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RESEARCH

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ABSTRACT

Seed P is predominantly bound in the organic compound phytate, which makes the bioavailability of P low for monogastric animals fed maize (*Zea mays* L.)-based diets. Decreasing phytate and increasing inorganic P (P_i, an available form of P) concentrations in maize grain would be desirable to help ameliorate environmental problems associated with high P in feces. Our objective was to investigate the potential of improving the P profile of maize grain through breeding and selection. Ninety S_1 families from the BS31 population were evaluated at two locations for phytate, P_i, and other grain quality and agronomic traits. Phytate concentrations ranged from 1.98 to 2.46 g kg^{-1}, and the broad-sense heritability (*H*) was relatively low (0.60). Both genetic variance and *H* (0.84) were much greater for P_i. Few unfavorable genetic correlations were observed between either P_i or phytate and other key economic traits. Also, selection differentials of multiple trait indices indicated that the P profile of maize grain and grain yield and moisture could be improved simultaneously. Many cycles of selection will be needed, however, to reach desirable phytate and P_i concentrations, especially when selecting for multiple traits. Regardless, our results are encouraging given that the families evaluated were related S_1 families and the number of families was relatively small.

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Abbreviations: *H*, broad-sense heritability; *lpa*, low phytic acid; P_i, inorganic phosphorus.

The bioavailability of P in maize (*Zea mays* L.)–soybean [*Glycine* max (L.) Merr.] diets is only 15% for monogastric animals because most plant seed P is bound in an organic compound known as phytate; monogastrics lack the enzyme phytase needed to utilize this form of P (Cromwell and Coffey, 1991). Therefore, most of the endogenous P in the diets of swine and poultry passes into the feces and onto the landscape, where it contributes to surface water pollution (Sharpley et al., 1994). The eutrophication of waterways from animal agriculture is a large problem in Europe and North America (Lott et al., 2000). Also, the diets of monogastric livestock must be supplemented with inorganic P or phytase to meet their nutritional requirements (Raboy, 2001), which is an additional expense for the producer.

It would be desirable to increase the concentration of inorganic P (P_i), an available form of P, and reduce the concentration of phytate in maize grain to help alleviate the associated nutritional and environmental problems simultaneously. Low phytic acid (*lpa*) mutant lines, which have up to a 66% reduction in phytate P and a molar equivalent increase in P_i (five- to 10-fold), have been developed in a variety of crops, including maize (Raboy et

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al., 2001). Animal feeding trials have shown that P availability is greatly improved in *lpa* maize compared with wild-type maize, leading to substantial decreases in feces P concentration (reviewed in Knowlton et al., 2004). Reductions in yield potential have been reported, however, for *lpa* maize (Ertl et al., 1998) as well as for *lpa* wheat (*Triticum aestivum* L.) (Guttieri et al., 2006). Pilu et al. (2005) showed that disruption of the gene that causes the *lpa1* mutation (the most common *lpa* mutant) has negative pleiotropic effects on kernel size and germination and thus breeding *lpa* cultivars equal in yield potential to their wild-type counterparts may not be possible.

Another approach to enhancing the P profile (decreased phytate, increased P_i) in maize grain is through recurrent selection that uses the indigenous quantitative genetic variation for these traits. Significant intraspecific genetic variation for both phytate and total P has been observed in wheat (Raboy et al., 1991), soybean (Israel et al., 2006), dry bean (*Phaseolis vulgaris* L.) (Lolas and Markakis, 1975) and oat (*Avena sativa* L.) (reviewed in Maga, 1982). In maize, Raboy et al. (1989) found a 2.5-fold difference in phytate concentration between Illinois high and low protein lines in their 83rd generation of divergent selection. Wardyn and Russell (2004) estimated a broad-sense heritability (*H*) of 0.82 for total P measured in a maize population of S_1 families. Both Raboy et al. (1989) and Wardyn and Russell (2004) concluded that P-related traits in maize could be easily modified through selection. No studies, however, have investigated the genetic variability of both phytate and P_i within a single maize breeding population to determine the potential of enhancing the P profile of maize grain through selection on both components simultaneously. Furthermore, phytate and P_i genetic variability should be studied using methods suitable for maize breeding projects, such as automated sampling during yield trial harvest and simple, rapid protocols for analysis of grain components.

Selection on single traits, such as insect and disease resistance, typically results in unfavorable changes in yield and other important agronomic traits (Hallauer et al., 1988). Yield, moisture, and root and stalk lodging are traits valued by producers and should be included in selection indices to prevent compromising the overall value of the germplasm. Therefore, studying the potential for improving the P profile of maize grain should be done in the context of multiple trait selection.

The objective of our research was to determine the potential of improving the P profile of maize grain alone and along with other economically important traits. This was accomplished by measuring several traits, including phytate and P_i, in a population of S_1 families and estimating variance components and *H*. Genetic correlations were calculated in addition to selection differentials obtained from various multiple-trait selection indices. Grain quality traits were measured on samples obtained through mechanical sampling of yield trial plots. The phytate and P_i laboratory protocol we used is relatively rapid and well suited for breeding (Lorenz et al., 2007).

MATERIALS AND METHODS

Germplasm, Field Design, and Evaluation

Randomly selected plants from the BS31(R)C1 population were self-pollinated to produce 90 S_1 families. The BS31 population was developed by five cycles of mass selection for earliness in FS8A(T)C4 (Horner, 1990). The BS31(*R*)C1 population was developed from BS31 by one cycle of reciprocal recurrent selection with a population derived from FS8B(T)C4 (Horner, 1990). The FS8A(T) population is a highly diverse population composed of germplasm from the southeastern U.S. (30%), Corn Belt (22%), and tropical (48%) sources (Horner, 1990). The 90 families were evaluated at Ames and Carroll, IA, in a randomized complete block design with two replications during the summer of 2004. Plots consisted of two rows 5.49 m long with 0.76 m between rows. The plots were overplanted by machine and thinned to a uniform plant density of 62,140 plants ha^{-1}. The lines were allowed to open pollinate and an ~454-g grain sample was obtained from each plot by an automated sampler during machine harvest. A subsample consisting of 30 whole kernels was taken from each field plot sample and milled until 70% of the millings passed through an 80-μm mesh screen.

Data collected on plots were machine-harvestable grain yield (megagrams per hectare, adjusted to 155 g kg^{-1} moisture), grain moisture (grams per kilogram), root lodging (percentage of plants leaning more than 30° from vertical), stalk lodging (percentage of plants broken at or below the primary ear node), test weight (kilograms per cubic meter), and silk emergence (days after planting in which 50% of the plants had emerged silks at Ames, IA). Data collected on field plot samples included kernel weight (grams per 250 whole kernels) and protein, oil, and starch (grams per kilogram; all predicted with near infrared spectroscopy of whole grain).

Phytate and Inorganic Phosphorus Analysis

The colorimetric assays of Vaintraub and Lapteva (1988) and Raboy et al. (2000) were modified as described by Lorenz et al. (2007) and used to measure phytate and P_i on the 30-kernel ground samples. Measurements were performed in triplicate by subsampling ground samples three times and blocking plots from each field replication on 96-well microtitre plates. Six standards were included on each phytate and P_i evaluation plate. Field plots and standards were randomized on each microtitre plate as a resolvable row–column design (John and Williams, 1995). The optical density of colorimetric reactions was quantified with a 96-well spectrophotometer. A single standard curve was developed within each field replication by regressing optical density means on known standard concentrations. The standard curves were used to predict phytate and P_i concentrations in each microtitre plate well. Standard curve R^2 values were >0.99.

Statistical Analysis

The number of microtitre plate measurements taken on phytate and P_i was 1080 each. Data from microtitre plate measurements were subjected to an outlier analysis according to Anscombe and

Tukey (1963), and outliers were removed from the data sets. The number of outliers removed for phytate and P_i were 14 (1.3%) and 10 (0.9%), respectively. Inorganic P concentration field plot least-squares means were calculated by fitting a mixed model including field plot (fixed) and plate (random). A similar model was used to calculate phytate concentration least-squares means of field plots with the exception of fitting row and column within plate as random effects due to the amount of variation accounted for by these variables. Phytate and P_i field plot least-squares means were subsequently treated as single measurements.

Field plot measurements were fit to a model that included family (random), location (fixed), family × location interaction (random), and field replication nested within location (random). Variance components were estimated using the MIXED (REML) procedure of SAS (SAS Institute, 2003) and tested for significance by the Wald *z*-test. Broad-sense heritability estimates on an S_1 family mean basis were calculated as

$$H^2 = \frac{\sigma_F^2}{\sigma_F^2 + \left(\sigma_{FL}^2/l\right) + \left(\sigma_e^2/lr\right)}$$

where σ_F^2 is the family variance, σ_{FL}^2 is the family × location interaction variance, σ_e^2 is the residual variance, l is the number of locations ($l = 2$), and r is the number of replications within locations ($r = 2$). The CV was calculated by dividing the square root of the residual variance by the experiment mean and was expressed as a percentage. Family means across locations were used to calculate phenotypic correlations. Genotypic correlations were calculated by equating mean cross products with their expected values (Bernardo, 2002).

Selection Indices

Selection differentials based on 10% selection intensity were calculated from a series of indices involving phytate, P_i, grain yield, grain moisture, root lodging, and stalk lodging. To avoid problems of scale, family means were transformed to standard deviation units before index values were calculated. Selection differentials are reported in the original units. The heritability index (Smith et al., 1981) and rank summation index (Mulamba and Mock, 1978; reviewed in Hallauer et al., 1988) were used to calculate family index values for various trait sets. The heritability index and rank summation index represent weighted and unweighted indices, respectively, and result in different selection differentials due to differences in *H*. Trait sets and their selection method are individually described here.

1. *All*: The transformed family means were ranked according to an index that included yield (1), moisture (−1), root lodging (−1), and stalk lodging (−1), where the numbers in parentheses are the assigned economic weights. The top 30 families (33% selection intensity) were truncated and reranked according to an index that included only P_i (1) and phytate (−1). The top nine families were selected.
2. *Phytate, P_i, Moisture, Yield*: Families were ranked according to an index that included these four traits. The assigned economic weights were equal to those in the "All" index. The nine families with the highest index values were selected.
3. *Phytate, P_i*: Families were ranked according to an index that included only these two P traits. Economic weights were equal to those in the "All" index. The nine families with the highest index values were selected.

Families were also selected based on phytate and P_i concentrations alone (i.e., the nine families with the lowest phytate concentration were selected and the nine families with the highest P_i concentrations were selected separately to produce two sets of selection differentials). Selection differentials were calculated by subtracting the population mean from the mean of the nine selected families for each trait.

RESULTS AND DISCUSSION

Family was a highly significant ($P < 0.01$) source of variation for all traits measured. Phytate concentrations were between 1.98 and 2.46 g kg^{-1}, whereas P_i concentrations were between 0.22 and 0.71 g kg^{-1} (Table 1). Both phytate and P_i values were within the range of values reported for wild-type maize hybrids in previous studies (Raboy et al., 1989, 2000; Shi et al., 2003). The range as a percentage of the mean was much greater for P_i than phytate (132 vs. 21%). We also found a larger amount of genetic variation for P_i relative to phytate in a previous study in which measurements were taken on inbred lines (Lorenz et al., 2007). Taken together, these results show a greater amount of genetic variation for P_i than for phytate both within breeding populations and among inbred lines of maize, but results in soybean were variable and suggest that significant differences among genotypes are more difficult to detect for P_i than phytate (Israel et al., 2006; Raboy and Dickinson, 1993; Pilu et al., 2005). Additional independent studies on the natural variation of phytate and P_i in maize are needed to validate our results.

Family × location interaction was not significant for either phytate or P_i. Because this study included only two locations, this result should be interpreted with caution. Our finding agrees with previous reports on the importance of genotype × environment interactions of P-related traits (Raboy and Dickinson, 1993; Wardyn and Russell, 2004; Israel et al., 2006) where the genotype × environment interaction was either nonsignificant or was largely due to changes in the magnitude of genotype differences rather than changes in genotype ranks. Broad-sense heritability values were high for all traits, suggesting a large amount of genetic diversity within the population studied (Table 1). The *H* of phytate was 0.60 and that of P_i was 0.84. The *H* values we found for phytate and P_i in this experiment were similar to previous findings (Lorenz et al., 2007) where the P_i measurements were more repeatable than phytate measurements (0.91 vs. 0.78). Because the CV of phytate was 5.2%, a search for more genetic variation for phytate may

Table 1. Variance component estimates from each source of variation, and population mean, minimum, maximum, broad-sense heritability, and coefficient of variation estimates for each trait. Measurements taken on 90 S_1 lines from the BS31(R)C1 population.

Variance component	df	Phytate	Pi†	Protein	Oil	Starch	Moist	Yield	RL†	SL†	TW†	KW†
		$-g^2\ kg^{-2} \times 10^{-3}$‡-			$g^2\ kg^{-2}$			$Mg^2\ a^{-2}$	$-\ \%^2\ -$		$kg^2\ m^{-6}$	g^2 (250 kernels)$^{-2}$
Family	89	5.26**	5.66**	32.65**	3.176**	36.78**	366.3**	0.81**	110.0**	4.71**	750.0**	31.72**
Family × location	89	0	0.44	2.02	0.261*	3.54*	49.0**	0.17*	36.4**	1.29*	14.0	0.71
Residual	178	14.11	3.42	9.58	1.294	13.02	86.9	0.62	37.1	4.68	296.8	6.05
		$g\ kg^{-1}$						$Mg\ ha^{-1}$	— % —		$kg\ m^{-3}$	g (250 kernels)$^{-1}$
Statistic												
Mean		2.24	0.37	90.1	39.1	591.4	200.9	6.69	9.2	2.5	757	66.1
Min.		1.98	0.22	78.3	35.3	576.0	164.5	3.49	0	0	669	51.5
Max.		2.46	0.71	107.6	45.6	605.8	264.9	9.25	56.9	15.9	837	79.2
Heritability		0.60	0.84	0.91	0.87	0.88	0.89	0.77	0.80	0.72	0.90	0.94
CV, %		5.2	15.8	3.4	2.9	0.6	4.6	11.8	65.2	86.6	2.3	3.7

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

†P_i = inorganic phosphorus, RL = root lodging, SL = stalk lodging, TW = test weight, KW = kernel weight.

‡Multiply the reported variance components by this to obtain the actual variance components. Statistics values are actual values.

be the most effective way to increase the heritability of this trait. Our results are encouraging given that the protocol used was designed primarily to be easy, inexpensive, and rapid and could easily be used to screen large numbers of families from many diverse populations.

Few statistically significant correlations were found between phytate or P_i and all other traits. As in many previous reports (Lorenz et al., 2007; Raboy et al., 1989, 1991), phytate was positively and significantly correlated with protein (0.37 phenotypic, 0.51 genotypic). A negative correlation occurred between phytate and starch (−0.33 phenotypic, −0.45 genotypic). This result reflects the seed deposition pattern of phytate, where approximately 90% of phytate is found in the germ (reviewed in Maga, 1982) and only trace amounts are in the endosperm. We expected a larger correlation given such a large discrepancy in phytate levels between these seed organs. The pattern of phytate deposition in the kernel was used to partially explain a negative correlation between phytate and kernel size in a previous experiment (Lorenz et al., 2007), but no significant correlation was found between phytate and kernel size within this population of S_1 lines. This may be due to less genetic variation for these traits among S_1 families than inbred lines derived from multiple backgrounds. The phenotypic and genotypic correlations between phytate and P_i were 0.05 and 0.15, respectively, which closely agree with the correlation between these P traits reported in Lorenz et al. (2007). In contrast, a lack of relationship between phytate and P_i differs with a positive correlation found among wild-type soybean lines (Israel et al., 2006) and the repartitioning of total P by the *lpa* mutations (Raboy et al., 2000). The *lpa* mutation confers a biochemical lesion in the phytic acid pathway and is inherited as a qualitative trait. Thus, the effects of the *lpa* mutation cannot be compared with the natural genetic variation of the phytate/P_i ratio. When measurements are taken among wild-type cultivars and breeding families, the correlation between phytate and total P is typically >0.90 (Raboy et al., 2001). This suggests that selection for reduced phytate would decrease total P without repartitioning the P bound in phytate to P_i, an undesirable outcome considering animal nutrition. Our findings reported here and those in Lorenz et al. (2007), however, suggest that phytate and P_i are independent and an improved P profile could be achieved through selection on both traits simultaneously.

Our results on selection indices that included phytate, P_i, grain yield, grain moisture, stalk lodging, and root lodging indicated that progress, albeit slow, could be made for each trait (Table 2). Selection differentials could have been increased, with no additional post-harvest labor, by evaluating more families for agronomic traits and truncating the best families before laboratory analysis. Truncating families once with an index including phytate, P_i, yield, and moisture did not achieve greater selection differentials for phytate and P_i compared to the "All" index, but greater pressure was placed on yield. Selection differentials calculated when selection was applied to phytate and P_i only indicates that the P profile could be altered in the desired direction, but yield and moisture are expected to decrease and increase, respectively. The differences observed between the heritability index and rank summation index were expected, with the heritability index placing more pressure on P_i. Realized gains in overall index value would probably be greater for the heritability index due to more emphasis being placed on traits with higher *H*, but it may not put enough pressure on phytate for significant reduction in the near future. Altogether, selection differentials for

Table 2. Selection differentials (10% selection intensity) for each trait when the selection indices were applied. High inorganic P (P_i) and yield values were considered favorable, while low phytate, moisture, root lodging, and stalk lodging values were considered favorable.

Selection method	Trait											
	Phytate	P_i	Yield	Moisture	Root lodging	Stalk lodging	Test weight	Protein	Oil	Starch	Kernel weight	Silk emergence
	—— g kg^{-1} ——		Mg ha^{-1}	g kg^{-1}	——% ——		kg m^{-3}	—— g kg^{-1} ——			g (250 kernels)$^{-1}$	DAP†
Heritability												
All‡	–0.05	0.09	0.36	–15.8	–4.5	–1.01	19.8	–2.19	0.08	2.29	5.06	–1.99
Phytate, P_i, moisture, yield	–0.06	0.09	0.57	–15.4	–1.5	0.04	13.7	–2.90	0.20	2.91	4.90	–1.42
Phytate, P_i	–0.09	0.13	–0.24	9.9	11.6	–1.30	–12.2	–0.37	–0.77	2.46	3.31	1.51
Rank summation												
All‡	–0.09	0.06	0.38	–13.1	–5.0	–0.61	9.5	–3.81	0.81	2.69	6.07	–1.26
Phytate, P_i, moisture, yield	–0.07	0.06	0.74	–14.9	–2.4	–0.22	8.3	–2.56	1.15	1.03	4.39	–1.61
Phytate, P_i	–0.12	0.08	0.05	9.8	4.5	–1.57	–12.4	–3.49	–0.11	4.53	4.58	0.99
Phytate only	–0.17	0	0.01	5.5	8.2	–1.55	–20.1	–4.25	–0.13	4.36	2.92	–0.20
P_i only	0.01	0.16	–0.34	–6.8	3.7	–0.65	15.1	1.49	–0.45	–0.08	1.06	0.57
Max.§	–0.17	0.16	1.71	–30.2	–9.0	–2.51	50.2	11.10	3.70	11.00	10.00	–3.60

†DAP, days after planting to midsilk emergence.

‡Thirty families (33% selection intensity) were first selected on index values that included yield, moisture, root lodging, and stalk lodging. The final nine families were selected on indices that included only phytate and P_i.

§Selection differential for each trait when selections are on that trait alone.

phytate were between 2.2 and 7.6% of the population mean when phytate was included in any of the selection methods used. On the other hand, selection differentials for P_i were between 16.2 and 43.2% of the population mean. Furthermore, a greater proportion of the selection differential would be expected to be inherited for P_i. Protein would be expected to decrease while starch is expected to increase for each selection method except when selection is applied to P_i only. An increase in seed phytate through direct selection on protein in maize has been reported (Raboy et al., 1989).

In summary, a large amount of intrapopulation genetic variance was found for P_i, amounting to more than a threefold difference between S_1 families. Less genetic variation for phytate resulted in a low *H* relative to the *H* values of all other traits measured, which agrees with a previous study conducted on a set of inbred lines (Lorenz et al., 2007). We are not aware of any other studies on the genetic variance of phytate and P_i within or among maize breeding populations and therefore cannot conclude whether there is truly less genetic variance for phytate than P_i. Selection differentials from the selection indices constructed indicate that there is enough genetic variation to enhance the P profile of maize grain through breeding and selection while maintaining or improving important agronomic traits. It should be noted that the population studied herein is a highly diverse composite of multiple germplasm sources and the variability and heritability of these traits is likely to be smaller within elite Corn Belt populations.

On average, swine need 2.35 g P_i kg^{-1} dietary dry matter to meet their nutritional requirements (Knowlton et al., 2004), and phytate levels should be as low as possible to minimize P pollution. Obviously, many cycles of selection would be needed to attain these goals, but the variation found among related S_1 families suggests genetic gain is possible, especially if a larger number of more diverse families is considered for selection and breeding. Nevertheless, any improvement in the P profile of maize grain would have a beneficial impact on the environment and farm economy.

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