and four checks evaluated across environments.

S/N	Hybrids	Zn mg kg^{-1}	Fe mg kg^{-1}	PVA µg g^{-1}	GY t ha^{-1}
1	1 × 9	30.86	25.22	6.84	5.80
2	2 × 9	28.31	23.23	6.78	4.99
3	3 ×9	27.79	22.96	8.00	4.70
4	4 × 9	30.41	23.95	7.98	5.50
5	1 ×10	32.22	25.27	6.19	4.67
6	2 × 10	30.16	25.31	8.36	3.16
7	3 × 10	26.22	22.01	6.72	4.51
8	4 × 10	30.67	22.36	7.65	5.79
9	1 × 11	30.30	29.65	7.00	4.00
10	2 × 11	28.08	24.39	6.53	4.49
11	3 × 11	28.25	25.89	6.62	3.48
12	4 × 11	29.78	24.94	7.83	4.92
13	1 ×12	31.45	26.63	8.61	5.57
14	2 × 12	23.52	22.21	8.18	4.94
15	3 × 12	27.82	23.59	7.23	5.86
16	4 × 12	31.97	23.74	8.73	4.65
17	9 × 5	30.90	25.77	6.35	5.74
18	10 × 5	29.36	23.47	6.00	5.11
19	11 × 5	30.86	25.52	6.07	4.36
20	12 × 5	29.04	24.27	7.52	5.35
21	9 × 6	30.38	25.16	9.72	4.90
22	10 × 6	31.65	23.55	9.71	5.37
23	11 × 6	29.91	25.48	8.38	4.40
24	12 × 6	31.42	23.80	11.08	5.82
25	9 × 7	30.60	23.36	8.26	4.90
26	10 × 7	31.64	24.48	6.86	5.21
27	11 × 7	30.19	24.65	7.09	4.65
28	12 × 7	29.95	23.51	8.49	4.91
29	9 × 8	28.10	20.84	7.04	6.00
30	10 × 8	30.50	21.58	6.87	5.69
31	11 × 8	29.89	24.08	7.81	5.91
32	12 × 8	28.23	20.69	7.35	6.04
33	17 × 1	29.72	26.21	7.37	4.58
34	18 × 1	30.12	25.93	6.76	5.62
35	19 × 1	29.80	26.75	10.00	4.93
36	20 × 1	28.64	22.37	6.44	5.32
37	17 × 2	25.97	23.55	8.02	4.32
38	18 × 2	25.54	20.94	7.02	5.66
39	19 × 2	26.67	22.88	11.32	5.54
40	20 × 2	26.26	21.00	9.25	4.85
41	17 × 3	29.79	21.81	6.85	4.94
42	18 × 3	22.51	20.88	9.46	5.48
43	19 × 3	26.33	23.24	9.61	4.33
44	20 × 3	24.81	22.61	8.65	3.89
45	17 × 4	27.05	22.33	8.39	4.62
46	18 × 4	27.44	21.95	7.89	5.64
47	19 × 4	27.92	22.46	11.99	5.57
48	20 × 4	28.03	22.49	9.93	4.33
49	13 × 21	28.99	23.50	6.18	4.08
50	14 × 21	28.72	24.13	7.02	4.34
51	15 × 21	30.06	24.83	4.69	4.60
52	16 × 21	31.19	25.64	8.75	4.31
53	13 × 22	29.78	25.72	7.32	4.58
54	14 × 22	29.52	24.20	8.68	5.00
55	15 × 22	29.14	24.25	5.40	5.20
56	16 × 22	29.08	26.43	9.62	5.15

Table 3. *Cont.*

S/N	Hybrids	Zn mg kg^{-1}	Fe mg kg^{-1}	PVA µg g^{-1}	GY t ha^{-1}
57	13 × 23	27.95	24.93	10.15	4.90
58	14 × 23	28.34	23.90	12.36	4.74
59	15 × 23	27.94	23.79	8.83	4.46
60	16 × 23	26.51	24.74	12.97	5.61
61	13 × 24	27.06	22.27	5.86	5.08
62	14 × 24	27.84	22.11	7.55	4.40
63	15 × 24	28.67	20.82	3.78	3.93
64	16 × 24	28.65	23.73	7.71	4.78
65	5 × 13	28.15	23.33	5.52	5.42
66	6 × 13	29.25	22.45	10.15	4.77
67	7 × 13	29.15	23.39	7.88	4.96
68	8 × 13	28.44	21.30	6.69	5.43
69	5 × 14	28.27	23.45	7.00	4.87
70	6 × 14	29.85	22.47	9.57	3.98
71	7 ×14	33.31	24.25	8.19	1.49
72	8 × 14	27.84	21.00	7.57	4.91
73	5 × 15	29.39	21.83	3.55	4.50
74	6 × 15	24.95	22.72	7.37	4.73
75	7 × 15	30.43	21.10	5.10	4.89
76	8 × 15	29.05	21.22	3.86	5.70
77	5 × 16	28.52	23.73	6.68	5.21
78	6 × 16	31.88	25.17	12.21	4.10
79	7 × 16	31.96	24.66	9.49	4.81
80	8 × 16	30.32	22.01	10.20	6.05
81	21 × 17	27.05	25.43	6.93	4.04
82	22 × 17	26.44	24.99	9.31	5.19
83	23 × 17	25.15	25.78	11.73	4.37
84	24 × 17	26.31	24.31	6.96	4.33
85	21 × 18	26.68	23.63	7.48	4.57
86	22 × 18	28.18	24.56	9.04	5.92
87	23 × 18	26.51	22.77	10.53	5.50
88	24 × 18	25.19	20.04	7.45	5.28
89	21 × 19	28.27	25.62	10.57	4.76
90	22 × 19	27.70	25.86	11.41	5.60
91	23 × 19	26.49	24.50	15.28	4.76
92	24 × 19	27.51	23.74	10.36	4.76
93	21 × 20	25.83	23.37	8.67	4.41
94	22 × 20	26.54	24.85	10.79	4.45
95	23 × 20	23.88	22.99	13.21	4.12
96	24 × 20	25.74	21.80	8.12	4.38
Ife Hybrid-3	check	27.21	21.40	10.59	4.70
Ife Hybrid-4	check	29.81	25.07	10.78	5.17
M1124-31	check	27.36	22.39	7.94	6.00
Oba Super 2	check	27.20	20.42	5.88	4.46
Grand mean		28.51	23.61	8.32	4.88
LSD ($p < 0.05$)		1.93	1.71	0.83	0.67

PVA: provitamin A, LSD: least significant difference.

3.2. Combining Ability Estimates

In the combined analysis, the effects of sets, female (sets), male (sets), and female × male (sets) interaction were also highly significant for all micronutrients including grain yield (Table 2). Environment × female (sets) interaction was significant for all micronutrients but not for grain yield, whereas environment × male (sets) interaction was significant for PVA and grain yield only. Environment × female × male (sets) interaction was not significant for Zn, Fe and grain yield. Heritability estimates in the hybrid trial were 0.88 for Zn, 0.87 for Fe, 0.92 for PVA, and 0.60 for grain yield. The additive variance ($\sigma^2_{Additive}$) was about

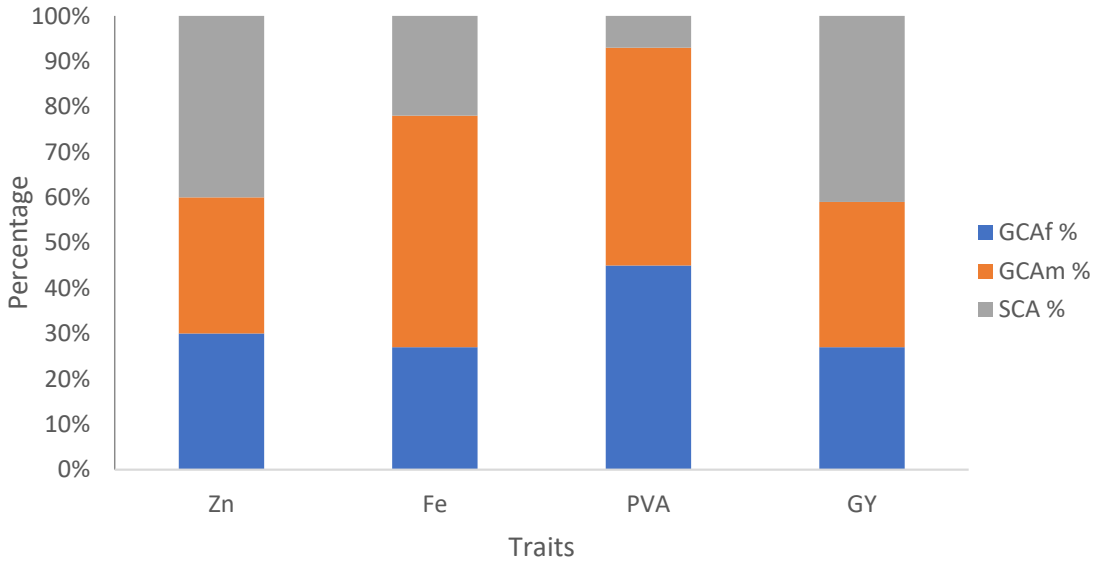
1.3 to 12 times higher than the non-additive variance($\sigma^2_{\text{Non-additive}}$) for Fe, Zn, PVA and grain yield. The predictability ratio was closer to one for the micronutrients and 0.76 for grain yield (Table 2).

The contribution of GCA male to the total sum of squares for hybrids was 30% for Zn, 51% for Fe, 48% for PVA and 32% for grain yield, while the contributions of GCA female was 30% for Zn, 27% for Fe, 45% for PVA and 27% for grain yield (Figure 1). The contribution of SCA effects to the total sum of squares was 40% for Zn, 22% for Fe, 7% for PVA, and 41% for grain yield. Some inbred lines combining positive GCA and SCA effects for multiple micronutrients and grain yield were identified (Tables 4 and S3). Inbred line 16 combined positive GCA effects on Fe, Zn, PVA contents and grain yield. This inbred line accumulated 35.33 mg kg^{-1} of Zn, 27.89 mg kg^{-1} of Fe and 9.31 µg g^{-1} of PVA (Table 1). Inbred lines 1 and 5 had positive GCA for Zn, Fe and grain yield, whereas inbred line 23 displayed positive GCA effects for PVA and Fe levels. In contrast, inbred lines 2, 15 and 24 combined negative GCA effects for the three micronutrients. Eleven hybrids combined positive SCA for Zn, Fe and PVA levels, whereas five hybrids combined positive SCA effects for Zn and Fe levels. Three hybrids combined positive SCA effects for Zn and PVA, while two hybrids combined positive SCA for PVA and Fe content.

Table 4. Estimates of general combining ability effects for Zinc, Iron, PVA and grain yield for 24 parental inbred lines used to form 96 hybrids evaluated across four environments.

		Fe (mg kg^{-1})		Zn (mg kg^{-1})		PVA (µg g^{-1})		GY (t ha^{-1})	
Parent	**GRP**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
1	I	1.39 *	1.79 **	0.87 *	1.87 **	-0.70	−0.82	0.08	0.02
2	I	−0.83	−0.10	−1.43 *	−0.66	0.41	−0.28	0.08	−0.14
3	I	−1.11 *	−0.21	−1.01 *	−1.00 *	0.22	−0.71	−0.15	−0.11
4	I	−0.96 *	−0.36	−0.19	1.26 *	0.93	0.26	0.11	0.19
5	II	0.81	0.06	0.29	0.02	−1.41 *	−2.08 **	0.04	0.12
6	II	0.46	0.10	1.24 *	−0.34	1.53 *	1.86 **	0.01	−0.19
7	II	0.39	0.13	0.94 *	1.84 **	−0.37	−0.35	−0.03	−0.37 *
8	II	−1.39 *	−1.53 **	−0.15	−0.14	−0.49	−0.54	0.43 *	0.32 *
9	III	−0.04	−0.05	0.26	0.99 *	−0.36	−0.19	0.21	0.20
10	III	−0.22	−0.52	0.42	0.80	−0.55	−0.47	−0.14	0.08
11	III	1.97 **	1.02 *	0.52	1.04 *	−0.87	−0.60	−0.26	−0.09
12	III	−0.07	−0.26	−0.19	0.57	0.19	0.54	0.14	0.21
13	IV	−0.63	0.05	−0.26	−0.47	−0.57	−0.51	0.21	−0.02
14	IV	−0.44	−0.15	0.59	−0.02	0.34	0.77	−0.47 **	−0.12
15	IV	−1.27 *	−0.43	−0.42	0.34	−2.60 **	−2.32 **	0.01	−0.12
16	IV	0.53	1.03 *	1.03 *	0.13	1.69	1.59 *	0.11	0.08
17	V	1.13 *	0.00	−0.76	−0.32	−0.19	−0.90	−0.16	−0.10
18	V	−0.75	−0.57	−0.50	−0.80	−0.44	−0.49	0.20	0.33 *
19	V	0.77	0.25	−0.07	−0.56	2.64 **	2.04 **	0.02	0.07
20	V	−0.48	−0.71	−1.16 *	−0.90	1.07 **	0.19	−0.25	−0.20
21	VI	0.53	0.55	0.76	−0.39	−1.50 *	−0.72	−0.16	−0.16
22	VI	0.78	0.82	0.38	−0.32	−0.16	1.03 *	0.04	0.13
23	VI	0.58	0.25	−0.56	−1.53 *	2.86 **	3.50 **	0.05	−0.04
24	VI	−1.15 *	−1.16 *	−0.58	−1.43 *	−1.68 *	−0.78	−0.11	−0.11
SE		0.56	0.53	0.60	0.66	0.61	0.60	0.20	0.19
GM		23.60	23.60	28.53	28.53	8.22	8.22	4.88	4.88

GRP: group, SE: standard error, GM: general mean, GY: grain yield, M: male, F: female, *: significant at $p < 0.05$, **: significant at $p < 0.01$.



GY: grain yield, SCA: specific combining ability, GCAm: general combing ability male, GCAf: general combining ability female, PVA: provitamin A.

Figure 1. The relative contribution of GCA of 24 inbred lines and SCA to the inheritance of Fe, Zn and grain yield in 96 F1 hybrids evaluated across environments.

Most inbred lines displayed positive GCA and SCA effects for at least one micronutrient (Tables 4 and S3). Significant and positive GCA effects on Zn were recorded in inbred lines 1, 7, 9, 11 used as females and lines 1, 6, 7 and 16 used as males. These inbred lines were parents of nine best 10% of the hybrids in Zn content, accumulating more than 31 mg kg^{-1} of Zn in their grains (Table 5). In contrast, inbred lines 3, 23 and 24 when used as females and 2, 3 and 20 used as males had significant negative GCA effects for Zn (Table 4). These inbred lines were either the male or the female parents to eight of the worst 10% of the hybrids in Zn content (Table 3). Of the 96 hybrids, SCA effect for Zn content was significant and positive for nine hybrids, while the effect was significant but negative for seven hybrids (Table S3). Inbred lines 1 and 11 when used as both male and female parents displayed significant and positive GCA effects for Fe content. Additionally, inbred line 16 used as female and 17 used as male had significant and positive GCA effects for Fe content (Table 4). These inbred lines were parents of eight of the best 10% of the hybrids in Fe content (Table 5). In contrast, inbred lines 8 and 24 had significant and negative GCA effects when used as females, while lines 4, 8, 15 and 24 had significant negative GCA effects when used males (Table 4). Only two hybrids had positive SCA effects for Fe content, whereas seven hybrids showed significant negative SCA effects for Fe content (Table S3). Inbred lines 6 and 19 showed significant positive GCA effects for PVA when used as males and female. Inbred lines 22 and 23 used as female and inbred line 20 used as male displayed significant positive GCA effects for PVA content (Table 4). These five inbred lines were parents of the best 10% of the hybrids in PVA content (Table 5). In contrast, inbred lines 5, 15, 21 and 24 had significant negative GCA for PVA content when used as both males and females (Table 4). Four hybrids had significant positive SCA effects for PVA content (Table S3).

Seventeen inbred lines displayed positive GCA for grain yield when used both as males and females. Inbred lines 8 and 18 showed significant positive GCA effects for grain yield when used both as males and females (Table 4). The largest GCA effect for grain yield was observed in inbred line 8 as a male parent, whereas the lowest GCA effect was observed in inbred line 14 as male parent (Table 4). The SCA effects for grain yield were positive for hybrids 8, 47, 86. (Table S3).

Table 5. Performance per se of hybrids and parental lines, GCA of parents for the top 10% ranking hybrids for micronutrients evaluated across environments.

H	Set	Parents		Performance per se			GCA		H	Set	Parents		Performance per se			GCA	
		M	F	F1	M	F	M	F			M	F	F1	M	F	M	F
Top 10% Hybrids Zn (mg kg^{-1})									Top 10% Hybrids Fe (mg kg^{-1})								
71	V	14	7	33.31	34.75	35.62	0.59	1.84 **	9	I	11	1	29.65	31.01	23.42	1.97 **	1.79 **
5	I	10	1	32.22	41.49	28.53	0.42	1.87 **	35	III	1	19	26.75	23.42	26.96	1.39 *	0.25
16	I	12	4	31.97	36.12	32.86	−0.19	1.26 *	13	I	12	1	26.63	24.03	22.73	−0.07	1.79 **
79	V	16	7	31.96	35.33	35.62	1.03 *	1.84 **	56	IV	22	16	26.43	26.16	27.89	0.78	1.03 *
78	V	16	6	31.88	35.33	32.61	1.03 *	−0.34	33	III	1	17	26.21	23.42	27.31	1.39 *	0.00
22	II	6	10	31.65	32.61	41.49	1.24 *	0.80	34	III	1	18	25.93	23.42	21.97	1.39 *	−0.57
26	II	7	10	31.64	35.62	41.49	1.24 *	0.80	11	I	11	3	25.89	31.01	22.69	1.97 **	0.21
13	I	12	1	31.45	36.12	28.53	−0.19	1.87 **	90	VI	19	22	25.86	26.96	26.16	0.77	0.82
24	II	6	12	31.42	32.61	36.12	1.24 *	0.57	83	VI	17	23	25.78	27.31	20.93	1.13 *	0.25
52	IV	21	16	31.19	37.12	35.33	0.76	0.13	17	II	5	9	25.77	23.06	24.29	0.81	−0.05
Top 10% Hybrids PVA (µg g^{-1})									Top 10% Hybrids β-Carotene (µg g^{-1})								
91	VI	19	23	15.28	10.88	15.15	2.64 **	3.50 **	91	VI	19	23	11.76	8.10	10.80	2.06 **	2.56 **
95	VI	20	23	13.21	11.25	15.15	1.07 **	3.50 **	60	IV	23	16	9.91	10.80	6.58	2.31 **	1.27 *
60	IV	23	16	12.97	15.15	9.31	2.86 **	1.59 *	58	IV	23	14	9.85	10.80	6.69	2.31 **	0.85
58	IV	23	14	12.36	15.15	8.39	2.86 **	0.77	47	III	4	19	9.49	7.34	8.10	1.06 *	1.36 *
78	V	16	6	12.21	9.31	5.85	1.69 *	1.86 **	90	VI	19	22	9.35	8.10	9.66	2.06 *	0.64
47	III	4	19	11.99	8.84	10.88	0.93	2.04 **	95	VI	20	23	9.1	7.34	10.80	0.49	2.31 *
83	VI	17	23	11.73	7..28	15.15	−0.19	3.50 **	78	V	16	6	8.93	6.58	3.37	1.38 **	1.42 **
90	VI	19	22	11.41	10.88	10.81	2.64 *	1.03 *	80	V	16	8	8.62	6.58	11.03	1.38 **	0.07
39	III	2	19	11.32	8.12	10.88	0.41	2.04 **	94	VI	20	22	8.29	7.34	9.66	0.49	0.64
24	II	6	12	11.08	5.85	8.33	1.53 *	0.54	87	VI	18	23	8.26	6.49	10.80	0.15	2.56 **

H: hybrids, GCA: general combining ability, F1: value of hybrid, M: male, F: female, PVA = provitamin A, *: significant at $p < 0.05$, **: significant at $p < 0.01$.

3.3. Effects of Mating Sets on Hybrid Zn, Fe and PVA Content

The mean performance of hybrids partitioned into six mating sets are shown in Table 6. Set I hybrids combined high levels of Zn (29.24 mg kg^{-1}) with high Fe content (24.45 mg kg^{-1}). Hybrids in set II combined highest Zn content (30.14 mg kg^{-1}) with average levels of PVA and Fe, and highest grain yield (5.23 t ha^{-1}). Hybrids in Set VI accumulated the highest levels of PVA (9.87 µg g^{-1}), but had the lowest Zn content (26.43 mg kg^{-1}).

Table 6. Mean values for Zn, Fe, PVA contents and grain yield for the six mating sets across environments.

Set	Description	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PVA (µg g^{-1})	GY (t ha^{-1})
1	GI × GIII	24.45	29.24	7.48	4.80
II	GIII × GII	23.74	30.14	7.80	5.23
III	GV × GI	22.97	27.33	8.67	4.98
IV	GIV × GVI	24.09	28.73	7.92	4.71
V	GII × GIV	22.70	29.43	7.54	4.74
V1	GVI × GV	24.04	26.43	9.87	4.78
	$LSD_{(0.05)}$	0.63	0.50	0.20	0.14

LSD: least significant difference, G: groups, PVA: provitamin A, GY: grain yield, see Table S1B for the description of sets.

3.4. Relationships among Micronutrients and Agronomic Traits

The correlation between PVA and Zn content was significant and negative but weak (r = −0.19, $p < 0.05$). While Zn content was positively correlated with Fe content ($p < 0.01$),

the correlation between Fe and PVA content was not significant (Table 7). In most cases, the correlations between agronomic traits and micronutrients were not significant (Table 8). PVA had significant negative correlation with ear aspect. Both Zn and Fe contents were positively correlated with flowering dates. Fe had significant and negative correlation with plant height and grain yield. While Zn had significant and positive correlation with ear height, its correlations with plant aspect and ear aspect were significant but negative. Zn had no association with grain yield.

Table 7. Pearson correlation coefficients among Fe, Zn and PVA contents in hybrids evaluated across environments.

	PVA (μg g^{-1})	**Fe (mg kg^{-1})**	**Zn (mg kg^{-1})**
PVA (μg g^{-1})	1	0.19	−0.19 *
Fe (mg kg^{-1})		1	0.42 **
Zn (mg kg^{-1})			1

*: significant at $p < 0.05$, **: significant at $p < 0.01$, PVA: provitamin.

Table 8. Pearson correlation coefficient between micronutrients content and agronomic traits averaged across environments.

	PVA (μg g^{-1})	**Fe (mg kg^{-1})**	**Zn (mg kg^{-1})**
Days to anthesis	0.12	0.21 *	0.56 **
Days to silking	0.10	0.21 *	0.56 **
ASI (days)	−0.13	0.06	0.06
Plant height (cm)	0.02	−0.33 *	0.13
Ear height (cm)	0.10	−0.09	0.37 **
Plant aspect	−0.11	0.14	−0.22 *
Ear aspect	−0.32 **	0.04	−0.23 *
Grain yield (tha^{-1})	0.02	−0.21 *	−0.05

* = significant at $p < 0.05$, ** = significant at $p < 0.01$, ASI: anthesis-silking interval.

4. Discussion

Combining ability studies provide information which aid in the understanding of the gene action involved in the inheritance of traits. This understanding will guide the design of breeding strategies for further enrichment of maize varieties with Zn, Fe and PVA, targeted to areas affected by deficiencies in micronutrients. This study was conducted to determine the gene actions involved in conditioning the inheritance of Zn, Fe, PVA contents and grain yield in maize.

In the present study, a wide range of variation was found among the parental lines and their hybrids for contents of Zn, Fe and PVA contents, as well as grain yield. The Zn and Fe contents reported in this study were slightly higher than the values reported by [15], and [10] but lower than the ranges reported by [40,41]. The PVA content reported in the present study was higher than the values reported by [16], but lower than the values reported by [28]. The similarities or differences in micronutrient content obtained in our study relative to values found in previous studies could arise due to differences in genetic backgrounds of the test materials, as well as the climatic and edaphic conditions prevalent at experimental sites. In general, micronutrient contents recorded in hybrids were lower than their parental lines, consistent with the results reported in previous studies for maize [15,28,42].

Increasing PVA, Zn and Fe content in maize may sometimes be associated with lower yields due to the dilution effects on micronutrients content caused by higher carbohydrates level in high yielding maize materials [2]. The absence of a significant correlation between grain yield and contents of Zn and PVA, and its weak association with Fe content in the present study, highlight the possibility of improving these nutrients in maize hybrids

without compromising grain yield. These findings are consistent with the reported low correlations between grain yield and Zn [14,15,42], and between grain yield and PVA content [24]. The significant positive correlation between Fe and Zn in the hybrid trial suggests that both micronutrients can be concurrently enhanced in maize, possibly due to tight linkages of the genes involved in accumulation of these micronutrients [43] and the similar physiological processes involved in nutrient uptake from the soil and their translocation to the grains [14,44]. As the vegetative parts of the maize plants serve as sink for Zn accumulation during the V7 to VT stages of development, taller plants could accumulate a higher content of Zn which is later remobilized to the grains. Therefore, plant height could be considered as a secondary trait for indirect selection of Zn [15]. However, in our study, Zn content and plant height had no significant correlation. This result is consistent with the findings of [14]. The significant positive correlation of Fe and Zn content with flowering dates reported in this study agrees with the findings of [15] suggesting that the longer the duration before flowering, the greater the content of minerals accumulated in vegetative parts of maize which may also be remobilized to the grains at maturity stage [45,46]. However, this association of flowering dates with Zn and Fe may be genotype-specific, as [14] reported significant positive correlation between mineral micronutrients and flowering dates in extra-early maturing maize while no significant associations were found in early-maturing maize in the same study. Although the correlation between PVA and Zn contents was significant and negative, it was too weak to hinder concurrent improvement of these nutrients. The fact that we found four hybrids that combined high contents of Zn and PVA levels supports this observation. The identification of inbred line 16 which had positive GCA effects for Fe, Zn and PVA gives additional credence to the possibility of enriching maize with high concentrations of the three micronutrients.

The significant male and female parent GCA and their SCA effects on PVA, Zn and Fe contents including grain yield imply the presence of both additive and non-additive gene effects controlling the inheritance these nutrients. However, the variance due to SCA represented a small fraction of the total variation in PVA and Fe contents, indicating the preponderance of additive gene effect over non-additive in the control of these nutrients. These results are consistent with results from previous studies [20,30,47] but contradicts with the findings reported by [24]. Inbred lines with the high significant and positive GCA effects for each of the micronutrients and grain yield are good combiners, highly adaptable and less influenced by environmental conditions. However, the low negative significant GCA effects for these micronutrients and grain yield displayed by some inbred lines in this study indicated their low combining ability for the traits and poor adaptability to varying environmental conditions. Hybrids with the high positive and significant SCA effects for the micronutrients content and grain yield implied desirable specific cross combinations for the traits. In this study, the PVA and Zn contents of the parental lines were encapsulated in their hybrids. For example, high-Zn × high-Zn crosses resulted in hybrids with high Zn content (Set I and II), whereas low-Zn × low-Zn crosses produced hybrids with low Zn content (Set V1). Similarly, high-PVA × high-PVA (Set III) and high-PVA × medium-PVA (Set VI) crosses produced hybrids with the highest PVA contents. Interestingly, low-Zn, high-PVA × high-Zn, high-PVA lines produced some hybrids with Zn content exceeding 29.0 mg kg^{-1}, implying that an inbred line with low Zn could have high genetic potential for use as a parent to develop hybrids combining high Zn with high PVA content. It is important to note that the best 10% of the hybrids for PVA, Zn and Fe contents had at least one parent with significant positive GCA effects for Zn, Fe and PVA, likewise the worst 10% of the hybrids for Zn and Fe content had at least one parent with significant negative GCA effects for these traits. Similar observations have been reported in previous combining ability studies for minerals in maize [15,25,48] and pearl millet [43,49]. The significant GCA × environment interaction effects on all three micronutrients including grain yield observed in at least one of the parents highlight the importance of selecting specific desirable inbred parents for a given environment.

The preponderance of additive gene action in the regulation of PVA and Fe contents implies that recurrent selection can significantly improve contents of the micronutrients in source populations which could be used to develop inbred lines with much higher levels of these nutrients. The superiority of GCA effects over SCA effects in this study for PVA and Fe is also an indication of the effectiveness of early generation testing to identify promising lines to develop hybrids with high micronutrients content. Since the SCA effects were also significant for the micronutrients and grain yield, predicting hybrid performance based on parents alone may not always be accurate. It was also observed that per se performance of inbred lines did not always correspond with their GCA effects on micronutrient content. For example, inbred line 1 which manifested large GCA effects for Zn and Fe content had relatively low content of the nutrients. This weak association between per se performance and GCA effects could be due to epistatic interactions [50] and thus selection of superior parents for micronutrient enrichment should be carried out in hybrid combinations.

Among the 12 inbred lines initially classified as high-Zn (GPI- GP III), seven inbred lines had positive GCA effects on Zn contents when used as males and as females. Positive GCA effects on Zn were also shown in inbred lines 21 and 22 (low-Zn group) as males. This result was consistent with the findings of [15] who found only three of the 10 inbred lines in the high-Zn group displaying positive GCA effects for Zn content. In contrast, [30] in their study reported that all inbred lines in the high-Zn group had positive GCA for Zn while all inbred lines in the low-Zn group display negative GCA for grain Zn content. Inbred lines 1, 5 and 16 combined positive GCA effects for Zn and Fe with positive GCA effects for grain yield. These lines could thus be used as potential parents for transferring favourable alleles to develop maize hybrids combining high Zn and Fe contents with high grain yield. Inbred line 16 which showed positive GCA effects for all three micronutrients and grain yield can be used as a parent for developing new maize hybrids. Some parental lines with significant positive GCA produced hybrids with significant positive SCA for a specific micronutrient. For instance, inbred line 7 with high GCA effects for Zn was a parent of hybrids 71, 26 and 27 showing positive SCA effect for Zn content. In contrast, some hybrids with significant positive SCA effects had parents with negative GCA for a trait. For example, inbred line 15 which had negative GCA effects for Zn was a parent of hybrids 73 and 76 with significant positive SCA effects for Zn content. Such contrasting potentials of parents could arise from differences in combination of dominant or recessive alleles from one of the parents [51].

5. Conclusions

Our study demonstrated that PVA and Fe content in maize were controlled predominantly by additive gene effects, whereas both additive and non-additive gene effects were important in the inheritance of Zn content and grain yield. Inbred lines with high GCA effects on micronutrients content and grain yield were identified for use as parents to develop synthetics, hybrids and new maize inbred lines enriched with high levels of micronutrients and superior agronomic performance. Hybrids with significant SCA effects on PVA, Fe and Zn were also identified. Further studies are necessary to investigate the potential of exploiting heterosis for PVA and mineral nutrients in maize.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13010266/s1, Table S1A: parental inbred lines used in factorial mating scheme and their groupings. Table S1B: description of the crossing scheme for each main set. Table S2: means squares from analysis of variance for Zn, Fe and PVA concentrations of 24 parental lines across environments. Table S3: estimates of specific combining ability (SCA) effects for micronutrients and grain yield in 96 hybrids evaluated across environments. Table S4: mean values for agronomic traits of 96 hybrids evaluated across environments. Table S5: the physical and chemical properties of soils (0–30 cm) of the study sites. Table S6: agroecological zones, average rainfall, temperature, relative humidity and soil type of the study locations.

Author Contributions: Conceptualization, A.M., A.A. and E.U.; methodology, E.U., A.M. and S.M.; data curation: E.U., A.M. and W.M.; writing-original draft preparation: E.U.; writing-review and

editing, E.U., A.M., A.A., S.M. and W.M.; supervision, A.M., A.A., S.M. and W.M., project administration, A.M.; fund acquisition, AM. All authors have read and agreed to the published version of the manuscript.

Funding: This work is part of a Ph.D research of the first author, funded by Bill and Melinda Gates Foundation (BMGF Chronos) grant number: OPP1019962), under the framework of HarvestPlus 3.

Data Availability Statement: Data presented in this study are available contained within the article or supplementary material.

Acknowledgments: The authors are grateful to the staff of Maize Improvement Program (MIP) and Food and Nutritional Sciences laboratory of the Institute of Tropical Agriculture (IITA) for their technical supports.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ekanem, S.F.; Audu, A.A.; Lieberman, M. Proximate Analysis of Grain Samples From Strategic Grain Reserve Facilities in Nigeria. *J. Chem. Soc. Niger.* **2022**, *47*, 592–602. [CrossRef]
2. Bänziger, M.; Long, J. The Potential for Increasing the Iron and Zinc Density of Maize through Plant Breeding. *Food Nutr. Bull.* **2000**, *21*, 397–400. [CrossRef]
3. Goredema-Matongera, N.; Ndhlela, T.; Magorokosho, C.; Kamutando, C.N.; van Biljon, A.; Labuschagne, M. Multinutrient Biofortification of Maize (*Zea mays* L.) in Africa: Current Status, Opportunities and Limitations. *Nutrients* **2021**, *13*, 1039. [CrossRef] [PubMed]
4. AREDS Research Group. Associations of Mortality with Ocular Disorders and an Interventionof High-Dose Antioxidants and Zinc in the Age-Related Eye Disease Study: AREDS Report No. 13. *Arch. Ophthalmol.* **2004**, *122*, 716–726. [CrossRef] [PubMed]
5. Rostan, E.F.; DeBuys, H.V.; Madey, D.L.; Pinnell, S.R. Evidence supporting zinc as an important antioxidant for skin. *Int. J. Dermatol.* **2002**, *41*, 606–611. [CrossRef]
6. Broadley, M.; Brown, P.; Cakmak, I.; Rengel, Z.; Zhao, F. Function of nutrients: Micronutrients. In *Marschner's Mineral Nutrition of Higher Plants*; Academic Press: Cambridge, MA, USA, 2012; pp. 191–248.
7. Awuchi, C.G.; Igwe, V.S.; Amagwula, I.O. Nutritional diseases and nutrient toxicities: A systematic review of the diets and nutrition for prevention and treatment. *Int. J. Adv. Acad. Res.* **2020**, *6*, 1–46. [CrossRef]
8. Amann, P.M.; Eichmuller, S.B.; Schmidt, J.; Bazhin, A.V. Regulation of gene expression by retinoids. *Curr. Med. Chem.* **2011**, *18*, 1405–1412. [CrossRef]
9. Shukla, A.K.; Behera, S.K.; Pakhre, A.; Chaudhari, S.K. Micronutrients in soils, plants, animals and humans. *Indian J. Fertil.* **2018**, *14*, 30–54.
10. Oikeh, S.O.; Menkir, A.; Maziya-Dixon, B.; Welch, R.; Glahn, R.P. Assessment of Concentrations of Iron and Zinc and Bioavailable Iron in Grains of Early-Maturing Tropical Maize Varieties. *J. Agric. Food Chem.* **2003**, *51*, 3688–3694. [CrossRef]
11. Chomba, E.; Westcott, C.M.; Westcott, J.E.; Mpabalwani, E.M.; Krebs, N.F.; Patinkin, Z.W.; Palacios, N.; Hambidge, K.M. Zinc Absorption from Biofortified Maize Meets the Requirements of Young Rural Zambian Children. *J. Nutr.* **2015**, *145*, 514–519. [CrossRef]
12. Maqbool, M.A.; Beshir, A. Zinc Biofortification of Maize (*Zea mays* L.): Status and Challenges. *Plant Breed.* **2019**, *138*, 1–28. [CrossRef]
13. Ortiz-Monasterio, J.I.; Palacios-Rojas, N.; Meng, E.; Pixley, K.; Trethowan, R.; Pena, R.J. Enhancing the Mineral and Vitamin Content of Wheat and Maize through Plant Breeding. *J. Cereal Sci.* **2007**, *46*, 293–307. [CrossRef]
14. Akinwale, R.O.; Adewopo, O.A. Grain Iron and Zinc Concentrations and Their Relationship with Selected Agronomic Traits in Early and Extra-Early Maize. *J. Crop Improv.* **2016**, *30*, 641–656. [CrossRef]
15. Mageto, E.K.; Lee, M.; Dhliwayo, T.; Palacios-rojas, N.; Vicente, S.; Burgueño, J.; Hallauer, A.R. An Evaluation of Kernel Zinc in Hybrids of Elite Quality Protein Maize (QPM) and Non-QPM Inbred Lines Adapted to the Tropics Based on a Mating Design. *Agronomy* **2020**, *10*, 695. [CrossRef]
16. Azmach, G.; Gedil, M.; Spillane, C.; Menkir, A. Combining Ability and Heterosis for Endosperm Carotenoids and Agronomic Traits in Tropical Maize Lines. *Front. Plant Sci.* **2021**, *12*, 674089. [CrossRef]
17. Asson-Batres, M.A.; Rochette-Egly, C. *Biochemistry of Retinoid Signaling II*; Springer: Berlin/Heidelberg, Germany, 2016.
18. Menkir, A.; Rocheford, T.; Maziya-Dixon, B.; Tanumihardjo, S. Exploiting natural variation in exotic germplasm for increasing provitamin-A carotenoids in tropical maize. *Euphytica* **2015**, *205*, 203–217. [CrossRef]
19. Nuss, E.T.; Arscott, S.A.; Bresnahan, K.; Pixley, K.V.; Rocheford, T.; Hotz, C.; Siamusantu, W.; Chileshe, J.; Tanumihardjo, S.A. Comparative intake of white-versus orange-colored maize by Zambian children in the context of promotion of biofortified maize. *Food Nutr. Bull.* **2012**, *33*, 63–71. [CrossRef]
20. Iseghohi, I.; Abe, A.; Meseka, S.; Mengesha, W.; Gedil, M.; Menkir, A. Assessing Effect of Marker-Based Improvement of Maize Synthetics on Agronomic Performance, Carotenoid Content, Combining Ability and Heterosis. *Agronomy* **2020**, *10*, 1625. [CrossRef]

21. Karlen, D.L.; Kramer, L.A.; Logsdon, S.D. Field-scale Nitrogen Balances Associated with Long-term Continuous Corn Production. *Agron. J.* **1998**, *90*, 644–650. [CrossRef]
22. Hindu, V.; Palacios-Rojas, N.; Babu, R.; Suwarno, W.B.; Rashid, Z.; Usha, R.; Saykhedkar, G.R.; Nair, S.K. Identification and Validation of Genomic Regions Influencing Kernel Zinc and Iron in Maize. *Theor. Appl. Genet.* **2018**, *131*, 1443–1457. [CrossRef]
23. Hallauer, A.R.; Filho, J.B.M.; Carena, M.J. Heterosis. In *Quantitative Genetics in Maize Breeding*, 3rd ed.; Prohens, J., Nuez, F., Carena, M.J., Eds.; Chapter 10; Springer Science+Business Media: NewYork, NY, USA, 2010; pp. 477–481.
24. Halilu, A.D.; Ado, S.G.; Aba, D.A.; Usman, I.S. Genetics of Carotenoids for Provitamin A Biofortification in Tropical-Adapted Maize. *Crop J.* **2016**, *4*, 313–322. [CrossRef]
25. Brkić, I.; Šimić, D.; Zdunić, Z.; Jambrović, A.; Ledenčan, T.; Kovačević, V.; Kadar, I. Combining Abilities of Corn-Belt Inbred Lines of Maize for Mineral Content in Grain. *Maydica* **2003**, *48*, 293–297.
26. Run, L.I.; Xiao, L.; Jing, W.; Lu, Y.; Rong, T.; Pan, G.; Wu, Y.; Qilin, T.; Hai, L.A.N.; Cao, M. Combining Ability and Parent-Offspring Correlation of Maize (*Zea may* L.) Grain β-Carotene Content with a Complete Diallel. *J. Integr. Agric.* **2013**, *12*, 19–26.
27. Obeng-Bio, E.; Badu-Apraku, B.; Ifie, B.E.; Danquah, A.; Blay, E.T.; Annor, B. Genetic Analysis of Grain Yield and Agronomic Traits of Early Provitamin A Quality Protein Maize Inbred Lines in Contrasting Environments. *J. Agric. Sci.* **2019**, *157*, 413–433. [CrossRef]
28. Maazou, A.-R.S.; Adetimirin, V.O.; Gedil, M.; Meseka, S.; Mengesha, W.; Menkir, A. Suitability of Testers to Characterize Provitamin a Content and Agronomic Performance of Tropical Maize Inbred Lines. *Front. Genet.* **2022**, *13*, 955420. [CrossRef]
29. El-Bendary, A.A.; El-Fouly, M.M.; Rakha, F.A.; Omar, A.A.; Abou-Youssef, A.Y. Mode of Inheritance of Zinc Accumulation in Maize. *J. Plant Nutr.* **1993**, *16*, 2043–2053. [CrossRef]
30. Long, J.K.; Bänziger, M.; Smith, M.E. Diallel Analysis of Grain Iron and Zinc Density in Southern African-adapted Maize Inbreds. *Crop Sci.* **2004**, *44*, 2019–2026. [CrossRef]
31. Akinwale, R.O.; Fadoju, A.O.; Sulola, B.H.; Oluwaranti, A.; Awosanmi, F.E. Inheritance of Seed Quality Traits and Concentrations of Zinc and Iron in Maize Topcross Hybrids. *Afr. Crop Sci. J.* **2021**, *29*, 119–131. [CrossRef]
32. Graham, R.D.; Rosser, J.M. Carotenoids in Staple Foods: Their Potential to Improve Human Nutrition. *Food Nutr. Bull.* **2000**, *21*, 404–409. [CrossRef]
33. García-Casal, M.N.; Leets, I.; Layrisse, M. β-Carotene and Inhibitors of Iron Absorption Modify Iron Uptake by Caco-2 Cells. *J. Nutr.* **2000**, *130*, 5–9. [CrossRef]
34. Paltridge, N.G.; Palmer, L.J.; Milham, P.J.; Guild, G.E.; Stangoulis, J.C.R. Energy-Dispersive X-ray Fluorescence Analysis of Zinc and Iron Concentration in Rice and Pearl Millet Grain. *Plant Soil* **2012**, *361*, 251–260. [CrossRef]
35. Howe, J.A.; Tanumihardjo, S.A. Carotenoid-Biofortified Maize Maintains Adequate Vitamin A Status in Mongolian Gerbils. *J. Nutr.* **2006**, *136*, 2562–2567. [CrossRef]
36. Rodriguez-Amaya, D.B.; Kimura, M. *HarvestPlus Handbook for Carotenoid Analysis*; International Food Policy Research Institute (IFPRI): Washington, MA, USA, 2004; Volume 2.
37. US Institute of Medicine. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*; The National Academies Press: Washington, DC, USA, 2001.
38. SAS Institute. *SAS System for Windows*; Release 9.4; SAS Institute Inc.: Cary, NC, USA, 2012.
39. Rodríguez, F.; Alvarado, G.; Pacheco, Á.; Crossa, J.; Burgueño, J. *AGR-R (Analysis of Genetic Designs in R)*; Version 5.0; International Maize and Wheat Improvement Center (CIMMYT): Mexico City, Mexico, 2018.
40. Prasanna, B.M.; Mazumdar, S.; Chakraborti, M.; Hossain, F.; Manjaiah, K.M.; Agrawal, P.K.; Gupta, H.S. Genetic variability and genotype × environment interactions for kernel iron and zinc concentrations in maize (*Zea mays*) genotypes. *Indian J. Agric Sci.* **2011**, *81*, 704–711.
41. Queiroz, V.A.V.; Guimarães, P.E.D.O.; Queiroz, L.R.; Guedes, E.D.O.; Vasconcelos, V.D.B.; Guimarães, L.J.; Ribeiro, P.E.D.A.; Schaffert, R.E. Iron and zinc availability in maize lines. *Food Sci. Technol.* **2011**, *31*, 577–583. [CrossRef]
42. Chakraborti, M.; Prasanna, B.M.; Hossain, F.; Singh, A.M. Evaluation of Single Cross Quality Protein Maize (QPM) Hybrids for Kernel Iron and Zinc Concentrations. *Indian J. Genet. Plant Breed.* **2011**, *71*, 312–319.
43. Kanatti, A.; Rai, K.N.; Radhika, K.; Govindaraj, M.; Sahrawat, K.L.; Rao, A.S. Grain iron and zinc density in pearl millet: Combining ability, heterosis and association with grain yield and grain size. *SpringerPlus* **2011**, *3*, 763. [CrossRef]
44. Oikeh, S.O.; Menkir, A.; Maziya-Dixon, B.; Welch, R.M.; Glahn, R.P.; Gauch, G. Environmental Stability of Iron and Zinc Concentrations in Grain of Elite Early-Maturing Tropical Maize Genotypes Grown under Field Conditions. *J. Agric. Sci.* **2004**, *142*, 543–551. [CrossRef]
45. Grzebisz, W.; Wronska, M.; Diatta, J.B.; Dullin, P. Effect of zinc foliar application at an early stage of maize growth on patterns of nutrients and dry matter accumulation by the canopy. Part I. Zinc uptake patterns and its redistribution among maize organs. *J. Elem.* **2008**, *13*, 17–28.
46. Sperotto, R.A. Zn/Fe remobilization from vegetative tissues to rice seeds: Should I stay or should I go? Ask Zn/Fe supply! *Front. Plant Sci.* **2013**, *4*, 464. [CrossRef]
47. Menkir, A.; Gedil, M.; Tanumihardjo, S.; Adepoju, A.; Bossey, B. Carotenoid Accumulation and Agronomic Performance of Maize Hybrids Involving Parental Combinations from Different Marker-Based Groups. *Food Chem.* **2014**, *148*, 131–137. [CrossRef]
48. Šimić, D.; Mladenović Drinić, S.; Zdunić, Z.; Jambrović, A.; Ledenčan, T.; Brkić, J.; Brkić, A.; Brkić, I. Quantitative Trait Loci for Biofortification Traits in Maize Grain. *J. Hered.* **2012**, *103*, 47–54. [CrossRef] [PubMed]

49. Velu, G.; Rai, K.N.; Muralidharan, V.; Longvah, T.; Crossa, J. Gene effects and heterosis for grain iron and zinc density in pearl millet (*Pennisetum glaucum* (L.) R. Br). *Euphytica* **2011**, *180*, 251–259. [CrossRef]
50. Vidhyavathi, R.; Manivannan, N.; Muralidharan, V. Line × tester analysis in sesame (*Sesamum indicum* L.). *Indian J. of Agric. Res.* **2005**, *39*, 225.
51. Chaudhari, G.R.; Patel, D.A.; Parmar, D.P.; Patel, K.C. Fe, Zn & Protein content in grain, per se performance, heterosis, combining ability of grain yield in bread wheat (*Triticum aestivum*) under normal & late sowing condition. *Res. Sq.* 2005, *pre-print*. [CrossRef]