

# Maize inbreds from different heterotic groups as favorable sources for increased potential bioavailability of magnesium, iron, manganese and zinc

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## ABSTRACT

Malnutrition, as a global problem, is mainly caused by low level of mineral elements in staple food (deficient soil). Biofortification is based on selection of genotypes with enhanced concentration of mineral elements in grain, as well as decreased concentration of substances which interfere bioavailability of mineral elements in gut (like phytic acid), and increased content of substances that increase availability (such as  $\beta$ -carotene). The experiment with 51 maize (*Zea mays* L.) inbred lines with different heterotic background was set up in order to evaluate chemical composition of grain and to determine the relations between phytic acid (PA),  $\beta$ -carotene, and mineral elements: Mg, Fe, Mn, and Zn. The highest average phytate,  $\beta$ -carotene, Fe, and Mn content was found in grain of inbreds from *Lancaster* heterotic group. The highest content of Mg was in grain of Independent source and Zn in grain of BSSS group. Increased level of Fe and Mn in *Lancaster* lines could be partially affected by higher PA content in grain, while increased  $\beta$ -carotene content could improve Mn and Zn availability from grain of BSSS genotypes and Mg availability from *Lancaster* inbreds. It is important to underline that PA reduction is followed by Zn content increase in grain of *Lancaster* heterotic group, as well as that variations in Mg, Fe, and Mn contents are independent on PA status in inbreds from Independent source, indicating that the genotypes with higher Mg, Fe and Mn status from this group could serve as favorable source for improved Mg, Fe, and Mn absorption.

**Key words:** Bioavailability,  $\beta$ -carotene, heterotic group, inbred line, mineral elements, phytic acid.

## INTRODUCTION

For optimal functioning, human body requires more than 22 mineral elements. Their intake could be restricted due to nutritional habits (i.e., vegetarian diets, avoidance of certain food type, etc.) or low level of mineral elements existing in staple food. Each of these factors could lead to malnutrition, having as a consequence numerous health problems, such as anemia, abnormal blood losses, chronic inflammatory stress, obesity, atherosclerosis, hypertension, osteoporosis, diabetes mellitus, and cancer (Hunt, 2003; White and Broadley, 2005; Nielsen, 2010).

Biofortification, aimed to enhance mineral elements concentrations and/or bioavailability in edible plant tissues, either agronomically or genetically using both conventional breeding and modern biotechnology, is considered to be the most promising and cost-effective approach to alleviate mineral malnutrition (Cakmak, 2008). The accumulation of minerals in seeds is a complex phenomenon, which is most likely controlled by a number of genes (Ghandilyan et al., 2009). The movement of mineral elements from soils to seeds involves their mobilization from soils, uptake by roots, translocation to the shoot, redistribution within the plant and deposition in seeds (Welch, 2003; White and Broadley, 2009).

Increase of mineral elements concentration in grain through breeding as a method for biofortification (White and Broadley, 2005), includes also a decrease of substances that interfere the absorption or utilization of mineral elements in gut. Namely, antinutrients (inhibitors), like phytate, polyphenolics etc., limit the absorption of mineral elements, whereas promoters (enhancing substances), such as ascorbate,  $\beta$ -carotene, S-containing amino acids, etc., promote mineral nutrients bioavailability or decrease the activity of inhibitors (Welch and Graham, 2004; Germano and Canniatti-Brazaca, 2011). Besides the processing for phytic acid (PA) reduction in foods (Bohn et al., 2008; Ramirez-Cardenas et al., 2008), one of the important goals of biofortification is selection of genotypes with low content of phytic acid (*lpa*). Naturally occurred mutations, like in maize (*Zea mays* L.), could have as a result normal level of total P but with significantly reduced PA level in grain, which in turn increases the level of inorganic P (P<sub>i</sub>) (Lönnerdal, 2003). For biofortification purposes, it is also important to identify genetic resources with high levels of the targeted mineral elements (especially when growing on soils with low level and/or reduced availability of targeted mineral elements), to consider the heritability of the targeted traits and to investigate Genotype  $\times$  Environment interactions (Ortiz-Monasterio et al., 2007).

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Although dietary phytate has received much attention as an antinutrient, more recent scientific studies support different beneficial properties of phytate in humans on several civilization diseases: antioxidative effect (particularly in regards to Fe), preventing pathological calcification, e.g. kidney stones and calcification in the heart vessels, cholesterol lowering effects and anticancer activity (Konietzny et al., 2006). Reported positive roles of phytic acid, along with the fact that is the major storage compound of P in cereals and legumes grains (Bohn et al., 2008; Branković et al., 2015), lead to necessity for achievement of homeostasis, i.e. for maintaining of optimal level between phytic acid and mineral elements.

Maize is a widely consumed multipurpose crop. Since nutrient quantities and bioavailability are, among other factors, influenced by genetics, information on genetic diversity and heterotic groups is very useful in evaluation for planning inbred lines crosses for hybrid cultivar development. All those findings prompt us to evaluate the variability of different maize genotypes for increased bioavailability of targeted mineral elements important to human nutrition. Considering the low level of some mineral nutrients caused by long-term inputs of NPK and N fertilizers, the experiment was conducted on 51 maize inbred lines with different heterotic background, in order to (i) evaluate chemical composition of grain and (ii) to determine the relations between phytic acid, and  $\beta$ -carotene, as factors affecting the absorption of mineral elements, i.e. Mg, Fe, Mn, and Zn.

## MATERIALS AND METHODS

The experiment was carried out in 2010 and 2011 in Zemun Polje (44°52' N, 20°19' E, 81 m a.s.l.), Serbia. The soil was slightly calcareous chernozem (Zivkovic et al., 1972), with: 53.0% sand, 30.0% silt, 17.0% clay, 3.3% organic matter, 7.17 pH KCl, and 7.40 pH H<sub>2</sub>O. The texture was silty clay

loam, containing: 37.45 mg kg<sup>-1</sup> of available N, 10.70 mg kg<sup>-1</sup> P, 107.40 mg kg<sup>-1</sup> K, 3270.00 mg kg<sup>-1</sup> Ca, 327.95 mg kg<sup>-1</sup> Mg, 0.65 mg kg<sup>-1</sup> Fe, 0.20 mg kg<sup>-1</sup> Mn, and < 0.02 mg kg<sup>-1</sup> Zn and 4.7% CaCO<sub>3</sub> in 0-30 cm layer. Available N was determined by the method of Scharpf and Wehrmann (1975), P was determined by method of Watanabe and Olsen (1965), and K, Ca, Mg, Fe, Mn and Zn by inductively coupled plasma-optical emission spectrometry (Spectroflame, 27.12 MHz and 2.5 kW, model P, Spectro Analytical Instruments, Kleve, Germany) after extraction by procedure of Mechlich (1984). CaCO<sub>3</sub> was determined by the method of Horváth et al. (2005). Fertilization was followed by 400 kg ha<sup>-1</sup> of NPK (15:15:15) in the autumn and 300 kg ha<sup>-1</sup> of urea in the spring, before sowing.

Fifty-one maize inbred lines from Maize Research Institute "Zemun Polje" were the objective of the present study. A randomized complete block design with four replicates was used in the experiment. Examined inbreds have different heterotic background: 20 inbreds (from L1 to L20) belong to *BSSS* heterotic group, 17 inbreds (from L21 to L37) belong to *Lancaster* heterotic group, while 13 inbreds (from L38 to L51) represent independent source, respectively. After harvesting and drying to 14% water content, chemical composition of grain was determined. Phytic (P<sub>phy</sub>) and inorganic P (P<sub>i</sub>) were determined spectrophotometrically by the method of Dragičević et al. (2011).  $\beta$ -Carotene was determined according to American Association of Cereal Chemists Method (AACC, 1995). Mineral elements (Mg, Fe, Mn, and Zn) were determined after wet digestion with HNO<sub>3</sub> + HClO<sub>4</sub>, by inductively coupled plasma-optical emission spectrometry.

## Statistical analysis

Analyses of chemical composition of grain were performed in four measurements (n = 4) and the experimental data were

**Table 1. Analysis of variance for the effect of genotype, year and Genotype  $\times$  Year interaction on phytic (P<sub>phy</sub>) and inorganic P (P<sub>i</sub>),  $\beta$ -carotene, Mg, Fe, Mn, and Zn contents in grain of 51 commercial maize inbred lines.**

Source of variation	df	P <sub>phy</sub>	P <sub>i</sub>	$\beta$ -carotene	Mg	Fe	Mn	Zn
		MS	MS	MS	MS	MS	MS	MS
		mg g <sup>-1</sup>			$\mu$ g g <sup>-1</sup>			
Replicate	3	0.060	0.000	0.582	1139.993	3.599	0.005	8.287
Inbred line (IL)	50	0.623**	0.066**	139.989**	35401.659**	212.892**	14.573**	256.311**
Year (Y)	1	5.678**	0.117**	1860.836**	17708854.170**	9008.256**	307.117**	1933.544**
IL $\times$ Y	50	0.644**	0.028**	26.990**	15388.952**	178.642**	5.607**	158.99**
Error	303	0.021	0.0001	0.479	38.897	0.812	0.066	4.730
CV, %		4.03	4.07	5.68	1.06	7.20	5.48	9.55
LSD (IL) <sub>0.05</sub>		0.146	0.010	0.695	6.263	0.905	0.258	2.184
Average	<i>BSSS</i>	3.57	0.45	12.86	584.68	12.61	4.52	23.87
Min		3.22	0.33	7.43	455.16	3.89	2.83	15.60
Max		4.20	0.64	20.26	687.97	20.30	7.24	40.70
Average	<i>Lanc.</i>	3.67	0.52	13.05	581.90	12.95	5.06	20.93
Min		3.04	0.35	3.56	520.00	3.81	2.68	13.57
Max		4.35	0.62	21.40	676.56	22.03	8.05	29.47
Average	Indep.source	3.56	0.51	10.16	594.25	11.85	4.47	23.43
Min		3.01	0.33	2.64	458.28	5.47	2.59	17.20
Max		3.84	0.68	17.55	731.72	28.09	6.75	34.93

\*Significant at 5% probability level; df: degrees of freedom; MS: mean squares.

subjected to two-way ANOVA. The coefficient of variation (CV) was determined for each trait, while significant differences between genotypes means were determined by the Fisher's least significant difference (LSD) test at the 0.05 probability level. Differences with  $P \leq 0.05$  were considered as significant. Ratios  $P_{phy}/P_i$ , phytic acid (PA)/ $\beta$ -carotene, PA/Mg, PA/Fe, PA/Mn, and PA/Zn were presented as mean  $\pm$  standard deviation (SD). For inbreds within each heterotic group, regression analysis and principal component analysis (PCA) were used for evaluation of interdependence between  $P_{phy}$  and mineral elements, as well as between  $\beta$ -carotene and mineral elements. Statistical analysis was performed by SPSS 15.0 (IBM Corporation, Armonk, New York, USA) for Windows Evaluation version.

## RESULTS AND DISCUSSION

In this study has been shown that effect of genotype (e.g., inbred line), year, and their interaction had significant impact on variation in  $P_{phy}$ ,  $P_i$ ,  $\beta$ -carotene, Mg, Fe, Mn, and Zn content in grain, as presented in Table 1. The highest variation between genotypes was observed for mineral elements contents, particularly for Fe and Zn, which could be reflected on different ability of the examined maize inbred lines to absorb and accumulate those elements (Kovačević et al., 2004). That is particularly important for genotypes growing on soils with low level and/or reduced availability of some mineral elements (Lynch and St.Clair, 2004). The highest average  $P_{phy}$ ,  $P_i$ ,  $\beta$ -carotene, Fe and Mn content was found in grain of inbreds from Lancaster heterotic group. The results obtained are opposed to the results of Mladenović-Drinić et al. (2013), who asserted that genotypes from *Lancaster* germplasm were low in P and Fe. However, the highest content of Mg was found in grain of inbreds belong to Independent source and Zn in grain of inbreds belong to *BSSS* group. This could mean that genotypes from *Lancaster* group are efficient to acquire Fe and Mn and from *BSSS* to acquire Zn from substrate poor in their content (Kovačević et al., 2004).

Based on the molar ratios between PA and mineral elements, such as PA/Zn and PA/Fe, it is possible to determine genotypes (or foods) with potentially high Fe and Zn bioavailability (Ma et al., 2007; Queiroz et al., 2011). According to relatively high content of examined mineral elements, lower mean values of PA/Fe and PA/Mn (98.59 and 221.94, respectively) were observed in grain of *Lancaster* group (Table 2). For inbreds belong to Independent source, lower  $P_{phy}/P_i$  and PA/Mg ratios were found (7.21 and 0.80, respectively). Among *BSSS* lines, the lowest PA/ $\beta$ -carotene was present in grain of L10 (491.3), which along with relative low values of PA/Mg and PA/Mn (0.73 and 180.7, respectively) could indicate their improved bioavailability (Queiroz et al., 2011). The lowest PA/Mn ratio among all examined lines was present in L27 (146.1), the inbred from *Lancaster* heterotic group with relatively low PA/ $\beta$ -carotene ratio (663.05), which could be reflected on better Mn availability. Among inbreds from Independent

**Table 2. Molar ratios between phytic P ( $P_{phy}$ )/inorganic P ( $P_i$ ), phytic acid (PA)/ $\beta$ -carotene, PA/Mg, PA/Fe, and PA/Zn.**

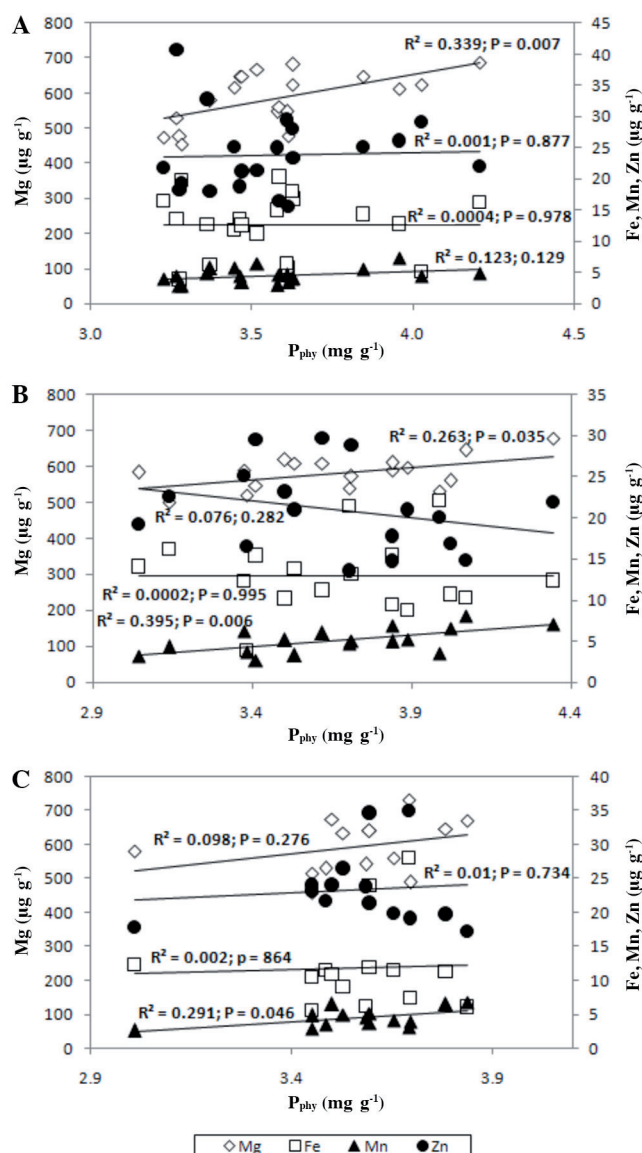
	$P_{phy}/P_i$	PA/ $\beta$ -carotene	PA/Mg	PA/Fe	PA/Mn	PA/Zn
<i>BSSS</i>						
L1	9.2 $\pm$ 0.3	957 $\pm$ 16	0.70 $\pm$ 0.006	77 $\pm$ 2	227 $\pm$ 3	64.8 $\pm$ 2.0
L2	7.0 $\pm$ 0.5	953 $\pm$ 9	0.70 $\pm$ 0.008	65 $\pm$ 24	259 $\pm$ 3	54.7 $\pm$ 2.1
L3	9.0 $\pm$ 0.3	950 $\pm$ 10	0.76 $\pm$ 0.006	60 $\pm$ 3	265 $\pm$ 3	45.6 $\pm$ 1.2
L4	7.5 $\pm$ 0.5	848 $\pm$ 13	0.86 $\pm$ 0.014	72 $\pm$ 2	359 $\pm$ 2	50.4 $\pm$ 2.6
L5	10.8 $\pm$ 1.5	1243 $\pm$ 106	0.83 $\pm$ 0.009	53 $\pm$ 4	224 $\pm$ 2	76.7 $\pm$ 2.0
L6	7.2 $\pm$ 3.0	1274 $\pm$ 45	0.90 $\pm$ 0.021	253 $\pm$ 17	342 $\pm$ 3	63.2 $\pm$ 2.6
L7	9.0 $\pm$ 1.6	808 $\pm$ 21	0.99 $\pm$ 0.021	187 $\pm$ 9	300 $\pm$ 7	81.5 $\pm$ 5.1
L8	8.1 $\pm$ 1.4	797 $\pm$ 10	0.76 $\pm$ 0.046	162 $\pm$ 12	176 $\pm$ 6	65.7 $\pm$ 6.5
L9	5.5 $\pm$ 1.3	1006 $\pm$ 15	0.69 $\pm$ 0.020	94 $\pm$ 6	152 $\pm$ 4	57.8 $\pm$ 4.8
L10	8.6 $\pm$ 1.1	491 $\pm$ 6	0.73 $\pm$ 0.023	88 $\pm$ 5	181 $\pm$ 5	48.4 $\pm$ 3.9
L11	6.3 $\pm$ 0.4	779 $\pm$ 19	0.81 $\pm$ 0.011	72 $\pm$ 3	212 $\pm$ 4	28.3 $\pm$ 1.2
L12	6.3 $\pm$ 0.4	622 $\pm$ 123	0.75 $\pm$ 0.013	80 $\pm$ 3	199 $\pm$ 5	36.1 $\pm$ 2.5
L13	8.4 $\pm$ 1.5	693 $\pm$ 126	0.70 $\pm$ 0.011	83 $\pm$ 3	287 $\pm$ 6	57.6 $\pm$ 2.3
L14	7.9 $\pm$ 0.3	645 $\pm$ 4	0.78 $\pm$ 0.006	81 $\pm$ 5	195 $\pm$ 5	53.9 $\pm$ 1.3
L15	7.1 $\pm$ 0.2	1027 $\pm$ 5	0.80 $\pm$ 0.004	78 $\pm$ 2	236 $\pm$ 1	67.1 $\pm$ 1.2
L16	8.9 $\pm$ 1.8	728 $\pm$ 7	0.86 $\pm$ 0.006	170 $\pm$ 2	231 $\pm$ 2	44.6 $\pm$ 0.5
L17	9.9 $\pm$ 0.5	1003 $\pm$ 6	0.84 $\pm$ 0.004	240 $\pm$ 3	263 $\pm$ 1	48.6 $\pm$ 1.4
L18	9.6 $\pm$ 1.2	723 $\pm$ 55	0.85 $\pm$ 0.007	93 $\pm$ 1	160 $\pm$ 1	53.3 $\pm$ 1.3
L19	9.3 $\pm$ 0.8	734 $\pm$ 46	0.94 $\pm$ 0.023	50 $\pm$ 6	333 $\pm$ 2	60.2 $\pm$ 3.1
L20	8.8 $\pm$ 0.4	605 $\pm$ 8	0.89 $\pm$ 0.015	59 $\pm$ 5	225 $\pm$ 1	52.1 $\pm$ 3.7
<i>Lancaster</i>						
L21	5.9 $\pm$ 0.4	864 $\pm$ 8	0.78 $\pm$ 0.011	97 $\pm$ 3	173 $\pm$ 2	43.0 $\pm$ 2.0
L22	11.1 $\pm$ 1.2	969 $\pm$ 12	0.85 $\pm$ 0.015	74 $\pm$ 2	225 $\pm$ 7	91.7 $\pm$ 3.7
L23	8.2 $\pm$ 4.0	700 $\pm$ 23	0.85 $\pm$ 0.022	267 $\pm$ 24	274 $\pm$ 4	72.4 $\pm$ 4.2
L24	8.8 $\pm$ 0.2	828 $\pm$ 6	0.85 $\pm$ 0.003	134 $\pm$ 1	202 $\pm$ 1	65.2 $\pm$ 0.9
L25	7.1 $\pm$ 0.2	710 $\pm$ 5	0.94 $\pm$ 0.006	113 $\pm$ 1	168 $\pm$ 1	84.3 $\pm$ 1.5
L26	7.9 $\pm$ 0.6	586 $\pm$ 6	0.84 $\pm$ 0.009	106 $\pm$ 2	174 $\pm$ 1	69.9 $\pm$ 2.1
L27	8.5 $\pm$ 0.1	663 $\pm$ 1	0.83 $\pm$ 0.004	119 $\pm$ 3	146 $\pm$ 1	96.5 $\pm$ 1.0
L28	5.9 $\pm$ 0.5	843 $\pm$ 15	0.74 $\pm$ 0.017	103 $\pm$ 5	194 $\pm$ 3	53.3 $\pm$ 3.4
L29	5.9 $\pm$ 0.7	2743 $\pm$ 255	0.75 $\pm$ 0.026	82 $\pm$ 5	157 $\pm$ 7	47.4 $\pm$ 4.2
L30	7.1 $\pm$ 1.2	608 $\pm$ 23	0.82 $\pm$ 0.017	123 $\pm$ 7	159 $\pm$ 2	76.1 $\pm$ 3.4
L31	6.9 $\pm$ 0.9	2162 $\pm$ 132	0.98 $\pm$ 0.022	54 $\pm$ 3	322 $\pm$ 2	70.2 $\pm$ 4.4
L32	5.2 $\pm$ 1.2	618 $\pm$ 6	0.82 $\pm$ 0.015	58 $\pm$ 5	208 $\pm$ 1	48.9 $\pm$ 3.6
L33	6.9 $\pm$ 1.1	745 $\pm$ 12	0.90 $\pm$ 0.015	52 $\pm$ 7	217 $\pm$ 1	96.1 $\pm$ 0.2
L34	6.5 $\pm$ 0.4	665 $\pm$ 8	0.76 $\pm$ 0.013	77 $\pm$ 10	311 $\pm$ 2	59.2 $\pm$ 1.9
L35	6.3 $\pm$ 0.6	587 $\pm$ 5	0.68 $\pm$ 0.011	65 $\pm$ 13	269 $\pm$ 1	55.7 $\pm$ 1.8
L36	6.8 $\pm$ 0.7	768 $\pm$ 15	0.81 $\pm$ 0.018	67 $\pm$ 3	357 $\pm$ 2	40.7 $\pm$ 1.5
L37	8.9 $\pm$ 1.1	2697 $\pm$ 70	0.85 $\pm$ 0.018	85 $\pm$ 10	217 $\pm$ 1	45.3 $\pm$ 2.5
Independent source						
L38	7.5 $\pm$ 0.5	980 $\pm$ 8	0.73 $\pm$ 0.009	91 $\pm$ 3	280 $\pm$ 6	59.1 $\pm$ 2.0
L39	9.6 $\pm$ 0.3	1874 $\pm$ 24	0.86 $\pm$ 0.012	91 $\pm$ 2	292 $\pm$ 7	56.7 $\pm$ 2.3
L40	6.1 $\pm$ 0.3	1005 $\pm$ 30	0.73 $\pm$ 0.010	117 $\pm$ 3	202 $\pm$ 4	46.9 $\pm$ 2.1
L41	5.1 $\pm$ 0.2	973 $\pm$ 28	0.68 $\pm$ 0.026	97 $\pm$ 6	149 $\pm$ 2	51.2 $\pm$ 4.3
L42	8.0 $\pm$ 1.4	697 $\pm$ 13	0.87 $\pm$ 0.017	175 $\pm$ 8	231 $\pm$ 5	52.9 $\pm$ 4.0
L43	7.2 $\pm$ 0.2	1990 $\pm$ 13	0.75 $\pm$ 0.009	190 $\pm$ 6	156 $\pm$ 1	78.5 $\pm$ 2.4
L44	6.2 $\pm$ 0.8	3778 $\pm$ 66	0.88 $\pm$ 0.022	99 $\pm$ 5	207 $\pm$ 2	52.3 $\pm$ 3.9
L45	8.1 $\pm$ 1.9	645 $\pm$ 7	0.77 $\pm$ 0.013	101 $\pm$ 27	159 $\pm$ 2	67.4 $\pm$ 3.6
L46	6.0 $\pm$ 0.3	592 $\pm$ 7	0.73 $\pm$ 0.021	45 $\pm$ 2	207 $\pm$ 3	36.4 $\pm$ 3.9
L47	7.9 $\pm$ 0.5	898 $\pm$ 5	0.99 $\pm$ 0.006	149 $\pm$ 2	265 $\pm$ 10	68.0 $\pm$ 1.2
L48	6.0 $\pm$ 1.5	959 $\pm$ 4	0.85 $\pm$ 0.006	96 $\pm$ 1	267 $\pm$ 4	64.8 $\pm$ 1.3
L49	10.4 $\pm$ 3.8	1063 $\pm$ 42	0.98 $\pm$ 0.024	190 $\pm$ 9	349 $\pm$ 8	50.5 $\pm$ 3.5
L50	6.0 $\pm$ 0.3	1215 $\pm$ 11	0.66 $\pm$ 0.016	39 $\pm$ 9	347 $\pm$ 2	37.2 $\pm$ 0.2
L51	6.8 $\pm$ 2.4	1222 $\pm$ 174	0.68 $\pm$ 0.031	74 $\pm$ 3	315 $\pm$ 4	59.4 $\pm$ 17.5

The results are mean  $\pm$  standard deviation of four measurements.

source, L41 has the lowest  $P_{phy}/P_i$  and PA/Mn ratios (5.13 and 148.8, respectively), signifying that some of the P in grain is not bound to PA (Lönnerdal, 2003) and that it could be absorbed in parallel with Mn. Additionally, L46 has the lowest PA/ $\beta$ -carotene together with PA/Fe ratio (591.5 and 45.0, respectively) indicating improved Fe availability. This could be considered as important, since it is well known



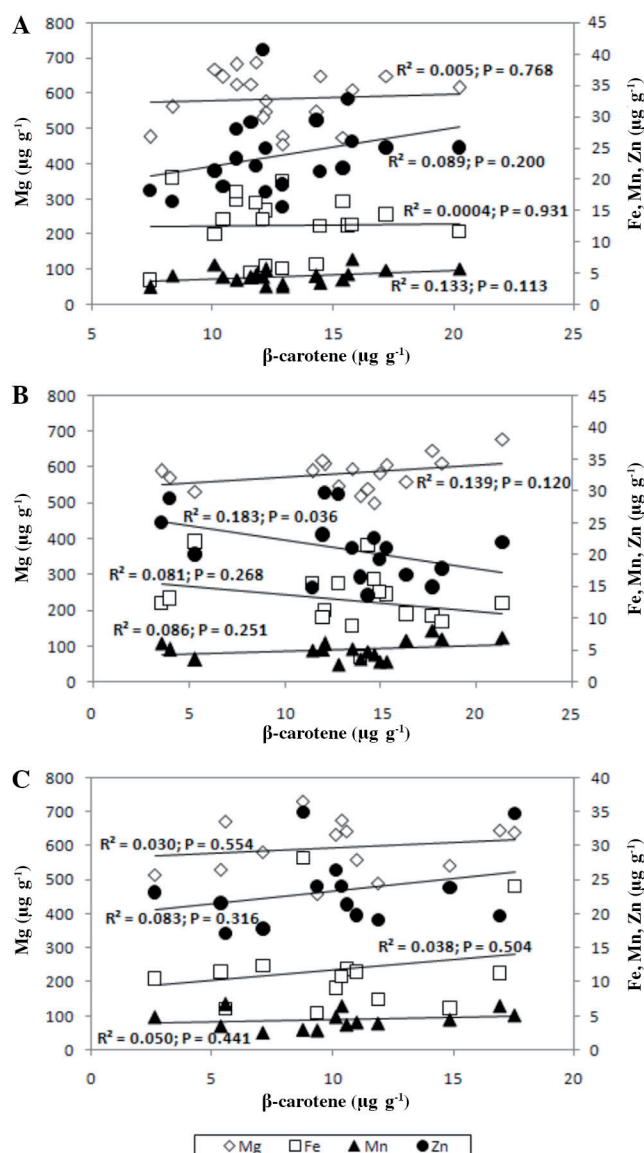
**Figure 1.** Interdependence between phytic P ( $P_{phy}$ ) and mineral elements in grain of maize lines belong to (A) *BSSS* heterotic group, (B) *Lancaster* heterotic group, and (C) Independent source.



that increased Fe absorption by humans is associated with increased  $\beta$ -carotene content in foods (Lönnerdal, 2003; Calheiros et al., 2011). The lowest PA/Mg ratio among all examined lines was found in L50 (0.66) from Independent group, the inbred with low  $P_{phy}/P_i$  ratio (6.0), while the lowest PA/Zn ratio was present in L11 (28.3), from *BSSS* group.

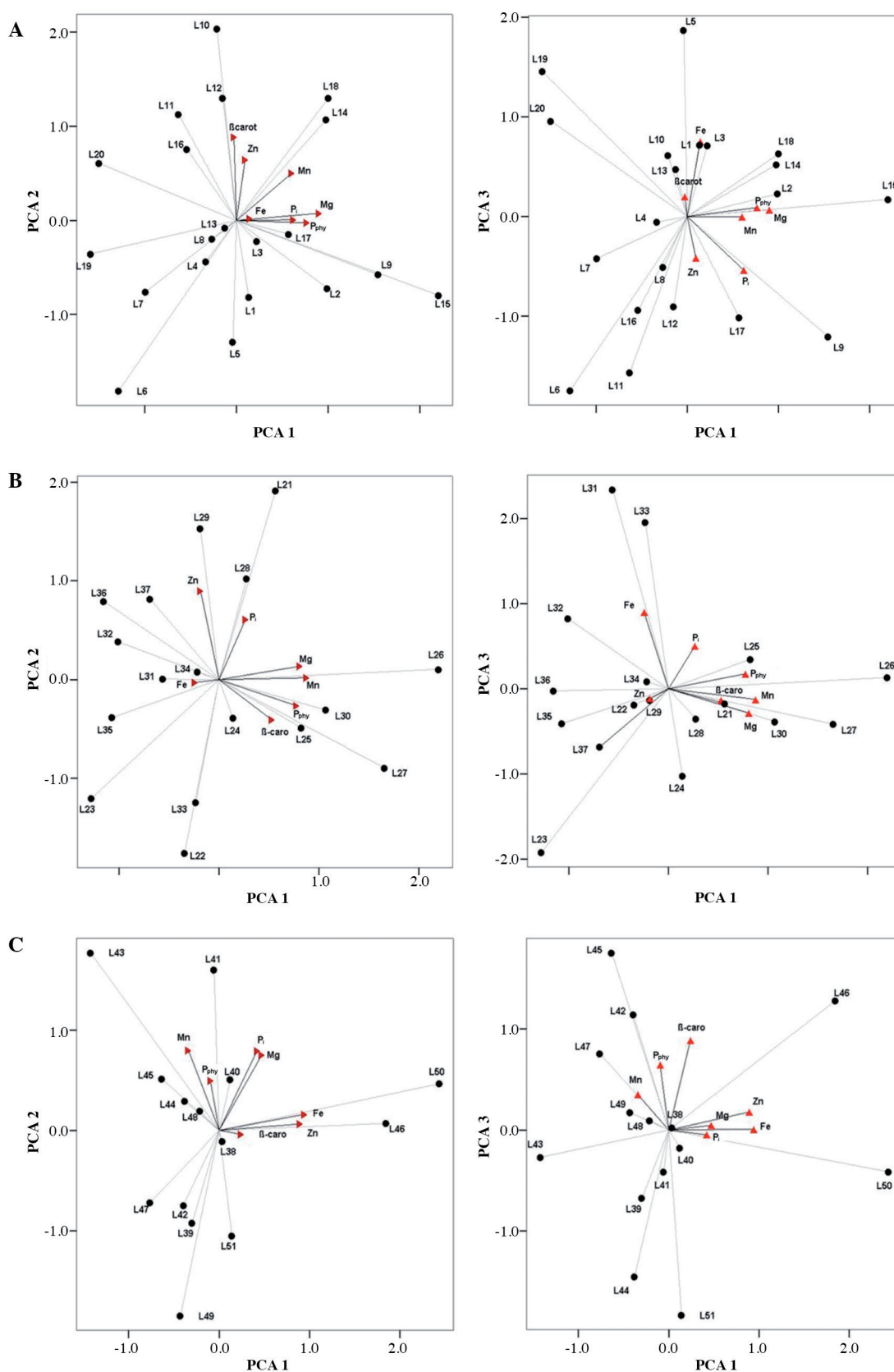
According to regression analysis, positive and significant interdependence between  $P_{phy}$  and Mg was observed in *BSSS* and *Lancaster* group ( $R^2 = 0.339$ ;  $P \leq 0.01$  and  $R^2 = 0.263$ ;  $P \leq 0.05$ , respectively; Figure 1). In all three heterotic groups, significant and positive correlation between  $P_{phy}$  and Mn was found, with the highest regression coefficient obtained in *Lancaster* group ( $R^2 = 0.395$ ;  $P \leq 0.01$ ). In addition, Ghandilyan et al. (2009) reported significant and negative correlation between PA content and Fe and Zn in *Arabidopsis*

**Figure 2.** Interdependence between  $\beta$ -carotene and mineral elements in grain of maize lines belong to (A) *BSSS* heterotic group, (B) *Lancaster* heterotic group, and (C) Independent source.



seeds, with increased impact of environment on variation of mineral elements in seeds, which is supported by the results presented in Table 1. Interdependence between  $\beta$ -carotene and examined mineral elements was expressed to a lesser extent (Figure 2), with positive, but not significant correlation between  $\beta$ -carotene and Mg in *Lancaster* group ( $R^2 = 0.139$ ), as well as between  $\beta$ -carotene and Mn in *BSSS* group ( $R^2 = 0.133$ ). The only significant and negative correlation was observed between  $\beta$ -carotene and Zn in *Lancaster* group ( $R^2 = 0.183$ ;  $P \leq 0.05$ ). This could denote that availability of Mg and Mn content, particularly from genotypes with higher content of Mg and Mn (from *Lancaster* group), could be restrained by increased PA content. According to the results, positive aspect of increased  $\beta$ -carotene content could be reflected on improved Mn and Zn availability from grain of *BSSS* genotypes and improved Mg from *Lancaster* inbreds.

Figure 3. Principal Component Analysis for phytic P ( $P_{phy}$ ), inorganic P ( $P_i$ ),  $\beta$ -carotene, Mg, Fe, Mn, and Zn content in examined maize inbred lines belong to (A) *BSSS* heterotic group, (B) *Lancaster* heterotic group, and (C) Independent source.



According to PCA, first three axes explained 67.309%, 73.166% and 81.812% of total variability for the variables set observed (for *BSSS*, *Lancaster*, and Independent source, respectively). Projection of the variables indicated that for *BSSS* heterotic group  $P_{phy}$  and Mg contents contributed mainly to PC1 (0.769 and 0.900, respectively; Figure 3A),  $\beta$ -carotene to PC2 (0.886), whereas PC3 was defined with Fe (0.750); for *Lancaster* group,  $P_{phy}$ , Mg, and Mn contributed mainly to PC1 (0.771, 0.806, and 0.875, respectively; Figure 3B), while PC2 and PC3 were determined by the contents of Zn and Fe (0.901 and 0.902, respectively). For the genotypes from Independent source, Fe and Zn contributed mainly to PC1 (0.940 and 0.893, respectively),  $P_i$ , Mg and Mn to PC2 (0.792, 0.749 and 0.799, respectively), whereas PC3 was determined with  $\beta$ -carotene content (0.887; Figure 3C). This could mean that factors which reduce  $P_{phy}$  content in grain induce significant decrease of Mg in grain of inbreds from *BSSS* heterotic group ( $r = 0.583$ ;  $P \leq 0.01$ ), as well as Mg ( $r = 0.513$ ;  $P \leq 0.05$ ) and Mn ( $r = 0.629$ ;  $P \leq 0.01$ ) in *Lancaster* group. Similar trend was observed for  $P_{phy}$  and Mn contents in grain of inbreds from Independent source ( $r = 0.540$ ;  $P \leq 0.05$ ). Only in grain of *Lancaster* heterotic group,  $P_{phy}$  reduction is followed by Zn content increase, while in grain of inbreds from Independent source, variations in Mg, Fe, and Mn contents are independent on  $P_{phy}$  status, indicating that the genotypes from this group (i.e. inbreds with higher Mg, Fe, and Mn status in grain), could serve as favorable source of improved Mg, Fe and Mn absorption (Ma et al., 2007; Queiroz et al., 2011).

## CONCLUSIONS

Evaluated maize inbred lines have different ability to acquire Fe, Mn, and Zn, when grown on calcareous soil with low content of examined mineral elements. The highest Fe and Mn was found in inbred lines belong to *Lancaster* heterotic group and Zn content in lines from *BSSS* group. Generally, increased level of Fe and Mn in *Lancaster* lines could be partially affected by higher PA content in grain, while increased  $\beta$ -carotene content could improve Mn and Zn availability from grain of *BSSS* genotypes and Mg availability from *Lancaster* inbreds. It is important to underline that  $P_{phy}$  reduction is followed by Zn content increase in grain of *Lancaster* heterotic group, as well as that variations in Mg, Fe, and Mn contents are independent on  $P_{phy}$  status in inbreds from Independent source, indicating that the genotypes with higher Mg, Fe, and Mn status from this group could serve as favorable source of improved Mg, Fe, and Mn absorption. Due to the lowest PA/Mn and PA/ $\beta$ -carotene ratios, inbred L10 from *BSSS* and L27 from *Lancaster* heterotic group could be considered as favorable sources for improved Mn bioavailability. Also, inbred L11, genetically related to *BSSS* heterotic group, could be considered as favorable source for higher Zn availability. Among genotypes belong to Independent source, inbred L46 could be considered as a desirable source for higher Fe availability, and maize inbred line L50 for higher Mg availability (due to lower PA content).

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