PHOSPHORUS FERTILIZATION IMPACTS ON WHEAT GROWTH AND SELENIUM BIOAVAILABILITY

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ABSTRACT

The objective of this study was to determine the effect of phosphorus (P) fertilization on selenium (Se) uptake by wheat (*Triticum aestivum* L. var. Oxen, Granger, Arapahoe, and Wendy) and the changes in soil Se fractionation. Soil (A horizon) from Presho, South Dakota, was used in a greenhouse experiment. The soil Se was consecutively fractionated into 0.07 mg Se kg^{-1} soluble Se (F1-Se); 0.08 mg Se kg^{-1} ligand exchangeable Se (F2-Se); 1.56 mg Se kg^{-1} acid extractable Se (F3-Se); 1.17 mg Se kg^{-1} oxidative acid decomposable Se (F4-Se); and 1.36 mg Se kg^{-1} residual Se (F5-Se). The main treatment was P fertilization at three different rates (0, 100, and 250 mg P kg^{-1}). Total biomass of spring and winter wheat was affected by a wheat variety $\times$ P application rate interaction. Phosphorus fertilization increased the available P in the soil and total P in stems and grain tissues of all wheat varieties. However, there was no statistical difference in total P concentration in wheat tissues between 100 and 250 mg P kg^{-1} fertilization rate. After wheat harvest, the only significant change in soil Se fraction measured was in the F2-Se. F2-Se significantly decreased with increasing P fertilizer application in both spring and winter wheat. However, total Se in the soil was unaffected by wheat varieties or P fertilizer application. Phosphorus fertilization increased the total absorbed Se in stems and grain in all wheat varieties.

Keywords

Selenium, Fractionation, Wheat, Phosphorus, Bioavailability

INTRODUCTION

Global interest in selenium (Se) has increased over the past few years due to its anti-oxidant and potential anti-cancer attributes in animal and human health (Xu et al., 2004; Yoshizawa et al., 1998). The main source of Se to humans and animals is through plants, although plants do not require Se to complete their life cycle. Wheat (*Triticum aestivum*) is considered one of the most efficient Se accumulators of the common cereal crops and is one of the most important Se sources for humans (Lyons et al., 2003). Global demand for food products naturally rich in Se is increasing due to consumer interest in reducing their cancer risk. Management of Se uptake by plants is necessary in high Se areas of the world (e.g., South Dakota) in order to obtain a steady supply of certifiable Se-rich food and feed products. Therefore, understanding crop management influence on Se uptake by wheat is a critical need if producers with seleniferous soil are to capitalize on this natural resource.

The level of Se accumulation by plants is governed by environmental factors such as amount and form of Se, plant species, and other nutrients (e.g. Cl^-, SO_4^{2-}, and PO_4^{3-}) (Mantgem et al., 1996; Johnson et al., 2000; Brown et al., 1982; Hooper et al., 1999). The successful management of Se uptake by plants requires a wide range of knowledge related to sustainable agronomic practices, for example, fertilizer application, irrigation, and crop rotation. Selenium can be adsorbed by the surface of soil humus or clay and can cause low Se availability for plant uptake (Neal, 1995). Selenium uptake by plants or transportation in the soil is highly related to its association with soluble and available Se rather than the total amount of soil Se. Chao et al. (1989) separated soil Se into five fractions: 1) soluble Se (available to plants), 2) ligand exchangeable Se (available to plants), 3) acid extractable Se (conditionally available to plants), 4) oxidative acid decomposable Se (unavailable to plants), and 5) residual Se (unavailable to plants). This procedure was used to determine changes in soil Se storage. In order to manage Se uptake by plants in high Se areas, the relationships between changes in plant available Se in soil and the total Se in plant tissues should be explained.

Therefore, this study was conducted to determine the effect of P fertilizer on Se uptake by selected wheat varieties and to investigate the changes in Se fractionation when P fertilizer is applied in naturally seleniferous soil.

METHODS

Soil naturally high in Se was collected from the A horizon (depth 0 - 15 cm) near Presho, in Lyman County, South Dakota (100°07'W longitude and 44°03'N Latitude) and used in a greenhouse study. The collected soil was the Promise soil series (very-fine, smectitic, mesic Typic Haplusterts). Selected physical and chemical properties are shown in Table 1. The soil sample was air dried, and crushed to pass through 6 mm screen for use in a greenhouse pot study. Nitrogen (200 mg N kg^{-1} using NH_4NO_3) and phosphorus (0, 100, and 250 mg P kg^{-1} using KH_2PO_4) fertilizer was pre-mixed with the soil before it was packed into pots. To improve the physical properties of the high clay soil, perlite was also pre-mixed with the soil at a 1:10 (v/w) perlite to soil ratio before packing into pots. Two different varieties of spring wheat (var. Oxen and Granger) and two varieties of winter wheat (var. Arapahoe and Wendy) were selected to investigate wheat variety differences. Pots were saturated with distilled water and kept in greenhouse for one day. Eight spring wheat seeds were directly planted in each pot, and later thinned to four seeds per pot. Winter wheat seeds were vernalized before planting. The greenhouse temperature was controlled at 25 ± 5 °C. Soil-moisture content was adjusted regularly to reach approximate field capacity and the plants were harvested after 12 weeks of growth. A completely randomized

Table 1. Physical and chemical properties of the Promise soil (A horizon) used in this Se bioavailability study.

	pH	EC†	TC	IC	TN	NO_3-N	Avail. P	SO_4-S	CEC	Particle size distribution (g kg^{-1})		
	(1:1)	*dS m^{-1}*	*g kg^{-1}*			*mg kg^{-1}*			*$cmol_c$ kg^{-1}*	*Sand*	*Silt*	*Clay*
Promise	7.9	0.57	32.7	12.1	2.5	2.91	14.3	6.9	49.7	14	365	621

†EC: Electrical Conductivity (1:1 extraction method), TC: Total Carbon, IC: Inorganic Carbon, TN: Total Nitrogen, Avail. P (extracted by sodium bicarbonate), SO_4-S (extracted by calcium phosphate), CEC: Cation Exchange Capacity (NaOAC method).

design (CRD) with four replications was used. Soil Se was sequentially fractionated into five fractions according to the stepwise procedure method described by Chao et al. (1989) (Table 2). The data were statistically analyzed using analysis of variance (ANOVA) with the JMP program. Spring and winter wheat data were treated separately in the statistical analysis due to different growth time periods (January to March for spring wheat, March to June for winter wheat). Least significant differences (LSD) were used to separate means when F-tests were statistically significant (P=0.05).

RESULTS

Phosphorus fertilizer application increased the grain yield in all wheat varieties tested (Table 3). Total biomass of both spring and winter wheat was affected by a variety × P application rate interaction. Total biomass and grain yield of wheat treated with P fertilizer increased significantly when compared to the no P application treatment. However, there was no difference between the 100 and 250 mg P kg^{-1} application rate at the 95% level.

Available P in the soil planted with spring wheat significantly increased with P fertilizer application (Figure 1). However, wheat varieties did not show an effect on available P since the absorbed P in wheat tissues was not significantly different between the wheat varieties. This result was same in the soil planted with winter wheat. The P fertilizer application significantly increased total P in wheat stems and grain when compared to the treatment with no P in all varieties

Table 2. Selenium fractionation in the Promise soil (A horizon) used in this study.

	F1-Se	F2-Se	F3-Se	F4-Se	F5-Se	Total Se
	mg Se kg^{-1}					
Promise	0.07	0.08	1.56	1.17	1.36	4.95

F1-Se: Soluble; Extracted by 0.25M KCl
F2-Se: Ligand exchangeable; Extracted by 0.1M KH_2PO_4
F3-Se: Acid extractable; Extracted by 4M HCl
F4-Se: oxidative acid decomposable; Extracted by $KClO_3$ + conc. HCl
F5-Se: Residual; Extracted by conc. HF + HNO_3 + $HClO_4$

Table 3. Phosphorus (P) fertilization impacts on total biomass and grain yield of wheat (*Triticum aestivum *L. var. Oxen, Granger, Arapahoe, and Wendy) grown in a Promise soil (A horizon).

P application (mg P kg^{-1})	SPRING WHEAT		WINTER WHEAT	
	Total biomass	Grain yield	Total biomass	Grain yield
	(g pot^{-1})			
	Oxen		*Arapahoe*	
0	6.97	2.94	6.20	1.08
100	12.72	5.35	11.97	2.72
250	12.02	4.88	12.58	2.66
Mean	10.57	4.39	10.25	2.15$^{A\Phi}$
	Granger		*Wendy*	
0	5.40	2.21	4.96	2.44
100	14.17	5.43	11.37	5.16
250	14.33	4.50	10.98	5.22
Mean	11.30	4.05	9.10	4.27^{B}
	P Means			
0	6.19^{a}	2.58^{a}	5.58^{a}	1.76^{a}
100	13.45^{b}	5.39^{b}	11.67^{b}	3.94^{b}
250	13.18^{b}	4.69^{b}	11.78^{b}	3.94^{b}
	LSD†			
Varieties	NS‡	NS	NS	0.78
P	1.30	0.87	1.54	0.95
Var. × P	1.83	NS	2.18	NS

†LSD$_{0.05}$ was used to separate means when F-tests were statistically significant.
‡NS = not significantly different at the 95% level.
$^{\Phi}$Mean comparisons between wheat varieties (capital letters), and between P applications (small letters). n=4 for each variety mean, n=8 for each P means.

(Figure 2). However, phosphorus fertilizer application of more than 100 mg P kg^{-1} did not increase the total P concentration in plant tissues.

The Se fraction distribution in the Promise soil (A horizon) sampled was: F3-Se (Acid extractable Se) > F5-Se (Residual Se) > F4-Se (oxidative acid decomposable Se) >> F2-Se (Ligand exchangeable Se) > F1-Se (Soluble Se) (Table 2). Using a classification of plant-availability, the order was: unavailable Se (F4-Se and F5-Se) > conditionally available Se (F3-Se) >> available Se (F1-Se and F2-Se). Table 4 shows the impact of P fertilizer on the sequential Se fractionation of the Promise soil after spring/winter wheat harvesting. In this study, F1-Se (soluble Se) was not significantly affected by wheat variety or P fertilizer application. On the other hand, the ligand exchangeable Se (F2-Se) concentration significantly decreased with P fertilization regardless of wheat variety. However, Se concentration in F2-Se was not significantly different between 100 and 250 mg P kg^{-1} fertilizer treatments in both spring and winter wheat cultivation. The other Se fractions (F3-Se through F5-Se) and total Se were not affected by P application or wheat variety.

Selenium concentration in stems and grain of spring or winter wheat was not significantly affected by P fertilizer application or wheat variety at the 95% level (Table 5). However, the total amount of Se absorbed by spring or winter wheat tissues was affected by P fertilizer application (Figure 3). The amount of Se absorbed by wheat in the treatments with P fertilizer was higher than the treatment with no P.

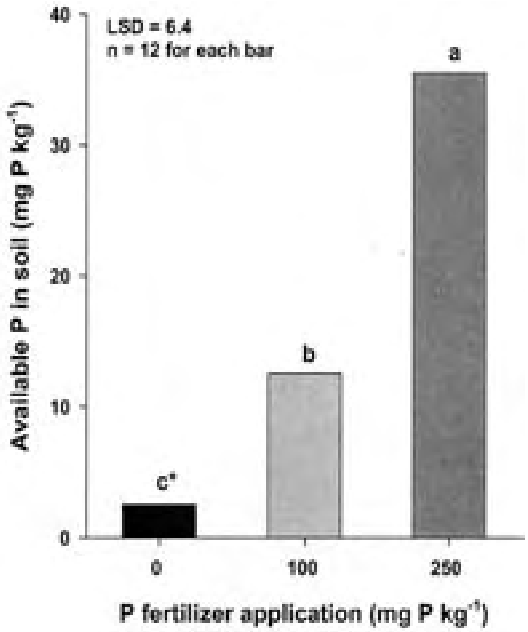

Figure 1. Available phosphorus (extracted by $NaHCO_3$) in a Promise soil (A horizon) treated with 0, 100, and 250 mg P kg^{-1} fertilizer application after harvesting of spring wheat (*Triticum aestivum *L. var. Oxen and Granger). *Bars with the same letter are not significantly different at 95% level.

DISCUSSION

Total biomass and grain yield increased significantly with P fertilizer application. Wheat yield was increased 55% because P encourages root growth and stimulates both tillering and seedling emergence. However, P fertilizer of more

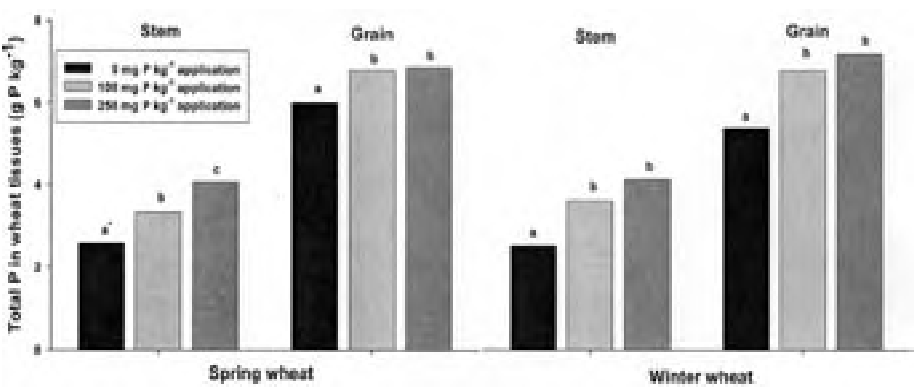

Figure 2. Phosphorus (P) fertilization impacts on total P content in stems mg P kg^{-1} and grain of wheat (*Triticum aestivum *L. var. Oxen, Granger, Arapahoe, and Wendy) grown on a Promise soil (A horizon). *Bars with the same letter are not significantly different at 95% level.

Table 4. Phosphorus fertilization impact on sequential Se fractionation in a Promise soil (A horizon) after harvesting of spring and winter wheat (*Triticum aestivum *L. var. Oxen, Granger, Arapahoe, and Wendy).

P application (mg P kg^{-1})	F1-Se	F2-Se	F3-Se	F4-Se	F5-Se	Total-Se
	mg Se kg^{-1}					
	Oxen / Arapahoe					
0	0.04/0.11	0.06/0.14	1.36/1.36	0.87/1.18	1.49/1.43	4.73/4.72
100	0.04/0.11	0.05/0.09	1.45/0.14	0.92/0.31	1.43/0.157	4.93/4.87
250	0.05/0.09	0.04/0.08	1.51/1.20	0.86/1.29	1.43/1.38	4.91/4.93
Mean	0.05/0.10	0.05/0.11	1.44/1.23	0.88/1.26	1.45/1.46	
	Granger / Wendy					
0	0.05/0.10	0.05/0.11	1.45/1.27	0.95/1.33	1.55/1.40	5.08/4.83
100	0.04/0.10	0.03/0.13	1.46/1.25	0.77/1.29	1.53/1.45	4.96/4.88
250	0.05/0.10	0.03/0.10	1.47/1.22	1.00/1.22	1.51/1.34	4.92/4.81
Mean	0.05/0.10	0.04/0.11	1.46/1.25	0.91/1.28	1.53/1.40	
	Control					
0	0.05/0.11	0.06/0.10	1.55/1.33	1.14/1.26	1.41/1.50	4.76/4.97
100	0.06/0.11	0.04/0.10	1.54/1.30	0.92/1.07	1.29/1.51	4.72/4.9
250	0.07/0.10	0.04/0.08	1.56/1.16	0.80/1.27	1.38/1.55	4.96/4.98
Mean	0.060.11	0.05/0.10	1.55/1.26	0.95/1.20	1.36/1.52	
	P Means					
0	0.05/0.11	0.06^{a}/0.12$^{a\Phi}$	1.46/1.32	0.99/1.26	1.48/1.44	4.86/4.84
100	0.05/0.11	0.04^{b}/0.11^{b}	1.48/1.23	0.87/1.22	1.42/1.51	4.87/4.88
250	0.06/0.10	0.04^{b}/0.09^{b}	1.51/1.19	0.89/1.26	1.44/1.42	4.93/4.92
	LSD†					
Varieties	NS/NS‡	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS
P	NS/NS	0.02/0.02	NS/NS	NS/NS	NS/NS	NS/NS
Var. × P	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS

†LSD was used to separate mean when F-tests were statistically significant (P=0.05).
‡NS = not significantly different at the 95% level.
$^{\Phi}$Mean comparisons between P applications. n=4 for each variety mean, n=8 for each P means.

than 100 mg P kg^{-1} did not increase or decrease the wheat yield. Phosphorus application was applied as high as 1,000 mg P kg^{-1} (data not shown) without a significant change in total biomass or grain yield beyond the 100 mg P kg^{-1} rate.

According to this study, total P concentration in wheat tissue was significantly increased the application of 100 and 250 mg P kg^{-1} of P fertilization. Phospho-

Table Table 5. Phosphorus fertilization impact on Se concentrations in stems and grain samples of spring and winter wheat (Triticum aestivum L. var. Oxen, Granger, Arapahoe, Wendy) grown on a Promise soil (A horizon). n=4 for each variety mean, n=8 for each P mean.

P application (mg P kg^{-1})	SPRING WHEAT		WINTER WHEAT	
	Stem	Grain	Stem	Grain
	mg Se kg^{-1}			
	Oxen		*Arapahoe*	
0	0.158	0.256	0.317	0.442
100	0.162	0.249	0.290	0.500
250	0.207	0.280	0.330	0.450
Mean	0.175	0.262	0.312	0.464
	Granger		*Wendy*	
0	0.157	0.239	0.243	0.452
100	0.168	0.242	0.260	0.418
250	0.206	0.277	0.328	0.512
Mean	0.177	0.253	0.277	0.461
	P Means			
0	0.158	0.248	0.280	0.447
100	0.165	0.246	0.275	0.459
250	0.207	0.279	0.329	0.481
	LSD†			
Varieties	NS‡	NS	NS	NS
P	NS	NS	NS	NS
Var. X P	NS	NS	NS	NS

†$LSD_{0.05}$ was used to separate mean when F-tests were statistically significant.
‡NS=not significantly different at the 95% level.

rus fertilizer application significantly increased plant-available P in soil, which would be available to interact with soil Se. An increase in plant-available P could cause an increase in Se uptake by wheat, since P and Se compete for the same soil adsorption sites and P might desorb or out-compete Se for the adsorption sites. This would make Se more available for plant uptake (Carter et al., 1972). Rajan et al. (1976) demonstrated that PO_4^{3-} was adsorbed three times more than SeO_3^{2-} at low concentrations, due to the PO_4^{3-} displacing more aqua groups and thus making the surface less positive. Therefore, more P in soil can be adsorbed on the surfaces of clay or humus, thus the P application increases the possibility for plant roots to absorb Se. In this study, F2-Se concentration decreased with increasing P fertilization. The decreased Se in F2-Se might be taken up by plants or move down with a percolating water front. Total Se concentration in soil was not significantly affected by wheat variety or P fertilizer application although F2-

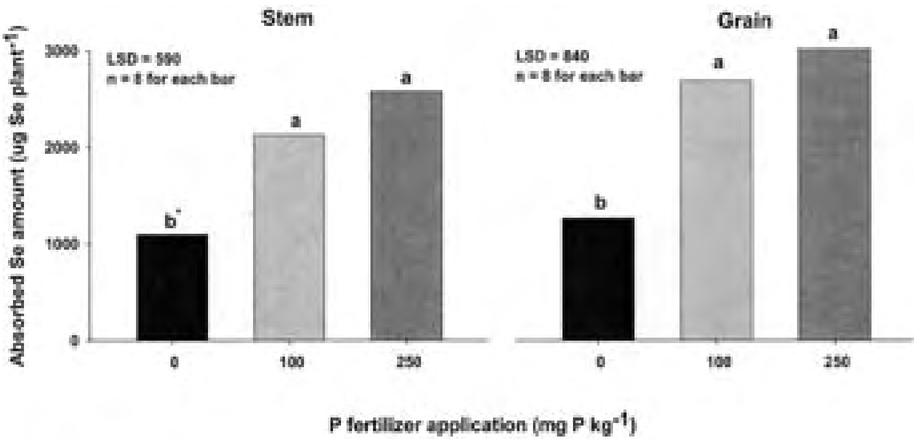

Figure 3. Phosphorus fertilization impacts on the total amount of Se absorbed in the stems and grain of winter wheat (*Triticum aestivum* L. var. Arapahoe and Wendy) grown on a Promise soil (A horizon). *Bars with the same letter are not significantly different (P=0.05).

Se concentration decreases with P fertilizer application. This may be due to the relatively small portion of F2-Se compared to the total Se concentration.

Selenium concentration in stems and grain of spring and winter wheat were not significantly affected by P fertilizer application or wheat varieties at the 95% level even though the F2-Se concentration in soil was significantly decreased. These data indicate that the amounts of Se absorbed by wheat were increased by P fertilizer application. However, Se concentrations in wheat tissues were not significantly different because the absorbed Se concentration was diluted by the wheat tissue. The Se concentration was higher in grain than stems in all varieties. This makes sense because most of the Se taken up by plant roots is associated with amino acids and proteins (Shrift, 1973), and protein content of grain is usually higher than in plant stems.

According to this study, we have found that the P fertilization should be a part of a management plan to control Se uptake by wheat. However, the soil Se concentration was significantly different at the different growing times which suggest a strong environmental influence. Selenium may be transferred into available form as a result of chemical weathering and changes in pH and redox potential (Chao et al., 1989). Therefore, selenium movement in soils or uptake by plants appears to be governed by multiple environmental factors (e.g., temperature, irrigation, and nutrition) which should be investigated further.

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