VARIATIONS OF PHYTIC AND INORGANIC PHOSPHORUS IN MAIZE GRAIN

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Summary

Maize is characterised with high diversity, which is at the same time maintained in present gene banks of plants. From that point, the application of the present ability of maize genetic variability could be used for creating of the genotypes with changed chemical composition of the grain. The aim of experiment was to examine the influence of different factors on variability of inorganic and phytic phosphorus in grain of nine ZP hybrids and their parental components. The experiment was performed during 2010 and 2011 on two locations of the Maize Research Institute.

The higher values of phytic and inorganic P were mainly present at lines in 2010, as well as at ZP 341 and ZP 434 hybrids, as well as at their parental components. Among all examined hybrids, the significant differences in phytic P, influenced by season were present at ZP 434 and ZP 555, as well as mother component of ZP 555. The both parental components show significant and positive imact on inorganic P increase in hybrid grain. The eligible increase of available P, with decrease of phytic P, could be effective obtained by using of the genetic resources in pre-breeding process4e, with recurent selection.

Key words*:* phytate, inorganic phosphorus, maize grain, variability

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Introduction

The maize grain has whole range of applications, such as feed, food or raw materials for many industrial and commercial products. Ability to use existing genetic variation could provide the basis for development of the next generation of specialty maize and new products from it (Balconi et al., 2007). From that point of view special attention was given to breeding programs aimed at lowering the phytate content in grains of different crops (Cichy and Raboy, 2009; Raboy, 2009).

Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakisphosphate) contains 75-80% of maize grain's total P (Raboy *et al.,* 2000). Primary function of phytate is P storage, together with maintaining integrity of mineral elements important for germination (Raboy *et al*, 1990). Phytate is rapidly utilizing during germination and early seedling development. Released inorganic P is present at high concentration in cell's nucleus, having a high metabolic activity, such as in maize embryo root cells (Deltour *et al*, 1981). Contrary to plants, that can readily decompose phytate by enzyme phytase, phytate cannot be efficiently used by monogastric animals and humans. It is excreted and pollutes the environment. According to Leytem *et al.* (2008), total P in excret was lowered when feeding poultry with low-phytate maize. Moreover, phytate is an efficient chelator of nutritionally important mineral cations and forms stable salts with Ca, Fe and Zn in the digestive tract (Raboy, 2009). From this perspective, phytate is considered as anti-nutrient, thus giving the advantage to lowering of phytate content in grains. According to Raboy *et al*. (2000), phytate metabolism during seed development is not solely responsible for P homeostasis. Lowering the phytic P in low-phytate mutants could be achieved by increase of inorganic P (P_i), so the sum of the total P in seed remains constant. Other important task of phytate is its antioxidant function. Therefore, a novel role in plant seed physiology can be assigned to phytate in protection against oxidative stress during the seed's life span. (Doria *et al*., 2009).

On the other hand, lowering phytate content in seeds has many negative effects, ie reduced plant growth, development and yield. Targeted engineering for lowering phytate content might avoid some or all of these negative impacts (Raboy, 2009). Disturbed inositol phosphate metabolism in four barley low-phytate mutants, which is connected to reduced crop yield (Bregitzer and Raboy, 2006), is much more dramatic in non-irrigated (stressful) *versus* irrigated (less stressful) environments. It is likely that this reduced stress tolerance occurs due to disturbed inositol phosphate metabolism in vegetative tissues. On the other hand, phytate content in maize grain haven't got necessary impact on variations in phytate content in maize leaves (Dragicevic *et al.*, 2010a), while protective characteristics against stress were underlined (Dragicevic *et al*., 2010b). Alterations of phytate content in seeds of different species was significant under the environmental influence (Kumar *et al*., 2005; Khan *et al*., 2007).

The objective of this study was to investigate variability in content of phytic (P_{phy}) and inorganic phosphorus (P_i) in some ZP hybrids, and their parental components.

Material and Methods

The trial was set up at two locations, in Sremska Mitrovica (44° 58' N, 19° 36' E) and Zemun Polje (44° 52' N 20° 20' E), on a slightly calcareous chernozem soil type, during the summer of 2010 and 2011 in rain-fed conditions. Higher average temperature with almost double lower sum of precipitation present during 2011 (Table 2) indicate dryer conditions, particularly in Jun (with 6 times lower sum of precipitation). Generally, July was characterised as a month with the highest average temperature and lowest precipitations, what could affect grain filling and imply yield losses.

Table 1. Average monthly air temperatures and monthly sums of precipitation for period April-September in 2010. and 2011.
Tabela 1. Prosečne mesečne temperature i sume padavina za period April-Septembar u 2010 i 2011.

	Temperature (°C)		Precipitation (mm)	
	2010	2011	2010	2011
April	13,2	13,4	44,0	14,9
May	17,5	16,8	64,1	89,6
Jun	21,0	21,5	167,3	26,2
July	23,2	23,3	35,6	44,0
August	23,1	23,9	68,2	66,0
September	17,6	21,6	68,0	32,6
Average (°C) /Sum (mm)	19,3	20,1	447,2	273,3

Sets of nine hybrids (FAO maturity groups 300-600) and their parental inbreds were used (Table 1). From the analysed inbreds, PL1 and PL2 were from independent source germplasm; PL3, PL4 and PL7 were from BSSS heterotic group, PL5, PL6 and PL8 were from Lancaster heterotic group. The experiment was conducted by RBCD design with four replications: main plots encompassed 4 rows of each genotype (each 6 m long), with distance between rows 0.75 m and plants in one row 0.24 m apart.

Table 2. The maize hybrids used in experiment and their parental inbreds
Tabela 2. Hibridi kukuruza i njihove roditeljske komponenete koji su korišćeni u ogledu

Hybrid	Female component (♀)	Male component (♂)
ZP 341	PL1	PL5
ZP 427	PL6 x PL8	PL2
ZP 434	PL1	PL6
ZP 555	PL3	PL5
ZP 560	PL7	PL6
ZP 600	PL7	PL8
ZP 606	PL3	PL8
ZP 666	PL3	PL6
ZP 684	PL8	PL4

The grain yield, 1000 grains weight, as well as contents of phytic (Pphy) and inorganic (Pi) phosphorus were analyzed in maize grain. Grain yield was measured at the end of a growing cycle and calculated with 14% of moisture.

Phytic and inorganic phosphorus were determined colorimetric by the method of Dragičević et al. (2011). Meal sample (0.25 g) for Pphy and Pi determination was treated with 5% TCA (trichloroacetic acid) for 1 h, at room temperature in a rotary shaker. The extract was centrifuged on 12,000 rpm for 15 min and the supernatant was decanted and diluted with double distilled H_2O (ratio 1:5). After adding the Wade reagent (for Pphy determination) or ammonium heptamolybdate + ammonium metavanadate (for Pi determination), the absorbance was read (λ = 500 nm for Pphy and λ = 400 nm for Pi).

The experimental data were statistically processed by analysis of the variance (ANOVA) with differences of means at the 0.05 probability level (LSD 0.05), together with coefficients of variation. Pearson's correlation between examined maize hybrids and their parental inbreds was calculated.

Beside the correlation, interdependence between hybrids and their parental inbreds were also presented through regression coefficients, calculated based on average values (for aboth years and locations) for each genotype.

Results and Discussion

The influences of year and location on grain yield (Table 3) and 1000 grains weight (Table 4) were insignificant, despite of lower average grain yield and lower variations in 2011, owing to unfavourable meteorological conditions. The exception was significantly higher yield in Zemun Polje than in Sremska Mitrovica. As it was expected, the significant differences were obtained between genotypes, where the majority of inbreds had significantly lower 1000 grains weight and yield in relation to hybrids. According to De Souza et al. (2009) the response of genotype to environment is complex trait, since the germplasm variability was underscored in presence of moderate stress. From that point, greater variability was observed between genotypes in this research, excluding the locations and years.

Table 3. Average grain yield (t ha^{-1}) of examined maize hybrids and their parental inbreds in 2010 and 2011 in Sremska Mitrovica (SM) and Zemun Polje (ZP)
Tabela 3. Prosečni prinos zrna (t ha^{-1}) ispitivanih hibrida kukuruza i nihovih roditeljskih linija u 2010 i 2011, kao i u Sremskoj Mitrovici (SM) i Zemun Polju (ZP)

Genotype Genotip	Year Godina		Location Lokacija		Average Prosek
	2010	2011	SM	ZP	
PL1	6,00	4,04	5,38	4,66	5,02
PL2	5,70	3,37	4,43	4,63	4,53
PL3	7,36	5,25	5,69	6,92	6,30
PL4	5,66	3,47	5,09	4,05	4,57
PL5	4,58	3,79	4,71	3,66	4,18
PL6	4,07	3,49	4,12	3,45	3,78
PL7	8,33	5,68	6,71	7,30	7,01
PL8	7,35	4,56	6,58	5,33	5,96
ZP 341	11,45	11,11	11,85	10,71	11,28
ZP 427	10,36	10,91	10,85	10,42	10,64
ZP 434	10,09	10,90	11,82	9,17	10,50
ZP 555	12,06	11,05	11,79	11,31	11,55
ZP 560	12,19	11,39	11,83	11,76	11,79
ZP 600	13,01	12,12	12,62	12,50	12,56
ZP 606	11,70	11,82	12,37	11,15	11,76
ZP 666	12,62	10,98	11,76	11,83	11,80
ZP 684	12,60	11,61	12,80	11,41	12,11
Average Prosek	9,13	7,97	8,85	8,25	8,55
LSD 0,05	Year	Gen.	Loc.	Y x G	L x G
	3,51	1,34	3,54	1,14	1,32

Opposite to insignificant influence of location or year, interactions between genotype and environment were underlined also by Čvarković et al. (2009). Interaction of year and genotype highlighted unfavourable conditions for grain filling in 2011, with significantly lower yield of inbreds during the same year (Table 3), compared to 2010. According to Tollenaar and Lee (2002), genetic improvement in maize yield and its components is associated neither with yield potential per se, nor with heterosis per se, but with increased stress tolerance, which is consistent with the improvement in the genotype × management interaction, also underlined in this research. Significant and the highest difference in 1000 grains weight (Table 4) between these two years was noticed for hybrids ZP 427 and ZP 600 and their parental components (PL2 and PL7). For grain yield, interaction of location and genotype was significant only for PL2, PL3, PL4, PL5 and PL6 (with highest difference obtained for PL5), while for 1000 grains weight it was significant for PL1, PL4, PL7 and PL8 (with highest difference obtained for PL7, as a female component of ZP 560 and ZP 600).

Table 4. Average 1000 grains weight (g) of maize hybrids and their parental inbreds in 2010 and 2011 in Sremska Mitrovica (SM) and Zemun Polje (ZP)
Tabela 4. Prosečna masa 1000 zrna (g) hibrida kukuruza i nihovih roditeljskih linija u 2010 i 2011, u Sremskoj Mitrovici (SM) i Zemun Polju (ZP)

Genotype Genotip	Year Godina		Location Lokacija		Average Prosek
	2010	2011	SM	ZP	
PL1	334,7	308,3	320,1	322,9	321,5
PL2	344,4	303,4	326,8	321,0	323,9
PL3	347,1	344,2	347,6	343,8	345,7
PL4	284,6	277,8	284,2	278,2	281,2
PL5	253,0	267,1	251,0	269,1	260,0
PL6	281,7	300,7	281,5	300,8	291,2
PL7	320,9	341,1	334,5	327,5	331,0
PL8	326,7	349,0	342,4	333,3	337,9
ZP 341	361,7	348,3	360,6	349,5	355,0
ZP 427	428,0	390,4	431,4	387,1	409,2
ZP 434	384,8	353,4	366,3	371,9	369,1
ZP 555	363,4	351,5	364,2	350,7	357,4
ZP 560	397,9	336,3	357,5	376,6	367,1
ZP 600	432,4	353,0	388,2	397,2	392,7
ZP 606	439,3	353,0	389,2	403,0	396,1
ZP 666	364,9	352,7	354,7	362,9	358,8
ZP 684	368,5	319,3	339,4	348,4	343,9
Average Prosek	354,9	332,3	343,5	343,8	343,6
LSD 0,05	Year	Gen.	Loc.	Y x G	L x G
	47,10	31,76	48,79	23,06	32,62

Similarly to results of yield and 1000 grains weight, variations in average content of Pphy and Pi were insignificantly affected by year and location (Tables 5 and 6). The only significant differences were obtained between genotypes, with higher average values of both parameters noticed at inbreds, compared to hybrids. Higher variations in Pphy were obtained on hybrid level, while the variations in Pi were obtained on inbred level. Significant and the highest average values of Pphy were noticed at PL1, PL2, PL3, PL4, PL7 and PL8 (Table 5). Significant and the highest average values of Pi were noticed at PL1, PL2 and PL5 (Table 6), parental components of hybrids ZP 341 and ZP 434, with also significantly higher Pi content among all hybrids. Obtained results differ to Raboy (2009); and Dragičević et al. (2010), who detected increased content of Pi in genotypes with lower Pphy contents.

Table 6. Average content of inorganic P (P_i - g kg^{-1}) in grain of maize hybrids and their parental inbreds in 2010 and 2011 in Sremska Mitrovica (SM) and Zemun Polje (ZP)
Tabela 6. Prosečan sadržaj neorganskog P (P_i - g kg^{-1}) u zrnu ispitivanih hibrida kukuruza i nihovih roditeljskih linija u 2010 i 2011, kao i u Sremskoj Mitrovici (SM) i Zemun Polju (ZP)

Genotype Genotip	Year Godina		Location Lokacija		Average Prosek
	2010	2011	SM	ZP	
PL1	2,16	2,27	2,18	2,25	2,22
PL2	2,57	1,62	2,09	2,10	2,10
PL3	1,66	1,37	1,37	1,66	1,51
PL4	1,16	1,35	1,18	1,32	1,25
PL5	1,95	2,11	1,94	2,11	2,03
PL6	1,46	1,70	1,58	1,58	1,58
PL7	1,87	1,38	1,46	1,79	1,63
PL8	1,39	1,64	1,53[a]	1,49	1,51
ZP 341	1,57	1,74	1,87	1,45	1,66
ZP 427	1,53	1,76	1,75	1,44	1,64
ZP 434	1,71	1,69	1,75	1,53	1,70
ZP 555	1,06	1,99	1,48	1,65	1,53
ZP 560	1,49	1,25	1,22	1,58	1,37
ZP 600	1,24	1,52	1,32	1,52	1,38
ZP 606	1,16	1,91	1,50	1,44	1,53
ZP 666	1,22	1,62	1,38	1,57	1,42
ZP 684	1,25	1,45	1,45	1,46	1,35
Average Prosek	1,56	1,67	1,59	1,64	
LSD 0,05	Year	Year x Genot.	Location	Locat. xGenot.	Genotype
	0,34	0,31	0,36	0,27	0,25

Significant alterations in Pphy and Pi content were obtained by interaction of location and genotype and in higher extent by interaction of year and genotype. This could indicate that variability in Pphy and Pi was highly influenced by meteorological factors. Higher values of both, Pphy and Pi, were mainly obtained at the level of inbreds in 2010 and on the level of hybrids in 2011 (Tables 5 and 6). Such situation could be connected to better tolerance of hybrids to poorer conditions in 2011 (with higher average temperature and lower level of precipitation).

Among examined genotypes, significant and the highest difference in P_{phy}, influenced by season, was obtained in grain of PL3, ZP 434 and ZP 555 (over 0.66 g kg^{-1}; Table 5). The interaction between location and genotype emphasized PL2, PL3, PL7, ZP 341 and ZP 555 as genotypes with significant difference in P_{phy} content (over 0.35 g kg^{-1}) between examined locations. Similarly, Kumar *et al.* (2005) and Khan *et al.* (2007) underlined significant effect of interaction between location and genotypes on variations in P_{phy} content soybean and wheat. The significant and the highest difference between examined years in P_i content was recorded at L2 (0.95 g kg^{-1}; Table 6), as well as at ZP 555 and ZP 606 (over 0.75 g kg^{-1}). Moreover, significant and the highest difference between tested locations in P_i content in seeds was noticed at PL7, ZP 341 and ZP 560 (over 0.32 g kg^{-1}). Among these genotypes, ZP 560 and its female component, PL7 achieved higher P_i content in Zemun Polje and lower coefficient of variation.

Table 6. Average content of inorganic P (P_i - g kg^{-1}) in grain of maize hybrids and their parental inbreds in 2010 and 2011 in Sremska Mitrovica (SM) and Zemun Polje (ZP)
Tabela 6. Prosečan sadržaj neorganskog P (P_i - g kg^{-1}) u zrnu ispitivanih hibrida kukuruza i nihovih roditeljskih linija u 2010 i 2011, kao i u Sremskoj Mitrovici (SM) i Zemun Polju (ZP)

Genotype Genotip	Year Godina		Location Lokacija		Average Prosek
	2010	2011	SM	ZP	
PL1	2,16	2,27	2,18	2,25	2,22
PL2	2,57	1,62	2,09	2,10	2,10
PL3	1,66	1,37	1,37	1,66	1,51
PL4	1,16	1,35	1,18	1,32	1,25
PL5	1,95	2,11	1,94	2,11	2,03
PL6	1,46	1,70	1,58	1,58	1,58
PL7	1,87	1,38	1,46	1,79	1,63
PL8	1,39	1,64	1,53[a]	1,49	1,51
ZP 341	1,57	1,74	1,87	1,45	1,66
ZP 427	1,53	1,76	1,75	1,44	1,64
ZP 434	1,71	1,69	1,75	1,53	1,70
ZP 555	1,06	1,99	1,48	1,65	1,53
ZP 560	1,49	1,25	1,22	1,58	1,37
ZP 600	1,24	1,52	1,32	1,52	1,38
ZP 606	1,16	1,91	1,50	1,44	1,53
ZP 666	1,22	1,62	1,38	1,57	1,42
ZP 684	1,25	1,45	1,45	1,46	1,35
Average Prosek	1,56	1,67	1,59	1,64	
LSD 0,05	Year	Year x Genot.	Location	Locat. xGenot.	Genotype
	0,34	0,31	0,36	0,27	0,25

Correlation between hybrids and their parental inbreds (Table 7) for grain yield was significant and negative for ZP 600 and its female components but significant and positive for ZP 434, ZP 555, ZP 560 and their male components. Generally, regression coefficient indicates significant influence of male component for hybrids (R^2=-0.113), irrespective to higher values of yield obtained by female component (Table 4). Higher influence of parental inbreds on hybrid's 1000 grains weight was noticed: it was significant for ZP 434, ZP 600, ZP 606, ZP 666, ZP 684 and their female components, as well as for ZP 427, ZP 600, ZP 666, ZP 684 and their male components. Regression coefficient also underlined significant and positive impact of male component.

Table 7. Correlation and regression coefficients for interdependence between maize hybrids and their parental inbreds (♀ - femalecomponents; ♂ - male components) for 1000 grains weight, grain yield, content of phytic and inorganic P - Pearson's correlation coefficients

Tabela 7. Korelacija i regresioni koeficijenti zavisnosti između hibrida i njihovih roditeljskih linija (♀ - komponenta majke; ♂ - komponenta oca) za masu 1000 semena, prinos zrna, sadržaj fitinskog i neorganskog P – Pirsonovi koeficijenti korelacije

Hybrid	Grain yield		1000 grains weight		Phytic P		Inorganic P	
	F1 x ♀	F1 x ♂	F1 x ♀	F1 x ♂	F1 x ♀	F1 x ♂	F1 x ♀	F1 x ♂
ZP 341	0,29	0,20	0,19	-0,25	-0,41*	-0,43*	0,27	0,32
ZP 427	-0,10	-0,18	0,08	0,55*	-0,14	-0,19	0,12	-0,40*
ZP 434	0,29	0,49*	0,80*	0,02	-0,30	-0,15	0,71*	-0,16
ZP 555	0,22	0,57*	0,04	0,25	-0,02	-0,57*	-0,03	-0,13
ZP 560	0,10	0,43*	0,10	-0,25	-0,28	-0,33	0,61*	0,34
ZP 600	-0,42*	-0,31	0,95*	-0,74*	-0,32	-0,17	-0,43*	0,59*
ZP 606	-0,02	0,06	-0,92*	-0,12	0,59*	-0,32	-0,55*	0,09
ZP 666	0,13	0,36	0,86*	-0,96*	-0,82*	-0,24	-0,58*	0,53*
ZP 684	0,19	0,32	-0,95*	-0,85*	0,26	-0,42*	-0,23	-0,27
Regression coefficients (R^2)	-0,005	-0,113	0,025	0,266	0,267	0,084	0,442	0,412

*Significant difference at the 0.05 level

Inversely proportional trend of correlations between hybrids and their parental inbreds in P_{phy} content (Table 7) indicates that inbreds with higher P_{phy} content haven't necessary got progeny with same trait. Significant correlation for ZP 341, ZP 606, ZP 666 and their female component was observed, while significant correlation for ZP 341, ZP 555, ZP 684 and their male component. The influence of female component was also emphasized through significantly higher regression coefficient, proposing new sight to breeding of maize hybrids with lower phytate content in grain.

On the other hand, both parental components had significant and positive impact on increase of P_i content in hybrid seeds, with R^2=0.442 for hybrid x female component and R^2=0.412 for hybrid x male component. Significant correlation was observed between ZP 434, ZP 560, ZP 600, ZP 606, ZP 666 and their female component and between ZP 427, ZP 60, ZP 666 and their male component.

Opposite to results of Bregitzer and Raboy (2006) who established that low-phytate barley mutants had dramatically low yield in stressed environment, in this research high-yielding maize hybrids had lover P_{phy} values. On the other hand, increased level of P_{phy} was obtained in seeds of hybrids that achieved higher yield during the unfavourable season such 2011, in relation to favourable 2010 (Table 5). Dragičević et al. (2010) previously established that maize populations with high yield had lower phytate content in grain that could be important from the aspect of sustainability and lowering of phosphorus impact on environment. This statement could be uphold by results present in tables 3 and 4, where hybrids with relatively high average grain yield (ZP 341 and ZP 684) had the lowest average P_{phy} content in grain.

Conclusion

Obtained results indicated that variability in Pphy and Pi contents were more susceptible to genotype × environment interactions (location and mainly year). Moreover, using of the inbreds with lower Pphy in grain as male component (as present in this research) haven't necessary result in lower Pphy content in hybrid grain. That opens the possibility of new breeding cycle, which would include these inbreds as female component. Stress factor, present during unfavourable seasons, affected Pphy and Pi accumulation, mainly in hybrid seeds, independently of their variations in parental inbreds. Inversely proportional trend in variations of Pphy and Pi in grain of inbreds and hybrids wasn't underlined, as it was expected. Generally, higher P_i accumulation in hybrid seeds could be allied with its content in parental inbreds. Such data could be important for achieving the high yielding maize hybrids with potentially low P_{phy} and/or high P_i content in grain.

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VARIJABILNOST FITINSKOG I NEORGANSKOG FOSFORA U SEMENU KUKURUZA

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Izvod

Kukuruz je vrsta koja se karakteriše velikim diverzitetom koji je ujedno dobro sačuvan u postojećim kolekcijama banki biljnih gena. Stoga postoji mogućnost korišćenja postojeće genetičke varijabilnosti kukuruza u cilju stvaranja genotipova sa izmenjenim hemijskim sastavom zrna. Cilj ovih istraživanja je bio da se ispita uticaj različitih faktora na varijabilnost neorganskog i fitinskog fosfora u semenu devet ZP hibrida kukuruza i njihovih roditeljskih komponenti. Ogled je bio postavljen tokom 2010 i 2011 na dve lokacije.

Veće vrednosti fitinskog i neorganskog P su uglavnom bile prisutne kod linija u 2010, kao i kod hibrida u 2011. godini. Značajne i najviše vrednosti neorganskog P su bile prisutne kod hibrida ZP 341 i ZP 434, kao i njihovih roditeljskih komponenti. Od svih ispitivanih hibrida, značajne i najveće razlike u sadržaju fitinskog P, a uzrokovane uticajem sezone, bile su prisutne kod hibrida ZP 434 i ZP 555, kao i majčinske komponente hibrida ZP 555. Obe roditeljske komponente su imale značajan i pozitivan uticaj na povećanje neorganskog fosfora u semenu hibrida. Poželjno povećanje sadržaja dostupnog fosfora uz smanjenje fitata se može efikasno postići korišćenjem genetičkih resursa u procesu predoplemenjivanja uz primenu rekurentne selekcije.

Ključne reči: fitat, neorganski fosfor, seme kukuruza, varijabilnost

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