# Phosphorus Fractions in the Grain of Diploid, Tetraploid, and Hexaploid Wheat Grown with Contrasting Phosphorus Supplies

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ABSTRACT

Phosphorus was extracted from 17 wheat genotypes (in three ploidy groups) grown at contrasting levels of phosphorus supply. Total phosphorus, which ranged from 0.14 to 0.71% P in dry mature grains, was highest in diploid and lowest in hexaploid wheats and was very highly correlated ($r = 0.99$) with phytate phosphorus. Wheat ploidy did not alter this relationship, so selection of hexaploid wheats for lower grain total phosphorus is suggested as a means to obtain lower grain phytate concentrations.


Several studies have shown that up to 90% of the total phosphorus in whole wheat grain is present as phytate, which is predominantly the potassium-magnesium salt (rarely the calcium salt) of myo-inositol hexakisphosphate (Lolas et al 1976, Nahapetian and Bassiri 1976, Lott and Spitzer 1980). Phytate occurs in the globoid crystals of protein bodies of the aleurone and embryo cells but not in the starchy endosperm (Tanaka et al 1974, Lott and Spitzer 1980).

Cosgrove (1980a) reviewed the suggested roles for phytate in seeds and listed five possible metabolic functions; namely: 1) a store of phosphorus; 2) an energy store; 3) a competitor for adenosine triphosphate as the grain approaches maturity, such that metabolism is inhibited and dormancy induced; 4) chelation and storage of multivalent cations; and 5) regulation of the concentration of inorganic phosphorus in the grain. This mechanism replaces that established for optimum metabolism in vegetative parenchyma cells where the level of inorganic phosphorus is buffered at 5–20 m$M$ by being isolated behind the membrane of the vacuole. In the developing seed the vacuole disappears, and phytate may assume the role of a buffering agent for phosphorus (Bieleski 1973, Heldt et al 1977, Michael et al 1980).

Phytate may have all or some of these functions in mature wheat grains. Information on the most likely role(s) can come from studies of the development of phytate in grains having different total supplies of phosphorus.

Other workers have demonstrated that in wheat grain the proportion of phosphorus found in phytate increases with the total phosphorus concentration (Fig. 1). If phytate is simply a storage form of phosphorus, it should be possible to produce zero-phytate grain by growing plants with a low total grain phosphorus concentration. Extrapolation of linear relationships shown in Figure 1 indicates that zero phytate could be achieved in plants that produce grain with a total phosphorus concentration of <0.1%.

The phytate content of wheat grains has been shown to vary between locations (Tabekhia and Donnelly 1982), seasons (Nahapetian and Bassiri 1976), and irrigation management (Bassiri and Nahapetian 1977, 1979), but these are all comparisons of grain grown with relatively similar supplies of phosphorus. Almost all phytate levels reported for wheat grain are also from a relatively narrow genetic range of *Triticum aestivum* genotypes.

The aim of the present study was to measure the major fractions of grain phosphorus in a genetically diverse range of wheats with a large range in grain phosphorus concentration.

## MATERIALS AND METHODS

The wheats used in this study, their origin, and ploidy are listed in Table I. These represent a portion of a larger study on the uptake and utilization of phosphorus by diploid, tetraploid, and hexaploid wheats (Batten 1986).

### Plant Growth

Wheat plants were grown one per pot in 1 kg of sand. Nutrients were supplied each morning as a Hoaglands no. 2 nutrient solution (Hewitt 1966, with modifications to the minor element), which contained either 1 m$M$ P (control) or 0.25 m$M$ P (low P). The nutrient solution for the low-P plants was changed 21 days after planting to one containing no phosphorus. Grain from two plants (replicates) of each of the 16 lines grown at each of the phosphorus regimes was collected at maturity.

### Phosphorus Analyses

Finely crushed grain samples (100 mg) were extracted twice with trichloroacetic acid (TCA) (5 ml, 0.3$M$) for 45 and 30 min, respectively, diluted to 0.1$M$ TCA with distilled water (10 ml each time), centrifuged (3,000 × $g$ for 10 min); the supernatant was filtered (Whatman No. 42 paper), and placed on an anion exchange

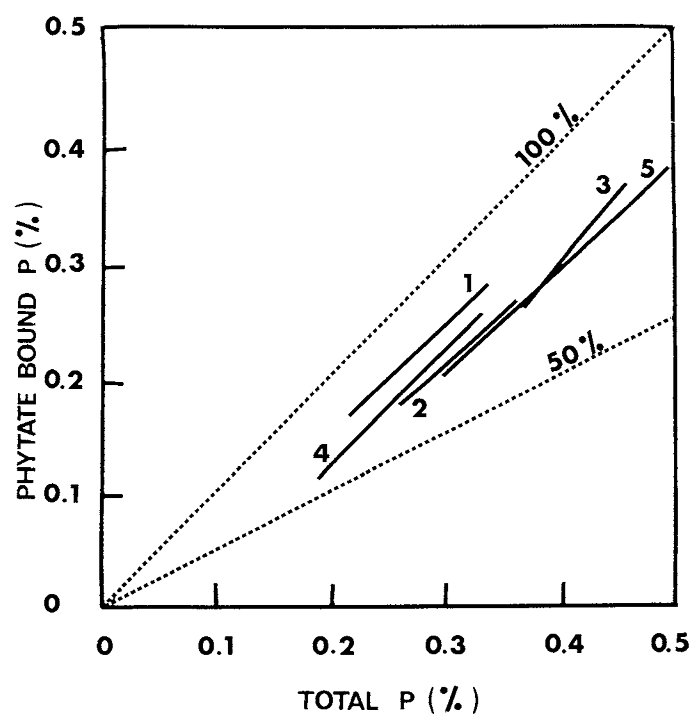


**Fig. 1.** Examples of the relationship between total phosphorus (TP) and phytate phosphorus (PP) in wheat. The theoretical 50 and 100% relationship lines have been included to emphasize that PP as a proportion of TP increases with TP and is usually more than 50%. Lines: **1** and **2**, wheat grown under dryland conditions at Wagga Wagga, Australia (Batten and Khan, *unpublished data*); **3** and **4**, wheat grown in Iran (Nahapetian and Bassiri 1976); **5**, wheat grown in Michigan and Kansas (Lolas et al 1976).

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resin column (Dowex AG1 X2 200–400 mesh; 5 × 1 cm). The column was eluted with TCA (70 ml, 0.1*M*) to recover inorganic and possibly inositol-1-phosphate; then eluted with HCl (49 ml, 1*M*) to recover phytate (Cosgrove 1980b). The grain residue that remained after the TCA extractions was digested by the Kjeldahl procedure (Williams and Twine 1967), while duplicate aliquots of the TCA and HCl elutants were digested in $HClO_4$, to mineralize phosphates which were then determined using the orthophosphate-molybdenum blue technique (John 1970). The phosphorus in the 0.1*M* TCA, 1*M* HCl, and 0.3*M* TCA-insoluble fractions are referred to here as inorganic, phytate, and residue phosphorus. Total grain phosphorus was calculated as the sum of these three determinations.

## RESULTS AND DISCUSSION

The total phosphorus concentration in the grain of wheat grown in the field varies between 0.11% (Batten and Khan, unpublished

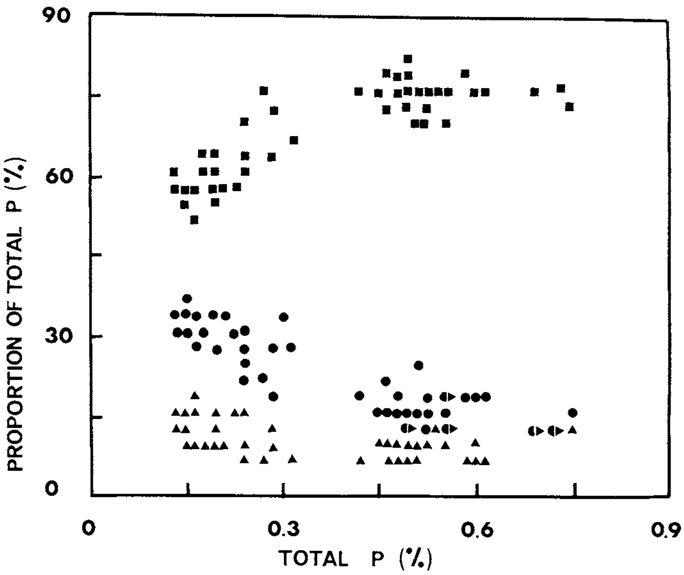


**Fig. 2.** Proportion of the total phosphorus in mature grain present in the inorganic (▲), phytate (■), or residue (●) fractions of grain with different total phosphorus concentrations.

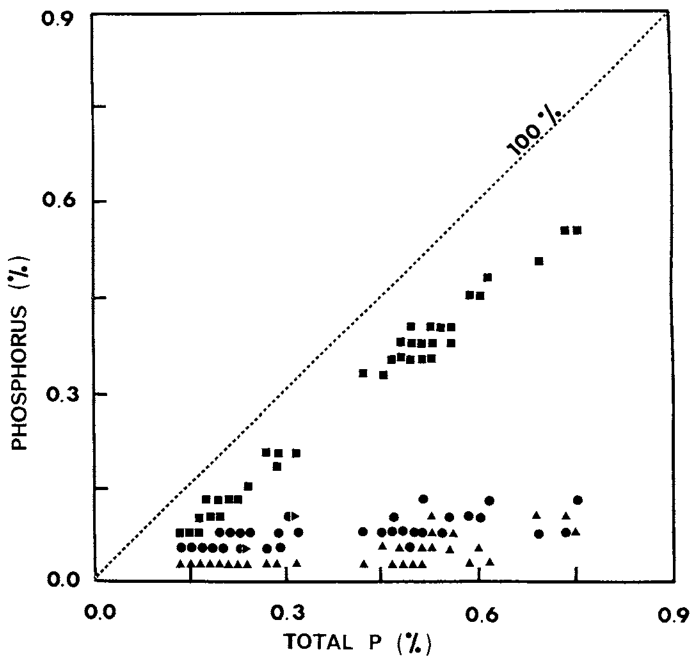


**Fig. 3.** Relationships between inorganic (▲), phytate (■), and residue phosphorus (●) in mature grains with different total phosphorus concentrations.

**TABLE I**
**Classification, Kernel Weight, Protein, and Total Phosphorus in Wheats Analyzed**

| Classification[a] | Genome | Line | Source of Seed[b] | Kernel Weight (mg) Control | Kernel Weight (mg) Low P | Protein (%N)[c] Control | Protein (%N)[c] Low P | Total Phosphorus (%P) Control | Total Phosphorus (%P) Low P |
|---|---|---|---|---|---|---|---|---|---|
| Diploid (2*n* = 14) | | | | | | | | | |
| *Triticum monococcum* ssp. *boeoticum* (Boiss) L. et L. | A | TBI | 1 | 15 | 12 | 4.07 | 4.05 | 0.640 | 0.274 |
| *Aegilops speltoides* (Tausch) | B | 6001 | 1 | 12 | ··· | 4.08 | ··· | 0.710 | ··· |
| Tetraploid (2*n* = 28) | | | | | | | | | |
| *Triticum turgidum* (L.) Thell. | | | | | | | | | |
| ssp. *dicoccoides* (Korn) Thell. | AB | W1043 | 1 | 52 | 29 | 3.89 | 3.81 | 0.580 | 0.211 |
| | AB | T6253 | 1 | 52 | 32 | 4.46 | 4.00 | 0.610 | 0.266 |
| ssp. *dicoccum* (Schrank) Thell. | AB | Khapli W12 | 1 | 53 | 37 | 3.21 | 3.51 | 0.496 | 0.196 |
| ssp. *turgidum* | | | | | | | | | |
| conv. durum (Desf.) MK | AB | Kubanka | 1 | 64 | 41 | 3.17 | 2.89 | 0.519 | 0.145 |
| | AB | Calibasan | AUS20678 | 98 | 78 | 3.76 | 2.85 | 0.498 | 0.170 |
| conv. polonicum | AB | Levissimum | AUS3823 | 98 | 87 | 3.66 | 3.30 | 0.470 | 0.264 |
| Hexaploid (2*n* = 42) | | | | | | | | | |
| *Triticum aestivum* (L.) Thell. | | | | | | | | | |
| ssp. *spelta* (L.) Thell. | ABD | H2 | 1 | 64 | 44 | 2.20 | 2.84 | 0.497 | 0.182 |
| ssp. *vulgare* (Vill.) MK | ABD | Gabo | 2 | 63 | 37 | 2.53 | 2.57 | 0.488 | 0.148 |
| | | Kite | 3 | 55 | 33 | 2.97 | 2.93 | 0.480 | 0.144 |
| | | Spica | AUS1303 | 59 | 39 | 2.97 | 2.97 | 0.463 | 0.277 |
| | | Keowechen | AUS402 | 69 | 45 | 2.65 | 2.84 | 0.483 | 0.156 |
| | | Gigue | AUS18135 | ··· | 55 | ··· | 2.59 | ··· | 0.136 |
| | | Ukraine | AUS3477 | 54 | 50 | 2.91 | 2.93 | 0.550 | 0.207 |
| | | Oligoculm 112-76 | AUS20431 | 79 | 50 | 3.65 | 3.29 | 0.545 | 0.180 |
| | | Carazinho | 4 | 62 | 49 | 2.84 | 3.27 | 0.536 | 0.188 |
| SE | | | | 4 | 3 | 0.16 | 0.14 | 0.050 | 0.024 |

[a] From Mac Key (1977).
[b] Seed sources: 1, Evans and Dunstone (1970); 2, Ian Wardlaw; 3, Previous glasshouse experiment; 4, Brendan Scott; AUS #, seed from the Australian Wheat Collection supplied by Ken Symes.
[c] Micro-Kjeldahl analyses (Williams and Twine 1967).

data for dryland wheat grown in New South Wales, Australia with a suboptimal input of phosphate fertilizer) and 0.55% P (Dikeman et al 1982, for wheat grown in Missouri, USA).

Under the low-P regime, the total phosphorus concentrations ranged from 0.14% P in bread wheats to 0.27% P in diploid wheats, and under the control-P regime ranged from 0.46% P to 0.71% P. Phosphorus concentrations in tetraploid wheats were intermediate between those of diploid and hexaploid wheats (Table I). By contrast, Lolas et al (1976) found for wheats grown in the field that two tetraploid wheats had total phosphorus concentrations which were one and two standard deviations lower than the mean for 36 bread wheats. This may reflect differences in yield, harvest index, or genotype (Batten 1986).

Phytate was the dominant phosphorus-containing compound in all wheats (Figs. 2 and 3). There was a highly linear relationship ($r = 0.99$) between phytate P and total P across all wheats grown in both phosphorus regimes (Fig. 3). This relationship is consistent with previous studies shown in Figure 1, but the range of total P obtained here, in a single controlled environment, exceeds the combined ranges for the total phosphorus of Figure 1.

It is interesting that neither phosphorus supply regime nor wheat ploidy significantly altered the relationship between total and phytate phosphorus. This is further evidence to support the suggestion that phytate phosphorus is a form of stored phosphorus that balances the level of metabolically active phosphorus in developing grain.

The concentrations of phosphorus in the inorganic and residue fractions also increased as total phosphorus increased, by threefold and twofold, respectively, over the lowest concentration in each phosphorus treatment. Such increases suggest that either there was incomplete extraction or separation of phosphorus fractions as the total grain phosphorus concentrations increased, or that phosphorus is partly stored in nonphytate forms similar to storage in organic compounds in vegetative tissues of *Spirodela* (Bieleski 1968), *Hordeum* spp. (Chapin and Bieleski 1982), and *T. aestivum* (Batten and Wardlaw, *unpublished*). However, the changes in nonphytate phosphorus were relatively small compared to that of phytate, as shown by the regression coefficients in equations 1, 2, and 3. Linear relationships were calculated between phosphorus fractions (Y) in wheat grain [Y = Tot P (± SE) + a (± SE); R2] for duplicate samples of 16 wheats grown at two phosphorus levels.

$$\text{Phytate P (\%)} = \text{Total P (\%)} \times 0.815\ (\pm 0.009) - 0.038\ (\pm 0.004)\ \frac{100\text{R2}}{99.2} \quad (1)$$

$$\text{Inorganic P (\%)} = \text{Total P (\%)} \times 0.093\ (\pm 0.009) - 0.002\ (\pm 0.004)\ 64.5 \quad (2)$$

$$\text{Residue P (\%)} = \text{Total P (\%)} \times 0.092\ (\pm 0.008) - 0.037\ (\pm 0.003)\ 69.6 \quad (3)$$

Michael et al (1980) reported similar relative changes in the forms of phosphorus in hexaploid wheat grains with between 0.266 and 0.426% total phosphorus. They used ferric chloride to separate phytate from inorganic plus ester phosphorus and, compared to the data in Figure 2, found 4–5% more phytate and hence 4–5% less inorganic plus ester phosphorus. Figure 2 also shows that the proportion of phytate phosphorus declines more rapidly when the total phosphorus content falls below about 0.3%.

Mineral deficiencies in animals have been associated with the intake of phytate (see reviews by Cheryan 1980 and Maga 1982). I consider the reduction and ultimate elimination of phytate from cereals to be a meritorious aim for nutritional reasons and because phytate phosphorus represents increased export of phosphorus from the cropping regions of the world, especially grain exporting areas, and must be replaced by fertilizer phosphorus.

Because of the linearity of the relationship and the inclusion of very low total P values in equation 1, it is possible to extrapolate and calculate that zero phytate would be expected to occur in mature grain with 0.047% total phosphorus. This concentration is smaller than the values of 0.064 to 0.139% P calculated using the data in Figure 1 for wheats grown in the field. In those studies, phytic acid was determined using iron precipitation methods (e.g., Wheeler and Ferrel 1971). Here phytate was isolated by ion exchange, and phosphorus was determined directly. Using either data set, the predicted value of total phosphorus at which a wheat will contain zero phytate is considerably lower than reported for commercial wheat grain, even that grown on very phosphorus-deficient soils. However, if a 40-mg wheat grain contained no phytate phosphorus at 0.047% total phosphorus, it would have only 18.8 $\mu$g P in structural and metabolic compounds (possibly in the ratio 3:1; Fig. 3). The germination and seedling vigor of a grain with such a low phosphorus content would need to be known.

Using a low-P regime similar to that in this study, Batten and Wardlaw (*unpublished*), found that phytate phosphorus began to accumulate as the grain approached physiological maturity—possibly because of the transfer of phosphorus from senescing vegetative tissues. However, at this stage of grain development, phytate could be forming to balance an increase in the concentration of inorganic phosphorus caused by the net loss of water from the grain and the declining rate of starch deposition. Any transfer of phosphate from nongrain tissues would enhance the accumulation of phytate. If the process of late retranslocation of phosphorus could be eliminated without any adverse effect on grain development, a mature grain with negligible, but not zero, phytate could be achieved.

The main factor that reduces total phosphorus in grain appears to be high yield (Lipsett 1969), especially if achieved via a high harvest index ratio (grain:shoot dry weight; Batten 1986). From the limited number of genotypes studied here, and work by Evans and Dunstone (1970) and Bamakhramah et al (1984), there appears to be little value in searching diploid or tetraploid wheats for higher harvest indexes and hence low total and phytate phosphorus contents. Selection for low total phosphorus at an equal (high) harvest index value, as suggested by Batten (1986), could produce low phytate concentrations in bread wheats, especially those grown with limited soil or fertilizer phosphorus.

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