A STUDY OF PHYTATE VARIABILITY IN WHEAT

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ABSTRACT

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By

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Phytic acid, a compound in seeds which chelates divalent metals, was measured in 30 soft winter wheat lines grown in five locations throughout southern Michigan and 42 winter wheats from the U.S.D.A. world wheat collection. Environmental and genetic controls on phytate were examined as a prelude to the inclusion of selection pressure for phytate into a wheat breeding program. Sufficient genetic variability was found to warrant further study, however, environmental influences were quite strong. Further studies should be conducted to examine the total variability available for phytic acid. Nevertheless, decreases of the magnitude found in this study would serve to substantially increase dietary mineral availability in populations whose primary food source is wheat.

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Walter David Worrall

A THESIS

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INTRODUCTION

Phytate, myo-inositol-1,2,3,4,5,6-Hexakis(dihydrogen phosphate), is a compound found in plant seeds and functions as a phosphorus-store for germination and early seedling growth. It may also function in the initiation of seed dormancy by chelating the divalent metal cations which are often cofactors in the enzymatic reactions involved in germination. It is this property that makes phytate a nutritional hazard to populations whose major food source is unleavened wheat products. An enzyme capable of breaking down the phytate molecule has been identified and is produced by a variety of microorganisms including bread yeasts. This enzyme is present in varying concentrations in the digestive systems of many animals, but at inadequate concentrations in the human gastrointestinal tract to facilitate substantial phytate breakdown. Consequently, alternative methods of decreasing the dietary intake of phytate must be examined. One possible method might be genetic manipulation of phytate levels in the wheat kernel if sufficient genetic variability could be identified. It was the purpose of this study to examine the genetic and environmental variation of a number of wheat genotypes grown in different locations and to determine the feasibility of genetically altering phytate content in wheat by plant breeding. This was accomplished by analyzing thirty high performing inbred wheat lines grown in a variety of Michigan environments as well as analyzing a sample of varieties from the world wheat collection. Due to

the small amount of seed available, lines from the world collection were planted in unreplicated headrows in only two environments, whereas, the thirty Michigan lines were planted in larger test plots in five environments. Consequently, the bulk of this study is concerned with results from the five-environment, thirty-line analysis.

LITERATURE REVIEW

Posternak, in 1903 first isolated and described a phosphorus containing compound found almost exclusively in the outer layers of seeds (McCollum, 1957). Due to its plant origin, Posternak named this substance phytic acid and assigned it the formula $C_2H_8O_9P_2$. Since then various other structural formulae have been assigned to phytic acid including $C_6H_{24}O_{27}P_6$, (Neuberg, 1908) and $C_6H_{18}O_{24}P_6$, (Anderson, 1914). X-ray crystallography and nuclear magnetic resonance have shown that the structure proposed by Anderson is the proper structure for naturally occurring phytic acid of plant origin, (Johnson and Tate, 1969), (Oberleas, 1971).

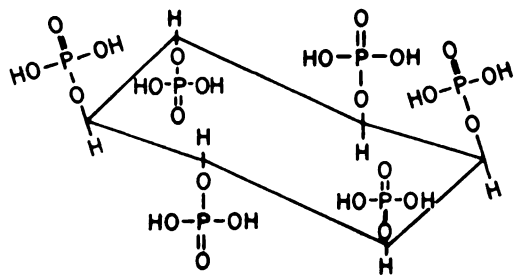

Figure 1. myo-Inositol-1,2,3,4,5,6-hexakis (dihydrogen phosphate)

The occurrence of phytate in diverse species of plants has been shown by many authors. Wheat contains 53% of its phosphorus as phytate (Williams, 1970), corn - 37%, rice - 59%, (Oke, 1965). Recent work with cereals has shown that the highest concentrations of phytate are found in the aleurone layer of seeds. O'Dell, et al. (1972), found that 87.1% of the phytate phosphorus in wheat was found in this seed fraction.

Nelson, et al. (1968), reported up to 77% of the total phosphorus present in wheat bran as phytate. This is in contrast to phytate-phosphorus concentrations of 12.9% and 2.2% for wheat germ and endosperm respectively, (O'Dell, et al., 1972). Scanning electron microscopy has shown a close association of phytate with the aleurone grains, (Stevens, 1973; Jones, 1969).

The above evidence indicates that phytate is a valuable storage form of phosphorus for seeds during germination when phosphorus serves as an energy source (Morton and Raison, 1963). In fact, phytate may have three additional physiological roles.

1. Experiments have shown that the phytate molecule is dephosphorylated in a stepwise fashion throughout germination and during the earliest stages of seedling growth (Mihailovic, et al., 1965), indicating that it provides the growing plant with a time-released pool of inorganic phosphorus (Hall and Hodges, 1966), (Abernathy, et al., 1973).

2. Phytate may also function in the onset of seed dormancy since the stepwise phosphorylation of the inositol molecule from inositol monophosphate to inositol hexaphosphate involves a drain on the ATP pool within the seed (Sobolev and Rodionova, 1966; Mandel and Biswas, 1970). Williams (1970), measured the concentration of ATP and found that it did not fall during the synthesis and phosphorylation of phytate during seed ripening. However, he did hypothesize an alternative mechanism through which phytate might facilitate seed dormancy. As shown in Figure 1, phytate has twelve replaceable hydrogen atoms. It is this characteristic which gives phytate the property of forming insoluble precipitates by chelating divalent metals such as calcium,

iron, zinc and manganese in the order of stability Zn^{++}, Mn^{++}, Fe^{++}, Ca^{++}, (Vohra, 1965). These metals are often cofactors in enzymatic reactions within the seed. If, during ripening, they are tightly chelated, then metabolic activities are reduced and dormancy may be initiated (Williams, 1970). Williams recognized, however, that this is a secondary mechanism possibly supplementing hormonal control of dormancy.

3. Finally, following the stepwise dephosphorylation during germination, the remaining myo-inositol may function as a precursor for cell wall polysaccharide biosynthesis (Loewus, 1974).

The physiological role of phytate is quite speculative and needs clarification.

Shortly after isolation and identification, phytate became of interest to nutritionists. Early in the 1900's phytin, the calcium-magnesium salt of phytic acid, was produced commercially and sold both as a tonic and as a source of what was believed to be readily utilizable phosphorus (McCance and Widdowson, 1935). However, considerable time elapsed before its anti-nutritional characteristics were elucidated. In 1949, Mellanby conducted nutritional experiments with diets consisting of high extraction cereal flours. Mellanby found these diets had a strong ricketogenic effect on puppies due to phytic acid complexes with calcium. He also recognized that the ricketogenic effects of white flour were significantly less than the respective whole flour counterparts. Later, chemical evaluations proved that lower extraction flours decreased the phytate concentration by as much as ninety percent (McCance and Widdowson, 1935). Hoff-Jorgensen, et al. (1946), carried out animal and human studies which introduced an age factor into

responses to dietary phytic acid. They first showed that puppies were less severely affected than older dogs by dietary phytic acid. Similar studies with human infants and older children followed. In both experiments, the results were similar; high levels of phytic acid decreased calcium availability more severely in older than in younger members of the same species. Recently, equivalent experiments were repeated with chickens to explain this differential age response. Older hens had a decreased activity of phytase, the enzyme responsible for phytate hydrolysis (Maddaiah, 1964).

One possible reason why nutritional work with phytate came to a near standstill in the 1950's may have been a report by Walker, et al. (1948). Walker and his coworkers designed an experiment in which four healthy European males were fed two experimental diets for periods ranging from four to nine weeks each. Following a control period, a diet consisting of whole wheat "war-bread" was fed for two to nine weeks followed by a 70% extraction white bread diet for a duration of one to nine additional weeks. Balance studies for calcium, magnesium and phosphorus were conducted. Walker's conclusions were that, 1) phytate was present in the intestine as phytin, 2) phytin was hydrolyzed at such a point in the digestive tract as to allow the absorption of calcium, magnesium and phosphorus, and 3) over time, the human gut can adapt itself to the presence of phytate and maintain nutrient equilibria.

The validity of these conclusions remains in question today although differences in opinion are not as great as they were then. McBean and Speckman (1974) claim that although very little is known about the interrelationships of different dietary components on mineral metabolism, the body can adapt to low levels of calcium availability

both by decreasing renal excretion and by increasing absorptive capacity of the gut. A number of questions arise from such a statement: 1) For how low a level of mineral availability can the body be expected to compensate? 2) Since phytate seems to be the complexing agent involved, how much dietary phytic acid is tolerable? 3) If a differential response to equivalent amounts of ingested phytate exists, when is the ability to adapt to high phytate intakes acquired, how long does it last and how is it manifested? 4) Is rickets the only nutritional malady upon which phytate exerts an effect or can other diseases be attributed to its ability to chelate metal ions?

In the past fifteen years scientists have begun investigating some of these questions. Iron deficiency anemia was identified in Iran in 1961 (Prasad, et al.). Over a period of two years Prasad and his coworkers studied eleven patients admitted to the Nemazee Hospital at the University of Shiraz, Iran for the following clinical features: short stature, hypogonadism, hepatosplenomegaly and iron deficiency anemia. Upon treatment with oral doses of iron, each patient promptly exhibited signs of recovery. Serum zinc was determined on only one patient and was found to be half the normal level. Prasad hypothesized that the same factors governing the absorption of iron also control the absorption of zinc. In fact, a number of the features which Prasad originally attributed to iron deficiency were probably caused by a deficiency in zinc. Additional evidence has since been added to this hypothesis by other workers in Iran (Reinhold, 1972; Reinhold, et al., 1973). It is now generally agreed that sexual dysfunction and dwarfism are manifestations of zinc deficiency (Halsted, 1972).

An interesting discovery by the researchers at Shiraz was the high incidence of geophagia, or clay eating, among the subjects they studied (Prasad, et al., 1961; Reinhold, 1972, Halsted, et al., 1972). Without exception, all subjects studied had, for an extended period of time, practiced geophagia. An American medical student living in a village in Iran in 1960 studied this phenomenon in 171 village residents (Greenwald, unpublished). He found no one in this village who had a history of prolonged geophagia without exhibiting mineral deficiency symptoms or deficiency symptoms without a history of geophagia (Prasad, et al., 1961).

Halsted, et al. (1974) claimed that geophagia is a result of zinc deficiency; that is, individuals whose tissue zinc concentrations have fallen below a given level develop a physiological craving for zinc which drives them to eat dirt. However, Reinhold (1972) and Prasad, et al. (1961), believe that geophagia, which is normally restricted to young children, is practiced because it relieves hunger and possibly because of the pleasing taste of clay. The latter explanation seems more reasonable in light of the work done by Byrd and Matrone (1965). This study provided chemical evidence to prove the theory that a triple-factor interaction exists between zinc, calcium and phytate. Other workers had noticed that mineral absorption was decreased in animals fed diets of high extraction flours and high levels of dietary calcium (O'Dell, 1969; Reinhold, et al., 1973; Oberleas and Prasad, 1969; Likuski and Forbes, 1965; Likuski and Forbes, 1964). Prasad attributed this to an interaction between phosphate and calcium, a fact indirectly correct since the phosphate content of high extraction flours can be traced to phytate. However, the clays typically eaten in Iran are highly

calcareous (Reinhold, 1972), which adds credence to the theory of Reinhold and Prasad. A third factor, zinc, is also involved. "Since zinc is a trace element, it does not supply enough cations to form a significant precipitation of zinc-phytate. However, calcium cations increase the total cationic environment sufficiently to initiate a co-precipitation with zinc to form insoluble phytates. Therefore, zinc in the presence of calcium would not be as available for absorption and zinc deficiency would be aggravated," (Byrd and Matrone, 1965). It is possible that zinc deficiency is even more complex since addition of histidine to low zinc soybean meal chick rations has been shown to cause remission of some zinc deficiency symptoms (Dahmer, et al., 1966).

The mystery of complexing factors in the human diet is far from being solved. Evidence indicates that high phytic acid levels indirectly affect many physiological activities of the body. For instance, Atkinson, et al., 1972, found lower carboxypeptidase activity in zinc deficient rats indicating an indirect effect of phytate on protein metabolism. This could possibly explain the malnourished state of the subjects studied in Iran, many of whom exhibited symptoms similar to kwashiokor.

Many approaches have been offered as solutions to the phytate problem. Phytase exists in insufficient quantities in the human gastrointestinal tract to handle the levels of dietary phytic acid characteristics of many areas of the developing world. However, phytase is produced by a number of fungi and therefore can be produced commercially and utilized as a food additive. This, however, has little applicability to the developing world where food processing is almost nonexistent. Reinhold (1975) found that significant destruction of phytate in

unleavened bread products could be achieved if the dough was allowed to stand for at least two hours. This would not be an insurmountable cultural change for the people involved. Finally, genetic manipulation of phytate within the seed might be possible if sufficient genetic variation exists. This seems a viable alternative since it is believed that different classes of wheat have different phytate contents (Mattern, pers. comm.), however, genetic manipulation may cause repercussions in terms of the physiological functions of the seed. Consequently, the physiological and genetic parameters of phytate must be studied concurrently.

MATERIALS AND METHODS

The wheat breeding program at Michigan State University is set up such that all lines under initial consideration are grown in preliminary comparison nurseries and, after evaluation for yield and quality characteristics, the most elite materials entered in advanced regional yield trial nurseries. Samples for this study were taken from the latter only.

Advanced regional yield trials were planted at five locations throughout southern Michigan. The sites chosen represent the heterogeneous nature of the environments in which Michigan wheats are grown. The 1974 nurseries were located in Monroe, Lenawee, Ionia, Tuscola, and Berrien Counties and are designated M31, L41, S51, T61 and B91, respectively. The thirty soft winter wheat entries included in each nursery were identical and consisted of 12 commercial varieties and 18 advanced selections from the Michigan State University wheat breeding project. Entries were grown as four-row plots, each row twelve feet long with twelve inches between rows. The nursery design was a 5 X 6 rectangular lattice with each entry replicated three times. Only the middle two rows of each plot were harvested, and after yield and test weight evaluations were made, a twenty gram subsample was taken from each plot for phytate analysis. In addition, 42 lines selected for winter hardiness characteristics from the U.S.D.A. world wheat collection were planted in unreplicated headrows in a completely randomized design in two locations. All samples were ground in a Udy Cyclone Sample Mill, stored for one week in the same room so that moisture levels were constant and then sealed in airtight glass containers until needed.

Oberleas (1971) has outlined a number of quantitative techniques for the determination of phytate and inositol phosphates. In order to analyze the large volume of material to be included in this study, a relatively rapid determination procedure was desirable. Consequently, the procedure decided upon was Oberleas' iron method. This technique makes use of the fact that phytate is soluble in 0.6% HCl. Extraction of phytic acid from the whole grain flour was accomplished by shaking 2.5 grams of flour in 1.2% HCl. Ten ml. of filtrate was diluted with deionized water to bring the acid concentration to 0.6% and ferric chloride added to facilitate the formation of the flocculent ferric-phytate precipitate. The precipitate was centrifuged at 4500 rpm, washed with a solution of 0.6% HCl and 4% Na_2SO_4, (sodium sulfate added to increase the firmness of the precipitate) and centrifuged again. The last step was repeated after which the precipitate was dissolved in 3 ml. of concentrated H_2SO_4 and brought to volume in 100 ml. volumetric flasks. Iron concentrations were determined on a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer. Finally, a conversion factor, based on the fact that iron binds to phytate in an ion-molecule ratio of 4 Fe:1 phytate, was used to convert ppm iron into percent phytate. This ratio does not agree with the structure of the phytate molecule shown in Figure 1. This is because a phytate molecule not only chelates iron, but may also become bound to other phytate molecules; a stereochemical process which overcomes the steric hindrances of the inositol hexaphosphate molecule.

Accuracy of the above procedure was determined daily by analyzing duplicate samples of a check variety from a commercial seed source. Also

a number of different concentrations of commercially prepared phytic acid were analyzed midway through the study to determine recovery rates which proved to be nearly 100%. Finally, all samples were replicated in the laboratory and reanalyzed if the values for percent phytate for the two replicates showed more than a 10% difference.

Chelation capability will be defined in this thesis as the maximum amount of iron capable of being bound by the phytate in a 100 gram sample of flour. Since the molecular weight of phytate is 468.05 and the atomic weight of iron is 55.8, the phytate in 100 grams of flour is capable of binding 223.2 grams of iron (4 x 55.8) once the flour is ingested. Since our values for phytate are expressed as percentages and chelation capabilities based on 100 grams of whole grain flour, multiplication of the percent phytate by a constant (0.4769) equals the chelation capability.

Analysis of variance was conducted within and between locations to determine the significance of entry (variety) differences and genotype X environment interaction. Analysis as randomized blocks was considerably more efficient than analysis as rectangular lattices. Therefore, appropriate adjustments in analysis procedures were made.

RESULTS

Analysis of variance tables for individual nurseries may be seen in Appendix B. Table 1 shows that mean values of phytate for entries within nurseries ranged from a high of M31 of 1.240% to a low in S51 of 0.819% with an overall mean of 1.03% and a standard deviation of 0.1220.

Table 1. Location means and ranges of percent phytate for 30 soft wheat lines grown in the 5 advanced regional yield trial nurseries for 1974.

Nursery	$\bar{X}$	High Line	Low Line
M31	1.045	1.240	0.842
L41	1.032	1.131	0.917
S51	0.955	1.045	0.819
T61	1.002	1.131	0.898
B91	1.112	1.222	0.964

Table 2, which summarizes the results of LSD tests performed on each nursery, shows that at the 5% level of probability, a large number of the varieties in each nursery had phytate values significantly different from the line with the highest percentage phytate. This was also true at the 1% level of probability.

Table 2. Numbers of lines significantly less than the highest line and their percentage of the total of 30 entries according to LSD tests on intranursery entry means for percent phytate.

	Locations									
	M31		L41		S51		T61		B91	
	# of sig. lines	% of total	# of sig. lines	% of total	# of sig. lines	% of total	# of sig. lines	% of total	# of sig. lines	% of total
$LSD_{.05}$	11	37	14	47	8	27	4	13	10	33
$LSD_{.01}$	2	7	8	27	3	10	1	3	4	13

Internursery entry means and their respective chelation capabilities are presented in tabular form in Table 3 and graphically in Figure 2. The highest internursery entry mean was found to be 1.093% and the lowest 0.955% giving a range of 0.138%.

Table 4 shows the rank for the means of individual entries over locations. Obviously, quite a bit of variability in rank exists, however, entry 2 was found to be stable for low phytate at all five locations and entries 6, 22 and 25 were relatively stable for high phytate at four of the five locations. Numerous other lines exhibited equivalent responses at three of the five nurseries. At first glance, the variability in rank might be indicative of an entry X location interaction. This was not the case as the internursery analysis of variance in Table 5 shows. Differences among entry means were found to be highly significant, but their interaction with location showed no significance ($P<.454$). Separation of variance components, shown in Table 6, further illustrates this fact. Locations, replications and entries account for over ninety-eight percent of the total variance while interaction of location X entry accounts for an insignificant 1.15%. Correlation coefficients were calculated between phytate and yield and phytate and test weight but no significant correlation was found.

Analysis of lines from the U.S.D.A. world wheat collection indicated a highly significant difference between environments, ($P<.0005$), as shown in Table 7, however, differences in entry mean values of phytate were less significant ($P<.077$) than differences between entry means in the regional yield trials. A slightly wider range in phytate values was observed in these lines as compared to the regional yield trials. This can be seen in Table 8 where the lowest line had a phytate value

(averaged over two locations) of 0.895% and the highest line 1.070% for a total range of 0.175%. This difference may be of no significance since a wider range would be expected with fewer observations per line and more lines.

Table 3. Mean phytate content and chelation capacities of the 30 lines included in the 1974 advanced regional yield trial nurseries.*

Entry #	% Phytate	Chelation Capacity (mg. Fe/100g. Flour)
1	1.075	512.7
2	0.968	461.6
3	1.016	484.5
4	1.012	482.6
5	0.955	455.4
6	1.078	514.1
7	1.027	489.8
8	1.002	477.9
9	0.967	461.2
10	1.011	482.1
11	1.043	497.4
12	1.093	521.3
13	1.049	500.3
14	0.999	476.4
15	1.021	486.9
16	1.053	502.2
17	0.997	475.5
18	1.058	504.6
19	1.056	503.6
20	1.023	487.9
21	1.029	490.7
22	1.044	497.9
23	1.052	501.7
24	1.057	504.1
25	1.044	497.9
26	1.025	488.8
27	1.070	510.3
28	0.969	462.1
29	1.049	500.3
30	1.038	495.0

Standard error entry mean = 0.1220

*Mean of 3 replications/location and 5 locations

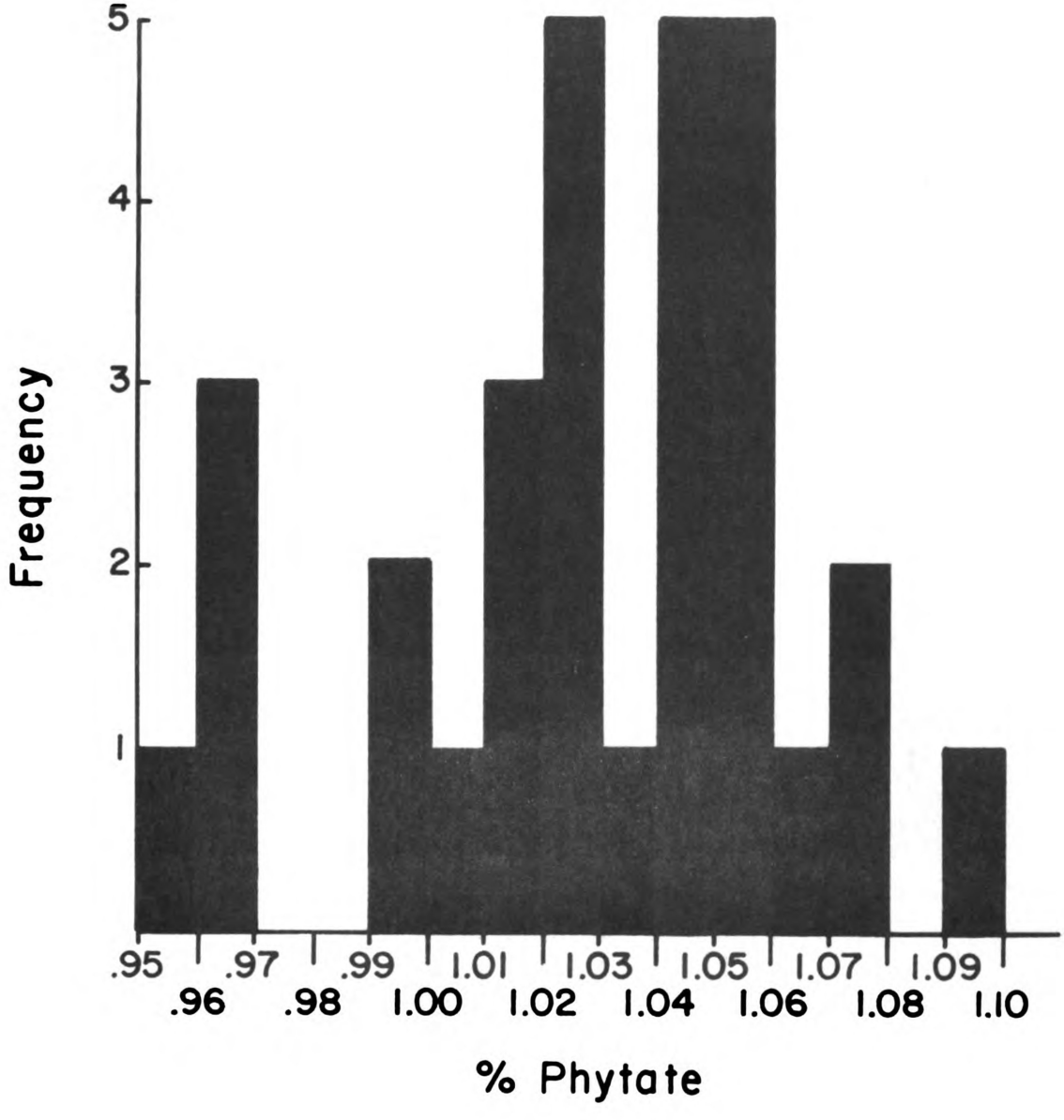

Figure 2. Distribution of internursery entry means of percent phytate for the 30 lines in the advanced regional yield trial for 1974.

Table 4. Ranks of entry means for percent phytate in descending order for the 30 lines included in the 1974 advanced regional yield trial **study.**

Variety Entry #	Nurseries M31	L41	S51	T61	B91
1	1	4	11	27	15
2	27	28	25	25	29
3	23	2	19	19	27
4	20	15	17	15	25
5	30	30	22	14	21
6	3	11	8	6	19
7	15	29	18	11	7
8	7	27	21	30	11
9	29	19	30	13	17
10	6	21	20	28	23
11	5	25	4	23	16
12	2	12	3	17	6
13	9	26	5	24	1
14	8	13	29	26	26
15	17	22	27	12	4
16	12	14	16	2	14
17	24	23	23	8	28
18	26	7	2	16	3
19	18	1	24	3	5
20	21	8	9	20	24
21	10	24	28	5	20
22	13	9	7	22	10
23	19	17	26	7	2
24	14	20	10	4	8
25	16	10	12	9	9
26	11	18	13	21	22
27	4	17	15	1	18
28	28	16	14	29	30
29	22	6	1	10	13
30	25	3	6	18	12

Table 5. Internursery analysis of variance for percent phytate in 30 lines of soft wheat included in the 1974 advanced regional yield trial study.

Source	df	SS	MS	Sig. of F Statistic
Location	4	1.2144	0.3036	0.066
Reps within location	10	0.9735	0.0973	
Entries	29	0.5303	0.0183	0.005
Locations X Entries	116	1.1432	0.0099	0.454
Residual Error	290	2.8172	0.0097	
Total	449	6.6785		

Table 6. Separation of variance components from mean squares derived from the internursery analysis of variance.

Source	Expected Mean Squares
Locations	$MS_1 = \sigma^2 + el\sigma_L^2 + reo_L^2$
Reps within location	$MS_2 = \sigma^2 + eo_R^2$
Entries	$MS_3 = \sigma^2 + r\sigma_{LE}^2 + rl\sigma_E^2$
Locations X Entries	$MS_4 = \sigma^2 + r\sigma_{LE}^2$
Residual Error	$MS_4 = \sigma^2$

L = Locations (5)
R = Replications (3)
E = Entries (30)

$$\sigma_L^2 = \frac{MS_1 - MS_2}{re} = 0.00229 \quad (39.23\%)$$

$$\sigma_R^2 = \frac{MS_2 - MS_5}{e} = 0.00292 \quad (50.03\%)$$

$$\sigma_E^2 = \frac{MS_3 - MS_4}{rl} = 0.00056 \quad (9.59\%)$$

$$\sigma_{LE}^2 = \frac{MS_4 - MS_5}{r} = 0.000067 \quad (1.15\%)$$

Table 7. Analysis of variance of percent phytate of 42 lines from the U.S.D.A. world wheat collection grown in unreplicated headrows at two locations in 1975.

Source	df	Mean Square	Significance of F Statistic
Locations	1	0.0677	0.0005
Entries	41	0.0026	0.077
Error	41	0.0016	
Total	83		

Table 8. Identification and origin of 42 lines from the U.S.D.A. World Wheat Collection grown in unreplicated headrows in two locations in 1975 and analyzed for percent phytate from which chelation capacity was computed.

I.D.#	Origin	% Phytate	Chelation Capacity (mg. Fe/100 g.)
CI012514	Texas	0.970	462.6
PI294987	Bulgaria	0.895	426.8
CI007166	China	0.913	435.4
PI285999	Poland	0.918	437.8
PI294992	Bulgaria	0.948	452.1
PI254839	Austria	0.965	460.2
CI011515	Nebraska	0.962	458.5
CI011724	Wisconsin	0.965	460.2
CI005086	China	0.979	466.9
PI167406	England	0.958	456.9
CI011676	Kansas	0.953	454.5
CI011879	Wisconsin	1.005	479.3
PI285974	Poland	0.975	465.0
*SD072447	South Dakota	0.939	447.8
*SD072448	South Dakota	0.953	454.5
CI007166	China	0.943	449.7
PI294987	Bulgaria	0.963	459.3
PI262661	U.S.S.R.	1.003	478.3
CI014486	Montana	0.973	464.0
CI006938	Canada	0.988	471.1
CI013670	Canada	1.018	485.5
CI008033	Montana	1.008	480.7
CI006914	U.S.S.R.	0.999	476.4
CI013547	Nebraska	1.030	491.2
PI262636	U.S.S.R.	1.020	486.4
CI014000	South Dakota	1.008	480.7
CI010722	China	1.013	483.1
**CI015327		1.054	502.7
CI012138	Minnesota	0.995	474.5
CI005149	Minnesota	0.980	467.4
PI167406	England	0.960	457.8
CI008034	Montana	0.970	462.6
CI005549	Montana	0.963	459.3
CI011676	Kansas	1.002	477.9
PI254839	Austria	1.036	494.1
CI012138	Minnesota	0.973	464.0
PI278467	U.S.S.R.	0.997	475.5
PI285974	Poland	1.008	480.7
PI294937	Bulgaria	0.977	465.9
PI285999	Poland	0.983	468.8
PI267143	U.S.S.R.	0.958	456.9
PI262632	U.S.S.R.	1.070	510.3

Standard error of entry mean = 0.054

*CI or PI numbers not available

**Variety Sundance, origin unknown

DISCUSSION

The thirty entries included in the advanced regional yield trial nurseries are soft winter wheats proven to be of good pastry quality, high yielding and well adapted to Michigan environments. These lines vary in insect resistance, disease resistance, height, yield, test weight and winter hardiness. Since no selection pressure has been exerted for phytate content they are assumed to be quite variable for this trait also. However, it can be seen in Table 9 that there is a preponderance of genes from the varieties Genesee, Arthur and Redcoat in the pedigrees of many of these lines. Therefore, one cannot assume that they represent a randomly distributed sample population of all wheats for phytate even though they have never been selected for this character. As an additional check on variability for phytate levels, winter wheats of widely divergent genetic origin from the U.S.D.A. world wheat collection were also tested (see Table 8). Significant variability for phytate was found in the advanced regional yield trials and is evidenced by the fact that an overall average of 31% of the lines in each nursery were statistically significant at the .05 level.

The inheritance of phytate was not examined but appears to be complex since in the absence of any genotype X environment interaction, only one genotype responded equally in all environments.

Although the possibilities for genetic manipulation of phytate are substantial, a more comprehensive survey of world wheat germplasm must be completed before conclusive statements may be made on the total amount of variability available.

Table 9. Names or accession numbers and pedigrees of the thirty varieties and lines included in the 1974 advanced regional yield trials.

Entry Numbers	Variety or Michigan Accession No.	Pedigree
1	Genesee	Yorkwin//Honor/Forward
2	Yorkstar	Genesee*3/3/Yorkwin//Brevor/Norin 10
3	Arrow	Avon sib.//Heines VII/Cornell
4	Ionia	Genesee*3/Redcoat
5	Frederick	Washington sel. 101/Genesee//C.D. 6707
6	Tecumseh	Minhardi/Wabash/5/Fultz sel./Hungarian/2/ W38/3/Wabash/4/Fairfield/6/Redcoat sib./Wisc. CI012633/7/Vigo/4/Trumbull/2/Hope/Hussar/3/ Fulhio/Purkof (Purdue 427a1-1-3)*3/5/Kenya Farmer
7	Ticonderoga	Genesee/4/82a1-2-4-7 (NY wheat-rye)/3/Genesee// Caldwell 8/ Cornell 595/5/Heines VII/6/ Genesee*2//Brevor/Norin 10/3/Avon sib. (NY 4848-2)
8	Arthur	Minhardi/Wabash/5/Fultz sel./Hungarian/2/W38/ 3/Wabash/4/Fairfield/6/Redcoat sib./Wisc. CI012633/7/Vigo/4/Trumbull/2/Hope/Hussar/3/ Fulhio/*Purkof/5/Kenya Farmer
9	Arthur 71	Arthur*5/3/Purdue 6028A2-15-9-2/2/Riley sib. *2/Riley 67
10	Abe	Arthur*4/3/Purdue 6028A2-15-9-2/2/Riley*2/ Riley 67
11	Oasis	Arthur 71/5/Arthur*3/3/Ribox/2/Riley*2/ Riley 67 (Purdue 6559 sel.)*2/4/Arthur*2/ 3/Riley 67*2/2/Riley/Bulgaria 88 (PI94407)
12	Logan	Vermillion/Lucas
13	A7170	Genesee*2/Redcoat//Talbot
14	B0272	Genesee/Redcoat//Genesee*2/Redcoat/4/Norin 10/Brevor//Yorkwin/3/2*Genesee
15	B0253	Genesee*2/Redcoat/3/Suwon 92/Brevor//5*Genesee
16	A9094	Asosan/3*Genesee
17	A9096	German MI/3*Genesee

Table 9. Continued

Entry Numbers	Variety or Michigan Accession No.	Pedigree
18	A7054	Genesee*2/Redcoat
19	A7055	Genesee*2/Redcoat
20	B0223	Purdue 5517-a1/3/Suwon 92/Brevor//5* Genesee
21	A8175	Suwon 92/Burt//3*Genesee/4/Norin 10/Brevor// Yorkwin/3/2*Genesee
22	B0215	Asosan/4*Genesee
23	B0261	Asosan/4*Genesee
24	B0216	Asosan/3*Genesee//Genesee/Redcoat
25	B0270	Asosan/3*Genesee/4/Norin 10/Brevor//Yorkwin/ 3/2*Genesee
26	A8177	German MI/3*Genesee
27	B0254	German MI/3*Genesee
28	B0255	German MI/3*Genesee
29	A9051	AC4835 (Durum Fly Resistance)/4*Genesee
30	B0240	AC4835 (Durum Fly Resistance)/5*Genesee

Although no significant genotype X environment interaction was identified, environment did play a role in determining phytate levels in wheat. This can be seen by the analysis of variance and separation of variance components for the regional yield trials and the analysis of variance of the U.S.D.A. lines. In both experiments macro- and micro-environments had an effect. The fact that variance due to replications in the regional yield trials accounted for about 50% of the total variability is an indication of the differential genetic response to soil heterogeneity. The extent of this heterogeneity follows from analyses of soil samples shown in Table 10. Unfortunately, these were gross samples representative of soil conditions for entire nurseries and therefore do not supply the information needed to precisely determine the soil factors responsible for the large variation between replications. Future studies should include plot-by-plot soil analyses to overcome this difficulty. Macro-environmental effects are shown by the significance levels of the location main effects in the analysis of variance. The contribution of individual main effects is substantial, explaining in part the variability of entry ranks between nurseries seen in Table 4. Apparently main effects compound one another in a random fashion resulting in a variety of entry responses but not necessarily in a genotype X environment interaction.

Lowering phytate a few tenths of a percent seemed of little consequence so a method was devised which brought this seemingly miniscule adjustment into proper perspective. Since the phytate determination procedure involved evaluation of the ability of the phytate in various lines of wheat to chelate iron, the method facilitated determination of the actual weight of iron, in milligrams chelated by 100 grams of whole grain

flour. This number was defined as an entry's chelation capacity. On the basis of the intranursery entry means mentioned previously, a difference of 0.421% phytate exists (1.240% - 0.819%). Phytate chelates iron in a molecular ratio of 1:4 (phytate:iron). Consequently, 1.240 grams of phytate is capable of binding 591 milligrams of iron, whereas, 0.819 grams of phytate has the capability of chelating only 391 milligrams; a difference of 66%. This decrease in phytate also means a decrease in phosphorus within the seed. This is apparently accomplished without any adverse effect on germination or seedling vigor since a comparison of ranks based on phytate and yield shows no relationship. The lines lowest in phytate are not necessarily the lines lowest in yield; in fact, the variety Yorkstar which has the lowest phytate content over the five nurseries had the highest yield over these nurseries. Nutritionally, the effect of lowering the chelation capability of wheat 66% may prove important to populations whose primary food source is wheat. Nevertheless, it would appear from the data that even the lowest level of phytate in the wheats analyzed is capable of chelating the total daily dietary intake of metal cations many times over. If this is so, what good would it do to lower phytate? There exists a multiplicity of answers to this question. Phytate is concentrated in the outer layers of the wheat kernel. In the rural areas of developing countries where phytate has the most significant nutritional impact, the crude milling practices employed fail to pulverize the bran layer into fine flour. This results in a number of phytate molecules, being closely associated with each other and probably partially bound together in the coarse bran fraction of the flour. Some of the binding sites may also have already been used to chelate metal ions in the intact seed. As the phytate enters the upper intestine,

Table 10. Results of analyses on soil samples taken in October 1973 for five of the advanced regional yield trial nurseries.

	pH	P*	K*	Ca*	Mg*	Zn**	Fe**
M31	5.7	115	180	1309	125	11.6	40
L41	6.6	273	660	3200	540	15.2	132
S51	6.8	30	154	4571	640	8.0	--
T61	7.2	128	324	5788	870	8.4	24
B91	6.4	121	348	3055	648	7.6	24

* Pounds per acre in upper 8" of soil
**Parts per million in upper 8" of soil

the primary absorption region for metal ions, lower phytate levels obviously indicate a greater dilution effect within the lumen. The probability of a phytate molecule and a metal cation meeting in exactly the proper orientation to form a chelation product is, therefore, greatly reduced. When all these factors are considered, even a relatively small reduction in phytate would have a marked effect on mineral availability.

The interrelationships of many physiological components of the seed must be studied before the nutritional impact of any one component may be fully understood. For example, research is presently underway to determine the feasibility of genetically decreasing the levels of certain protease inhibitors in seeds which have the effect of lowering the digestibility of plant proteins. However, if this is accomplished the results might be indirectly related to mineral availability since phytate is closely associated with plant protein. The sooner bran proteins are hydrolyzed in the upper part of the gastrointestinal tract, the sooner free phytate will become available for chelation of metals.

Since so little is understood about the physiological role of phytate within the seed it would be naive to attempt to genetically alter phytate levels without conducting concurrent studies correlating germination, dormancy and seedling vigor with various phytate levels.

Before plant breeders undertake a detailed genetic analysis of phytate a new determination procedure must be made available. The iron method in current vogue is too time consuming to accommodate the large volume of material plant breeders must consider. A coloremetric quantification procedure for phosphorus is available (Oberleas, 1971), which

improves the accuracy of phytate determinations but is more lengthy than the iron method. There is reason to believe, however, that a gel electrophoresis procedure will soon be published which will satisfy the requirements of both time and accuracy (Purvis, pers. comm.).

CONCLUSIONS

Genetic variability for phytate in wheat lines included in the advanced generation yield trials was identified and found to be of sufficient magnitude to warrant further investigation. Analyses of forty-two lines obtained from the U.S.D.A. small grain collection indicate that additional variability may be available.

Environmental influences on phytate were also quite strong and should be investigated further. Obviously, detailed studies of soil components both in the field, greenhouse and possibly hydroponics are needed to elucidate these effects.

Although phytate exerts little influence on the health of people eating highly diversified diets, it has profound effects on the nutritional well being of a considerable portion of the developing world's population. A decrease in phytate levels of the extent described in this study would be a big step toward bettering the diet of the world's poor.

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APPENDICES

Appendix A: Entry means of percent phytate for the 30 entries included in the 1974 advanced regional yield trial study.*

Table A1

	Nurseries				
Entry #	M31	L41	S51	T61	B91
1	1.240	1.088	0.983	0.932	1.115
2	0.977	0.965	0.894	0.956	1.014
3	0.983	1.127	0.933	0.978	1.055
4	0.997	1.031	0.957	1.005	1.065
5	0.842	0.917	0.926	1.007	1.096
6	1.158	1.058	0.996	1.060	1.105
7	1.051	0.961	0.936	1.019	1.151
8	1.091	0.965	0.929	0.898	1.129
9	0.900	1.017	0.819	1.007	1.112
10	1.127	1.004	0.932	0.915	1.090
11	1.133	0.975	1.015	0.962	1.113
12	1.224	1.050	1.023	0.991	1.152
13	1.083	0.969	1.014	0.962	1.222
14	1.087	1.050	0.874	0.934	1.064
15	1.033	0.999	0.891	1.018	1.169
16	1.061	1.039	0.961	1.094	1.117
17	0.980	0.995	0.914	1.042	1.053
18	0.977	1.084	1.025	1.002	1.171
19	1.023	1.131	0.902	1.093	1.157
20	0.997	1.081	0.995	0.970	1.080
21	1.079	0.988	0.887	1.085	1.105
22	1.059	1.064	1.006	0.969	1.130
23	1.015	1.087	0.893	1.043	1.213
24	1.055	1.006	0.987	1.090	1.151
25	1.045	1.063	0.978	1.023	1.141
26	1.066	1.024	0.977	0.970	1.092
27	1.135	1.026	0.962	1.131	1.105
28	0.953	1.031	0.969	0.904	0.964
29	0.995	1.084	1.045	1.021	1.118
30	0.980	1.107	1.012	0.981	1.124

*Means over three replications.

Appendix B: Intranursery analyses of variance of percent phytate with LSD and coefficients of variation for the 5 nurseries of the advanced regional yield trial studies in 1974.

Table B1

Location M31:

Source	df	SS	MS
Replications	2	0.10023	0.05011
Entries	29	0.64109	0.02211
Error	58	1.16478	0.02008
Total	89	1.90610	

Least Significant Differences:

P<.05 LSD = 0.2314
P<.01 LSD = 0.3008

Coefficient of Variation = 13.56%

Table B2

Location L41:

Source	df	SS	MS
Replications	2	0.16694	0.08347
Entries	29	0.23937	0.00825
Error	58	0.29825	0.00514
Total	89	0.70456	

Least Significant Differences:

P<.05 LSD = 0.1043
P<.01 LSD = 0.1356

Coefficient of Variation = 6.1838%

Appendix B: Continued

Table B3

Location S51:

Source	df	SS	MS
Replications	2	0.15522	0.07761
Entries	29	0.25772	0.00889
Error	58	0.31611	0.00545
Total	89	0.72905	

Least Significant Differences:

P<.05 LSD = 0.1206
P<.01 LSD = 0.1567

Coefficient of Variation = 7.7346%

Table B4

Location T61:

Source	df	SS	MS
Replications	2	0.33793	0.16897
Entries	29	0.30969	0.01068
Error	58	0.67988	0.01172
Total	89	1.32750	

Least Significant Differences:

P<.05 LSD = 0.1768
P<.01 LSD = 0.2298

Coefficient of Variation = 10.8040%

Appendix B: Continued

Table B5

Location B91:

Source	df	SS	MS
Replications	2	0.21317	0.10658
Entries	29	0.22557	0.00778
Error	58	0.35814	0.00617
Total	89	0.79687	

Least Significant Differences:

P<.05 LSD = .1252
P<.01 LSD = .1627

Coefficient of Variation = 6.8908%