The presence and role of phytic acid in the alfalfa root and crown
by Mark Robert Campbell

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

Abstract:
Phytic acid, myo-inositol 1,2,3,4,5,6 hexakisphosphate, is the major storage form of phosphorus (P) in seeds, comprising 60 to 90% of total seed P. Phytic acid has also been identified in pollen, vegetative tissues of some aquatic angiosperms, and underground storage structures including roots. No studies have unequivocally demonstrated that phytic acid is indeed present in roots.

Three methods (ferric precipitation, ion-exchange chromatography, and high voltage paper electrophoresis) were used to demonstrate that phytic acid is a P-containing compound within the root and crown tissue of alfalfa (Medicagq sativa L.). Phytic acid P was found to represent from 5 to 15% of total root and crown P. The effect of varying frequencies and intensity of shoot harvest on root and crown phytic acid P were next studied. More frequent and intense shoot harvests clearly depleted root and crown phytic acid P as compared with less frequent and intense harvest. Phytic acid was also observed to increase substantially in this tissue following hardening off.

These results indicate that phytic acid may be an important P storage compound in alfalfa root and crown that is mobilized to provide P for subsequent shoot growth.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Phytic acid, myo-inositol 1,2,3,4,5,6 hexakisphosphate, is the major storage form of phosphorus (P) in seeds, comprising 60 to 90% of total seed P. Phytic acid has also been identified in pollen, vegetative tissues of some aquatic angiosperms, and underground storage structures including roots. No studies have unequivocally demonstrated that phytic acid is indeed present in roots.

Three methods (ferric precipitation, ion-exchange chromatography, and high voltage paper electrophoresis) were used to demonstrate that phytic acid is a P-containing compound within the root and crown tissue of alfalfa (Medicago sativa L.). Phytic acid P was found to represent from 5 to 15% of total root and crown P. The effect of varying frequencies and intensity of shoot harvest on root and crown phytic acid P were next studied. More frequent and intense shoot harvests clearly depleted root and crown phytic acid P as compared with less frequent and intense harvest. Phytic acid was also observed to increase substantially in this tissue following hardening off.

These results indicate that phytic acid may be an important P storage compound in alfalfa root and crown that is mobilized to provide P for subsequent shoot growth.

INTRODUCTION

Phytic acid (myo-inositol hexakisphosphate) is the major storage form of phosphorus (P) in the plant seed. It is commonly deposited as the mixed "phytate" salts of several physiologically important mineral cations. Upon germination, it serves as a source of P, mineral cations and myo-inositol needed for seedling growth.

Historically, the greatest interest in seed phytic acid has been in its role as an "antinutrient". Mixed phytate salts of mineral cations are often insoluble and are not readily absorbed across the intestinal mucosa of non-ruminants, leading to reduced availability of both P and minerals such as zinc, calcium and iron. Problems associated with high phytate intake may be most severe in populations where cereal and legume grains comprise a major portion of the diet.

The deposition of phytic acid is not limited to seeds alone. Other tissues containing phytic acid include pollen, floral structures, corms, roots, tubers, and other vegetative tissues. Phytic acid is most commonly deposited as discrete inclusions referred to as "globoids". Often the observation of "globoid-like" structures using microscopy is the only evidence provided to indicate the presence of phytic acid. Additional analytical evidence is required to unequivocally

demonstrate the presence of phytic acid.

Shoot P in the perennial forage crop alfalfa (Medicago sativa L.) is important for its nutritional quality and productivity. Phosphorus concentration in this forage has been found to be a limiting component for maximal animal productivity. The alfalfa crown and root tissue serve as a nutrient reserve important for its overwinter survival and subsequent regrowth in the spring. Studies were conducted to determine if phytic acid was present in the alfalfa root and crown tissues and if levels were substantial enough to represent a store of P. The effects of simulated grazing treatments and hardening off on root and crown phytic acid levels were also determined.

REVIEW OF LITERATURE

Seed Phytic Acid

Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate) is the storage form of phosphorus (P) in seeds of nearly all higher plants, and typically represents 60 to 90% of the total seed P (Raboy, 1990). Numerous studies have demonstrated that mature seeds contain a substantial amount of the hexakisphosphate ester of myo-inositol (phytic acid), but at most contain trace levels of the "lower" myo-inositol polyphosphates (myo-inositol bis-, tris-, tetrakis-, and pentakis phosphate, which contain two through five phosphate esters, respectively). These studies used a variety of chromatographic techniques which distinguish between the various phosphate esters of myo-inositol. Recently nuclear magnetic resonance (NMR) methods have also been used to both identify and quantitate phytic acid (Jackson et al., 1982). The "ferric-precipitation method" for quantifying phytic acid can be used for routine analyses, once it is demonstrated that a tissue contains phytic acid and not substantial levels of other myo-inositol phosphates. Since all other myo-inositol polyphosphates are effectively precipitated as ferric salts, this method cannot distinguish among these and alone cannot provide evidence that the tissue contains phytic acid.

Phytic acid is deposited in single-membrane storage microbodies referred to as protein bodies (Lott, 1980). Typically, seed phytic acid is not found uniformly within the proteinaceous matrix of these protein bodies, but rather occurs in discrete electron dense particles known as globoids (Lott, 1986). In seeds, phytic acid is deposited as the mixed "phytate" salt of several physiologically important cations. Energy dispersive X-ray (EDX) analysis has revealed that globoids of both monocots and dicot species predominantly contain potassium and magnesium phytates. Other elements such as calcium, iron, zinc, barium and manganese may also be found in phytate, depending on the tissue region and soil conditions (Lott, 1986). Phytate functions as a source of inorganic phosphorus (P_i), myo-inositol and mineral cations needed for seedling growth (Lott, 1986).

Phytic acid contained in seed-based diets of non-ruminants results in reduced bioavailability of dietary minerals (Graf, 1986). Insoluble phytate salts, formed in the intestinal tract, are not readily absorbed across the intestinal mucosa. Reduced retention of P and mineral cations by phytic acid may lead to mineral deficiencies (Solomons, 1982; Morris, 1986).

Phytic Acid Deposition in Non-Seed Tissues

Phytic acid has been identified in pollen using both chromatographic and NMR methods. Pollen phytic acid is thought to account for its accumulation in soils, especially pine forests (Jackson and Linskens, 1982). Three gymnosperms and 25 angiosperms were assayed for pollen phytic acid. All three gymnosperms were found to contain detectable levels (1.0 to 5.9 mg phytic acid/g dry weight). Angiosperms having mean style lengths greater than 5 mm also contained phytic acid (0.5 to 21.0 mg/g dry weight). Pollen phytate was suggested to function as a source of myo-inositol, a precursor to cell wall polysaccharide synthesis (Jackson et al, 1982).

Floral structures of Petunia hybrida L. also contain phytic acid and its levels in tissues has been shown to be associated with gametophytic incompatibility genes (Jackson and Kamboj, 1983). Phytic acid was identified in stigmas (0.3 to 0.6 mg phytic acid/g dry weight), upper style ($\leq$ 0.05 mg phytic acid/g dry weight) and lower style ($\leq$ 0.01 mg phytic acid/g dry weight). Among three P. hybrida clones, floral phytic acid accumulation varied according to the incompatibility allele (S) present.

Various studies have identified phytic acid in vegetative tissues. Two aquatic angiosperms of the Lemnaceae family, Wolffiella floridana L. and Lemna gibba L., were found to synthesize phytic acid in their vegetative tissues when grown in an axenic culture including myo-inositol (Roberts and

Loewus, 1968). Phytic acid accumulation was thought to be related to turion (resting frond) formation. Suspension cultures of rice cells (Oryza sativa L.) were found to contain relatively constant amounts of myo-inositol monophosphate while phytic acid levels increased with the length of the culture period (Igaue et al., 1980). Both studies used widely accepted chromatographic methods.

Root phytic acid was first identified in some selected food crops by McCance and Widdowson (1935). Roots of carrot (Dacaus carota L.), parsnip (Pastinaca sativa L.), and tubers (modified stems) of jerusalem artichoke (Helianthus tuberosus L.) and the potato (Solanacia tuberosum L.), were found to contain 15.8, 31.4, 25.0 and 23.0% respectively, of their total P in the form of phytic acid. Samotus (1965) found the potato tuber to contain 0.08 to 0.55 mg phytic acid/g. In these studies, phytic acid content was quantified using the ferric-precipitation method. Unequivocal evidence that these tissues solely contained phytic acid and not other myo-inositol phosphate esters was not provided.

Corms and tubers of two granite outcrop inhabiting species Stylidium petiolare Sond. and Philydrella pygmaea (R. Br.) Caruel., are almost fully dehydrated upon entry into summer dormancy and retain the ability to rehydrate and grow after autumn rains. Light microscopy revealed that these corms and tubers contained protein bodies superficially similar to those described for seeds (Pate and Dixon, 1982).

These protein bodies contained globoid-like inclusions rich in P, calcium, magnesium, zinc and, manganese (Dixon et al., 1983).

Inclusions observed in the elongating root tip of Deschampsia caespitosa (L.) Beauv. also are similar to those of phytate containing globoids (Van Steveninck et al., 1987). Electron probe microanalyses have shown that the zinc, potassium, and magnesium contained in these inclusions is in appropriate proportion to P if they were contained as phytate salts. As above, no additional evidence was provided that unequivocally demonstrated the presence of phytic acid or the deposition of minerals as phytate salts. Additionally, vacuolar bodies in the zinc tolerant ecotype of D. caespitosa contained almost three times more zinc than the zinc sensitive ecotype. Globoids appear more frequent in the tolerant ecotypes when exposed to high zinc concentrations. It was suggested that phytate may play a role as a chelator involved in zinc detoxification.

Factors Associated with Phytate Accumulation

Studies of factors affecting phytic acid accumulation have mostly concerned seed phytic acid. Identification of lines differing in phytic acid accumulation may be useful in selecting grain cultivars low in phytic acid. Levels of groat phytic acid among four cultivars of oats (Avena sativa L.) ranked the same over years and locations, suggesting that

phytic acid production is similarly affected by environment (Miller et al., 1980a). Statistically significant differences in groat phytic acid among cultivars were seen, although differences represented only a 0.1% separation between highest and lowest means. In contrast, substantial variation in seed phytic acid content was observed in 163 soybean lines, ranging from 13.9 to 23.0 mg/g dry weight (Raboy and Dickinson, 1984). Batton (1986) found that diploid wheats (Triticum aestivum L.) contained higher seed phytic acid concentrations than tetraploid and hexaploid wheats when grown in nutrient culture under two levels of P. Levels of phytic acid were found to be greater in six varieties of the hexaploid oat than two varieties of diploid oat (A. strigosa and A. strigosa X A. brevis) (Ashton and Williams, 1958). No substantial variation was found within a species.

In seeds, a high positive correlation between phytic acid P and total P has been observed. Lolas and Markakis (1976) observed correlation coefficients (r) between total P and phytic acid P in soybeans {Glycine max L. (Merr.)}, oats, barley (Hordeum vulgar L.) and wheat to be 0.98, 0.91, 0.96 and 0.97, respectively. Additional studies with wheat (Miller et al., 1980a), soybeans (Raboy and Dickinson, 1984), and oats (Ashton and Williams, 1958) have confirmed these findings.

Several studies have demonstrated that variation in seed phytic acid closely parallel levels of vegetative P during the period of seed development (Raboy, 1990). Vegetative P in

turn closely parallels levels of nutrient P available to the growing plant. Miller et al. (1980b) found that among seven oat cultivars, groat phytic acid significantly increased with a given increase in available soil P. Similar findings have been observed in other species (Asada et al., 1969; Batten, 1986; Michael et al., 1980; Raboy and Dickinson, 1984).

Several lines of evidence indicate that phytic acid metabolism plays a role in the regulation of cellular P concentration. Early and DeTurk (1944) found that during the cell expansion phase of the developing maize (Zea mays L.) kernel, additional P translocated to the developing kernel was converted to phytic acid while total non-phytic acid P levels remained constant. Similar patterns of total P and phytic acid P accumulation during seed developments were found in wheat (Abernathy et al., 1973; Nahapetian and Bassiri, 1975) rice (Ogawa et al., 1979), soybean (Raboy and Dickinson, 1987) and bengal gram (Verma and Lal, 1966). Variation in phytic acid P usually accounts for nearly all variation in seed total P resulting from differences in nutrient P levels (Raboy, 1990). Dmitrieva and Sobolev (1984) found that during the sixth to eighth day of castor bean (Ricinus communis L.) germination, mobilization of the main stores of phytic acid in the endosperm coincided with phytic acid accumulation in the cotyledons. It was suggested that P_i from phytic acid breakdown in the endosperm resulted in a transiently high P_i concentration in the cotyledons leading to phytic acid

synthesis. Transient cotyledon phytic acid was then mobilized to supply the developing seedling with P. Samotus (1965), suggested a mechanism for regulating organic P in potato, whereby increased deposition of phytic acid lowers levels of P_i.

Alfalfa Root and Crown Tissue, P and Forage Quality

The root and crown of alfalfa contain nutrient stores which are mobilized to support shoot regrowth in its vegetative cycle. High levels of root and crown soluble carbohydrates, starch, NH^+_4, organic P and P_i have been shown to be associated with harvest tolerance (Chatterton et al., 1977). During the fall hardening period alfalfa undergoes biochemical, biophysical, and morphological changes which enables the root and crown tissue to survive low temperatures. Factors including light, temperature, and soil conditions decrease total water content and increase intracellular sugars and starch in alfalfa root and crown tissue during fall hardening (McKenzie et al., 1988). Although limited data exists on P metabolism and storage in root and crown tissue, much of the P has been found to be combined into organic forms immediately after entry into the root (Rhykerd and Overdahl, 1972).

Phosphorus deficiency in forage is of economic importance as it can be a limiting component for forage and animal productivity in many areas of the United States. Low plant P

in alfalfa may be associated with production problems such as short stand persistency, reduced nitrogen fixation and low seed yield (Griffith, 1978; Pedersen et al., 1972; Rhykerd and Overdahl, 1972). In dairy cattle, low P in forage may result in reduced milk production, depressed appetite and problems in reproduction (Butler and Jones, 1973). Selecting for high P in forage varieties may require a broader understanding of P metabolism throughout the plant.

MATERIALS AND METHODS

Analytical Methods

Phytic Acid Determination in Alfalfa Root and Crown

A modification of the methods of Early and Deturk (1944) was used for the determination of phytic acid. Samples of dry alfalfa root and crown flour (1.0 g) were extracted in 0.4 HCl containing 0.7 M Na_2SO_4 (15 ml) by stirring 18 to 24 hours at room temperature. Samples were centrifuged (10000 g for 15 min.), supernatant decanted into a second tube and centrifuged again (10000 g for 15 min.). Ten ml of the supernatant were placed in a 30 ml Corex glass centrifuge tube and diluted with 10 ml of glass distilled H_2O and 5 ml of 15 mM $FeCL_3$ in 0.2 N HCl containing 0.35 M Na_2SO_4. Samples were heated for 1.5 h in a boiling H_2O bath. The ferric phytate precipitate obtained after centrifugation (10000 g for 10 min.) was washed twice with 10 ml of 0.2 M HCl, completely digested on a hot plate (215° C) with 2 ml concentrated H_2SO_4, H_2O_2 (as needed) and diluted to 12.5 ml with glass distilled H_2O. Phosphorus in the digests was determined colorimetrically (Chen, 1956). Phytic acid P data were expressed on a dry weight basis.

Determination of Total P

Samples of alfalfa root and crown flour (0.2 g) were placed in 50 ml Nessler tubes and digested on a hot plate with 2 ml concentrated H_2SO_4 and H_2O_2 (as needed) to give complete digestion. Samples were diluted to 12.5 ml with glass distilled H_2O, and P determined colorimetrically as before. Total P data were expressed on a dry weight basis.

Ion-Exchange Chromatography

Analysis of alfalfa root and crown myo-inositol phosphates was conducted using a modification of the method of Saio (Saio, 1964). Samples of alfalfa root flour (2.0 g) were extracted in 0.4 M HCl (15 ml) by stirring 18 to 20 hours at room temperature. Following centrifugation, as before, 10 ml of supernatant was diluted 5-fold with glass-distilled water, and passed through a 0.7 X 13 cm column containing 5 ml of AG 1-X8 (Cl^-) resin, pH 5.2. The column was then eluted with a linear gradient of HCl (0.0 to 1.0 N, 1 ml min^{-1}, total 400 ml). Five ml fractions were collected and 2 ml aliquots were dried and digested with 1 ml H_2SO_4, H_2O_2 (as needed) and diluted with 5.5 ml glass distilled H_2O and 5.5 ml 5 N NaOH. Fraction P was determined colorimetrically (Chen, 1956). Inorganic P was determined directly from selected undigested fractions (Chen, 1956). Elution profiles were compared with those of authentic phytic acid (Sigma) and myo-inositol pentakisphosphate (Calbiochem).

High Voltage Paper Electrophoresis

Crude extracts and portions of selected ion-exchange chromatography peaks were lyophilized, dissolved in glass distilled water, and subjected to paper electrophoresis (0.1 M oxalic acid, pH 1.6; 23 V/cm for 2 hr: Whatman No.1 paper). Standards were phytic acid and a chemical hydrolysate of phytic acid containing a mixture of myo-inositol bisphosphate through myo-inositol pentakisphosphate, prepared as previously described (Desjobert and Peteck, 1956). Phosphorus containing spots were visualized using Hains Isherwood reagent (Bandusrk, 1951) followed by ultraviolet irradiation.

Experiments

Study #1: Phytic Acid Quantification and Confirmation in Alfalfa Root and Crown.

Root and crown tissue from eight alfalfa cultivars (Anstar, Apollo II, Baker, Centurion, Elevation, Ladak-65, Spredor II and Vernal) were collected (October 27, 1988) from a cultivar trial established May 16, 1986 at the Arthur H. Post Experiment Station near Bozeman, MT. These were kindly provided by Dr. Ray Ditterline (Dept. of Plant and Soil Science, MSU). Alfalfa seed was planted at a rate of 11.2 kg ha^{-1} on a Bozeman silt loam (Agric Pachic Cryoborals) with 515 kg ha^{-1} P_2O_5. The trial was irrigated as needed to avoid moisture stress. This field study used a randomized design with four blocks. Root and crown samples were collected

following the first hard fall frost (-4.0° C) (October, 27). Plants samples were collected, washed, crowns trimmed to 2.5 cm and roots trimmed to 20 cm. Samples were microwave irradiated 2 minutes (high power), turned and heated for an additional two minutes to inactivate hydrolytic enzymes. Samples were oven dried (50° C for 5 days), a bulk sample representing five or more plants was prepared for each replication and ground in a Thomas-Wiley mill to pass 20 mesh. Phytic acid P and total P were determined as described previously.

Study #2: Effect of Simulated Grazing Regimes and Hardening Off on Root and Crown Phytic Acid

Alfalfa root and crown samples from a simulated grazing field study were obtained from Shaun Townsend (Dept. of Plant and Soils, MSU). The study was established on May 1986, and located at the Arthur H. Post Experiment Station. A 2 X 3 X 3 (2 cultivars, 3 harvest frequencies, and 3 clipping intensities) complete factorial randomized complete block design with four replications was used (Agustinho, 1988). Alfalfa was seeded, P_2O_5 applied, and irrigated as described previously. Root and crown tissue from 3 replications were collected following the final seasonal harvest (mid-August) from outside rows of selected plots. Only treatments involving the cultivar Spredor II (fall dormancy 1) were analyzed. Plants were harvested under three clipping intensity treatments (33, 50 and 67% top growth removal as

Table 1. Selected factorial treatments from a simulated grazing study including a graduated treatment.

Treatment	Clipping interval	Clipping Intensity
	FACTORIAL	
	days	% removal
* 1	8	33
2	8	50
* 3	8	67
4	16	33
* 5	16	50
6	16	67
* 7	32	33
8	32	50
* 9	32	67

* Graduated:

Clipping interval	intensity
days	% removal
8	33
8	33
16	33
16	50
32	50
Fall Management	67

* Selected treatments included for analysis.

measured by plant height), and three clipping frequencies (8, 16, and 32 day clipping intervals) (Table 1). Of nine factorial treatments, five representing high, low and intermediate harvest severities were selected for all analyses. A graduated (GRAD) treatment was also included with an early season harvest at 8 d frequency with 33% forage removal. Harvest intensity as well as cutting interval were increased with progression of growing season (Table 1). A control plot (hay) was harvested at 10% bloom to a 10 cm stubble height. Management treatments were not imposed the establishment year. Five plant samples per replication were collected in 1988, and processed as before. The flour was bulked, and stored in a desiccator until analysis. For 1989, 10 samples were collected per replication and processed as before, with the exception of grinding with a Thomas-Wily Mill, (Model 4, Arthur H. Thomas Co., Philadelphia, PA) and reground through a Cyclone Sample Mill (UD Corporation, Boulder, CO) using a 0.5 mm size screen. Phytic acid P (ferric-phytate precipitation) and total P were determined using methods previously described.

Alfalfa root and crown tissue from the same study was collected on a second date (September, 16) in 1989 following a hard frost (-4.0° C, September, 11). Ten plants per replication were collected and tissue processed as before. Phytic acid and total were P determined as described previously.

In an additional study, the root and crown tissue of five cultivars (Ladak-65, Arrow, Vernal, Garst-636 and Thor) was obtained from two intra-state variety trials located at the Arthur H. Post Experiment Station established by Dr. Ray Ditterline. The trials were planted in 1989 (location 1) and 1988 (location 2) in a randomized block design with four replications. Ten plants were collected from each replication 2 days prior to the final seasonal harvest (August 3), approximately 3 weeks following (August 23), and after a hard frost (-4° C) (October 8). Plants were trimmed and processed. Total P and phytic acid P were analyzed as previously described. Treatment effects were determined using a split plot test over date.

RESULTS AND DISCUSSION

Phytic Acid Quantification and Confirmation in Alfalfa Root and Crown

Samples of alfalfa root and crown tissue of the eight cultivars contained phytic acid P, as determined by the ferric-phytate precipitation method (Table 2). Means ranged from 0.22 to 0.29 mg phytic acid P per g dry tissue. Mean root and crown total P ranged between 1.91 to 2.54 mg per g dry tissue. Phytic acid P as a percent of total P ranged from 10.5 to 14.1%. Phytic acid P was found to be positively correlated with total P ($r = 0.41$; $P = 0.045$). Although statistically significant differences in traits measured were not observed between cultivars a trend for higher levels of phytic acid P, total P and phytic acid P as percent of total P was seen with cultivars of higher dormancy rating (3 and 4) as compared with cultivars of lower dormancy rating (1 and 2).

Three cultivars (Vernal, Spredor II, Ladak-65), representing the range of phytic acid P levels observed using the ferric precipitation method, were selected for further chromatographic analyses. Ion-exchange chromatography identified a compound (fractions 50 to 65) with an elution profile similar to authentic phytic acid present in the extracts of each cultivar (Figure 1). A second peak

Table 2. Mean[a] phytic acid P, total P and phytic acid P as the percent of total P in alfalfa root and crown tissue as determined using the ferric precipitation method.

Cultivar	Dormancy Rating	Phytic Acid P	Total P	Phytic acid P as the percent of Total P
		---mg/g dry tissue---		%
ANSTAR	4	0.29	2.03	14.1
APOLLO II	4	0.29	2.28	13.0
ELEVATION	3	0.29	2.17	13.4
CENTURION	3	0.29	2.54	11.5
VERNAL	2	0.22	1.91	11.2
BAKER	2	0.27	2.13	12.8
LADAK-65	1-2	0.23	2.19	10.5
SPREDOR II	1	0.25	2.21	11.2
Mean		0.23	2.14	11.0
LSD (0.05)		0.08	0.41	4.1
CV%		16.3	3.9	18.4

[a] Data represents the mean of three replications.

occurring at approximately the 5th to 11th tubes represented inorganic P (P_i) and was confirmed by direct colorimetric determination of P_i from selected column fractions. Elution profiles from each cultivar indicated no substantial levels of other partially phosphorylated inositol polyphosphate intermediates (myo-inositol bisphosphate to myo-inositol pentakisphosphate) in the root and crown extracts. Ion-exchange chromatography fractions representing phytic acid peaks from the three cultivars were pooled and concentrated (Figure 1). High voltage paper electrophoresis of each pooled sample, as well as crude extracts yielded a single spot co-migrating with standard phytic acid (Figure 2). No lower myo-inositol polyphosphates were observed.

Table 3. Comparison of root and crown phytic acid P quantified by ion-exchange chromatography verses the ferric precipitation method.

Cultivar	Phytic Acid P		Percent Recovery
	Column Chromatography	Ferric Precipitation	
	---mg. phytic acid P/g tissue----		%
Vernal	0.15	0.18	83.3
Spredor II	0.16	0.18	88.8
Ladak-65	0.08	0.10	80.0
Mean			84.3