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Kernel Iron and Zinc Concentration in Maize Double Haploid Lines and their Bioavailability

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ABSTRACT: Micronutrient deficiency is one of the major widespread problem in the population caused by hidden hunger, in order to address these challenges various biofortification programmes have been designed worldwide. In this study kernel concentrations of iron and zinc were evaluated in two MPS population consisting of 193 double haploid lines using Atomic Absorption Spectrophotometer (AAS) method. Bioavailability of iron and zinc in these double haploid lines were found using the phytate concentration obtained through wade assay method. The Phy/Fe and Phy/Zn molar ratios in MPS 1 population ranged from 0.33 to 63.93 and from 0.53 to 80.99, respectively. In MPS 2 population Phy/Fe and Phy/Zn molar ratios ranged from 0.28 to 73.34 and from 0.30 to 83.80, respectively. According to the phy/Fe and phy/Zn molar ratios, the lines ZL19406, ZL19365 and Zl19412 were found to have potential for the development of cultivars of maize containing high zinc and iron concentration.

Keywords: Iron, zinc, phytic acid, multiparent synthetic populations, maize.

INTRODUCTION

Iron and zinc perform a variety of functions in the human body, and their deficiencies have serious implications that have a significant impact on a country's health and economic development. The development of an efficient breeding program to increase minerals concentration in maize depends on the presence of genetic variability in this species (Menkir, 2008). Iron deficiency, anaemia is the most common nutritional deficiency, affecting around 4 to 5 billion people worldwide, with high-risk populations including children, pregnant women, nursing mothers, and the elderly. Fe related deficiencies affecting the cognitive development, growth and reproductive performance. And there is a high prevalence of zinc deficiency, with billions of people at risk, particularly in developing countries. Zn deficiencies leads to impaired growth, altered reproductive biology and gastrointestinal problems. Mineral deficiencies have been reduced using the low-cost and very easy approaches such as the provision of medical supplements, food fortification, and post-harvest changes in eating patterns. However these approaches are not found to be reliable as they are not inherent. The nutritional value of selected maize varieties will depend not only on the micronutrient concentration in the kernel but to a large extent on the bioavailability of the micronutrients to humans after consumption. Development of micronutrient enriched staple foods through breeding techniques hold significant promise to tackle the problem of malnutrition (Banziger and Long 2000; Pfeiffer and McClafferty 2007). Biofortification is advocated as the most appropriate, cost-effective and sustainable intervention that has widespread coverage in minimizing nutrient deficiencies globally (Cakmak and Kutman 2018).

Maize is the most important food and feed crop in the developing world; together with rice and wheat, maize provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries (Shiferaw *et al.*, 2011). Apart from its use as human food, maize contributes significantly to the livestock-to-meat cycle across the world and has various industrial purposes, including ethanol and biofuel production (Prasanna *et al*., 2020). The concentrations of various nutrients in maize kernels depend on the genetic background or the genotype, agronomic management, interaction between genotype and the environment, and post-harvest handling (Ekpa *et al.*, 2019). Analysis of genetic diversity in maize germplasm for kernel micronutrients and their potential for use in breeding programme assumes significance. However such studies undertaken in India were very few (Prasanna *et al.,* 2011; Chakraborti *et al.,* 2011; Agrawal *et al.,* 2012). The present study was undertaken to identify the genetic variability for kernel iron and zinc concentration in Indian germplasm.

MATERIAL AND METHODS

A subset of DH lines derived from two MPS population was used for the biochemical analysis. About 193 DH lines were used for iron, zinc and phytic acid estimation. Out of 193, 101 DH lines were from MPS 1 and 92 DH lines were from MPS 2.DH lines were raised during summer 2020 and self-pollinated and the self-pollinated fresh seeds were subjected for biochemical analysis. The contaminants were removed from the field samples. The grains were washed for few seconds with flowing water in a plastic sieve and are dried enough using towel paper. The grain samples were transferred to bags and placed immediately in an hot air oven with forced air circulation at 80°C for 3-4 days. After drying, the grain samples were powdered using Willey mill (Marconi, model MA 020) with a 20 mesh screen. The powdered samples were stored in polyethylene capped bottles.

A mixture of concentrated nitric acid and hydrochloric acid in the ratio of 9:1 was used for digestion. About 0.5 g of the finely ground seed sample was added to the 10 ml of acid mixture. The solutions were kept in the microwave digestion system at a temperature of about 180ºC and the digestion was carried out till a colourless solution was obtained. The digested solution was made up to 50 mL and filtered through Whatmann's filter paper number 40 into a 100 mL volumetric flask to obtain a clear colourless solution. The colourless solution obtained from the digestion was analysed for estimation of iron and zinc in AAS (thermo scientific) and their concentrations were determined using standards.

Phytic acid (myoinositolhexa-phosphoric acid, IP6) concentration was determined by modified Wade assay method according to Lorenz *et al.*, (2007). Ten milligrams (±0.2 mg) ground whole kernels from each sample was placed in assigned wells and 200 µL of 0.65 M HCl was added to each well. The 96-well plates were shaken at room temperature overnight (~12 h) and then centrifuged at 3000 rpm for 20 min. Thirty microliters of extract were transferred to each evaluation plate, maintaining randomized sample position. Equal volumes of the phytate quantitative standards were placed in assigned wells. Phytic acid dodecasodium salt from corn (Sigma P-8810) and KH_2PO_4 (Sigma P-5379) were used for the phytate stock solution. Phytate standard was prepared by dissolving 10 mg per 1 mL of 0.65 *M* HCl. For the measurement of phytate, 200 µL Wade reagent (2.5 g 5-sulfosalycyclic acid, 0.25 g $FeCl_3$. $6H_2O$, and 150 mL deionized H_2O, can be stored for one month at 4°C) was added to each well and allowed to react for 15 min at room temperature. Absorbance of the reaction mixture was measured at 490 nm. They phytic acid concentration was determined by using standard curve. A linear standard curve was obtained by plotting the decrease in absorbance at 490 nm against phytate concentration. The value of the correlation coefficient (r = 0.9777) of the calibration curve represented a strong negative relationship between absorbance and concentration of phytate. As the value of absorbance increased, the amount of phytate decreased. The total content pfphytic acid in maize grain as determined using the linear equation, Y= - 0.103 + 3.3868.

The Fe and Zn availability was estimated using the phytic acid/Zn (Phy/Zn) and phytic acid/Fe (Phy/F) molar ratios, calculated according to the Equation 1 described below (Harland *et al.*, 2004)

MR= (Phy/ MW of Phy)/(Min/ AW Min)

MR = molar ratio; Phy = phytic acid in the sample (mg.kg-1); MW Phy = phytic acid molecular weight (660 Da); Min = Fe or Zn in the sample (mg.kg-1); AW Min = Fe (56 Da) or Zn (65 Da) atomic weight.

RESULT AND DISCUSSION

The zinc, iron and phytic acid concentrations of two MPS population studied are in Table 1. The ANOVA was significant and showed genetic variability between two MPS population for iron, zinc and phytic acid. In MPS 1, iron concentration ranged from 13.45 mg to 72.92 mg, zinc concentration ranged from 10.79 mg to 39.23 mg and phytic acid concentration ranged from 0.13 mg to 19.29 mg. In MPS 2, iron concentration ranged from 7.07 mg to 102.6 mg, zinc concentration ranged from 3.34 mg to 36.97 mg and phytic acid concentration ranged from 0.10 mg to 14.32. Prasanna *et al.* (2011) recorded the similar variation in a set of 30 diverse genotypes were evaluated for kernel iron and zinc concentration. It was found that kernel iron and zinc concentration ranged from 11.28 mg to 60.11mg and 15.14 mg to 52.95 mg/kg, respectively. Menkir (2008) in his study noticed analysis of variance revealed a significant variation in kernel iron and zinc concentration among inbred lines which was similar to significant difference noticed between the DH lines of two MPS population.

The obtained phytic acid concentrations in the study were in accordance with the findings of Mikulski and Klosowski (2014). They reported the concentration of phytic acid in maize ranged from 1.86 to 10.78 mg/g, which was similar to the range obtained by this study. The variability in phytic acid levels in this study corroborated the results of Chandana (2018), which reported significant variations among the genotypes similar to the DH lines from two MPS populations.

The Phy/Fe and Phy/Zn molar ratios in MPS 1 population ranged from 0.33 to 63.93 and from 0.53 to 80.99, respectively. In MPS 2 population Phy/Fe and Phy/Zn molar ratios ranged from 0.28 to 73.34 and from 0.30 to 83.80, respectively. The lines ZL19406, ZL19365, ZL19412 showed lower values for Phy/Fe molar ratio in MPS 1 population. The lines ZL19493, ZL19481, ZL19567 showed lower values for Phy/Fe molar ratio in MPS 2 population. Regarding Phy/Zn molar ratio best results were found in DH lines ZL19406, ZL19365, ZL19412 of MPS 1 population. In case of MPS 2 population, best results were found inZL19493, ZL19481, ZL19567.Abebe*et al.* (2007) found Phy/Zn and Phy/Fe average molar ratio of 35.4 and 27.8, respectively, in maize consumed in south eastern Ethiopia - values a bit higher to those found in this study. Queiroz *et al.* (2011) found Phy/Zn and Phy/Fe average molar ratio 30.2 and 28.95 respectively in 22 maize tropical inbred lines with different genetic background were found to be little higher compared to average molar ratios obtained in this study.

Table 1: Iron, zinc, phytic acid and molar ratios of MPS 2 populations.

Line MPS1	Phytic acid (mg/g)	iron mg/kg	zinc	phy/iron	phy/zinc
ZL19296	13.86	25.86	24.59	45.46	55.47
ZL19297	12.02	28.97	30.95	35.19	38.22
ZL19298	9.55	31.91	27.71	25.39	33.92
ZL19299	3.45	24.68	22.84	11.85	14.86
ZL19300	0.92	29.92	27.79	2.60	3.25
ZL19301	6.32	29.00	22.65	18.47	27.44
ZL19302	10.79	55.28	23.99	16.55	44.26
ZL19304	5.34	33.76	33.79	13.43	15.56
ZL19307	15.73	41.96	27.58	31.78	56.11
ZL19309	19.29	35.44	30.17	46.15	62.90
ZL19312	8.78	35.45	21.60	20.99	39.98
ZL19313	11.23	31.35	26.10	30.37	42.34
ZL19314	9.29	34.80	23.19	22.63	39.40
ZL19315	16.67	38.73	26.20	36.48	62.60
ZL19316	0.97	37.82	36.05	2.17	2.65
ZL19317	8.24	48.18	28.83	14.50	28.12
ZL19318	4.74	28.42	20.37	14.15	22.91
ZL19319	10.02	31.13	34.19	27.31	28.85
ZL19320	8.47	20.81	23.52	34.52	35.44
ZL19321	2.83	31.05	19.61	7.72	14.19
ZL19323	1.35	29.56	25.68	3.88	5.18
ZL19325	6.25	31.69	25.59	16.73	24.04
ZL19326	0.75	32.92	21.67	1.93	3.40
ZL19329	2.20	27.78	17.11	6.71	12.65
ZL19330	4.81	23.01	24.61	17.74	19.25
ZL19332	4.72	23.11	19.92	17.31	23.30
ZL19333	6.06	24.20	18.27	21.22	32.62
ZL19334	12.52	23.65	15.71	44.89	78.42
ZL19335	3.37	34.68	25.08	8.24	13.22
ZL19336	4.71	30.31	30.23	13.18	15.33
ZL19337	4.40	21.18	16.51	17.62	26.22
ZL19339	1.11	24.15	21.45	3.88	5.07
ZL19341	6.83	25.12	17.36	23.07	38.74
ZL19342	4.12	26.54	19.94	13.15	20.30
ZL19343	11.77	30.45	24.61	32.76	47.04
ZL19344	2.59	14.63	20.57	15.00	12.38
ZL19345	2.48	16.69	19.07	12.59	12.79
ZL19348	1.38	31.84	20.60	3.67	6.58
ZL19351	1.21	29.09	18.04	3.53	6.60
ZL19356	9.68	34.95	27.01	23.47	35.25
ZL19357	7.97	33.93	18.23	19.91	42.99
ZL19358	5.47	35.76	17.31	12.98	31.11
ZL19359	13.33	32.39	26.70	34.89	49.12
ZL19362	9.16	23.32	21.25	33.30	42.41
ZL19363	6.52	31.14	39.23	17.75	16.34
ZL19364	0.92	18.25	17.15	4.26	5.27
ZL19365	0.28	40.13	20.58	0.59	1.34
ZL19366	10.50	13.93	12.76	63.93	80.99
ZL19367	8.83	13.45	12.04	55.65	72.14
ZL19369	2.35	29.89	23.14	6.68	10.01
ZL19370	4.09	24.44	20.25	14.19	19.88
ZL19371	3.99	30.84	29.36	10.98	13.38
ZL19374	9.87	56.93	31.53	14.70	30.80
ZL19376	11.78	30.05	24.61	33.24	47.10
ZL19380	13.80	72.92	20.17	16.05	67.33
ZL19381	9.85	31.57	25.31	26.46	38.30
ZL19382	2.40	22.74	13.55	8.95	17.43
ZL19384	10.00	19.96	13.25	42.48	74.25
ZL19387	0.85	41.29	13.78	1.74	6.04
ZL19389	2.70	27.82	25.68	8.22	10.34
ZL19390	0.73	30.53	15.36	2.03	4.68
ZL19393	7.79	20.67	11.17	31.94	68.56
ZL19396	4.44	19.20	15.20	19.60	28.74
ZL19401	1.12	23.43	15.08	4.05	7.30
ZL19406	0.13	32.51	23.71	0.33	0.53
ZL19411	7.60	14.11	10.80	45.70	69.29

ZL19412	0.38	23.79	19.45	1.36	1.93
ZL19413	3.51	26.73	26.15	11.13	13.20
ZL19414	1.95	24.43	24.04	6.76	7.97
ZL19415	2.39	27.60	25.42	7.33	9.24
ZL19416	11.97	31.18	29.14	32.56	40.42
ZL19418	4.65	30.98	23.12	12.72	19.78
ZL19420	1.01	20.50	23.21	4.17	4.28
ZL19421	5.55	31.10	20.63	15.12	26.45
ZL19422	1.63	29.90	16.37	4.62	9.80
ZL19 423	1.14	34.40	26.76	2.80	4.18
ZL19424	5.30	38.80	24.01	11.58	21.72
ZL19425	9.54	36.60	21.98	22.11	42.71
ZL19426	1.20	35.45	15.94	2.88	7.43
ZL19427	2.66	33.58	15.82	6.71	16.54
ZL19428	0.65	37.79	27.02	1.45	2.35
ZL19429	2.43	40.00	24.12	5.16	9.92
ZL19431	7.97	56.65	21.21	11.92	36.95
ZL19432	5.16	48.67	23.80	9.00	21.35
ZL19433	6.61	48.35	25.29	11.60	25.73
ZL19434	2.04	48.91	16.51	3.53	12.14
ZL19435	11.27	56.43	18.85	16.93	58.82
ZL19436	0.70	39.41	20.25	1.52	3.42
ZL19437	10.91	36.16	16.11	25.57	66.60
ZL19438	4.61	44.45	16.28	8.80	27.89
ZL19439	1.50	54.45	22.74	2.34	6.49
ZL19440	6.84	24.93	17.95	23.27	37.51
ZL19441	5.59	29.33	32.25	16.16	17.06
ZL19443	3.22	27.54	28.06	9.92	11.30
ZL19444	3.57	54.96	37.22	5.51	9.44
ZL19445	3.01	34.10	25.76	7.50	11.52
ZL19447	1.05	32.45	22.66	2.75	4.58
ZL19448	10.40	36.80	27.38	23.97	37.38
ZL19449	7.27	20.94	13.84	29.46	51.71
ZL19450	8.63	33.79	29.39	21.67	28.91
ZL19451	7.75	22.97	18.59	28.62	41.03

Table 2: Iron, zinc, phytic acid and molar ratios concentration of MPS 2 populations.

Line MPS 2	Phytic acid (mg/g)	iron mg/kg	zinc	phy/iron	phy/zinc
ZL19457	1.51	50.19	25.61	2.55	5.79
ZL19472	6.26	46.89	19.69	11.32	31.27
ZL19473	10.72	27.59	14.38	32.96	73.36
ZL19476	4.70	22.10	19.61	18.05	23.60
ZL19477	0.88	16.01	14.13	4.65	6.12
ZL19478	6.05	17.85	16.34	28.74	36.45
ZL19479	7.13	27.40	22.04	22.07	31.84
ZL19480	3.03	46.19	31.12	5.56	9.57
ZL19481	0.18	41.71	27.07	0.37	0.65
ZL19482	2.79	42.23	23.50	5.60	11.67
ZL19484	3.45	49.12	22.87	5.95	14.84
ZL19485	5.30	43.85	21.78	10.25	23.94
ZL19486	0.92	41.74	17.65	1.88	5.15
ZL19487	0.45	46.56	18.28	0.82	2.43
ZL19489	1.87	45.71	26.56	3.47	6.93
ZL19490	2.32	36.74	20.30	5.36	11.26
ZL19491	0.78	42.95	16.96	1.54	4.54
ZL19492	3.00	41.41	20.48	6.15	14.43
ZL19493	0.21	38.21	17.12	0.47	1.22
ZL19495	6.91	39.62	17.43	14.79	39.02
ZL19498	0.87	53.23	28.87	1.39	2.97
ZL19500	2.73	32.23	20.78	7.18	12.93
ZL19501	2.85	7.07	3.34	34.13	83.80
ZL19503	9.81	40.23	33.10	20.68	29.16
ZL19504	5.14	41.66	31.08	10.47	16.29
ZL19505	0.92	30.17	21.74	2.58	4.15
ZL19506	2.74	24.56	16.50	9.45	16.31
ZL19507	1.14	44.47	33.42	2.18	3.37
ZL19508	4.72	30.18	31.22	13.26	14.87
ZL19512	5.35	7.46	9.24	60.87	57.01
ZL19513	3.20	16.25	19.66	16.71	16.03
ZL19514	2.67	23.80	25.08	9.52	10.48
ZL19516	0.69	28.79	34.58	2.04	1.97
ZL19518	4.32	24.44	26.05	14.97	16.30

ZL19519	2.62	21.14	22.54	10.51	11.44
ZL19521	7.01	28.97	24.08	20.52	28.64
ZL19522	14.32	16.56	18.52	73.35	76.11
ZL19533	10.10	44.71	25.53	19.16	38.94
ZL19534	10.52	51.07	33.64	17.47	30.78
ZL19535	9.64	46.83	22.35	17.46	42.46
ZL19536	4.72	51.02	33.57	7.85	13.85
ZL19539	13.14	23.80	25.56	46.81	50.58
ZL19540	6.90	28.02	25.20	20.88	26.94
ZL19541	11.77	32.95	32.10	30.28	36.06
ZL19542	0.62	38.76	36.43	1.36	1.67
ZL19543	4.75	30.88	29.32	13.04	15.94
ZL19545	12.02	27.88	29.97	36.57	39.46
ZL19548	6.06	20.76	20.61	24.74	28.92
ZL19550	3.46	54.73	30.38	5.35	11.19
ZL19551	5.20	42.62	27.77	10.34	18.41
ZL19559	4.71	55.45	23.17	7.20	20.01
ZL19564	8.23	22.20	24.09	31.42	33.60
ZL19565	1.76	20.80	27.00	7.17	6.41
ZL19567	0.10	31.10	33.40	0.28	0.30
ZL19569	0.42	35.50	20.94	1.00	1.97
ZL19570	1.13	40.26	27.94	2.38	3.98
ZL19571	2.72	38.50	27.87	5.98	9.59
ZL19572	1.42	36.50	26.10	3.29	5.34
ZL19573	6.74	38.75	25.11	14.76	26.42
ZL19574	1.20	45.28	32.62	2.24	3.61
ZL19575	2.44	34.53	31.32	5.99	7.66
ZL19576	1.99	39.03	31.89	4.31	6.13
ZL19577	4.72	46.09	28.61	8.69	16.24
ZL19578	5.04	50.41	24.65	8.48	20.12
ZL19579	2.10	42.34	25.76	4.21	8.03
ZL19583	1.05	29.37	25.80	3.02	3.99
ZL19585	3.02	10.21	21.87	25.09	13.59
ZL19587	1.14	15.73	18.25	6.13	6.14
ZL19588	9.03	26.12	21.31	29.33	41.72
ZL19589	0.88	30.86	25.49	2.42	3.39
ZL19590	0.76	40.00	19.85	1.62	3.78
ZL19591	0.57	39.45	24.57	1.24	2.30
ZL19592	4.23	35.50	29.10	10.09	14.29
ZL19593	0.46	29.90	23.90	1.32	1.91
ZL19594	5.29	36.64	20.89	12.24	24.90
ZL19595	1.88	26.54	18.59	5.99	9.93
ZL19596	3.45	31.56	26.89	9.27	12.62
ZL19597	11.26	32.59	28.55	29.30	38.80
ZL19598	4.72	39.00	17.20	10.27	27.03
ZL19602	9.31	27.01	22.86	29.24	40.09
ZL19611	10.32	102.60	20.41	8.53	49.76
ZL19617	4.51	27.96	23.95	13.68	18.53
ZL19620	1.91	42.75	20.06	3.78	9.36
ZL19621	1.31	45.98	23.73	2.42	5.44
ZL19622	5.29	39.90	14.56	11.25	35.76
ZL19624	7.63	40.95	20.98	15.80	35.80
ZL19625	2.65	49.88	28.55	4.50	9.12
ZL19631	1.15	34.75	23.84	2.81	4.75
ZL19632	4.82	27.42	27.04	14.91	17.55
ZL19634	1.20	33.56	28.16	3.04	4.20
ZL19635	8.20	30.19	22.52	23.03	35.84
ZL19636	4.63	35.08	36.98	11.18	12.31

CONCLUSION

According to the phy/Fe and phy/Zn molar ratios, the lines ZL19406, ZL19365 and Zl19412 were found to have potential for the development of cultivars of maize containing high zinc and iron concentration and they could help in overcoming the micronutrient deficiencies in the population to certain extent and enrich the staple diet with nutrients. However, further evaluation needs to be conducted for the environmental effects on the availability of iron and zinc in different DH lines.

Conflict of Interest. None.

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