UDC 575:633.15
DOI: 10.2298/GENSR1003555D
Original scientific paper

THE VARIATION OF PHYTIC AND INORGANIC PHOSPHORUS IN LEAVES AND GRAIN IN MAIZE POPULATIONS

Vesna DRAGIČEVIĆ, Dragan KOVAČEVIĆ, Slobodanka SREDOJEVIĆ, Zoran DUMANOVIĆ and Snežana MLADENOVIĆ DRINIĆ

Maize Research Institute "Zemun Polje", Belgrade, Serbia

Dragičević V., D. Kovačević, S. Sredojević, Z. Dumanović and S. Mladenović Drinić (2010): *The variation of phytic and inorganic phosphorus in leaves and grain in maize populations* - Genetika, Vol 42, No. 3, 555 -563.

The phytate function in plants is still not completely understood: it is the primary storage P form in seeds that is utilized during germination and early seedling development. Approaches to resolve problem of the bad nutritive quality of grain phytate include engineering of crops with reduced levels of seed phytic acid. The objective of this study was to investigate genetic variability and correlation of phytic (P_{phy}) and inorganic phosphorus (P_i) and soluble proteins among 28 maize populations, consisted into three groups: low-, intermediate- and high-phytic populations, with the aim to determine the potential of enhancing the P profile of maize plants and high grain yield through selection. The highest genetic variability of P_i and P_{phy}

Corresponding author: Vesna Dragićević, e-mail: vdragicevic@mrizp.rs
Phone +381113756704, Fax: +381113756707

content in leaves was expressed in group with intermediate P_{phy} content in grain. Meanwhile, leaves of low-phytic populations were characterized with low P_{phy}, too (averagely 18%) and high content of soluble proteins (averagely 15%) in relation to high- and intermediate-phytic populations. Additionally, the lowest genetic variability of protein content was also noticeable in leaves of low-phytic populations. Positive correlation between P_i and protein content was observed in leaves of low- and high-phytic populations. The negative correlation between P_{phy} and P_i was detected in maize grain, but correlation was significant only in intermediate-phytic group. The highest, but not significant, average yield was observed in group of low-phytic populations, as well as its relative high genetic variability. That indicates that development of high yielding genotypes with lower phytate in grain is reasonable, and could be potentially useful in enhancing the sustainability and decreasing of environmental impact in agricultural production.

Key words: inorganic phosphoorus, maize populations, phytic phosphorus, soluble proteins

INTRODUCTION

Phytic acid is a good chelator of iron ions and from that point of view it could have an antioxidant function (GRAF *et al,* 1987). In the cereal grains most of phytic acid is located in the germ, as well as aleurone layer (O'DELL *et al*, 1972). In maize, more of 80% of grain phytate is placed in the germ, with the remainder in the aleurone layer, what may connected to its antioxidative function, important to seed viability (GLASS and GEDDES, 1959; RABOY, 2009). The role which phytate plays in plants is still not completely understood. In seeds, it is the primary storage P form that is utilized during germination and early seedling development. The P released from phytate during germination is very important to early seedling growth. Approaches to resolve problems of the bed nutritive quality of grain phytate include engineering of crops to express high levels of *phytase* enzyme in seeds (BRINCH-PEDERSEN *et al*, 2002), or breeding crops with reduced levels of seed phytic acid: low-phytate or high-available P (RABOY, 2009).Although low phytate maize offers major environmental and nutritional benefits, phytates are regarded as important to dry seeds in protecting and maintaining the integrity of mineral elements until needed for germination (RABOY *et al,* 1997). In seeds homozygous for the maize lpa1-241 allele, which control 90% reduction in seed phytic acid (O'DELL *et al*, 1972) germination rates are reduced. Phytic acid itself is incorporated in the core of a protein that functions in auxin signaling, the auxin receptor (TAN *et al,* 2007). It is not known if phytic acid has a structural function or both: structural and regulatory functions. Disturbance of phytic acid synthesis have many downstream impacts. Several of these downstream effects may result in reduced plant growth, development and yield. Targeted engineering of the low-phytate content might avoid some or all of these negative impacts (RABOY, 2009). Reduce in the crop yield of four barley low-phytate mutants, connected to disturbed Ins phosphate metabolism, (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 is due to disturbed Ins phosphate metabolism in vegetative tissues. The objective of this study was to investigate genetic variability and correlation of phytic (P_{phy}) and inorganic phosphorus (P_i) and soluble proteins between 28 maize populations, separated into three groups: low-, intermediate- and high-phytic populations, with the aim to determine the potential of enhancing the P profile of maize plants and high grain yield through the process of selection.

MATERIAL AND METHODS

Sets of 8 low-phytic, 10 intermediate- and 10 high-phytic local populations from Maize Research Institute Genebank, analysed in this study was grown in a randomized complete block design (RCBD) with two replications at Zemun Polje, during the summer of 2008. The populations were allowed to open pollinate, and both rows were hand-harvested to estimate yield and collect grain samples. Phytic phosphorus (P_{phy}), inorganic phosphorus (P_i) and soluble protein content were measured in maize leaves (two leaves per plant, above and below ear) during silking period, as well as from grain, after harvesting. To determine P_{phy}, P_i and soluble proteins, a 0.25 g sample was treated with bi-distilled water for 1 h at room temperature in a rotary shaker. The extract was centrifuged on 14000 rpm for 15 min and the supernatant was decanted and diluted. P_{phy} was determined by the method of LATTA and ESKIN (1980), modified by SREDOJEVIC *et al* (2009). P_{phy} was estimated colorimetrically, based on the pink color of the Wade reagent, which is formed upon the reaction of ferric ion and sulfosalicilic acid, and has an absorbance maximum at λ=500 nm. Pi was determined from the same extract colorimetric ally, according to POLLMAN (1991), while content of soluble proteins was determined by LOWRY (1951).

RESULTS AND DISCUSSION

Because this study has been performed only in one environment, we can make only limited conclusions about the performance of examined populations. Several authors reported insignificant interactions of genotype and environment, or little variations among genotypes across environments on phytate content in grain (RABOY and DICKINSON, 1984; WARDYN and RUSSELL, 2004). Based on that, we supposed that measurements in one environment should separate high, intermediate and low phytic genotypes. Maize leaves are characterized with relative low P_{phy} content, since it presents storage P form and it haven't got active function in metabolism, opposite to P_i, as form which actively participates in plant metabolism (RABOY, 2009). The observed maize populations, separated into three groups express relative low variations of P_i content in leaves (Table 1), as well as high variations of P_{phy} content (compared to its lower content).

Table 1. The content of phytic (P_{phy}) and inorganic (P_i) phosphorus and soluble proteins in maize leaves and grain of low-, intermediate- and high-phytic populations

	Leaves			Grain			
Popul.	P_i (µg g^{-1})	P_{phy} (mg g^{-1})	Sol. prot. (mg g^{-1})	P_i (µg g^{-1})	P_{phy} (mg g^{-1})	Sol. prot. (mg g^{-1})	Yield (t ha^{-1})
Low-phytic							
1	138.95	2.01	122.94	0.16	2.46	98.33	4.53
2	144.43	2.28	124.55	0.18	2.06	98.13	3.86
3	158.89	1.50	132.13	0.17	2.35	95.58	3.01
4	189.64	1.76	142.57	0.22	2.17	104.53	4.07
5	160.22	1.66	124.90	0.20	1.58	93.79	4.74
6	183.66	1.51	124.55	0.25	2.07	95.23	4.32
7	131.30	1.87	129.83	0.15	2.40	96.27	6.08
8	151.08	2.80	126.04	0.17	2.06	104.81	5.77
Aver.	157.27	1.92	128.44	0.19	2.14	98.33	4.55
Intermediate-phytic							
9	126.81	3.37	93.44	0.14	3.70	97.16	4.76
10	162.72	2.55	147.63	0.17	3.75	98.13	3.23
11	138.28	2.13	138.21	0.17	3.71	87.04	4.46
12	129.31	1.52	116.52	0.16	3.72	98.61	3.87
13	208.26	2.44	83.91	0.17	3.65	108.94	4.89
14	181.16	1.59	129.83	0.11	4.10	96.47	3.70
15	152.91	2.25	72.09	0.11	3.81	99.16	4.70
16	151.41	2.80	91.26	0.06	4.12	87.11	4.34
17	230.53	2.49	121.57	0.09	4.04	98.88	4.50
18	156.07	1.50	90.11	0.11	3.66	102.26	4.92
Aver.	163.75	2.26	108.46	0.13	3.83	97.38	4.34
High-phytic							
19	179.50	1.62	130.87	0.19	5.09	103.22	6.30
20	145.10	2.64	127.31	0.11	5.55	121.54	5.59
21	150.08	3.06	111.01	0.11	4.83	109.22	2.75
22	142.60	2.60	105.95	0.10	4.86	106.12	4.11
23	136.62	2.77	63.37	0.08	5.72	114.04	4.63
24	173.35	2.32	94.02	0.18	5.61	112.45	3.75
25	145.43	1.40	94.71	0.14	4.78	112.80	4.09
26	159.89	2.18	92.41	0.11	4.70	115.90	3.96
27	165.04	2.54	140.28	0.10	5.12	112.18	4.83
28	168.20	2.85	140.05	0.16	4.75	103.91	2.94
Aver.	156.58	2.40	110.00	0.13	5.10	111.14	4.29
LSD 5%	25.10	1.82	22.60	0.07	2.20	18.16	0.83

The highest genetic variability in P_i and P_{phy} content in leaves was expressed in group with intermediate P_{phy} content in grain (up to 1.8 and 2.2 times between maximal and minimal value, respectively), regardless to fact that average values had not significant differences. Meanwhile, leaves of low-phytic populations were characterized with low P_{phy}, too (averagely 18%) and high content of soluble proteins (averagely 15%) in relation to high- and intermediate-phytic populations. Additionally, the lowest genetic variability in protein content was also noticeable in leaves of low-phytic populations. Opposite to previous data about positive correlation between phytate and protein content in maize grain (LORENZ *et al*, 2008), there was no significant correlation observed between them in maize leaves. Meanwhile, positive correlation between P_i and protein content was determined in leaves of low- and high-phytic populations (r=0.52, Figures 1 and 3).

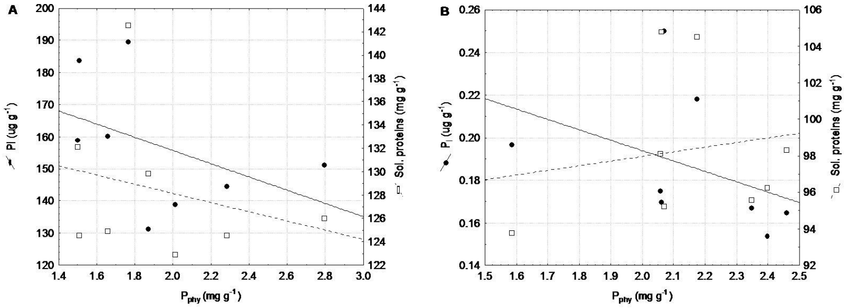

Figure 1. Correlation between phytic (P_{phy}), inorganic (P_i) and soluble proteins in leaves (A) and grain (B) of low-phytic populations

The relative high genetic variability in P_i content was present in maize grain, regardless to its relative low content (in comparison to maize leaves): the highest variability was noticed in intermediate-phytic group (up to 2.8 times between maximal and minimal value, Table 1). Moreover, high level of P_i, as digestible P form (BRINCH-PEDERSEN *et al*, 2002) has been determined in low-phytic populations, indicated their higher nutritional quality. The lower genetic variability in P_{phy} content, in relation to P_i, in grain of all three groups was in accordance with results of LORENZ *et al* (2008) and VANČETOVIĆ *et al* (2010). As it was expected, the low-phytic populations had lower P_{phy} content in grain (averagely 58% lower to high-phytic and 44% lower to intermediate-phytic populations), expressing the highest genetic variability (up to 55% between maximal and minimal value) among all three groups. The high-phytic populations, with low P_i level in grain (32% in relation to low-phytic populations), express additional high genetic variability of P_{phy} and soluble protein content, which varied parallel in range between maximal and minimal value of 119 and 121%, respectively (Table 1).

The negative correlation between P_{phy} and P_i was detected in maize grain, but insignificant in high and low-phytic populations (r=-0.05 and r=-0.41, respectively, Figures 1 and 3), while in intermediate-phytic group correlation was significant (r=-0.77, Figure 2).

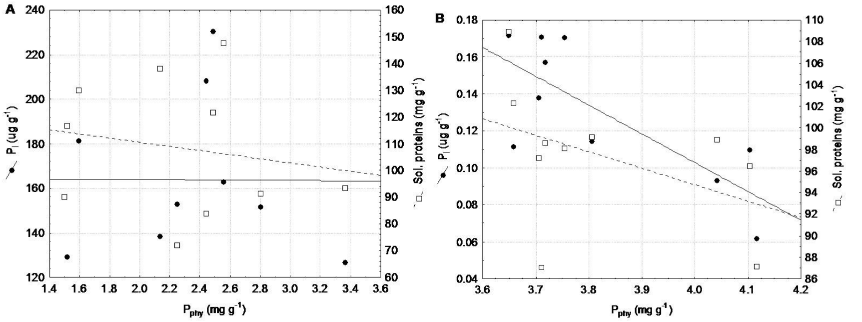

Figure 2: Correlation between phytic (P_{phy}), inorganic (P_i) and soluble proteins in leaves (A) and grain (B) of intermediate-phytic populations

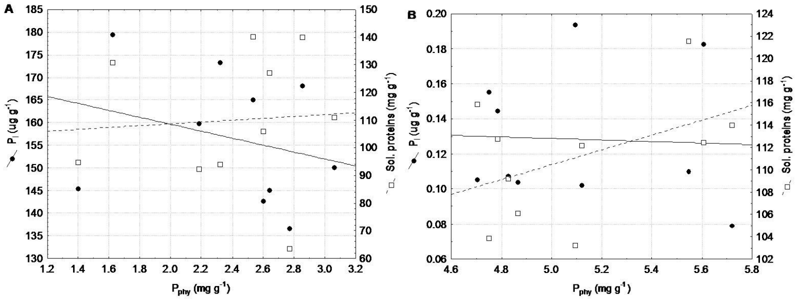

Figure 3: Correlation between phytic (P_{phy}), inorganic (P_i) and soluble proteins in leaves (A) and grain (B) of high-phytic populations

The interdependence between phytate and protein content can be explaned by the correlation between them (RABOY *et al.* 1991). As in many previous reports phytate was positively and significantly correlated with proteins (LORENZ *et al*, 2008), while DRINIC *et al* (2009) found positive, but insignificant correlation between phytate and protein content in 60 maize populations. In this study, correlation between P_{phy} and soluble proteins was positive, but not significant in high- and low-phytic groups (r=0.46 and r=0.17, respectively, Figures 1 and 3), while in intermediate group correlation was negative (r=-0.44, Figure 2). Based on the results of COELCHO et al. (2002) the chemical fertilization is practice, which have high impact on correlation between phytate and protein: the higher dose of P fertilizer as the consequence has high correlation between phytate and protein, while the lower P dose have no significant impact on correlation in dry bean genotypes. The opposite trend was observed in soybean genotypes (ISRAEL *et al.* 2006), where seed protein content did not differ significantly between normal and low phytic lines.

The genetic variability in content of soluble proteins (Table 1) was narrow in all three groups of populations; the highest variability, of 25% was noticed in intermediate-phytic group. The highest, but not significant, average yield was observed in group of low-phytic populations (5% in relation to high- and intermediate- group), together with its relative high genetic variability (Table 1). The obtained data were in accordance with results about negative correlation between grain yield and phytate content reported by BREGITZER and RABOY, 2006; RABOY, 2009; DRINIĆ *et al*, 2009. That indicates that development of high yielding genotypes with lower phytate is desirable breeding program.

Population 5 have the lowest P_{phy} content of 1.58 mg g^{-1} and lowest protein content of 93.79 mg g^{-1}, as well as higher yield (4% higher to average of low-phytic group), what makes it favorable for further breeding genotypes with low phytate content and good agronomic traits.

The phytate, generally considered as a bad nutrient is assigned as a protector against oxidative stress, too (GLASS and GEDDES, 1959; RABOY, 2009), what gives novel approach in considering of general P utilization. Developing of the high yielding genotypes with low phytate content in grain could be potentially useful in enhancing the sustainability and decreasing of environmental impact in agricultural production.

ACKNOWLEDGEMENT

This research was supported by the Ministry of Science and Technology, Republic of Serbia, through Project TR 20114.

Received May 04th2010
Accepted December 6th, 2010

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VARIRANJE SADRŽAJA FITINSKOG I NEORGANSKOG FOSFORA U LISTOVIMA I SEMENU POPULACIJA KUKURUZA

Vesna DRAGIČEVIĆ, Dragan KOVAČEVIĆ, Slobodanka SREDOJEVIĆ, Zoran DUMANOVIĆ i Snežana MLADENOVIĆ DRINIĆ

Institut za kukuruz “Zemun Polje”, Beograd, Srbija,

Izvod

Uloga fitata u biljkama jož nije potpuno razjašnjena: on predstavlja prevashodno skladišnu P formu u semenu, koja se koristi tokom klijanja i ranog rasta klijanaca. Antinutritivni kvalitet fitata iz zrna je inicirao rad na inženjeringu biljnih vrsta sa smanjenim nivoom fitinske kiseline u zrnu. Cilj ovog rada je da se ispita genetska varijabilnost i korelacije između fitinskog (P_{phy}) i neorganskog fosfora (P_i), kao i rastvorljivih proteina kod 28 populacija kukuruza, podeljenih u tri grupe: nisko, srednje i visoko fitinske, da bi se utvrdio potencijal poboljšanja P profila kod kukuruza, uz visok pronos preko selekcije. Najveća genetska varijabilnost P_i i P_{phy} je bila ispoljena u listovima kukuruza populacija sa srednjim nvoom fitata u zrnu. Međutim, nisko fitinske populacije su pored niskog sadržaja P_{phy} u listovima (prosečno za 18%) imale i viši nivo rastvorljivih proteina (prosečno za 15%), u odnosu na visoko i srednje fitinske populacije. Takođe, najniža genetska varijabilnost u sadržaju proteina je bila prisutna u listovima nisko fitinskih populacija. Pozitivna korelacija između P_i i proteina je bila uočena u listovima nisko i visoko fitinskih populacija. Negativna korelacija između P_{phy} i P_i je bila uočena u zrnu kukuruza, ali je bila značajna samo u grupi srednje fitinskih populacija. Najveći prosečan prinos, ali ne značajno, je bio prisutan kod nisko fitinskih populacija, uz najveću genetsku varijabilnost. Ovo ukazuje na mogućnost razvijanja visokoprinosnih genotipova sa niskim nivoom fitata u zrnu, što bi moglo biti potencijalno korisno sa aspekta održivosti i smanjenja uticaja okoline na poljoprivrednu proizvodnju.

Primljeno 04.V. 2010.
Odobreno 06.XII. 2010.