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Research article

Heterosis and combining ability of iron, zinc and their bioavailability in maize inbred lines under low nitrogen and optimal environments

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A B S T R A C T

Iron (Fe) and zinc (Zn) nutrient enrichment of staple crops through biofortification can contribute to alleviating micronutrient deficiency in sub-Saharan Africa. A line × tester mating design was used to determine the general combining ability (GCA), specific combining ability (SCA) and heterosis for grain yield, iron, Zn and phytic concentration of six lines crossed with three testers. Lines and testers were selected for high, intermediate and low mineral content. The F1 hybrids and parental lines were evaluated under low nitrogen (N) and optimum conditions across four environments over two seasons. Under low N conditions, Fe and Zn concentration in grain, and grain yield of genotypes were reduced by 9%, 9%, and 59%, respectively. However, phytic acid concentration in grain was increased by 10% under low N conditions. Both additive and non-additive gene effects were important in controlling Fe, Zn and phytic acid concentration in grain and grain yield of maize under both N conditions. The preponderance of GCA effects indicates the importance of additive gene effects in the inheritance of grain yield. Line GCA effects were more sensitive to N conditions across the environments than the tester GCA. High and significant positive SCA effects for grain yield, Fe and Zn content under low N conditions, would be a good indicator of possible heterosis in these traits. Hybrid CBY101 LM-1600 × CBY358 LM-1857 had high and significant positive SCA for grain yield under low N conditions and is a promising candidate for production in low N environments. CBY358 LM-1857 (tester) and CBY102 LM-1601 (line) are a good general combiners for Fe, Zn and GY can be used as parents in future maize hybrid breeding programs to develop high-yielding maize genotypes with high Fe and Zn content.

1. Introduction

Micronutrients are required in small amounts [1] but are essential for normal functioning of several metabolic processes in the human body [2]. Fe and Zn are vital micronutrients that play a role in transporting oxygen in blood and regulation of Zn-containing

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enzymes, respectively, in people of all ages, but especially in women, and children under five years of age [3–5]. Hidden hunger (particularly in the case of minerals such as Fe and Zn) affects every third person in the world, which amounts to two billion people, particularly in low-income countries [6]. Provitamin A, iron and zinc deficiency negatively affects the physical well-being, eyesight and cognitive development of people and can be leads to birth defects and child mortality, and due to this, those traits are the major nutritional quality traits for maize grain SSA [7]. Enrichment of staple food crops with Fe and Zn through breeding is the most effective way to overcome deficiencies of these mineral nutrients, as biofortification is a once-off intervention, and rural populations can access biofortified crop varieties [3,8–10]. Bioavailability (the ability of Fe and Zn to be digested, absorbed and utilized by the body) should also be considered in a breeding program, because some compounds such as phytate, hinders the absorption of micronutrient (such as Fe and Zn) in the human body [10,11]. Calculations of molar ratios of Fe and Zn with phytic acid (PA) is an initial screening for the prediction of Fe and Zn bioavailability [12].

In sub-Saharan Africa, low soil nitrogen (N) is the major maize production threat [13]. A 90–120 kg N/ha fertilizer rate in SSA is generally recommended for maize production [14]. Nevertheless, fertilizer application rates in the region still need to catch up to the recommended doses because of the high cost of fertilizer relative to the extra yield gained from fertilizer application and making it uneconomical for farmers to apply and the non-availability of fertilizer at the time it is needed [13,14]. Therefore, low N remains a great challenge to maize production in resource-poor farmers' fields. Because of these production challenges in the SSA, about 10%–50% of annual losses in maize production are attributable to the impact of low N in the soil [13]. Therefore, developing and using superior maize hybrids for low N tolerance is crucial for increasing production and productivity across SSA. In maize breeding strategy, information on combining ability and heterosis are the most important factors for developing high-yielding maize hybrids for tolerance to low N offers the most economical and sustainable approach to increasing yielding maize hybrids by small-scale farmers who normally only apply small inputs [15]. Analysis of combining ability of inbred lines for types of gene action controlling quantitative traits, which are used for genetic diversity analysis, inbred selection for N tolerance with high grain yield, estimation of heterosis, and hybrid development [16].

Maize is one of the most commonly grown cereals in the world and is the staple food of millions of people in Asia, Latin America and Africa. It has high levels of genetic variability and has the ability to grow in tropical, sub-tropical and temperate agroecological conditions [17]. The area of maize production has been consistently increasing. Therefore, there is need to develop maize hybrids with higher levels of bioavailable Fe and Zn, and good yield potential. Investigation of the amount of genetic variation present and the combining ability effects is necessary for efficient use of such parental materials in breeding. Combining ability is an important tool for selecting potential parental lines and determining the type of gene action involved in required traits, which helps plant breeders plan hybrid breeding programs [18]. Line × tester analysis[19] has been used extensively by plant breeders in the world, also in maize breeding, to obtain reliable information on GCA and SCA of large numbers of parents and their hybrid combinations for yield and other traits [20–22]. Crossed 14 South African adapted white maize inbred lines using a diallel scheme and generated seven high and seven low Fe and Zn containing hybrids and evaluated them at six locations with two replicates under optimal (five sites) and low N (one site) conditions in Zimbabwe [23]. They concluded that GCA effects were significantly more important than SCA effects for Fe and Zn content under optimal conditions. One line with low Fe and Zn was found to have significant and positive GCA effects for Fe with no effect for Zn contents. Lines with low Fe and Zn emerged as promising lines for hybrid development. The objective of this study was to determine the combing ability of maize genotypes, and the nature, magnitude and direction of gene action governing the expression of grain yield, Fe, Zn and PA content of inbred lines and their hybrids under low nitrogen and optimum environments, and to identify suitable hybrids for the target environments.

2. Materials and methods

2.1. Germplasm and environments

The plant material consisted of nine parents, including six females and three males (Table 1) selected after screening 215 South African maize inbred lines obtained from the Agricultural Research Council - Grain Crops (ARC-GC) for concentration (low, medium and high) of Fe and Zn. The parents were planted in July 2016 at Makhathini Research Station located in the far northern area of Kwazulu Natal Province and crossed following a line by tester (6 × 3) scheme and 18 F_1 maize hybrids (Table 2) were generated. The

Table 1
List of parental material selected from 215 maize germplasm lines.

Parents	Pedigree	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Concentration
Line-1 (F1)	CBY075 LM-1574	340.0	105.0	High
Line-2 (F2)	CBY101 LM-1600	287.0	103.0	High
Line-3 (F3)	CBY102 LM-1601	116.5	49.5	Medium
Line-4 (F4)	CBY359 LM-1858	101.0	47.5	Medium
Line-5 (F5)	CBY017 LM-1516	55.5	18.0	Low
Line-6 (F6)	CBY014 LM-1513	45.0	10.5	Low
Tester-1 (M1)	CBY358 LM-1857	139.5	56.0	High
Tester-2 (M2)	CBY104 LM-1603	121.5	35.0	Medium
Tester-3 (M3)	CBY013 LM-1512	44.5	9.0	Low

F= Female, M = Male.

hybrids along with the nine parents were planted in December 2016 and 2017 at Potchefstroom, Cedara and Vaalharts following a randomised complete block design with two replicates under optimal and low nitrogen (N) conditions.

Potchefstroom is in the Northwest province and lies at −26.73° latitude, 27.08° longitude, at an altitude of 1349 m above sea level (masl), with brown sandy loam soils (Table 3). Low N conditions were created by depleting soil of N, by planting maize for several years without N fertilization and removing all stover from the field. The fertilizer regime for optimal conditions was compound fertilizer 3:2:1 (25) + Zn applied as a basal application at planting at a rate of 200 kg NPK ha^{-1} to optimum N plots. Limestone ammonium nitrate (LAN) with 28% N was used for top-dressing in two equal splits at 28 and 56 days after emergence at a rate of 100 kg ha^{-1} each only in optimum N plots. In low N plots, NPK was applied at a rate of 100 kg ha^{-1}.

Cedara is in the KwaZulu-Natal province and lies at −29.54° latitude, 30.26° longitude, at an altitude of 1066 masl, with reddish brown clay soils (Table 3). Fertilizer used was monoammonium phosphate (MAP), 250 kg ha^{-1} at planting, for optimum N environments, 30 kg ha^{-1} in the low N environment and LAN given at 150 kg ha^{-1} in two equal splits of 75 kg ha^{-1} for only the optimum N sites at 28 and 56 days after emergence. Vaalharts is in the Northern Cape province at −28°06′56.84″ S 24°55′32.50″ E at an altitude of 1192 masl. The fertilizer was applied at the same rate as at Potchefstroom. All standard agronomic practises were applied under both growing conditions. Under low N conditions no N was applied. Trials were grown under dryland conditions, which is the norm for the trial areas. The distance between plants and rows were 0.25 m and 0.75 m, respectively at all locations. In each plot there were two rows of 4 m length. The plot size was 6 m^2. Five healthy plants from the middle of each plot were selected for data collection.

2.2. Data collection

2.2.1. Fe and Zn analysis

Five plants per plot for all plots were self-pollinated at all locations to generate seed for laboratory analysis. Selfing was done to eliminate the possibility of pollination with foreign pollen, which may influence results. These samples were oven dried and milled using an IKA, A10 Yellowline grinder (Merck Chemicals Pty Ltd) and sieved with a 1 mm screen mesh. The extraction steps of Fe and Zn were done according to the dry-ashing method outlined by the AOAC (2000). Approximately 2 g of flour was weighed into glazed, high-form porcelain crucibles and ashed in a furnace at 550 °C for 3 h. One mL nitric acid (HNO_3, 55%) was added to the samples for digestion. The samples were then placed in a hot sand-bath until they were completely dry, after which they were returned to the oven for 1 h at 550 °C for further ashing. After cooling, 10 mL of 1:2 HNO_3 was added to the samples for further digestion. The samples were returned to the hot sand-bath until they became warm. The samples were then transferred to 100 mL volumetric flasks using Whatman # 4 filter paper and filled to the mark with distilled water. Mineral concentrations were measured in triplicate using an Atomic Absorption Spectrophotometer (Agilent Technologies 200 Series AA).

2.2.2. Phytic acid analysis

Phytic acid analysis was done following the method of [24] with some modifications. Finely ground maize flour (0.25 g) was weighed into a 15 mL Falcon tube. Ten mL freshly prepared 5% Tri-chloroacetic acid (TCA) was added to the samples and placed on a mechanical shaker for 1 h and vortexed at 10 min intervals to extract PA. Five mL of PA extract was then transferred to another 15 mL Falcon tube and centrifuged at 12 000 rpm for 10 min (at 4 °C). After centrifugation, 0.5 mL of the supernatant and 1.5 mL of the Wade reagent were transferred into 2 mL Eppendorf tubes and centrifuged in an Eppendorf 5417C centrifuge, at 12 000 rpm for 10 min. Double distilled water was used as a blank and absorbance of the Wade reagent and samples were recorded at 500 nm in a Jenway 7315 spectrophotometer. The absorbance of the samples was subtracted from the absorbance of the Wade reagent. A standard curve for PA determination was prepared in MS Excel and calculations were done according to Ref. [25]. Moles of Fe and Zn and Pa were calculated by dividing the weight of PA and Fe and Zn by their atomic weights and then molar ratios of Fe and Zn were calculated by dividing

Table 2
List of 18 hybrids generated from the line × tester crosses.

Hybrids	Pedigree
Line-1 × Tester-1	CBY075 LM-1574 × CBY358 LM-1857
Line-1 × Tester-2	CBY075 LM-1574 × CBY104 LM-1603
Line-1 × Tester-3	CBY075 LM-1574 × CBY013 LM-1512
Line-2 × Tester-1	CBY075 LM-1574 × CBY358 LM-1857
Line-2 × Tester-2	CBY075 LM-1574 × CBY104 LM-1603
Line-2 × Tester-3	CBY075 LM-1574 × CBY013 LM-1512
Line-3 × Tester-1	CBY075 LM-1574 × CBY358 LM-1857
Line-3 × Tester-2	CBY075 LM-1574 × CBY104 LM-1603
Line-3 × Tester-3	CBY075 LM-1574 × CBY013 LM-1512
Line-4 × Tester-1	CBY075 LM-1574 × CBY358 LM-1857
Line-4 × Tester-2	CBY075 LM-1574 × CBY104 LM-1603
Line-4 × Tester-3	CBY075 LM-1574 × CBY013 LM-1512
Line-5 × Tester-1	CBY075 LM-1574 × CBY358 LM-1857
Line-5 × Tester-2	CBY075 LM-1574 × CBY104 LM-1603
Line-5 × Tester-3	CBY075 LM-1574 × CBY013 LM-1512
Line-6 × Tester-1	CBY075 LM-1574 × CBY358 LM-1857
Line-6 × Tester-2	CBY075 LM-1574 × CBY104 LM-1603
Line-6 × Tester-3	CBY075 LM-1574 × CBY013 LM-1512

Table 3
Soil analysis of experimental sites used.

Minerals	Soil Depth	Potchefstroom		Cedara		Vaalharts	
		Optimal	Low N	Optimal	Low N	Optimal	Low N
		2016–17	2016–17	2016–17	2016–17	2016–17	2016–17
Fe (mg kg^{-1})	30 cm	11.9	10.0	13.5	9.6	7.0	5.9
	60 cm	10.6	8.4	11.9	10.1	6.6	5.9
Zn (mg kg^{-1})	30 cm	9.4	9.0	1.3	3.2	3.3	2.5
	60 cm	8.6	5.6	1.4	2.2	2.9	2.3
P (mg kg^{-1})	30 cm	27.9	15.5	11.7	12.8	52.3	32.4
	60 cm	35.7	12.6	10.5	10.1	44.7	29.3
K (mg kg^{-1})	30 cm	278.5	198.4	77.0	174.5	123	163
	60 cm	314.9	209.7	70.5	120.0	114	149
Ca (mg kg^{-1})	30 cm	830.0	666.0	513.0	699.0	436	535
	60 cm	952.0	887.0	511.0	694.0	402	500
Mg (mg kg^{-1})	30 cm	384.9	328.5	99.0	166.0	141	174
	60 cm	440.7	438.9	99.5	154.0	128	169
Mn (mg kg^{-1})	30 cm	38.9	35.1	3.6	3.4	11.1	13.2
	60 cm	43.8	26.9	3.6	2.3	9.2	13.1
Soil pH	30 cm	6.5	6.1	4.3	4.4	6.0	6.3
	60 cm	6.6	6.0	4.4	4.5	6.1	6.3

moles of PA with moles of minerals [26].

2.2.3. Grain yield

Grain yield (GY) was measured as kg $plot^{-1}$ using an electronic balance and then converted to ton per hectare (t ha^{-1}) at 12.5% moisture. Grain moisture was measured using moisture meter for maize in the field at harvesting.

2.3. Data analysis

Genotypes (lines, testers and hybrids) and environments were treated as fixed, whereas interactions (site × line, site × tester, line × tester, and site × line × tester), and replication were treated as random effects. Combining ability effects and variances were determined using Analysis of Genetic Designs with R for Windows [27]. The effects of lines and testers and SCA effects of hybrids were estimated using line × tester analysis [19,28].

2.3.1. Heterosis analysis

Heterosis was estimated as:

High parent heterosis (%): [(F1 – HPV)/HPV] × 100

where: F1 is the mean performance of the cross and HPV is the mean value of the highest performing parent.

Mid parent heterosis (MPH) = [(F1 – MPV)/MPV] × 100

where MPV is mean of the two parents.

2.3.2. GCA and SCA variance estimates

The variances for GCA (lines and testers) and SCA effects were calculated according to Ref. [29]. Narrow sense heritability (h^2) was calculated using the formula described by Ref. [30] as follows:

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2} \times 100$$

where: h^2 = Heritability in the narrow sense; σ_p^2 = Phenotypic variance; σ_a^2 = Additive variance.

3. Results

3.1. Mean performance of genotypes

The effects of genotypes and environment were significant for all traits in all environments (Tables 4 and 5). Under optimum conditions, the grain yield varied from 2.46 to 9.33 t ha^{-1}, with a mean of 6.14 (t ha^{-1}). The top five yielding genotypes were CBY101 LM-1600 × CBY358 LM-1857, CBY102 LM-1601 × CBY358 LM-1857, CBY359 LM-1858 × CBY104 LM-1603, CBY359 LM-1858 × CBY013 LM-1512 and CBY017 LM-1516 × CBY013 LM-1512. The mean of Fe ranged from 16.33 to 20.44 mg kg^{-1}, with an average of

18.02 mg kg^{-1}, while Zn content varied from 18.36 to 23.79 mg kg^{-1} with an average of 20.39 mg kg^{-1}. The top five genotypes for Fe content comprised of four hybrids and one line (CBY017 LM-1516 × CBY013 LM-1512, CBY017 LM-1516, CBY017 LM-1516 × CBY358 LM-1857, CBY017 LM-1516 × CBY104 LM-1603 and CBY359 LM-1858 × CBY358 LM-1857) while the top five genotypes for Zn content comprised of five hybrids (CBY075 LM-1574 × CBY358 LM-1857, CBY102 LM-1601 × CBY358 LM-1857, CBY359 LM-1858 × CBY358 LM-1857, CBY101 LM-1600 × CBY358 LM-1857 and CBY358 LM-1857). In terms of the molar ratio of Fe with PA (Fe:PA), the mean Fe:PA varied from 21.99 to 30.52, while the molar ratio of Zn with PA (Zn:PA) varied from 22.92 to 30.45. PA varied from 4.63 to 6.06 mg kg^{-1} with an average of 5.36 mg kg^{-1}. The five genotypes with the highest PA content, including one line, were CBY075 LM-1574 × CBY013 LM-1512, CBY075 LM-1574, CBY075 LM-1574 × CBY358 LM-1857, CBY075 LM-1574 × CBY104 LM-1603 and CBY359 LM-1858 × CBY358 LM-1857.

Under low N conditions, mean grain yield varied from 1.81 to 3.74 t ha^{-1}, with an average of 2.50 t ha^{-1}. The top five genotypes for grain yield, including one tester were CBY102 LM-1601 × CBY358 LM-1857, CBY014 LM-1513 × CBY358 LM-1857, CBY017 LM-1516 × CBY358 LM-1857, CBY358 LM-1857 and CBY101 LM-1600 × CBY358 LM-1857. The mean Fe content varied from 11.80 to 19.44 mg kg^{-1}, with an average of 16.23 mg kg^{-1}. The top five genotypes for Fe content comprised of five hybrids (CBY075 LM-1574 × CBY104 LM-1603, CBY101 LM-1600 × CBY358 LM-1857, CBY102 LM-1601 × CBY358 LM-1857, CBY359 LM-1858 × CBY013 LM-1512 and CBY102 LM-1601 × CBY013 LM-1512). The Zn content varied from 15.11 to 22.15 mg kg^{-1}, with an average of 18.39 mg kg^{-1}.

Of the top five genotypes for Zn content genotypes, only one was a line (CBY101 LM-1600 × CBY358 LM-1857, CBY359 LM-1858 × CBY104 LM-1603, CBY359 LM-1858 × CBY358 LM-1857, CBY359 LM-1858 and CBY017 LM-1516 × CBY358 LM-1857). Fe:PA varied from 25.27 to 43.96, with an average of 32.84, while Zn: Pa varied from 26.83 to 39.31 mg kg^{-1}, with an average of 32.25 mg kg^{-1}. The mean PA ranged from 5.05 to 6.58, with a mean of 5.89 mg kg^{-1}. Of the top five genotypes, only one line was a line (CBY075 LM-1574 × CBY104 LM-1603, CBY017 LM-1516 × CBY358 LM-1857, CBY359 LM-1858 × CBY358 LM-1857, CBY101 LM-1600 × CBY358 LM-1857 and CBY075 LM-1574).

3.2. GCA and SCA variances

Under optimum conditions, the effect of environment was highly significant ($P \leq 0.01$) for all traits (Table 6). The effect of the cross was highly significant for all traits except for Fe:PA. GCA variance for lines were highly significant ($P \leq 0.01$) for all traits except Zn:PA, while GCA variance for testers was highly significant for grain yield, Zn:PA and Zn. The line by tester interaction was highly significant for grain yield and Zn ($P \leq 0.01$) and significant ($P \leq 0.05$) for Zn:PA and PA). The env × GCA_{line} × GCA_{tester} interaction was a highly significant ($P \leq 0.01$) for all traits (Table 6).

Table 4
Mean performance of 18 hybrids and nine parents for six traits across optimum environments.

	Genotypes	Traits					
		GY (t ha^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	Fe:PA	Zn:PA
Crosses	CBY075 LM-1574 × CBY358 LM-1857	4.80	18.71	23.79	5.82	29.02	23.73
	CBY075 LM-1574 × CBY104 LM-1603	5.45	17.10	19.23	5.72	29.20	29.38
	CBY075 LM-1574 × CBY013 LM-1512	6.07	17.57	20.08	6.06	30.02	30.45
	CBY101 LM-1600 × CBY358 LM-1857	9.33	16.91	21.93	5.10	26.41	23.68
	CBY101 LM-1600 × CBY104 LM-1603	4.04	16.91	21.26	5.56	29.02	26.68
	CBY101 LM-1600 × CBY013 LM-1512	7.18	16.53	20.01	4.95	26.33	24.31
	CBY102 LM-1601 × CBY358 LM-1857	8.20	15.93	22.99	5.09	30.52	22.92
	CBY102 LM-1601 × CBY104 LM-1603	5.20	18.66	19.05	5.42	24.56	28.01
	CBY102 LM-1601 × CBY013 LM-1512	5.42	18.06	19.91	5.57	28.95	28.09
	CBY359 LM-1858 × CBY358 LM-1857	5.05	18.99	22.73	5.69	26.99	25.88
	CBY359 LM-1858 × CBY104 LM-1603	8.16	17.60	20.02	5.33	26.39	28.05
	CBY359 LM-1858 × CBY013 LM-1512	7.58	16.33	19.55	5.54	28.95	28.52
	CBY017 LM-1516 × CBY358 LM-1857	7.09	19.80	19.66	5.36	23.12	27.43
	CBY017 LM-1516 × CBY104 LM-1603	4.30	19.20	18.36	5.11	23.77	28.34
	CBY017 LM-1516 × CBY013 LM-1512	7.55	20.44	19.82	5.25	23.70	27.50
	CBY014 LM-1513 × CBY358 LM-1857	6.07	18.60	19.63	5.11	24.77	27.11
	CBY014 LM-1513 × CBY104 LM-1603	6.62	18.12	20.33	4.63	21.99	23.76
	CBY014 LM-1513 × CBY013 LM-1512	2.46	18.84	18.64	5.24	24.31	27.04
Lines	CBY075 LM-1574	5.44	17.79	21.03	5.87	29.41	27.85
	CBY101 LM-1600	6.85	16.78	21.06	5.21	27.25	24.89
	CBY102 LM-1601	6.27	17.55	20.65	5.36	28.01	26.34
	CBY359 LM-1858	6.93	17.64	20.77	5.52	27.44	27.48
	CBY017 LM-1516	6.31	19.81	19.28	5.24	23.53	27.76
	CBY014 LM-1513	5.05	18.52	19.53	4.99	23.69	25.97
Testers	CBY358 LM-1857	6.76	18.16	21.79	5.36	26.80	25.12
	CBY104 LM-1603	5.63	17.93	19.71	5.29	25.82	27.37
	CBY013 LM-1512	6.04	17.96	19.67	5.44	27.04	27.65
	Mean	6.14	18.02	20.39	5.36	26.56	26.71

Fe = Iron, Zn = Zinc, PA = Phytic acid, Fe:PA = Molar ratio of Fe with Pa, Zn:PA = Molar ratio of Zn with PA, GY = Grain yield.

Table 5
Mean performance of 18 hybrids and nine parents for six traits under low nitrogen conditions.

Genotypes		GY (t ha^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	PA (mg g^{-1})	Fe:PA	Zn:PA
Crosses	CBY075 LM-1574 × CBY358 LM-1857	2.68	16.74	19.10	5.86	33.05	32.29
	CBY075 LM-1574 × CBY104 LM-1603	1.81	19.44	17.39	6.58	29.85	35.07
	CBY075 LM-1574 × CBY013 LM-1512	2.43	15.40	17.69	5.96	35.07	32.70
	CBY101 LM-1600 × CBY358 LM-1857	2.84	18.22	22.15	6.21	30.47	29.43
	CBY101 LM-1600 × CBY104 LM-1603	2.06	14.96	18.64	6.01	37.42	32.98
	CBY101 LM-1600 × CBY013 LM-1512	2.61	11.80	17.08	5.87	43.96	30.72
	CBY102 LM-1601 × CBY358 LM-1857	3.74	17.93	19.73	5.94	31.29	31.27
	CBY102 LM-1601 × CBY104 LM-1603	2.61	13.56	15.11	5.81	36.55	39.31
	CBY102 LM-1601 × CBY013 LM-1512	2.15	17.67	17.63	5.69	28.66	33.51
	CBY359 LM-1858 × CBY358 LM-1857	2.10	15.92	20.24	6.25	35.62	31.78
	CBY359 LM-1858 × CBY104 LM-1603	2.04	16.43	21.34	6.04	32.28	28.34
	CBY359 LM-1858 × CBY013 LM-1512	2.51	17.78	19.03	5.95	29.24	30.01
	CBY017 LM-1516 × CBY358 LM-1857	3.30	14.96	19.89	6.31	35.94	32.61
	CBY017 LM-1516 × CBY104 LM-1603	1.99	17.31	17.97	5.25	26.58	31.58
	CBY017 LM-1516 × CBY013 LM-1512	2.48	17.21	17.98	5.05	25.27	26.83
	CBY014 LM-1513 × CBY358 LM-1857	3.40	15.81	17.86	5.75	31.14	30.86
	CBY014 LM-1513 × CBY104 LM-1603	2.58	15.00	16.38	5.92	38.83	36.41
	CBY014 LM-1513 × CBY013 LM-1512	1.65	16.05	15.87	5.48	29.90	34.80
Lines	CBY075 LM-1574	2.31	17.20	18.06	6.13	32.66	33.35
	CBY101 LM-1600	2.51	14.99	19.29	6.03	37.28	31.04
	CBY102 LM-1601	2.83	16.39	17.49	5.81	32.17	34.70
	CBY359 LM-1858	2.22	16.71	20.21	6.08	32.38	30.04
	CBY017 LM-1516	2.59	16.49	18.61	5.54	29.26	30.34
	CBY014 LM-1513	2.54	15.62	16.71	5.72	33.29	34.02
Testers	CBY358 LM-1857	3.01	16.60	19.83	6.05	32.92	31.37
	CBY104 LM-1603	2.18	16.12	17.81	5.94	33.59	33.95
	CBY013 LM-1512	2.30	15.99	17.55	5.67	32.02	31.43
	Mean	2.5	16.23	18.39	5.89	32.84	32.25

Fe = Iron, Zn = Zinc, PA = Phytic acid, Fe:PA = Molar ratio of Fe with PA, Zn:PA = Molar ratio of Zn with PA, GY = Grain yield.

Under low N conditions, the effect of environment and genotype (cross) was highly significant ($P \le 0.01$) for all traits (Table 6). The effect of line GCA was not significant for grain yield and Fe, while the tester GCA effect was highly significant ($P \le 0.01$) for grain yield, PA and Zn. The line by tester interaction was highly significant for Fe, grain yield, Fe:PA, Zn:PA and PA, and significant for Zn ($P \le 0.05$). The env × GCA $_{line}$ × GCA $_{tester}$ interaction was highly significant ($P \le 0.01$) for all traits, except PA and Zn (Table 6).

Table 6
Analysis of variance for GCA effects of lines and testers and SCA effects of crosses for grain yield and other studied traits under optimum and low N conditions across environments.

Source of variation	DF	GY	Fe	Zn	PA	Fe:PA	Zn:PA
	Optimal conditions						
Environments (Env)	3	370.33**	101.46**	198.08**	16.11**	815.79**	1530.04**
Rep(Env)	4	0.34	0.60	1.48	0.08	4.76	5.65
Cross	17	33.47**	14.52**	30.56**	1.18**	70.58	44.99**
GCA $_{line}$	5	17.78**	28.99**	38.10**	2.58**	181.90**	23.61
GCA $_{tester}$	2	19.41**	3.22	103.26**	0.30	13.15	151.16**
GCA $_{line}$ × GCA $_{tester}$	10	44.12**	9.54	12.25**	0.66*	26.40	34.44*
Env × Cross	51	5.85**	21.19**	11.88**	0.77**	80.36**	37.27**
Env × GCA $_{line}$	15	1.82	17.84*	17.61**	0.85**	66.96	40.82**
Env × GCA $_{tester}$	6	2.95	12.76	11.23*	0.36	72.76	21.84
Env × GCA $_{line}$ × GCA $_{tester}$	30	8.44**	24.55**	9.14**	0.81**	88.58**	38.58**
Residuals	116	3.17	9.10	3.87	0.26	45.38	14.83
	Low N conditions						
Environments (Env)	3	15.85**	111.94**	165.77**	8.51**	788.49**	625.59**
Rep(Env)	4	0.54	0.58	3.51	0.37	10.16	12.16
Cross	17	3.08**	29.04**	31.95**	1.41**	218.90**	73.59**
GCA $_{line}$	5	0.78	19.43	52.52**	2.18**	258.53**	97.82**
GCA $_{tester}$	2	11.90**	0.70	74.76**	1.72**	60.17	109.97*
GCA $_{line}$ × GCA $_{tester}$	10	2.46**	39.51**	13.11*	0.97**	230.84**	54.20*
Env × Cross	51	1.60**	23.67**	10.14*	0.59**	119.69**	60.40**
Env × GCA $_{line}$	15	1.98**	13.12	8.08	0.54	76.75	20.37
Env × GCA $_{tester}$	6	0.50	20.38	26.41**	0.82*	147.07*	158.74
Env × GCA $_{line}$ × GCA $_{tester}$	30	1.64**	29.60**	7.91	0.57*	135.69**	60.74**
Residuals	116	0.57	10.18	6.20	0.34	60.29	25.62

* $P \le 0.05$,
** $P \le 0.01$.

3.3. GCA and SCA variance components

Under optimum conditions, the GCA variance (lines) was higher than SCA variance for all traits except Zn and Zn:PA. The variance of GCA (testers) was lower than the variance of SCA for all traits except Zn (Table 7). The variance ratio of GCA/SCA was less than one for all traits except Zn.

Under low nitrogen conditions, the variance of GCA (lines) was higher than SCA variance for Fe, Fe:PA, PA, and Zn, while the variance due to GCA (lines) was less than SCA variance for grain yield and Zn:PA (Table 7). Except for GCA (testers) for Zn, the variance of GCA (testers) was less than SCA variances for all traits. The GCA/SCA ratio was more than one for Zn, PA and Zn:PA (Table 7).

In terms of the contribution by lines, testers, and lines × testers to the total genetic variance for the studied traits under optimum conditions, the order was lines × testers> lines > testers for all traits except for Zn and, testers > lines × testers > lines for Zn. The contribution of line × tester interaction was more than 55% for all traits except Zn (Table 8). Under low nitrogen conditions, the order was lines × testers > lines > testers for all traits except for grain yield, Zn and Zn:PA, and lines × testers> testers > lines for all traits except for Fe, PA, and Fe:PA (Table 8). The contribution by lines × testers was higher than 71% for Zn, PA and Zn:PA.

3.4. Specific combining ability effects

Under optimum N conditions, crosses CBY075 LM-1574 × CBY358 LM-1857, CBY101 LM-1600 × CBY104 LM-1603, CBY102 LM-1601 × CBY013 LM-1512, CBY017 LM-1516 × CBY358 LM-1857, CBY017 LM-1516 × CBY104 LM-1603 and CBY014 LM-1513 × CBY013 LM-1512 had highly significant positive SCA effects for Fe while CBY075 LM-1574 × CBY104 LM-1603 and CBY014 LM-1513 × CBY358 LM-1857 had highly significant negative SCA effects (Table 9). CBY101 LM-1600 × CBY358 LM-1857, CBY102 LM-1601 × CBY358 LM-1857, CBY359 LM-1858 × CBY104 LM-1603 and CBY359 LM-1858 × CBY013 LM-1512 had highly significant positive SCA effects for grain yield. However, CBY075 LM-1574 × CBY358 LM-1857, CBY101 LM-1600 × CBY104 LM-1603, CBY359 LM-1858 × CBY358 LM-1857, CBY017 LM-1516 × CBY104 LM-1603 and CBY014 LM-1513 × CBY013 LM-1512 had highly significant negative SCA effects. SCA effects for Fe:PA were positive and highly significant for CBY102 LM-1601 × CBY358 LM-1857 but negative and significant for CBY075 LM-1574 × CBY104 LM-1603, CBY101 LM-1600 × CBY104 LM-1603 and CBY102 LM-1601 × CBY104 LM-1603. CBY075 LM-1574 × CBY358 LM-1857 and CBY014 LM-1513 × CBY104 LM-1603 had significant and positive SCA effects for Zn.

Under low N conditions, CBY102 LM-1601 × CBY358 LM-1857 and CBY014 LM-1513 × CBY358 LM-1857 showed highly significant and positive SCA effects for grain yield (Table 9). Highly significant positive SCA effects for Fe:PA were observed for CBY101 LM-1600 × CBY013 LM-1512, CBY359 LM-1858 × CBY358 LM-1857. However, CBY017 LM-1516 × CBY358 LM-1857. CBY101 LM-1600 × CBY358 LM-1857 showed significant positive SCA effects for Zn (Table 9).

3.5. General combining ability effects

Under optimum conditions, CBY017 LM-1516 for Fe, CBY359 LM-1858 for grain yield and Fe:PA had significant positive GCA effects. Regarding testers GCA effects, CBY358 LM-1857 had significant and positive effects for Fe, Zn, and grain yield while CBY358 LM-1857 had negative GCA effects for Zn:PA. CBY013 LM-1512 had positive GCA effects for Fe:PA, Zn:PA and PA. CBY104 LM-1603 had negative and non-significant effects for Fe and Zn (Table 9). Under low N conditions, CBY102 LM-1601 had positive GCA effects for Fe, grain yield and Zn:PA. CBY075 LM-1574 and CBY101 LM-1600 had positive GCA effects for Fe: Zn while CBY359 LM-1858 had

Table 7
Estimates of genetic components of studied traits of maize hybrids under optimum and low nitrogen conditions across environments.

Variance components	GY	Fe	Zn	PA	Fe:PA	Zn:PA
	Optimal conditions					
δ^2 GCA $_{line}$	0.12	1.21	0.02	0.06	4.91	1.09
δ^2 GCA $_{tester}$	0.06	0.49	1.32	0.01	0.98	1.59
δ^2 SCA $_{line \times tester}$	5.32	2.32	0.41	0.19	8.06	8.73
δ^2 GCA/δ^2 SCA ratio	0.03	0.73	3.25	0.36	0.73	0.31
Error mean squares	1.58	4.70	1.26	0.13	22.27	7.42
δ^2g	3.59	3.84	1.35	0.23	12.53	71.70
δ^2 a	0.26	15.37	5.40	0.10	50.12	4.20
δ^2d	21.29	9.29	1.65	0.78	32.22	34.91
h^2	0.12	0.53	0.65	0.15	0.48	0.20
	Low N conditions					
δ^2 GCA $_{line}$	0.07	0.34	1.05	0.03	1.65	1.50
δ^2 GCA $_{tester}$	0.11	0.08	0.93	0.02	0.67	7.35
δ^2 SCA $_{line \times tester}$	0.44	5.92	0.46	0.04	30.98	7.80
δ^2 GCA/δ^2 SCA ratio	0.41	0.07	4.29	1.08	0.07	1.13
Error mean squares	0.28	5.04	1.15	0.07	29.96	12.99
δ^2_g	0.57	6.29	2.23	0.08	32.96	14.64
δ^2 $_a$	2.26	25.15	8.93	0.30	131.83	58.55
δ^2_d	1.75	23.67	1.85	0.16	123.91	31.20
h^2	0.53	0.47	0.75	0.57	0.47	0.43

h^2 = narrow sense heritability, δ^2_a = additive variance, δ^2_d = dominance variance, δ^2_g = genotype variance.

Table 8
Contribution of lines, testers, and lines × testers to the total genetic variance for the studied traits in maize inbred lines under optimum and low nitrogen conditions across environments.

Traits	Lines	Contribution (%)	
		Testers	Lines × Testers
	Optimal conditions		
GY	2.25	1.03	96.71
Fe	30.04	12.19	57.77
Zn	1.03	75.43	23.54
PA	24.38	1.90	73.72
Fe:PA	35.23	7.00	57.77
Zn:PA	9.56	13.92	76.53
	Low N conditions		
GY	30.12	21.69	48.19
Fe	43.08	38.00	18.92
Zn	5.39	1.22	93.39
PA	11.09	17.76	71.15
Fe: PA	9.00	44.16	46.84
Zn: PA	4.95	2.03	93.03

Table 9
Specific combining ability effects for the maize hybrid cross for grain yield and other studied traits under optimum and low nitrogen conditions across environments.

Hybrid crosses	GY	Fe	Zn	PA	Fe:PA	Zn:PA
	Optimal conditions					
CBY075 LM-1574 × CBY358 LM-1857	−1.10**	1.10**	0.66*	0.01	1.28	−0.19
CBY075 LM-1574 × CBY104 LM-1603	−0.52	−0.73**	−0.24	−0.05	−1.33*	1.82
CBY075 LM-1574 × CBY013 LM-1512	−0.03	−0.39	0.02	0.16	−0.36	1.07
CBY101 LM-1600 × CBY358 LM-1857	2.55**	−0.94	0.01	−0.07	0.75	−0.72
CBY101 LM-1600 × CBY104 LM-1603	−1.74**	0.50**	0.42	0.06	−0.95*	0.16
CBY101 LM-1600 × CBY013 LM-1512	0.85	−0.75**	0.01	−0.29	0.44	0.00
CBY102 LM-1601 × CBY358 LM-1857	1.66**	−0.27	0.36	−0.23	1.94*	−2.26
CBY102 LM-1601 × CBY104 LM-1603	−0.71	0.90	−0.24	0.16	−0.35*	1.86
CBY102 LM-1601 × CBY013 LM-1512	−0.58	0.19*	0.03	0.17	−1.59	0.53
CBY359 LM-1858 × CBY358 LM-1857	−0.89**	0.34	0.32	0.21	−1.06	−0.43
CBY359 LM-1858 × CBY104 LM-1603	1.62**	−0.35	0.02	−0.07	−0.19	−1.84
CBY359 LM-1858 × CBY013 LM-1512	1.17**	−0.81	−0.10	0.02	−1.25	0.25
CBY017 LM-1516 × CBY358 LM-1857	0.78	0.81**	−0.47	0.06	−0.67	1.35
CBY017 LM-1516 × CBY104 LM-1603	−1.52**	0.67*	−0.37	−0.03	1.28	0.26
CBY017 LM-1516 × CBY013 LM-1512	1.14	−0.27	0.13	−0.03	−1.33	−1.14
CBY014 LM-1513 × CBY358 LM-1857	−0.02	−0.75*	−0.50	0.00	−0.36	−0.16
CBY014 LM-1513 × CBY104 LM-1603	0.41*	−0.17	0.23*	−0.39	0.75	−2.02
CBY014 LM-1513 × CBY013 LM-1512	−3.08**	0.93**	−0.28	0.21	−0.95	1.46
SE	0.69	0.74	0.55	0.01	1.61	1.39
	Low N conditions					
CBY075 LM-1574 × CBY358 LM-1857	−0.06	0.12	−0.10	0.20	0.14	0.00
CBY075 LM-1574 × CBY104 LM-1603	−0.10	0.65	−0.09	0.04	−1.24	0.13
CBY075 LM-1574 × CBY013 LM-1512	0.04	−0.17	0.08	0.34	0.97	0.03
CBY101 LM-1600 × CBY358 LM-1857	−0.01	0.40	0.65**	−0.14	−0.98	−0.19
CBY101 LM-1600 × CBY104 LM-1603	−0.05	−0.33	0.05	0.23	2.35	0.05
CBY101 LM-1600 × CBY013 LM-1512	0.08	−0.90	−0.41	−0.20	4.74**	−0.06
CBY102 LM-1601 × CBY358 LM-1857	0.21**	0.34	0.24	−0.15	−0.63	−0.20
CBY102 LM-1601 × CBY104 LM-1603	0.08	−0.55	−0.74	−0.02	1.60	0.53
CBY102 LM-1601 × CBY013 LM-1512	−0.07	0.36	0.24	0.05	−2.09	0.06
CBY359 LM-1858 × CBY358 LM-1857	−0.23	−0.08	−0.25	0.12	1.44**	0.17
CBY359 LM-1858 × CBY104 LM-1603	−0.03	0.04	0.74	−0.08	−0.21	−0.43
CBY359 LM-1858 × CBY013 LM-1512	0.07	0.31	0.04	0.07	−1.50	−0.08
CBY017 LM-1516 × CBY358 LM-1857	0.11	−0.26	0.06	0.09	1.34**	0.21
CBY017 LM-1516 × CBY104 LM-1603	−0.08	0.27	−0.04	−0.13	−3.15	−0.11
CBY017 LM-1516 × CBY013 LM-1512	0.04	0.19	0.05	−0.08	−3.18	−0.44
CBY014 LM-1513 × CBY358 LM-1857	0.14**	−0.09	−0.16	−0.01	−0.70	−0.22
CBY014 LM-1513 × CBY104 LM-1603	0.09	−0.25	−0.10	−0.29	2.56	0.23
CBY014 LM-1513 × CBY013 LM-1512	−0.21	−0.05	−0.27	−0.03	−1.46	0.30
SE	0.21	0.72	0.59	0.25	2.18	0.77

SE = standard error.

positive GCA effects for Zn content (Table 10).

3.6. Heterosis

Under optimum conditions, only five crosses had positive and significant mid-parent and high parent heterosis values for yield (Table 11). However, CBY075 LM-1574 × CBY358 LM-1857, CBY101 LM-1600 × CBY104 LM-1603, CBY359 LM-1858 × CBY358 LM-1857, CBY359 LM-1858 × CBY358 LM-1857, and CBY014 LM-1513 × CBY013 LM-1512 had negative significant mid-parent heterosis values for grain yield. CBY075 LM-1574 × CBY358 LM-1857, CBY101 LM-1600 × CBY104 LM-1603, CBY102 LM-1601 × CBY104 LM-1603, CBY102 LM-1601 × CBY013 LM-1512, CBY359 LM-1858 × CBY358 LM-1857, CBY017 LM-1516 × CBY104 LM-1603 and CBY014 LM-1513 × CBY013 LM-1512 had negative significant high parent heterosis values for grain yield (Table 11).

Under low N conditions, CBY102 LM-1601 × CBY358 LM-1857 and CBY014 LM-1513 × CBY358 LM-1857 had positive significant mid-parent and high parent heterosis values for grain yield. However, CBY014 LM-1513 × CBY013 LM-1512 had negative significant mid-parent heterosis values for grain yield, and CBY075 LM-1574 × CBY104 LM-1603, CBY102 LM-1601 × CBY013 LM-1512, CBY359 LM-1858 × CBY358 LM-1857, CBY017 LM-1516 × CBY104 LM-1603 and CBY014 LM-1513 × CBY013 LM-1512 had the negative significant high parent heterosis values for grain yield (Table 12).

4. Discussion

4.1. Mean performance of genotypes

In the present investigation, the overall effect of genotype was significant for grain yield performance under both growing conditions. The other measured traits had varied genotype responses to the two N levels in the soil. Similar results on maize inbred and hybrid performance under contrasting environments [31,32].

Low N decreased grain yield, grain Fe and Zn concentration of crosses by 59%, 9% and 9%, respectively. This implies that the effect of N was significant on grain yield, and grain Fe and Zn concentration, because low N in maize plants interferes with the absorption of the Fe and Zn; in addition, lack of these elements can because a deformed grain structure and shrinked grain [33].

On the other hand, PA, the molar ratio of Zn with PA and the molar ratio of Fe with PA were increased by 10%, 21% and 24%, respectively, under low N conditions, indicating increased bioavailability of the minerals under low N conditions. Phytic acid is a major anti-nutritional factor in maize gains that significantly reduces the bioavailability of minerals such as Fe and Zn, leading to micronutrient malnutrition [34].

In this study, the mean of grain yield, Fe, and Zn concentration was 6.14 t ha^{-1}, 18.02 mg kg^{-1} and 20.39 mg kg^{-1}, respectively, under optimum conditions, compared to 2.50 t ha^{-1}, 16.23 mg kg^{-1} and 18.39 mg kg^{-1}, respectively, under low N conditions. This indicated that low N conditions significantly influenced the expression of the measured traits. In addition, their genetic potential markedly influenced the hybrid performance in varying N conditions, as also reported by Ref. [35].

Table 10
General combining ability effects on the maize hybrid parents for grain yield and other studied traits under optimum and low nitrogen conditions across environments.

	Parents	GY	Fe	Zn	PA	Fe:PA	Zn:PA
		Optimal conditions					
Lines	CBY075 LM-1574	−0.18	−0.06	0.02*	0.36	1.20	0.46
	CBY101 LM-1600	0.08	−0.46	0.02	−0.10*	0.23	−0.49
	CBY102 LM-1601	0.28	−0.16	0.01	0.00	0.44	−0.08*
	CBY359 LM-1858	0.83*	−0.10	0.01	0.12	0.14*	0.62
	CBY017 LM-1516	−0.09	0.60*	−0.03	−0.11	−1.37	−0.13
	CBY014 LM-1513	−0.92*	0.18	−0.02	−0.27	−0.64	−0.37
	SE	0.35	0.48	0.89	0.13	0.38	0.80
Testers	CBY358 LM-1857	0.48	0.88*	1.21*	−0.03	−0.03	−1.14*
	CBY104 LM-1603	−0.37	−1.42	−0.58	−0.06	−0.24	0.48
	CBY013 LM-1512	−0.11	0.54	−0.63**	0.09	0.27	0.66**
	SE	0.36	0.65	0.50	0.11	1.14	0.65
		Low N conditions					
Lines	CBY075 LM-1574	−0.06	−0.71	−0.24	0.10	1.97	0.54
	CBY101 LM-1600	−0.02	−0.96	0.64	0.07	1.25	−0.64
	CBY102 LM-1601	0.20	0.78	−0.57	−0.03	−0.92	1.26
	CBY359 LM-1858	−0.25	0.61	1.22	0.09	−1.42	−1.10
	CBY017 LM-1516	0.09	0.75	0.16	−0.16	−1.56	−1.07
	CBY014 LM-1513	0.04	−0.46	−1.21	−0.07	0.69	1.01
	SE	0.24	0.87	0.61	0.12	2.10	1.01
Testers	CBY358 LM-1857	0.61	0.34	0.89	0.08	−0.91	0.77
	CBY104 LM-1603	−0.22	−0.17	−0.36	0.02	0.88	0.29
	CBY013 LM-1512	−0.10	−0.17	−0.53	−0.10	0.02	−1.06
	SE	0.35	0.48	0.62	0.10	1.40	0.95

SE = standard error.

Table 11
Mid parent heterosis and high parent heterosis for grain yield and other traits of crosses among 18 inbred lines under optimum conditions across environments.

Crosses	Mid Parent Heterosis (mph)						High Parent Heterosis (hph)					
	Fe	Gy	Fe: Pa	Zn: Pa	Pa	Zn	Fe	Gy	Fe: Pa	Zn: Pa	Pa	Zn
CBY075 LM-1574 × CBY358 LM-1857	4.09	−21.31*	60.46	10.87	3.65	11.12	3.03	−28.99**	−1.33	10.87	−0.85	9.18
CBY075 LM-1574 × CBY104 LM-1603	−4.26	−1.54	66.67	1.75	2.51	−5.60	−4.63	−3.20	−0.71	1.75	−2.56	−8.56
CBY075 LM-1574 × CBY013 LM-1512	−1.71	5.75	69.37	0.72	7.16	−1.33	−2.17	0.50	2.07	0.72	3.24	−4.52
CBY101 LM-1600 × CBY358 LM-1857	−3.21	37.11**	55.31	−0.92	−3.50	2.36	−6.88	36.20**	−3.08	−0.92	−4.85	0.64
CBY101 LM-1600 × CBY104 LM-1603	−2.56	−35.26**	76.52	−9.06	5.90	4.29	−5.69	−41.02**	6.50	−9.06	5.10	0.95
CBY101 LM-1600 × CBY013 LM-1512	−4.84	11.40	58.19	−9.98	−7.04	−1.74	−7.96	4.82	−3.38	−9.98	−9.01	−4.99
CBY102 LM-1601 × CBY358 LM-1857	−10.78	25.86**	75.55	4.86	−5.04	8.34	−12.28	21.30**	8.96	4.86	−5.04	5.51
CBY102 LM-1601 × CBY104 LM-1603	5.19	−12.61	46.02	−3.76	1.78	−5.60	4.07	−17.07**	−12.32	−3.76	1.12	−7.75
CBY102 LM-1601 × CBY013 LM-1512	1.72	−11.94	70.04	−4.74	3.15	−1.24	0.56	−13.56**	3.36	−4.74	2.39	−3.58
CBY359 LM-1858 × CBY358 LM-1857	6.09	−26.22**	57.84	9.39	4.60	6.81	4.57	−27.13**	−1.64	9.39	3.08	4.31
CBY359 LM-1858 × CBY104 LM-1603	−1.04	29.94**	59.60	0.40	−1.39	−1.09	−1.84	17.75**	−3.83	0.40	−3.44	−3.61
CBY359 LM-1858 × CBY013 LM-1512	−8.26	16.89*	72.94	−0.61	1.09	−3.31	−9.08	9.38*	5.50	−0.61	0.36	−5.87
CBY017 LM-1516 × CBY358 LM-1857	4.29	8.49	52.66	10.51	1.13	−4.26	−0.05	4.88	−1.74	10.51	0.00	−9.78
CBY017 LM-1516 × CBY104 LM-1603	1.75	−27.97**	63.03	1.42	−2.94	−5.82	−3.08	−31.85**	1.02	1.42	−3.40	−6.85
CBY017 LM-1516 × CBY013 LM-1512	8.23	22.27**	60.30	0.40	−1.69	1.77	3.18	19.65**	0.72	0.40	−3.49	0.76
CBY014 LM-1513 × CBY358 LM-1857	1.42	2.79	62.69	3.38	−1.26	−4.99	0.43	−10.21*	4.56	3.38	−4.66	−9.91
CBY014 LM-1513 × CBY104 LM-1603	−0.58	23.97*	50.00	−5.12	−9.92	3.62	−2.16	17.58	−7.18	−5.12	−12.48	3.15
CBY014 LM-1513 × CBY013 LM-1512	3.29	−55.64**	63.54*	−6.08	0.48	−4.90	1.73	−59.27	2.62	−6.08	−3.68	−5.24
Maximum	8.23	37.11	76.52	10.87	7.16	11.12	4.57	36.20	8.96	10.87	5.10	9.18
Minimum	−10.78	−55.64	46.02	−9.98	−9.92	−5.82	−12.28	−59.27	−12.32	−9.98	−12.48	−9.91
Mean	−0.06	−0.45	62.26	0.19	−0.07	−0.09	−2.13	−5.57	0.01	0.19	−2.12	−2.56

4.2. Combining ability

General combining ability and SCA of lines and testers were influenced by the environmental conditions, which was also reported by [32,36].

Line and tester analysis indicated that mean squares attributable to GCA were significant for grain yield and most measured traits under low and optimum N conditions. The GCA effects of line and testers were significant, indicating that either the line or tester could produce valuable hybrids for improvement programs for yield, Fe, and Zn concentration through selection under low N and optimum conditions. Similar results on maize hybrids under contrasting conditions [32,37]. The significance of variance for GCA and SCA of lines and testers for grain yield, Fe, and Zn imply that both additive and non-additive gene action are involved in the inheritance of these traits under low N and optimum conditions.

The parents with favourable GCA effects can be used to generate superior hybrids for high grain yield, Fe and Zn concentration in grain because additive variance is associated with an effective response to selection. Good lines that had the highest and most significant positive GCA effects for grain yield under low pH conditions [32].

Line CBY102 LM-1601 had positive GCA effects for grain yield and Fe concentration under low N conditions. It can, therefore, be used to develop hybrids and as a potential donor for development of low N tolerance and Fe-rich maize grain. Tester CBY358 LM-1857 was the best general combiner under low and optimum N conditions for all measured traits. The significant contribution of both parents (lines and testers) to grain yield, Fe and Zn concentration indicated that additive gene effects were important for these traits and that good response to selection is possible for these traits. Similarly, significant line and tester parent GCA effects for grain yield [38].

The significance of SCA effects for grain yield and Zn concentration under low N conditions indicated dominance effects. Understanding the SCA of inbred lines is vital for a hybrid-based program to determine the best parental cross combinations under low

Table 12
Mid parent heterosis and high parent heterosis for grain yield and other traits of crosses among 18 inbred lines under low N conditions across environments.

Crosses	Mid Parent Heterosis (mph)						High Parent Heterosis (bph)					
	Fe	Gy	Fe: Pa	Zn: Pa	Pa	Zn	Fe	Gy	Fe: Pa	Zn: Pa	Pa	Zn
CBY075 LM-1574 × CBY358 LM-1857	−0.95	0.75	0.79	−0.22	−3.78	0.82	−2.67	−10.96	0.39	−3.18	−4.40	−3.68
CBY075 LM-1574 × CBY104 LM-1603	16.69	−19.38	−9.89	4.22	9.03	−3.04	13.02	−21.65*	−11.13	3.30	7.34	−3.71
CBY075 LM-1574 × CBY013 LM-1512	−7.20	5.42	8.44	0.96	1.02	−0.65	−10.47	5.19	7.38	−1.95	−2.77	−2.05
CBY101 LM-1600 × CBY358 LM-1857	15.35	2.90	−13.19	−5.69	2.81	13.24	9.76	−5.65	−18.27	−6.18	2.64	11.70*
CBY101 LM-1600 × CBY104 LM-1603	−3.83	−12.15	5.60	1.49	0.42	0.49	−7.20	−17.93	0.38	−2.86	−0.33	−3.37
CBY101 LM-1600 × CBY013 LM-1512	−23.82	8.52	26.87	−1.65	0.34	−7.27	−26.20	3.98	17.92	−2.26	−2.65	−11.46*
CBY102 LM-1601 × CBY358 LM-1857	8.70	28.08*	−3.86	−5.34	0.17	5.73	8.01	24.25**	−4.95	−9.88	−1.82	−0.50
CBY102 LM-1601 × CBY104 LM-1603	−16.58	4.19	11.16	14.52	−1.11	−14.39	−17.27	−7.77	8.81	13.29	−2.19	−15.16*
CBY102 LM-1601 × CBY013 LM-1512	9.14	−16.18	−10.70	1.35	−0.87	0.63	7.81	−24.03**	−10.91	−3.43	−2.07	0.46
CBY359 LM-1858 × CBY358 LM-1857	−4.41	−19.69	9.10	3.50	3.05	1.10	−4.73	−30.23**	8.20	1.31	2.80	0.15
CBY359 LM-1858 × CBY104 LM-1603	0.09	−7.27	−2.14	−11.42	0.50	12.26	−1.68	−8.11	−3.90	−16.52	−0.66	5.59
CBY359 LM-1858 × CBY013 LM-1512	8.75	11.06	−9.19	−2.36	1.28	0.79	6.40	9.13	−9.70	−4.52	−2.14	−5.84
CBY017 LM-1516 × CBY358 LM-1857	−9.58	17.86	15.60	5.69	8.89	3.49	−9.88	9.63	9.17	3.95	4.30	0.30
CBY017 LM-1516 × CBY104 LM-1603	6.16	−16.56	−15.42	−1.76	−8.54	−1.32	4.97	−23.17**	−20.87	−6.98	−11.62*	−3.44
CBY017 LM-1516 × CBY013 LM-1512	5.97	1.43	−17.53	−13.13	−9.90	−0.55	4.37	−4.25	−21.08	−14.64	−10.93*	−3.39
CBY014 LM-1513 × CBY358 LM-1857	−1.86	22.52*	−5.94	−5.61	−2.29	−2.24	−4.76	12.96*	−6.46	−9.29	−4.96	−9.93
CBY014 LM-1513 × CBY104 LM-1603	−5.48	9.32	16.12	7.14	1.54	−5.10	−6.95	1.57	15.60	7.03	−0.34	−8.03
CBY014 LM-1513 × CBY013 LM-1512	1.55	−31.82**	−8.44	6.34	−3.78	−7.36	0.38	−35.04**	−10.18	2.29	−4.20	−9.57
Maximum	16.69	28.08	26.87	14.52	9.03	13.24	13.02	24.25	17.92	13.29	7.34	11.70
Minimum	−23.82	−31.82	−17.53	−13.13	−9.90	−14.39	−26.20	−35.04	−21.08	−16.52	−11.62	−15.16
Mean	−0.07	−0.61	−0.14	−0.11	−0.07	−0.19	−2.06	−6.78	−2.76	−2.81	−1.89	−3.44

and optimum N conditions to develop superior hybrids. CBY358 LM-1857 had high and positive GCA effects for most traits, including grain yield, and significant SCA effects for grain yield and Zn concentration in most crosses, which indicated the importance of non-additive gene action for these traits. This results in agreement with [32,38], who reported a combination of desirable SCA effects and high per se grain yield performance under contrasting environments. In general, the present study demonstrated that both additive and non-additive gene actions are involved in the expression of grain yield, Fe, Zn and PA concentration in maize grain in the two different N conditions.

The relative magnitude of GCA:SCA mean square rations was high (>1) for PA, the molar ratio of Zn with PA and Zn concentration in grain under low N conditions, indicating the predominance of GCA and additive gene action in the expression of these traits, which was similar to findings of [38]. However, the ratio of GCA to SCA was less than one for grain yield, Fe concentration, and the molar ratio of Fe with PA, indicating non-additive gene action for these traits under low N conditions.

4.3. Heterosis estimates

In maize breeding, yield heterosis is the most important trait for identifying populations that may be valuable sources for inbred line development and is dependent on the level of dominance and differences in gene frequency in the parents. The quantitative genetic theory states that heterosis is a function of increasing genetic diversity among the two parents within a specific range [30]. Still, that heterosis declined in extremely divergent crosses [39].

Under low N conditions, the range of mid and high parent heterosis for grain yield was −31.82%–28.08% and −35.04%–24.25%, respectively. Seven and 11 hybrids had positive high and mid-parent heterosis for grain yield. This indicates substantial heterosis in some of the hybrids and the potential of these inbred lines for hybrid development for low N conditions. Likewise, mid and high-parent heterosis for grain yield of 29.14 and 19.81%, respectively, under low N conditions [40]. In this study, cross combinations CBY102 LM-1601 × CBY358 LM-1857 had good mid and high parent heterosis under low N conditions, for grain yield. However, some hybrids had negative heterosis for grain yield, indicating that the parents combined poorly under low N conditions, and that these hybrids will not perform under these conditions. Mid-parent heterosis for yield ranged from −3.6% to 72.0% while high parent heterosis ranged from −9.9% to 43.0% [41].

Mid and high parent heterosis varied from −23.82/% to16.69 and −26.20%–13.02% for Fe and −15.16%–11.70% and −14.39%–13.24% for Zn, respectively, under low N conditions. CBY075 LM-1574 × CBY104 LM-1603 and CBY101 LM-1600 × CBY358 LM-1857 had high mid and high parent heterosis; this indicated that these hybrids had combined well for Fe and Zn concentration in maize grain. Heterosis expressed by the hybrids is mainly dependent on the genetic diversity of the parental genotypes. The parental inbreds utilized should be grouped into heterotic pools to serve as a source population for developing high yielding and Fe and Zn rich maize grain hybrids. Developing a heterotic pool along with identified hybrids could be maintained as a reference those hybrids in the biofortification of maize [42].

4.4. Gene action under low nitrogen conditions and implications for maize biofortification

Nitrogen use efficiency is therefore an important objective for breeding programs in sub-Saharan Africa. The present study suggested that when improving maize for Fe and Zn content, and low PA content, breeders could use the same materials for low and optimum N conditions if it has sufficient genetic variation for grain yield. Apart from drought stress, a major challenge to smallholder farmers in sub-Saharan Africa (SSA) is low fertility soils with poor N-supply capacity [43].

Positive values with a high contribution of GCA to total variation and heritability consistently higher than 50% under low and optimum N conditions, suggested that breeding methods that exploit additive gene action will be effective in developing hybrids with high Fe and Zn content and low PA for low N soil conditions. Maize breeding methods that exploit additive genetic variance will be effective in developing high provitamin A hybrids under low N, and drought stress conditions [44]. In addition, the relative magnitude of GCA:SCA mean squares of the traits (grain yield, Fe, and Zn) is greater than one under low N conditions, indicating the predominance additive gene action in the expression of these traits. This indicates that there should be a good response to selection for these traits, and that Fe and Zn content as well as yield can be improved under low N conditions. The maize hybrids could be developed which performed better in N-depleted soils [45].

5. Conclusions

Nitrogen deficiency is major abiotic stress limiting Fe and Zn and grain yield of maize genotypes in dryland areas of southern Africa. Developing and evaluating maize genotypes that perform well under low N conditions can improve maize productivity and increase the bioavailability of Fe and Zn in the endosperm of maize grain. In the present study, both parents and their hybrids showed a broad range of diversity in grain yield, Fe, Zn and PA concentration under optimum and low N conditions. Genotypes responded differently to N levels across the environments. Both additive and non-additive gene effects were important in controlling Fe, Zn and PA concentration and grain yield under both N conditions. The bioavailability of Fe and Zn as measured by their molar ration with PA, was higher under low N than optimum N conditions, indicating that the N deficiency decreased anti-nutritional (phytic acid) factors in maize grain. Line CBY359 LM-1858 and tester CBY358 LM-1857 had positive GCA effects and can be recommended for improvement of yield, Fe and Zn. Hybrid CBY101 LM-1600 × CBY358 LM-1857 had high and significant positive SCA for grain yield under low N conditions and is a promising candidate for production in low N environments.

Author contribution statement

Tesfaye Walle Mekonnen: Performed the experiments; Conceived and designed the experiments; analysed and interpreted the data; Wrote the paper.

Sajjad Akhtar; Kingstone Mashingaidze; Gernot Osthoff; Maryke Labuschagne: Performed the experiments; Contributed analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

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Additional information

No additional information is available for this paper.

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