MAIZE LOCAL LANDRACES AS SOURCES FOR IMPROVED MINERAL ELEMENTS AVAILABILITY FROM GRAIN

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Abstract

The aim of this study was to investigate thirteen maize local landraces from Maize Research Institute (MRIZP) Gene Bank drought tolerant mini-core collection in respect to Fe, Mn and Zn content in grain. In addition, phytate (Phy) and β-carotene contents were determined. According to the results obtained, the highest Fe content was found in grain of LL3, whereas LL1and LL13 were the genotypes with the highest Mn, i.e. Zn content, respectively. However, due to the lowest level of P_{phy}, along with relatively higher level of Fe, Mn and Zn contents in grain, LL2 could be considered as valuable source in further breeding programs for improved mineral nutrient contents, particularly for Fe. Possible availability of investigated mineral elements was determined according to molar ratio between phytate as inhibiting factor and β-carotene as promoting factor for their absorption. Accordingly, genotype LL2, being with the lowest P_{phy} content, and genotype LL3, being with the highest β-carotene content (25.63 μg g^{-1}) and the lowest phytate/β-carotene ratio, could be considered as potential sources of favorable genes for further breeding programs for improved nutritional quality, such as enhanced availability of investigated mineral elements.

***Key words**:* β-carotene, iron, manganese, phytate, zinc, *Zea mays* L.

Introduction

Mineral elements such as phosphorus (P), iron (Fe), manganese (Mn) and zinc (Zn) perform a variety of functions in plant cells, and are essential for growth and development in plants as well as in animals and humans (White and Broadley, 2005; Menkir, 2008). It is estimated that over three billion people are currently malnourished because of lack of minerals, especially iron and zinc, in their diet (Welch and Graham, 2004). Malnutrition, resulting from deficiency of mineral nutrients

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in food, could cause serious health problems. The highest importance was given to Fe and Zn in vegetarian diets, since elimination of meat and increased intake of legumes and whole grains rich in phytate, decreases the Fe and Zn absorption (Hunt, 2003). This could lead to anemia (Fe deficiency), as well as to problems with immune system or abnormal blood (Zn deficiency). Manganese is also essential for humans; it is a part of the antioxidant system in mitochondria, and is involved in metabolism, bone development and wound healing (Underwood and Suttle, 1999).

The accumulation of minerals in seeds is a complex phenomenon, which is most likely controlled by a number of genes. 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 (White and Broadley, 2009).

Not all mineral elements in plant foods are bio-available to humans and animals. Plant foods can contain inhibitors - anti-nutrients (e.g., phytate, polyphenolics, etc.), which obstruct the absorption or utilization of these nutrients. Moreover, there are enhancing substances - promoters (e.g., ascorbic acid, β-carotene, S-containing amino acids, etc.) that promote bioavailability of micronutrient or decrease the effects of anti-nutrient substances (Luo and Xie, 2012). Thus, it is essential to decrease the content of various anti-nutrients in foods and to increase the content of promoters (Graham et al., 2007).

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).

Local landraces are considered to be the most significant genotypes, since they represent the original biological material developed by the process of natural selection and adapted to local growing conditions (Camacho Villa et al., 2005). Since exploring natural biodiversity, as a source of novel alleles to improve the productivity, adaptation, quality and nutritional value of crops is of prime importance in 21st century breeding programs (Ortiz-Monasterio et al., 2007), the aims of this study were (i) to evaluate chemical composition of grain in investigated maize local landraces and (ii) to determine relations between phytic acid, inorganic phosphorus and β-carotene, as factors affecting the absorption of important mineral elements, i.e. Fe, Mn and Zn.

Material and methods

Plant material - After two-year of screening for drought tolerance in Egypt under managed stress environment (MSE) conditions, a core collection of 9.8 % accessions from Maize Research Institute Zemun Polje (MRIZP) gene bank was created and further tested in temperate climate conditions (Serbia and Macedonia). Based on field trials´ results and general combining ability, a drought tolerant mini-core collection of 41 accessions (15 inbred lines, 13 local and 13 introduced landraces) was established (Babic et al., 2011). A set of thirteen maize local landraces was the objective of the present study. Country of origin and kernels characteristics of investigated genotypes were given in Table 1.

Table 1. Country of origin and kernels characteristics of investigated maize local landraces
Tabela 1. Zemlja porekla i karakteristike zrna ispitivanih domaćih sorti kukuruza

Genotype / Genotip	Country of origin / Zemlja porekla*	Kernel type / Tip zrna	Kernel colour / Boja zrna
LL1	SRB	Semiflints/Polutvrdunac	White / Bela
LL2	BIH	Dent / Zuban	Yellow / Žuta
LL3	BIH	Flint / Tvrdunac	Orange / Narandžasta
LL4	SRB	Semiflints/Polutvrdunac	White / Bela
LL5	SLO	Semident / Poluzuban	Yellow / Žuta
LL6	BIH	Semiflints/Polutvrdunac	Yellow / Žuta
LL7	SRB	Semiflints/Polutvrdunac	Orange / Narandžasta
LL8	SRB	Dent / Zuban	White / Bela
LL9	BIH	Semiflints/Polutvrdunac	Yellow / Žuta
LL10	MKD	Dent / Zuban	White / Bela
LL11	BIH	Flint / Tvrdunac	Yellow / Žuta
LL12	BIH	Semiflints/Polutvrdunac	Yellow / Žuta
LL13	CRO	Flint / Tvrdunac	Yellow / Žuta

* *Abbreviation for country of origin: SRB - Serbia; BIH - Bosnia-Herzegovina; SLO - Slovenia; CRO - Croatia; MKD - Macedonia*
Skraćenica za zemlju porekla: SRB - Srbija; BIH - Bosna i Hercegovina; SLO - Slovenija; CRO - Hrvatska; MKD - Makedonija

Field trial and laboratory experiments - For this study, the experiment was carried out in 2011, at Zemun Polje, Serbia (44˚52´N, 20˚19´E, 81 m asl). The soil was slightly calcareous chernozem (with low content of investigated mineral elements). A randomized block design with two replications was used in the experiment. The plants were harvested manually and after drying to 14% of moisture content, grain was used for chemical analyses.

Chemical composition of grain was determined colorimetrically. Phytic (P_{phy}) and inorganic phosphorus (P_i) were determined by the method of Dragičević et al. (2011). β-carotene was determined according to American Association of Cereal Chemists Method (1995). Mineral elements (Fe, Mn and Zn) were determined after wet digestion with HNO_3 + $HClO_4$, by Inductively Coupled Plasma - Optical Emission Spectrometry (Spectro Analytical Instruments, Germany).

Statistical analysis - All analyses were performed in four replicates (n = 4) and the results were statistically analyzed and presented as mean ± standard deviation (SD). Coefficient of variation (CV) for each chemical parameter was determined. Significant differences between genotypes means were determined by the Fisher's least significant difference (LSD) test at the 0.05 probability level, after the analysis of variance (ANOVA) using one-factorial completely randomized block design. Differences between means values with P values ≤ 0.05 were considered significant.

Results and discussion

Many of the problems associated with

phosphorus (P) in maize grain are not due to the concentration of total P *per se*, but rather to the fact that most of the P is bound in phytate (Raboy, 2001). Therefore, it would be desirable to increase the amount of available P and reduce the amount of phytate in maize grain.

Although low phytate maize offers major environmental and nutritional benefits, phytates are considered as important to non-germinating seeds in protecting and maintaining the integrity of mineral elements until needed for germination (Raboy, 1997). In seeds, it is the primary storage form of P that is utilized during germination and early seedling establishment. The P released from phytate (K and Mg salt of phytate) during germination is very important to early seedling growth.

In this study, genetic variability in phytic P (P_{phy}) content was observed, with values ranging from 3.5476 (LL2) to 3.9006 mg g^{-1} (LL13), with an averaged being 3.8060 mg g^{-1}, as presented in Table 2.

Inorganic P (P_i) is important form of phosphorus in grains, although it is present in

Table 2. Chemical composition of grain for investigated maize local landraces
Table 2. Hemijski sastav zrna ispitivanih domaćih sorti kukuruza

Genotype/ Genotip	P_{phy} (mg g^{-1})	P_i	β-carotene (µg g^{-1})	Fe (mg g^{-1})	Mn	Zn
LL1	3.8888 ^{ab}	0.2847 ^{l}	3.1115 ^{ef}	10.7813 ^{b}	2.8750 ^{a}	15.4063^{bc}
LL2	3.5476 ^{cd}	0.3425 ^{k}	10.3866 ^{d}	12.0000 ^{a}	2.6250 ^{cd}	15.0313^{c}
LL3	3.8770 ^{ab}	0.3723 ^{h}	25.6283 ^{a}	12.4375 ^{a}	2.3438 ^{gh}	11.6875^{def}
LL4	3.8810 ^{ab}	0.3615 ^{i}	10.0259 ^{d}	10.0625 ^{cd}	2.2813 ^{hi}	14.0313^{cd}
LL5	3.7241 ^{bc}	0.3615 ^{i}	15.2868 ^{c}	11.8438 ^{a}	2.5625 ^{de}	14.1250^{cd}
LL6	3.9163 ^{a}	0.4166 ^{e}	20.8784 ^{b}	11.7500 ^{a}	2.2188 ^{i}	15.2188^{bc}
LL7	3.9045 ^{ab}	0.4796 ^{a}	19.4204 ^{b}	10.7188 ^{bc}	2.7500 ^{b}	10.4063^{ef}
LL8	3.8378 ^{ab}	0.4553 ^{c}	1.9090 ^{f}	12.1250 ^{a}	2.6250 ^{cd}	10.1563^{f}
LL9	3.8182 ^{ab}	0.4630 ^{b}	15.9331 ^{c}	11.9688 ^{a}	2.5000 ^{ef}	17.8438^{ab}
LL10	3.5320 ^{d}	0.3913 ^{f}	4.0735 ^{e}	9.9063 ^{d}	2.2813 ^{hi}	12.7813^{cdef}
LL11	3.7829 ^{ab}	0.3878 ^{g}	19.9315 ^{b}	11.7500 ^{a}	2.4063 ^{fg}	13.0000^{cde}
LL12	3.8692 ^{ab}	0.4411 ^{d}	15.8730 ^{c}	10.8438 ^{b}	2.6875 ^{bc}	12.0938^{def}
LL13	3.9006 ^{ab}	0.3449 ^{j}	16.1135 ^{c}	10.9688 ^{b}	2.5313 ^{de}	20.5313^{a}
X	3.8060	0.3925	13.7363	11.3197	2.5144	14.0240
SD	0.11	0.01	0.72	0.59	0.10	1.92
CV (%)	3.38	2.00	8.19	4.28	3.37	13.65
$LSD_{0.05}$	0.1870	0.0014	1.612	0.6925	0.1200	2.746

The results are represented as mean of four replicates. Means followed by the same letter within the same column are not significantly different (P < 0.05). P_{phy} - phytic phosphorus; P_i - inorganic phosphorus; SD - standard deviation; CV - coefficient of variation; LSD - least significant difference.

Rezultati predstavljaju prosečne vrednosti dobijene iz četiri ponavljanja. Vrednosti praćene istim slovima unutar jedne kolone nisu statistički različite (P < 0.05). P_{phy} - fitinski fosfor; P_i - neorganski fosfor; SD - standardna devijacija; CV - koeficijent varijacije; $LSD_{0.05}$ - najmanje značajna razlika, P < 0.05.

relatively low concentrations and thus constitutes a small fraction of the total P of grains. High levels of inorganic P are regarded as desirable from a nutritional standpoint. P_i concentration was in a range from 0.2847 (LL1) to 0.4796 mg g^{-1} (LL7), with an average of 0.3925 mg g^{-1}. Both P_{phy} and P_i values were within the range of previously reported values for wild-type maize hybrids (Raboy et al., 2000) and S1 populations (Lorenz et al., 2008). In compare to P_{phy}, a higher genetic variation was observed for level of P_i content. The results obtained are in line with Lorenz et al (2007), who observed a higher amount of genetic variation for P_i than for P_{phy} and phytate, respectively, both in landraces and inbred lines.

β-carotene positively affects absorption of mineral nutrients (Lönnerdal, 2003). High variations in level of β-carotene were observed among the investigated drought tolerant maize local landraces, which was in consistance to Safawo et al. (2010). In this study, β-carotene content ranged from 1.9090 μg g^{-1} (LL10) to 25.6283 μg g^{-1} (LL3), respectively. The majority of the landraces revealed β-carotene content > 14 μg kg^{-1} and $P_{phy} \leq 3.9$ mg g^{-1}. This could be important, since the increased β-carotene concentration in maize grain enables better absorption of mineral nutrients by forming a complex with Fe that keeps it soluble in the intestinal lumen and prevents the deleterious effect of phytic acid on Fe assimilation (Walter Lopez et al., 2002). Moreover, the highest β-carotene content in three genotypes (LL3, LL6 and LL11) could also have a positive effect on mineral nutrients absorption, possibly by decreasing negative effect of phytic acid (Lönnerdal, 2003; Luo and Xie, 2012). This could be facilitated by exibited the lowest P_{phy}/β-carotene ratio (i.e., < 550) in those three local landraces, as presented in Figure 1.

The highest values for Fe content (> 12 mg g^{-1}) were obtained in grain of LL2, LL3,

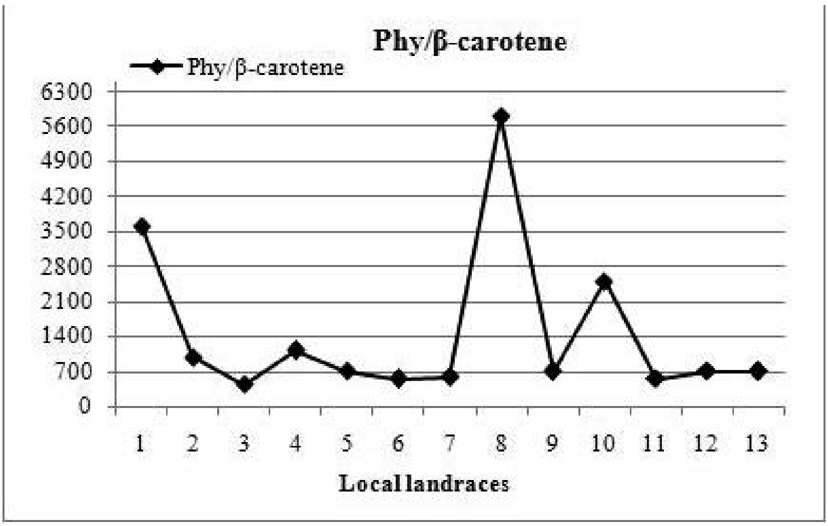

Figure 1. Molar ratio between phytic acid and β-carotene for investigated maize local landraces
Grafik 1. Molarni odnos između fitinske kiseline i β-karotena kod ispitivanih domaćih sorti kukuruza

LL8 and LL9 local landraces, respectively, as presented in Table 2. Also, these genotypes exhibited the lowest ratio between phytic acid (Phy) and Fe (< 95.2; Figure 2) indicating higher potential for Fe availability. However, Fe contents higher than those reported by Queiroz et al. (2011), indicated to relatively higher content of phytic acid in investigated local landraces. In addition, LL8 and LL9 were with the highest P_i concentration (Table 2), thus could be considered as potentially favourable sources of P and Fe in further breeding programs for improved mineral elements availability. Since β-carotene enables better Fe absorption from food (Lönnerdal, 2003; Luo and Xie, 2012; Walter Lopez et al., 2002), higher contents of both components, particularly presented in grain of LL2, LL3 and LL9 (Table 2), provide certainly increased nutritional quality of such food.

Variations of Mn concentration in grain ranged from 2.2188 mg g^{-1} to 2.8750 mg g^{-1}, with the highest values (≥ 2.5 mg g^{-1}) found in grain of LL1, LL2, LL5, LL7, LL8, LL9, LL12 and LL13 (Table 2). Ratio between phytic acid and Mn could be concidered as favourable (< 400) only in grain of LL1 and LL2 (Figure 2). However, due to relatively higher β-carotene content and the lowest Phy/Mn ratio observed, drought tolerant LL2 could be considered as valuable source in breeding for improved Mn assimilation. Underwood and Suttle (1999) also obtained that phytate breakage in rumen increases Mn assimilation in ruminants.

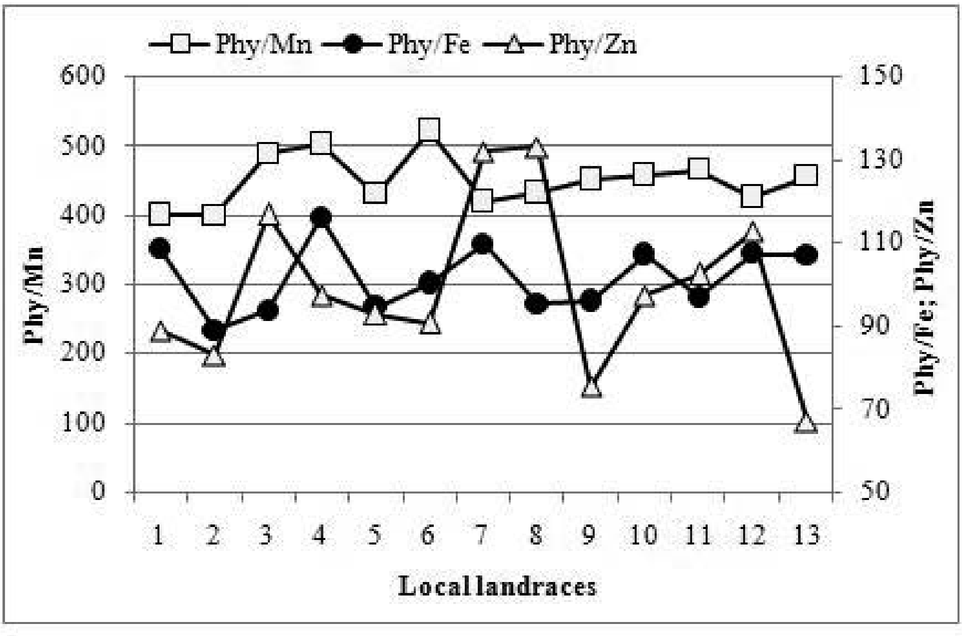

Figure 2. Molar ratios between phytic acid (Phy) and mineral elements for investigated maize local landraces
Grafik 2. Molarni odnosi između fitinske kiseline (Phy) i mineralnih elemenata kod ispitivanih domaćih sorti kukuruza

Zn concentration in grain of investigated maize landraces ranged from 10.1563 mg g^{-1} to 20.5313 mg g^{-1}. The highest values for Zn content (≥ 15 mg g^{-1}, Table 2), along with the lower Phy/Zn ratio (≤ 90, Figure 2), observed in grain of LL1, LL2, LL6, LL9 and LL13, could characterized them as potential sources of available Zn, which is in line with the results of Queiroz et al. (2011). High concentrations of both, β-carotene and Zn, are desirable characteristics (Luo and Xie, 2012). Moreover, Zn absorption highly depends upon β-carotene content in food (Oikeh et al., 2003). Accordingly, high Zn and β-carotene contents found in grain of LL2, LL6, LL9 and LL13 (Table 2), indicated that those genotypes could be considered as potential sources in further breeding programs for improved Zn availability.

Conclusion

To fully and effective utilize the genetic variability of local maize germplasm for enrichment of maize seeds with bioavailable mineral elements, it is necessary to study and evaluate the variations for mineral traits of maize germplasm from different origins and to identify genotypes from which elite inbred lines with high mineral elements content could be created. Based on results obtained, it could be concluded that investigated drought tolerant maize local landraces exhibited high variability in concentration of important mineral elements (i.e., Fe, Mn and Zn), phytic acid (as suppressor of mineral elements availability) and β-carotene (which enables better absorption of mineral element and minimize negative effect of phytic acid). Due to the highest β-carotene and Fe contents, as well the lower Phy/Fe ratio, LL3 could be considered as favourable source for improved ability of Fe absorption. Genotype LL9, being with relatively high Pi, Fe and Zn contents, and favourable Pphy/Pi ratio, could be considered as a good source of P, Fe and Zn. According to relatively high concentrations of all four factors observed (β-carotene, Fe, Mn and Zn), as well as the lowest ratios between Phy and all examined mineral elements, drought tolerant maize local landrace LL2 is the most promising genotype in further breeding programs for improved nutritional quality, such as enhanced availability of investigated mineral elements.

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LOKALNE SORTE KUKURUZA KAO IZVORI POBOLJŠANE DOSTUPNOSTI MINERALNIH ELEMENATA IZ ZRNA

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Sažetak

Cilj ovog istraživanja je bilo ispitivanje trinaest domaćih sorti kukuruza iz *mini-core* kolekcije za tolerantnost prema suši banke gena Instituta za kukuruz Zemun Polje (MRIZP), u pogledu sadržaja gvožđa (Fe), mangana (Mn) i cinka (Zn) u zrnu. Pored toga, određen je i sadržaj fitina (Phy) i β-karotena. Na osnovu dobijenih rezultata, najveći sadržaj gvožđa u zrnu imala je sorta LL3, dok su LL1 i LL13 bili genotipovi sa najvećim sadržajem mangana i cinka. Međutim, na osnovu utvrđenog najnižeg sadržaja fitinskog fosfora (P_{phy}), kao i relativno visokog sadržaja Fe, Mn and Zn, sorta LL2 bi se mogla smatrati izvorom poželjih gena za buduće programe oplemenjivajna na povećan sadržaj mineralnih elemenata, naročito gvožđa. Moguća dostupnost ispitivanih mineralnih elemenata je određena na osnovu molarnih odnosa između fitina kao inhibitornog faktora i β-karotena kao promoterskog faktora za njihovu apsorpciju. Shodno tome, sorta LL2, kao genotip sa najnižim sadržajem fitinskog fosfora (P_{phi}), i sorta LL3, kao genotip sa najvišim sadržajem β-karotena (25.63 µg g^{-1}) i najnižom vrednošću za odnos fitina i β-karotena, mogu se smatrati izvorima poželjih gena za buduće programe oplemenjivajna na poboljšani nutritivni kvalitet zrna, kao što je povećana dostupnost ispitivanih mineralnih elemenata.

Ključne reči: β-karoten, cink, fitin, gvožđe, mangan, *Zea mays* L.

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