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Original scientific paper

GENETIC VARIATION OF PHYTATE AND IONORGANIC PHOSPHORUS IN MAIZE POPULATION

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Analysis of 60 maize populations was conducted to identify genotypes that had either low or high concentration of phytate. Genetic variability in seed phytate content was observed, with values ranging from 1,147 to 4, 13 g kg^{-1}. Inorganic phosphorus (Pi) concentrations were between 0, 35 and 1, 29 and averaged 0, 65 g kg^{-1}. Three groups of populations were identified as having low, intermediate and high phytate content. The low phytate concentration was measured in eight, intermediate in 25 and high in 27 populations. Positive correlation was found between phytate and protein. Population 216 had the lowest phytate

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concentration of 1, 14 gkg^{-1}, and a Pi concentration 40% greater than Pi mean but lower than average protein content. This population will be used for further breeding genotypes with low phytate content and good agronomic traits.

Key words: iongarnic phosphorus, phytate, maize population

INTRODUCTION

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. One approach is based on development of low phytic acid (*lpa*) mutant lines in a variety of crop species, including maize, that greatly reduces amounts of phytate and provides valuable information regarding the biochemical, physiological and nutritional roles of phytate (RABOY et al., 2001, RABOY, 2002). PILU et al. (2005) showed that disruption of the gene that causes the *lpa* mutation has negative pleiotropic effects on kernel size and germination and concluded that breeding *lpa* genotypes equal in yield potential to their wild-type counterparts may not be possible. Another approach to decrease phytate and increase Pi in maize grain is through recurrent selection that uses the indigenous quantitative genetic variation.

Although low phytate maize offers major environmental and nutritional benefits, phytates are regarded as important to non-germinating seeds in protecting and maintaining the integrity of mineral elements until needed for germination (RABOY *et al.*, 1997). The role which phytate plays in plants is still not completely understood. 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. Low phytate maize is also of interest to feeders because it makes more phosphorus available to the animal, lowering the phosphorous content in animal waste.

The objective of this study was to investigate genetic variability of phytate and inorganic phosphorus (Pi) among sixty maize populations with the aim to determine the potential of enhancing the P profile of maize grain through selection.

MATERIAL AND METHODS

A set of 60 populations from Maize Research Institute Genebank, included 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. Grain yield, kernel weight (mass of whole kernel), phytate, Pi, soluble protein content, phenols and PSH were measured. To determine phytate, the method of LATTA and ESKIN (1980), modified by SREDOJEVIC (1989, 2009) was employed. A 500 mg sample was treated with 0, 15% sodium citrate for 1 h at room temperature in a rotary shaker. The extract was centrifuged on 12000 rpm for 15 min and the supernatant was decanted and diluted. Phytate was determined calorimetrically, 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 500 nm. In the presence of phytate, the iron is sequestered and unavailable to react with sulfosalicylic acid, resulting in a decrease in pink color intensity. The extraction procedure employed for determination of Pi was according to POLLMAN (1991). Reduced PSH was determined as given in DE KOK et al. (1981) and total phenols according to Prais method of Prussian Blue (BUDINI et al., 1980) modified by SIMIC et al, 2004. Protein content was determined by LOWERY (1951).

RESULTS AND DISCUSSION

Inorganic P (Pi) is important form of phosphorus in grains, although it is present in 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. Pi concentration was between 0, 35 and 1, 29 and averaged 0, 65 g kg^{-1}, Table 1. Genetic variability in phytate contents was observed, with values ranging from 1,147 to 4, 13 and averaged 2, 91 g kg^{-1}. Both phytate and Pi values were within the range of values reported for wild –type maize hybrids in previous studies (RABOY et al., 1989, 2000) and S1 populations (LORENZ et al., 2008). A larger amount of genetic variation for Pi relative to phytate was found. The results of previous studies (LORENZ et al, 2007, LORENZ et al, 2008) showed a greater amount of genetic variation for Pi than for phytate both with populations and inbred lines. But for soybean significant differences among genotypes are more difficult to detect for Pi than phytate (ISRAEL et al, 2006, RABOY and DICKINSON, 1993). Three groups of populations were identified as having low, intermediate, and high phytate content. The low phytate concentration was measured in eight, intermediate in 25 and high in 27 populations (Table 2).

Grain yields were between 1,326 t/ha and 6,298 t/ha and averaged 4,226 t/ha (Table 1). Because this study included only one environment, we can make only limited conclusions on the relative performance of these populations. As several authors reported insignificant genotype x environment interactions or little to no rank changes among genotypes across environments (RABOY et al., 1984, WARDYN and RUSSELL, 2004), we supposed that measurements in one environment should separate high and low genotypes.

Population 216 was determined to have the lowest phytate concentration of 1, 14 gkg^{-1}, and a Pi concentration 40% greater than Pi mean but lower than average protein content. This population ranked as 19th among the 60 populations with respect to yield. Population 216 will be used for further breeding genotypes with low phytate content and good agronomic traits.

Table 1. The grain yield, phytate, Pi and protein content

population	Grain yield tha^{-1}	Kernal weight mg	Pi gkg^{-1}	Phytate gkg^{-1}	Protein content gkg^{-1}
4	1,326	186,14	0,984	2,609	97,16
20	4,126	243,64	0,568	2,564	97,57
33	3,466	291,56	0,690	2,502	89,65
79	4,939	293,31	0,565	2,591	96,26
90	4,763	342,45	0,608	2,681	97,16
103	3,234	210,21	0,752	2,717	98,12
121	4,458	173,39	0,755	2,685	87,03
128	3,868	293,02	0,693	2,690	98,60
132	4,532	333,63	0,727	1,780	98,33
138	3,867	297,43	0,77	1,488	98,12
186	3,011	181,22	0,736	1,698	95,57
190	4,066	251,79	0,963	1,573	104,53
216	4,737	303,25	0,868	1,147	93,78
262	4,318	281,47	1,103	1,497	95,23
268	6,083	323,28	0,678	1,734	96,26
280	5,769	339,10	0,749	1,492	104,80
376	6,298	292,95	0,856	3,686	103,22
399	5,585	245,78	0,486	4,013	121,54
408	2,899	225,98	0,622	3,363	108,04
419	3,76	300,23	0,746	3,291	107,42
477	4,569	245,34	0,831	3,376	108,93
485	4,398	235,31	0,669	3,407	102,87
558	2,75	151,23	0,473	3,492	109,21
559	2,605	322,38	0,626	3,174	113,00
588	4,296	263,51	0,586	3,170	118,85
606	4,111	354,52	0,658	3,520	106,11
641	4,627	286,21	0,348	4,138	114,03
674	4,113	296,74	0,522	3,187	121,68
676	4,885	333,92	0,557	2,640	108,93
690	3,745	292,63	0,806	4,058	112,45
692	4,088	377,95	0,638	3,461	112,79
707	3,962	359,00	0,464	3,403	115,89
709	3,697	325,93	0,484	2,969	96,472
710	3,426	338,17	0,607	2,817	119,75
754	4,695	383,39	0,505	2,754	99,15
770	4,34	300,00	0,273	2,978	87,10
785	4,504	321,63	0,412	2,924	98,88
794	4,818	448,43	0,709	2,893	99,29

802	4,918	296,83	0,692	2,646	102,25
807	3,528	457,03	0,572	3,001	93,09
826	4,268	328,60	0,643	3,288	99,22
832	4,426	290,09	0,598	3,297	113,55
850	4,842	263,40	0,576	3,211	111,28
887	5,359	386,52	0,738	3,086	97,50
891	4,828	298,41	0,450	3,705	112,17
900	2,937	164,50	0,686	3,436	103,91
902	3,478	338,75	0,377	3,195	101,56
909	4,338	414,41	0,610	3,285	100,94
914	5,066	289,62	0,749	3,177	101,08
921	2,291	187,15	0,433	3,060	97,64
937	5,246	346,39	0,570	3,020	89,65
965	5,043	338,82	0,508	3,195	101,77
968	3,952	351,18	0,638	3,334	94,88
984	3,526	312,84	0,366	3,132	98,12
5006	2,057	267,69	0,742	2,760	86,76
5011	5,472	280,02	0,518	2,876	92,34
5027	5,368	359,81	0,867	3,123	99,5
5030	4,88	389,90	1,048	3,002	92,82
6739	4,687	366,59	1,079	2,706	85,72
6741	4,309	257,95	0,949	3,146	83,45

The interdependence between phytate and protein can be observed by the correlation between these two variables (RABOY et al. 1991). Positive but non significant correlations was found (r=0, 42). High correlation between phytate and protein was observed under the higher dose of P fertilization, while under the lower P dose no significant correlation was observed (COELCHO et al., 2002). As in many previous reports (LORENZ et al, 2007, 2008) phytate was positively and significantly correlated with proteins (r=0, 72). LORENZ et al. (2008) found negative correlation between phytate and starch indicating the seed deposition of phytate in the germ (90%). Phytate and Pi content differed among analyzed populations and phytate was negatively correlated with Pi. A negative correlation between yield and phytate indicates that development of high - yielding genotypes with lower phytate should be an attainable goal.

An important relationship to consider, before selecting for decreased whole kernel phytate, is that between phytate and kernel size. We measured phytate concentration in ground whole kernels and, as large portion of phytate is found in germs, kernels with larger endosperm should have a diluted concentration of phytate. In our study phytate/kernel weight correlation was near zero, which contrasts significant negative correlation between these traits found by LORENZ et al. (2007) for maize inbred lines. The authors postulated that whole-kernel phytate concentration decreases as seed size increases, based on the presumption that kernel size is largely a function of endosperm size. This was not the case in our study as the populations 807, 794, and 909 with high kernel weight had phytate concentration above mean value.

Table 2. Phytate value of populations selected to represent low, intermediate, and high level of phytate concetration.

Population				group	Group meean
132	138	186	190	low	
216	262	268	280	low	1,55 g kg^{-1}
4	20	33	79	intermediate	
90	103	121	128	intermediate	
676	709	710	754	intermediate	
770	785	794	802	intermediate	
807	887	921	937	intermediate	
5006	5011	5027	5030	intermediate	
6739				intermediate	2,82 g kg^{-1}
376	399	408	419	high	
477	485	558	559	high	
588	606	641	674	high	
690	692	707	826	high	
832	850	891	900	high	
902	909	914	965	high	
968	984	6741		high	3,41 g kg^{-1}

The phytate is generally regarded as an antinutrient. On the other hand, phytate may play an important role as an antioxidant by complexing iron (COELHO et al, 2002, DORIA et al, 2009). In our study the populations with low phytate also had low antioxidants PSH and phenol (date not shown). The correlation between phytate and phenols, as well as between phytate and PSH was positive (r=0, 30 and r=0, 37, respectively). Therefore, a novel role in plant seed physiology can be assigned to phytate, i.e. protection against oxidative stress during the seed's life span. As the greater part of phytate (and thus of metal ions) is concentrated in the embryo of maize kernels, its antioxidant action may be of particular relevance in this crop.

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GENETIČKA VARIJABILNOST FITINA I NEORGANSKOG FOSFORA U POPULACIJAMA KUKURUZA

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I z v o d

Analizirano je 60 popualacija kukuruza u cilju identifikacije genotipova koji iamju nizak ili visok sadržaj fitina. Utvrđena je genetička varijabilnost u sadržaju fitina u opsegu od 1,147 do 4,13 g kg^{-1}. Sadržaj neorganskog fosfora je bila između 0,35 i 1,29, prosečno 0,65 g kg^{-1}. TTri grupe populacija su identifikovane koje sadrže nizak, srenji I visok sadržaj fitina. U osam populacija izmerena je niska koncentracija fitina, u 25 srednja i u 27 visoka. Pozitivna korelacija je utvrđena između sadržaja fitina i proteina. Populacija 216 ima najnižu koncetraciju fitina 1,14 gkg^{-1}, i Pi koncetraciju 40% veću nego prosek Pi ali niži od proseka sadržaj proteina. Ova populacija će se koristiti za dalju selekciju genotipova sa niskim sadržajem fitina i dobrim agronomskim osobinama.

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