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Xenia and Deficit Nitrogen Influence the Iron and Zinc Concentration in the Grains of Hybrid Maize

Sajjad Akhtar [1], Maryke Labuschagne [1], Gernot Osthoff [2], Kingston Mashingaidze [3] and Akbar Hossain [4,*]

[1] Department of Plant Sciences, University of the Free State, Bloemfontein 9300, South Africa; akhtarsajjad21@gmail.com (S.A.); labuscm@ufs.ac.za (M.L.)
[2] Department of Microbial Biochemical and Food Biotechnology, University of the Free State, Bloemfontein 9300, South Africa; OsthoffG@ufs.ac.za
[3] ARC–Grain Cops Institute, Potchefstroom 2520, South Africa; mashingaidzek@arc.agric.za
[4] Department of Agronomy, Bangladesh Wheat and Maize Research Institute, Dinajpur 5200, Bangladesh
* Correspondence: akbarhossainwrc@gmail.com

Abstract: Xenia is the immediate effect of pollen on seed development after pollination. This study was conducted to investigate xenia's effects on Fe and Zn concentration in self- and open-pollinated maize seeds. Eighteen maize hybrids derived from parents with varying concentrations of Fe and Zn were planted at Potchefstroom, Cedara and Vaalharts in South Africa for two seasons, 2017 and 2018. Open- and self-pollinated seeds were compared. Self-pollination of five selected healthy maize hybrids of uniform height from each plot was performed. Fe, Zn and phytic acid (Pa) analysis was conducted on self -and open-pollinated maize seeds, and the molar ratios of Fe and Zn to Pa were also calculated to assess the bioavailability of Fe and Zn. The ranges of Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in self-pollinated maize seeds were 15.93–21.36 mg kg^{-1}, 18.50–24.34 mg kg^{-1}, 4.63–5.84 mg g^{-1}, 21.97–31.22 and 23.53–30.16 under high N, and 12.76–19.29 mg kg^{-1}, 16.78–23.50 mg kg^{-1}, 5.05–6.48 mg g^{-1}, 26.12–44.54 and 25.44–35.94 under low N conditions, respectively. The ranges of Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in open-pollinated seeds were 14.34–19.12 mg kg^{-1}, 17.27–23.27 mg kg^{-1}, 4.96–5.89 mg g^{-1}, 20.90–28.22 and 22.92–29.37 under high N, and 12.54–18.39 mg kg^{-1}, 16.94–20.93 mg kg^{-1}, 5.30–6.17 mg g^{-1}, 27.90–38.75 and 27.95–36.34 under low N conditions, respectively. The bioavailability of Fe and Zn was high in self-pollinated seeds under high N conditions, while Fe was higher in self-pollinated seeds, and Zn was higher in open-pollinated seeds under low N conditions. Combined over seasons, there were no significant differences between the values of Fe and Zn in self- and open-pollinated seeds under both high N and low N conditions. This indicated that unknown males had no influence on Fe and Zn concentration in maize. Therefore, either self- or open-pollinated seeds can be used for Fe and Zn determination in maize crosses.

Keywords: maize; hybrids; self-pollinated; open-pollinated; bioavailability

1. Introduction

Maize originated from the domestication of teosinte (*Zea mays* subspecies *parviglumis*) in mid-altitude regions of South-Central Mexico [1]. It has a large amount of genetic variability and is adapted to tropical, subtropical and temperate agroecological conditions [2]. It is the third most important cereal crop worldwide [3] and a staple food of millions of people in Asia, Latin America and Africa. It is the main staple and cash crop for about 300 million smallholder farmers in sub-Saharan Africa (SSA) [4,5] and is grown in diverse regions of SSA under various climatic and ecological conditions due to its extensive adoption and adaptation [6].

In many parts of the world, micronutrient deficiency and poor dietary quality are more widespread problems than low energy intake [7]. Factors responsible for micronutrient deficiency include poverty, lack of access to a balanced diet and variety of foods (such as meat and fish), plant-based foods with a low bioavailability of minerals, unawareness of good nutritional practices and a high incidence of infectious diseases [8]. The problem

is exacerbated by a lack of stable government policies, safe distribution schemes and satisfactory investment [9]. The populations in developing countries are at a risk of developing zinc (Zn) and iron (Fe) deficiencies due to their reliance on cereals as a staple in the diet [10]. Fe and Zn are most important for human health, especially for children and pregnant women. Fe is the main part of haemoglobin in red blood cells and transports oxygen from lungs to tissues. It is involved in energy production, immunity and thyroid function, and is the main component of cytochromes and ferredoxins [11,12]. Zn is mostly present in bones and skeletal muscles. It is responsible for the efficient functioning of more than 300 enzymes and acts as a stabiliser and protects membrane structure and cell components [13]. Deficiencies of Zn and Fe are in the fifth and sixth position among the top 10 most important risk factors responsible for causing illnesses and diseases in developing countries [12]. Fe and Zn deficiencies cause severe health problems such as impaired immune response and physical growth, impaired cognitive development, anaemia and morbidity in women and children under 5 years [14]. Fe and Zn deficiency affects more than 2 million people in the world, especially in low-income countries [15]. In cereals such as maize, 80% of the grain phosphorous is in the form of phytic acid and can be used as an indicator of the bioavailability of Fe and Zn [16]. The bioavailability of Fe decreases when the molar ratio of phytate/Fe is more than 1, and the bioavailability of Zn decreases when the molar ratio of phytate/Zn is more than 6 [17].

Nitrogen (N) is a crucial nutrient needed for normal plant growth and development. It is the main component of chlorophyll, the photosynthetic pigment present in the leaves, which helps in the photosynthesis process [18]. Low levels of N in tropical soils constitute a major constraint to high crop productivity. Several decades of farming without the application of adequate fertiliser has resulted in depletion of the essential soil nutrients required to support plant growth [18–20]. Maize grain yield losses reach up to 10–50% annually due to low soil N in SSA [19,20]. Soil N stress prior to anthesis and silking decreases leaf area development, the photosynthesis rate and the number of ear rows, while nutrient stress during the anthesis and silking periods cause kernel and ear abortion, and, during grain-filling, enhances leaf senescence and lessens crop photosynthesis and kernel weight [21]. Biofortification of maize for Fe and Zn concentration can be done through breeding, but then enough genetic variation of these minerals in the seeds is necessary. Due to the large intake of maize in the human diet, biofortification for elements such as Fe and Zn is becoming crucial. Biofortification is a sustainable option to increase the nutritional value of maize for all consumers [22,23].

Xenia is the immediate effect of pollen on seed development after pollination. Earlier researchers observed that xenia's effects on Fe and Zn concentration in self- and open-pollinated maize seeds [24,25]. Xenia also had an influence (immediate effect of pollen source) on seed size and nitrogen (N) content in pearl millet and suggested that selfed seeds should be used for correct determination of the mineral content in cross-pollinated crops such as pearl millet and maize [26]. Selfing is the most cost-effective method for seed production; however, it is time-consuming and affects seed setting, depending on the genotype and environment [27]. Genotype by environment (G × E) interactions also affect grain Fe and Zn concentrations in maize. A significant G × E interaction for both grain Fe and Zn content was observed in a multiple-location trial with 20 early and 49 late maturing maize cultivars [28,29]. Since the effect of xenia on the concentration of Fe and Zn in maize grain has not been determined yet, this study aimed to compare open- and self-pollinated seed from the same hybrids, derived from crosses of parents varying in Fe and Zn content.

2. Materials and Methods

2.1. Germplasm and Locations

The plant material consisted of 9 parents, including 6 females and 3 males (Table 1) selected after screening 215 South African maize inbred lines obtained from the Agricultural Research Council (ARC)–Grain Cops Institute (ARC-GCI) for their concentration (low, medium and high) of Fe and Zn. The parents were planted in July 2016 at Makhathini

Research Station and crossed following a line by tester (6×3) scheme, and 18 F_1 maize hybrids (Table 2) were generated. The hybrids, along with the 9 parents, were planted in December 2016 and 2017 at Potchefstroom, Cedara and Vaalharts in South Africa, following a randomised complete block design with 2 replicates under high N and poor N conditions.

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 (F_1)	CBY075 LM-1574	340.0	105.0	High
Line-2 (F_2)	CBY101 LM-1600	287.0	103.0	High
Line-3 (F_3)	CBY102 LM-1601	116.5	49.5	Medium
Line-4 (F_4)	CBY359 LM-1858	101.0	47.5	Medium
Line-5 (F_5)	CBY017 LM-1516	55.5	18.0	Low
Line-6 (F_6)	CBY014 LM-1513	45.0	10.5	Low
Tester-1 (M_1)	CBY358 LM-1857	139.5	56.0	High
Tester-2 (M_2)	CBY104 LM-1603	121.5	35.0	Medium
Tester-3 (M_3)	CBY013 LM-1512	44.5	9.0	Low

F = Female, M = Male.

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

Hybrids	Codes	Pedigree
Line-1 × Tester-1	SMH1	CBY075 LM-1574 × CBY358 LM-1857
Line-1 × Tester-2	SMH7	CBY075 LM-1574 × CBY104 LM-1603
Line-1 × Tester-3	SMH13	CBY075 LM-1574 × CBY013 LM-1512
Line-2 × Tester-1	SMH2	CBY075 LM-1574 × CBY358 LM-1857
Line-2 × Tester-2	SMH8	CBY075 LM-1574 × CBY104 LM-1603
Line-2 × Tester-3	SMH14	CBY075 LM-1574 × CBY013 LM-1512
Line-3 × Tester-1	SMH3	CBY075 LM-1574 × CBY358 LM-1857
Line-3 × Tester-2	SMH9	CBY075 LM-1574 × CBY104 LM-1603
Line-3 × Tester-3	SMH15	CBY075 LM-1574 × CBY013 LM-1512
Line-4 × Tester-1	SMH4	CBY075 LM-1574 × CBY358 LM-1857
Line-4 × Tester-2	SMH10	CBY075 LM-1574 × CBY104 LM-1603
Line-4 × Tester-3	SMH16	CBY075 LM-1574 × CBY013 LM-1512
Line-5 × Tester-1	SMH5	CBY075 LM-1574 × CBY358 LM-1857
Line-5 × Tester-2	SMH11	CBY075 LM-1574 × CBY104 LM-1603
Line-5 × Tester-3	SMH17	CBY075 LM-1574 × CBY013 LM-1512
Line-6 × Tester-1	SMH6	CBY075 LM-1574 × CBY358 LM-1857
Line-6 × Tester-2	SMH12	CBY075 LM-1574 × CBY104 LM-1603
Line-6 × Tester-3	SMH18	CBY075 LM-1574 × CBY013 LM-1512

SMH = Sajjad maize hybrid.

Potchefstroom is in the Tlokwe municipality of the Northwest (NW) province, and lies at $-26.73°$ latitude and $27.08°$ longitude at an altitude of 1349 m above sea level (m asl), with brown sandy loam soils. Low N conditions were created by depleting the soil of N, by planting maize for several years without N fertilisation and removing all stover from the field. Under managed conditions, the soil was considered low N when it had approximately 7 ppm N (translating to 54 kg ha^{-1}) in the upper 60 cm soil depth, allowing the soil to still supply some N to plants [30]. The fertiliser regime for high N conditions was a compound fertiliser (3:2:1 + Zn) applied as a basal application at planting at a rate of 200 kg NPK ha^{-1} to optimum N plots. Calcium ammonium nitrate (CAN) with 28% N was used for topdressing in 2 equal splits at 28 and 56 days after emergence at a rate of 100 kg ha^{-1} each in optimum N plots only. In low N plots, NPK was applied at the rate of 100 kg ha^{-1}.

Cedara is in the uMngeni municipality of the KwaZulu-Natal (KZN) province, and lies at $-29.54°$ latitude and $30.26°$ longitude at an altitude of 1066 m asl, with reddish-brown clay soils. The fertiliser used was mono-ammonium phosphate (MAP) at 250 kg ha^{-1} at planting for the optimum N environments or at 30 kg ha^{-1} in the low N environment, and CAN was given at 150 kg ha^{-1} in 2 equal splits of 75 kg ha^{-1} for the optimum N plots

only at 28 and 56 days after emergence. The Vaalharts is in the Northern Cape Province at 28°06′56.84″ S 24°55′32.50″ E at an altitude of 1192 m asl. Fertiliser was applied at the same rate as in Potchefstroom. All standard agronomic practices were applied in the trials. The total amounts of mineral N (basal + topdressing) applied in the 3 locations under optimum and poor conditions. Trials were grown under dryland conditions, which is the norm for the trial areas. The distances between plants and rows were 0.25 m and 0.75 m, respectively at all locations. In each plot, there were two rows 4 m in length. The plot size was 6 m². Five healthy plants from the middle of each plot were selected for data collection. Soil analysis data are presented in Table 3.

Table 3. Soil analysis of the experimental sites used.

Minerals	Soil Depth (cm)	Potchefstroom		Cedara		Vaalharts	
		High N	Low N	High N	Low N	High N	Low N
		2016–17	2016–17	2016–17	2016–17	2016–17	2016–17
Fe (mg kg^{-1})	0–30	11.9	10.0	13.5	9.6	7.0	5.9
	30–60	10.6	8.4	11.9	10.1	6.6	5.9
Zn (mg kg^{-1})	0–30	9.4	9.0	1.3	3.2	3.3	2.5
	30–60	8.6	5.6	1.4	2.2	2.9	2.3
P (mg kg^{-1})	0–30	27.9	15.5	11.7	12.8	52.3	32.4
	30–60	35.7	12.6	10.5	10.1	44.7	29.3
K (mg kg^{-1})	0–30	278.5	198.4	77.0	174.5	123	163
	30–60	314.9	209.7	70.5	120.0	114	149
Ca (mg kg^{-1})	0–30	830.0	666.0	513.0	699.0	436	535
	30–60	952.0	887.0	511.0	694.0	402	500
Mg (mg kg^{-1})	0–30	384.9	328.5	99.0	166.0	141	174
	30–60	440.7	438.9	99.5	154.0	128	169
Mn (mg kg^{-1})	0–30	38.9	35.1	3.6	3.4	11.1	13.2
	30–60	43.8	26.9	3.6	2.3	9.2	13.1
Soil pH	0–30	6.5	6.1	4.3	4.4	6.0	6.3
	30–60	6.6	6.0	4.4	4.5	6.1	6.3

2.2. Self- and Open-Pollinated Seeds

The selected cobs of 5 healthy hybrid plants of uniform height from each plot were closed for self-pollination. The cobs of the rest of the plants in each plot were left open for cross-pollination with unknown males. On selected plants, the silk was covered with a small polythene bag after emergence to avoid contamination, and the tassel was covered with a brown paper bag for pollen collection. The next morning, pollen was collected in the brown paper bag and the silk was self-pollinated and covered again with the same brown paper bag. At maturity, the self- and open-pollinated ears (with 12.5% moisture) were harvested and shelled manually. Shelled seeds were dried in the shade and then weighed and ground for Fe and Zn analysis.

2.3. Biochemical Analysis

The analysis was performed according to the dry-ashing method outlined by the AOAC [31] in the Soil Science Laboratory, University of the Free State, Bloemfontein, South Africa. Phytic acid analysis was performed following the method of Dragičević et al. [32] with some modifications. A standard curve for phytic acid determination was prepared in MS Excel and calculations were carried out according to Latta and Eskin [33]. Moles of Fe, Zn and Pa were calculated by dividing the weight of Pa, Fe and Zn by their atomic masses and then the molar ratios of Fe and Zn were calculated by dividing the moles of Pa by the moles of the minerals [34].

2.4. Grain Yield

Grain yield (GY) was measured as kg plot^{-1} using an electronic balance and then GY was converted to tons per hectare (t ha^{-1}) at 12.5% moisture by using the following Equation [35]:

$$\text{Grain yield (t ha}^{-1}) = (\text{Shelled grain weight}/1000) \times (\text{Adjusted grain moisture at 12.5}) \times (\text{Area factor})$$

where shelled grain weight (kg)/1000 is the weight converted into tons and the adjusted standard factor of grain moisture at 12.5% was the 100-grain moisture measured at harvest (%)/(100 − 12.5).

Grain moisture was measured using a moisture meter for maize in the field at harvesting time.

2.5. Statistical Analysis

Analyses of variance for single and combined locations were performed using Agrobase software [36]. Please note that due to the unavailability of self-data at 1 location in the first year and of the second location in the second year, the data of 2 locations were used for the final analysis of variance (ANOVA) and the degrees of freedom of location is 1 instead of 2.

3. Results

3.1. Season 1 (2016–2017)

High N conditions: The ranges of Fe, Zn, Pa, Fe:Pa and Zn:Pa in self-pollinated maize seeds were 15.11–21.49 mg kg^{-1}, 18.20–25.38 mg kg^{-1}, 4.18–5.83 mg g^{-1}, 19.64–28.73 and 17.82–27.64, respectively. The ranges of Fe, Zn, Pa, Fe:Pa and Zn:Pa in open-pollinated maize seeds were 12.18–18.19 mg kg^{-1}, 16.22–21.73 mg kg^{-1}, 4.35–5.68 mg g^{-1}, 19.99–26.72 and 21.14–28.95, respectively (Table S1).

The hybrid, location and hybrid × location effects were highly significant for all characteristics (except for Fe and Zn to Pa molar ratios in the case of location) in both types of seed (Table S2).

Low N conditions: The ranges of Fe, Zn, Pa, Fe:Pa and Zn:Pa in self-pollinated maize seeds were 12.88–22.11 mg kg^{-1}, 14.98–21.34 mg kg^{-1}, 4.86–6.63 mg g^{-1}, 23.46–38.62 and 27.99–40.35, respectively. The ranges of Fe, Zn, Pa, Fe:Pa and Zn:Pa in open-pollinated maize seeds were 11.58–20.77 mg kg^{-1}, 12.51–18.86 mg kg^{-1}, 4.47–6.01 mg g^{-1}, 24.67–39.23 and 28.63–45.54, respectively (Table S3).

The hybrid effect was highly significant for Fe, Zn, Pa, Fe:Pa and Zn: Pa in both types of seed. The location effect was highly significant in self- (significant for Pa) and open-pollinated (except for Zn) maize seeds for the measured characteristics. The hybrid × location effect was highly significant for Fe, Zn, Fe:Pa and Zn:Pa, and significant for Pa in both types of maize seed (Table S4).

3.2. Season 2 (2017–2018)

High N conditions: The ranges of Fe, Zn, Pa, Fe:Pa and Zn:Pa in self-pollinated maize seeds were 12.64–21.93 mg kg^{-1}, 17.95–23.30 mg kg^{-1}, 4.98–6.30 mg g^{-1}, 21.07–41.40 and 25.62–34.71, respectively. The ranges of Fe, Zn, Pa, Fe:Pa and Zn:Pa in open-pollinated maize seeds were 15.77–21.94 mg kg^{-1}, 18.33–24.80 mg kg^{-1}, 5.18–6.29 mg g^{-1}, 20.33–31.74 and 23.45–31.50, respectively (Table S5). The hybrid, location and hybrid × location effects were highly significant for all characteristics in both types of maize seed (Table S6).

Low N conditions: The ranges of Fe, Zn, Pa, Fe:Pa and Zn:Pa in self-pollinated maize seeds were 10.87–17.76 mg kg^{-1}, 15.90–27.58 mg kg^{-1}, 5.19–6.89 mg g^{-1}, 26.83–55.64 and 22.49–36.85, respectively. The ranges of Fe, Zn, Pa, Fe:Pa and Zn:Pa in open-pollinated maize seeds were 12.46–18.64 mg kg^{-1}, 17.90–23.56 mg kg^{-1}, 5.65–6.99 mg g^{-1}, 30.08–48.82 and 26.56–35.39, respectively (Table S7). The hybrid, location and hybrid × location effects were highly significant for Fe, Zn, Pa, Fe:Pa and Zn: Pa in both types of maize seed (Table S8).

3.3. Both Seasons Combined

High N conditions: The ranges of Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in self-pollinated maize seeds were 15.93–21.36 mg kg^{-1}, 18.50–24.34 mg kg^{-1}, 4.63–5.84 mg g^{-1}, 21.97–31.22 and 23.53–30.16, respectively. The ranges of Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in open-pollinated seeds were 14.34–19.12 mg kg^{-1}, 17.27–23.27 mg kg^{-1}, 4.96–5.89 mg g^{-1}, 20.90–28.22 and 22.92–29.37, respectively (Table 4). The effects of location, entry, season (non-significant for Fe), location × entry, location × season, entry × season (non-significant for Zn:Pa) and location × entry × season were highly significant for all characteristics in self- as well as in open-pollinated maize seeds under high N conditions (Table 5).

Table 4. Mean performance for Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in self- and open-pollinated maize seeds under high N conditions during both seasons.

Hybrids	Pollination	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Pa (mg g^{-1})	Fe:Pa	Zn:Pa
SMH1	Self	21.36	22.53	5.68	22.70	24.89
	Open	16.84	21.22	5.89	25.56	26.75
SMH2	Self	16.91	21.93	5.10	26.63	23.85
	Open	17.29	20.85	5.11	21.71	23.57
SMH3	Self	15.93	22.99	5.28	31.22	23.66
	Open	19.12	21.18	5.53	23.85	25.11
SMH4	Self	20.14	24.34	5.56	24.34	23.72
	Open	17.67	23.27	5.53	21.94	22.92
SMH5	Self	19.80	19.66	5.24	22.65	27.05
	Open	17.61	21.06	5.14	22.07	23.34
SMH6	Self	18.60	19.63	5.11	24.77	27.11
	Open	15.50	20.00	5.35	26.31	25.50
SMH7	Self	17.10	19.23	5.84	29.79	30.08
	Open	15.65	17.92	5.42	27.04	28.54
SMH8	Self	19.11	19.31	5.39	24.16	29.29
	Open	15.89	17.27	5.00	25.52	27.69
SMH9	Self	18.66	19.05	5.42	24.45	27.93
	Open	16.24	19.50	5.52	24.28	26.84
SMH10	Self	17.60	20.02	5.20	25.81	27.56
	Open	17.26	19.19	5.32	24.14	26.54
SMH11	Self	19.25	18.50	5.26	24.41	28.87
	Open	18.12	18.36	5.15	22.20	26.92
SMH12	Self	18.12	20.33	4.63	21.99	23.76
	Open	18.23	18.88	5.31	23.23	26.98
SMH13	Self	17.57	20.08	5.81	29.07	29.31
	Open	15.25	20.01	5.87	28.22	28.17
SMH14	Self	16.53	20.01	4.83	25.75	23.53
	Open	14.34	17.99	4.96	25.23	26.26
SMH15	Self	16.97	19.12	5.75	29.23	30.16
	Open	16.70	17.99	5.49	27.58	29.37
SMH16	Self	16.33	19.55	5.54	28.96	28.66
	Open	15.66	19.92	5.70	25.95	27.79
SMH17	Self	20.44	19.82	5.12	23.20	26.89
	Open	16.33	18.43	5.26	24.39	27.57
SMH18	Self	19.72	19.62	5.10	21.97	25.84
	Open	19.08	20.08	5.15	20.90	24.72
Mean	Self	18.34	20.32	5.33	25.62	26.79
	Open	16.82	19.62	5.37	24.45	26.37
LSD$_{0.05}$	Self	0.60	0.73	0.37	2.44	2.25
	Open	0.65	0.88	0.17	1.47	1.38

SMH = Sajjad maize hybrid; Fe = iron; Zn = zinc; Pa = phytic acid; Fe:Pa = molar ratio of Fe to Pa; Zn:Pa = molar ratio of Zn to Pa; LSD = least significant difference.

Table 5. Analysis of variance showing mean squares for Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in self and open-pollinated maize seeds under high N conditions during both seasons.

Source	df	Pollination	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Pa (mg g^{-1})	Fe:Pa	Zn:Pa
Block in	4	Self	1.00	0.59	1.01	36.51	27.76
(L × Y)		Open	0.96	1.16	0.12	3.37	5.46
Location	1	Self	6.27 ***	259.13 ***	10.82 ***	104.24 ***	1743.20 ***
(L)		Open	56.32 ***	114.92 ***	11.81 ***	125.28 ***	724.93 ***
Entry (E)	17	Self	19.85 ***	19.67 ***	0.88 ***	67.27 ***	45.55 ***
		Open	21.42 ***	20.20 ***	0.89 ***	55.45 ***	40.40 ***
Year (Y)	1	Self	0.01	22.35 ***	48.28 ***	1351.05 ***	2214.33 ***
		Open	1158.42 ***	108.08 ***	24.58 ***	538.72 ***	599.33 ***
L × E	17	Self	23.25 ***	7.67 ***	0.45 **	55.23 ***	28.86 ***
		Open	17.77 ***	12.21 ***	0.52 ***	27.75 ***	22.59 ***
L × Y	1	Self	350.19 ***	384.98 ***	13.40 ***	1631.62 ***	2257.99 ***
		Open	22.24 ***	63.96 ***	6.34 ***	168.81 ***	515.03 ***
E × Y	17	Self	13.41 ***	3.22 ***	0.53 **	37.21 ***	10.37
		Open	12.23 ***	6.73 ***	0.29 ***	34.25 ***	15.46 ***
L × E × Y	17	Self	18.00 ***	16.28 ***	0.51 **	61.63 ***	46.30 ***
		Open	15.72 ***	5.89 ***	0.56 ***	31.03 ***	20.42 ***
Residual	68	Self	0.51	0.77	0.20	8.58	7.28
		Open	0.91	1.69	0.06	4.68	4.17
R^2 value		Self	0.98	0.97	0.90	0.92	0.94
		Open	0.97	0.91	0.95	0.91	0.93

** $p \leq 0.01$; *** $p \leq 0.001$; df = degrees of freedom; Fe = iron; Zn = zinc; Pa = phytic acid; Fe:Pa = molar ratio of Fe to Pa; Zn:Pa = molar ratio of Zn to Pa.

Low N conditions: The ranges of Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in self-pollinated maize seeds were 12.76–19.29 mg kg^{-1}, 16.78–23.50 mg kg^{-1}, 5.05–6.48 mg g^{-1}, 26.12–44.54 and 25.44–35.94, respectively. The ranges of Fe, Zn, Pa and the molar ratios of Fe and Zn to Pa in open-pollinated seeds were 12.54–18.39 mg kg^{-1}, 16.94–20.93 mg kg^{-1}, 5.30–6.17 mg g^{-1}, 27.90–38.75 and 27.95–36.34, respectively (Table 6). The effects of location, entry, season (significant for Pa), location × entry, location × season, entry × season (significant for Pa) and location × entry × season were highly significant for all characteristics in self- as well as in open-pollinated maize seeds under high N and low N conditions (Table 7).

Table 6. Mean performance for Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in self- and open-pollinated maize seeds under low N conditions during both seasons.

Hybrids	Pollination	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Pa (mg g^{-1})	Fe:Pa	Zn:Pa
SMH1	Self	15.11	20.21	6.37	44.54	33.19
	Open	18.39	19.20	5.81	27.90	31.98
SMH2	Self	19.12	23.50	6.13	26.51	28.20
	Open	15.96	20.93	6.13	33.89	30.74
SMH3	Self	16.83	20.19	5.89	32.82	30.45
	Open	13.59	19.81	6.00	37.52	30.96
SMH4	Self	14.72	21.37	6.39	38.32	30.38
	Open	17.80	19.70	6.08	31.67	32.61
SMH5	Self	15.80	20.71	6.48	39.51	32.41
	Open	16.29	18.97	6.13	34.72	35.15
SMH6	Self	15.85	18.41	5.62	28.88	29.55
	Open	16.66	18.97	5.30	29.44	27.95
SMH7	Self	19.29	17.49	6.29	27.46	33.40
	Open	13.69	18.58	6.17	38.57	33.39
SMH8	Self	13.28	18.30	5.95	38.76	33.42
	Open	12.81	18.04	6.14	38.75	33.91

Table 6. *Cont.*

Hybrids	Pollination	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Pa (mg g^{-1})	Fe:Pa	Zn:Pa
SMH9	Self	13.56	17.37	5.70	35.03	34.37
	Open	16.11	16.94	5.74	33.65	33.43
SMH10	Self	17.63	21.01	6.21	31.79	29.64
	Open	15.20	18.15	5.94	35.59	32.30
SMH11	Self	15.64	20.72	5.05	30.63	27.70
	Open	17.18	18.15	5.81	31.36	31.78
SMH12	Self	15.26	17.14	5.80	38.03	34.04
	Open	15.12	17.67	5.48	31.15	31.28
SMH13	Self	15.18	17.35	5.87	36.82	33.01
	Open	15.88	18.47	6.13	33.74	33.39
SMH14	Self	12.76	17.63	5.83	42.11	29.41
	Open	12.54	17.04	5.47	34.76	31.91
SMH15	Self	14.03	17.11	5.84	31.82	35.94
	Open	14.40	18.13	6.07	38.53	34.09
SMH16	Self	18.32	21.07	5.97	29.63	27.19
	Open	15.38	17.82	6.16	33.86	36.34
SMH17	Self	16.81	19.42	5.14	26.12	25.44
	Open	17.21	18.39	5.45	28.20	29.82
SMH18	Self	14.83	16.78	5.27	32.32	30.56
	Open	14.64	18.01	5.48	33.06	31.11
Mean	Self	15.78	19.21	5.88	33.95	31.02
	Open	15.49	18.50	5.88	33.69	32.34
LSD$_{0.05}$	Self	1.09	1.14	0.38	3.20	2.93
	Open	0.57	1.05	0.33	2.55	2.82

SMH = Sajjad maize hybrid; Fe = iron; Zn = zinc; Pa = phytic acid; Fe:Pa = molar ratio of Fe to Pa; Zn:Pa = molar ratio of Zn to Pa; LSD = least significant difference.

Table 7. Analysis of variance showing the mean squares for Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in self- and open-pollinated maize seeds under low N conditions during both seasons.

Source	df	Pollination	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Pa (mg g^{-1})	Fe:Pa	Zn:Pa
Block in (L × Y)	4	Self	5.33	4.27	0.36	51.68	9.78
		Open	0.51	1.05	0.10	9.69	8.91
Location (L)	1	Self	1.60	74.29 ***	2.47 ***	1.59	25.58
		Open	249.80 ***	245.53 ***	15.85 ***	763.17 ***	1260.10 ***
Entry (E)	17	Self	29.16 ***	30.41 ***	1.38 ***	237.70 ***	65.42 ***
		Open	33.32 ***	11.44 ***	1.09 ***	135.20 ***	46.77 ***
Year (Y)	1	Self	244.98 ***	400.10 ***	1.10 *	1780.21 ***	704.77 ***
		Open	24.71 ***	1060.63 ***	62.44 ***	3034.43 ***	436.82 ***
L × E	17	Self	21.97 ***	20.94 ***	0.81 ***	87.00 ***	58.69 ***
		Open	13.60 ***	13.83 ***	0.63 ***	86.05 ***	65.11 ***
L × Y	1	Self	572.80 ***	34.98 ***	11.12 ***	1130.02 ***	137.93 ***
		Open	6.74 ***	75.21 ***	1.43 **	111.27 ***	156.08 ***
E × Y	17	Self	33.59 ***	19.39 ***	0.42 *	234.87 ***	40.66 ***
		Open	26.65 ***	18.64 ***	0.53 **	158.26 ***	96.74 ***
L × E × Y	17	Self	13.34 ***	6.73 ***	0.75 ***	123.43 ***	74.89 ***
		Open	24.20 ***	12.87 ***	0.69 ***	119.07 ***	78.21 ***
Residual	68	Self	1.71	1.88	0.21	14.69	12.32
		Open	0.71	2.42	0.24	14.16	17.30
R^2 value		Self	0.96	0.94	0.84	0.94	0.86
		Open	0.98	0.93	0.87	0.92	0.86

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; df = degrees of freedom; Fe = iron; Zn = zinc; Pa = phytic acid; Fe:Pa = molar ratio of Fe to Pa; Zn:Pa = molar ratio of Zn to Pa.

4. Discussion

The concentration of Fe and Zn and the molar ratio of Fe to Pa were slightly higher in self-pollinated than cross-pollinated seeds under high N as well as low N conditions. The concentration of Pa was lower in self-pollinated maize seeds under high N but higher under low N conditions. The molar ratio of Zn to Pa was lower in open-pollinated maize seeds under high N conditions while higher under low N conditions, indicating higher Zn absorption from the soil by open-pollinated seeds. A low seed set might increase seed size due to assimilate (products of photosynthesis, food) distribution into fewer grains as compared with good seed setting (open pollination). However, in this study, selfed seeds with a small size and even shrivelled seeds were also observed, indicating the additional effect of the small seed size in the high concentration of Fe and Zn in self-pollinated seeds [27]. The farmers' fields are deficient in N in western and central Africa [37]. Soil N leaches down to below the root zone of plants during periods of enough rainfall and results in N stress. Extensive removal of crop residues for animal feed and fuel, and inadequate weed control further compound soil N depletion [19,38]. Additionally, small landholder farmers apply N fertiliser in inadequate amounts due to the high prices of inorganic N fertiliser [37]. One effective strategy available for reducing fertiliser costs is to develop maize genotypes with combined high N use efficiency and high grain yield potential. Genotypes with high grain yield potential under low N are also needed to support the rapidly growing human population and provide incentives to farmers who mostly apply low to modest amounts of N to their maize fields. Improved maize varieties that tolerate low N will help maize farmers in soil N stress-prone areas to obtain better harvests [39]. Low N significantly affected the concentration and bioavailability of Zn in both types of hybrid seed in both seasons. During the first season, there was a large difference between the values of Fe and Zn in self- and open-pollinated seeds under high N as well as low N conditions. This indicated that the unknown males had a large effect on the values of Fe and Zn. However, during the second season and in the combined seasons, there were no significant differences between the values of Fe and Zn in self- and open-pollinated seeds under both high N and low N conditions. This indicated that the unknown males did not influence the Fe and Zn concentrations in maize. Therefore, either self- or open-pollinated seeds can be used for Fe and Zn determination in maize. This will make it much easier, as selfing is tedious and time-consuming. The difference in the values of Fe and Zn during the first season may have been due to dry environmental conditions. Rain was sufficient during the second season. Hybrid, location and location $\times$ hybrid effects (significant for Pa in the first season under low N) highly significantly affected the concentrations of Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in self- as well as in open-pollinated hybrids during both seasons under high N and low N conditions. Genetic differences were found for Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa among self- and open-pollinated hybrids under high N and low N conditions across locations. Highly significant hybrid (genotype) and location (environment) effects on Fe, Zn and Pa, and the molar ratios of Fe and Zn to Pa in maize were also reported in the literature [40,41]. A highly significant genotype $\times$ environment interaction effect was reported for Fe and Zn contents in the grain of 20 tropical maize genotypes [42,43], and a significant genotype $\times$ environment interaction effect was reported for Fe in the grain of 49 tropical maize varieties in six environments in Africa [29]. Breeding for tolerance to low soil N offers the most appropriate and sustainable approach for increased maize grain yields by small-scale farmers who apply low agricultural inputs in maize production in SSA.

5. Conclusions

Poor N significantly affected the concentration and bioavailability of zinc during both seasons. Environment played a crucial role in the expression of Fe and Zn in maize. Unknown males had no significant influence on Fe and Zn concentrations in maize when measured over two seasons. Therefore, maize breeders can use open-pollinated seeds for Fe and Zn determination in maize in future. This will make it much easier, as selfing is tedious and time-consuming.

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