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Phosphorus Fertilization Differentially Influences Fatty Acids, Protein, and Oil in Soybean

Xinhua Yin[1*], Nacer Bellaloui[2], Angela M. McClure[1], Don D. Tyler[3], Alemu Mengistu[4]

[1]Department of Plant Sciences, The University of Tennessee, Jackson, TN, USA
[2]Crop Genetics Research Unit, USDA-ARS, Stoneville, MS, USA
[3]Crop Genetics Research Unit, USDA-ARS, Jackson, TN, USA
[4]Department of Biosystems Engineering and Soil Science, The University of Tennessee, Jackson, TN, USA
Email: xyin2@utk.edu

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Abstract

Information is limited about phosphorus (P) fertilization effects on soybean seed composition. A field experiment was conducted to investigate the effects of P application rates on the concentrations of various fatty acids, protein, and oil in soybean under no-tillage on low and high testing P soils at Jackson and Milan, Tennessee from 2008 through 2011. Five P rates 0, 10, 20, 30, and 40 kg·P·ha^{-1} plus the recommended P fertilizer rate based on soil P testing results were arranged in a randomized complete block design with four replicates. Protein, oil, and fatty acid concentrations in seed responded differently to P fertilization. In general, protein concentrations were enhanced but oil levels decreased with increased P application rate. Palmitic and oleic concentrations responded positively to P application rate up to a certain level. However, the response of linolenic acid concentration was inconsistent (negative or positive). Stearic concentration was not influenced by P fertilization. Application of 10 kg·P·ha^{-1} resulted in higher production of protein and palmitic, oleic, and linolenic acids than zero P and the higher P application rates as well on the P deficient soil. Excessive P application rates could lower seed yield and the quality of some attributes in seed. In conclusion, linoleic acid concentration, a key quality attribute in soybean seed for human and animal consumption, can sometimes be enhanced by P fertilization; the indigenous soil P level and P application rate should be taken into account in breeding soybean cultivars with low linolenic acid level.

Keywords

Phosphorus, Fatty Acids, Protein, Oil, Yield, Soybean

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is traditionally grown for protein and oil in the seed. Soybean seed grown in the United States contains approximately 41% protein and 21% oil on a dry weight basis [1]. In addition, soybean contains about 33% carbohydrates [2] and secondary metabolites such as phenolics including isoflavones [3]. There are five major fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid) and three major isoflavones (daidzein, genistein, and glycitein) in soybean seed [4] [5].

Soybean seed quality attributes are affected by both genetics [1] [6] and the environment [7] [8]. Genotype and maturity [9]-[11], planting date [12] [13], temperature [7], drought [10] [14], and nutrients in soil and seed [15]-[17] are among the genetic and environmental factors. For instance, Helms *et al.* (1990) [13] found that soybean seed attributes varied in the same cultivar grown in different years or under different environments in the same year, and seed protein concentration increased as planting date was delayed. Burton (1985) [18] and Shannon *et al.* (1972) [19] reported a negative relationship of seed protein concentration with seed yield of soybean.

Although nutrient management effects on soybean seed yield and protein and oil concentrations have been examined in previous studies [17] [20]-[22], its impacts on the concentrations of fatty acids in soybean seed have been scarcely investigated [23]. Since N fertilization is usually not needed, primarily P and K have been studied. Fertilization with P and K can influence soybean yield and various physiological processes that, in turn, could affect seed yield and protein and oil concentrations [24]. Seed composition can be altered with P fertilization although these alterations have been reported to be moderate and inconsistent [20] [23] [25] [26]. Abbasi *et al.* (2012) [27] observed that P application increased both protein and oil concentrations in soybean seed. However, Haq and Mallarino (2005) [20] found that P fertilization increased protein and oil concentrations in some trials but decreased protein and oil levels in some other trials. Krueger *et al.* (2013) [22] reported that protein and oil concentrations in soybean seed were not affected by P fertilization at most locations. Gaydou and Arrivets (1983) [25], Israel *et al.* (2007) [28], and Krueger *et al.* (2013) [23] confirmed that linoleic acid concentration decreased with P fertilization. Gaydou and Arrivets (1983) [25] and Israel *et al.* (2007) [28] also observed an increase in oleic acid concentration at higher P application rates. Krueger *et al.* (2013) [23] reported that linolenic acid concentration increased with excessively high P fertilization.

With the increased interest in eliminating trans fatty acids in the diets of Americans, soybean breeders continue to place more emphasis on developing varieties which can produce seed with elevated levels of desired fatty acids such as oleic acid. If a market premium is offered for specific seed quality attributes, soybean growers will need information on how to improve their seed quality. The main objective of this study was to evaluate P application rate effects on the concentrations and production of palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, protein, and oil of soybean under no-tillage on low to high testing P soils.

2. Materials and Methods

2.1. Site Description and Experimental Design and Implementation

The P fertilization effects on seed quality attributes and yields of soybean were investigated in a small plot field experiment that was conducted on the University of Tennessee's West Tennessee Research and Education Center at Jackson and University of Tennessee's Research and Education Center at Milan from 2008 to 2011. The fields used for this study were classified as Memphis silt loam at Jackson and Dexter loam at Milan, which had been under continuous no-till production for more than 10 years before experiment establishment. The previous crop was soybean at both locations in 2007.

A randomized complete block design was used with six P treatments repeated four times at both locations each year. The first five treatments consisted of the following five P application rates: 0, 10, 20, 30, and 40 $kg \cdot P \cdot ha^{-1}$ (equal to 0, 20, 40, 60, and 80 lb P_2O_5 a^{-1}). The last treatment was the recommended P application rate based on soil P testing results every year. The recommended P rates for Milan were 22, 0, 10, and 0 $kg \cdot ha^{-1}$ and the rates for Jackson were 15, 0, 0, and 10 $kg \cdot ha^{-1}$ for 2008, 2009, 2010, and 2011, respectively. The P fertilizer was uniformly applied on the soil surface by hand as triple superphosphate (0N-20P-0K). Plots were 9.1 m long and 3.0 m wide. A full season soybean "Pioneer 94M80" crop was no-till planted in 76-cm rows at both locations in all four seasons. No winter crop was grown in any year in this study. The planting dates were 6 May 2008, 12 May 2009, 6 May 2010, and 11 May 2011 at Jackson and 23 May 2008, 20 May 2009, 13 May 2010, and 2 June 2011 at Milan. A uniform rate of potash fertilizer was applied as needed according to the soil K testing results each year [29]. Pests, weeds, and diseases were controlled with the University of Tennessee Extension Service's recommended management practices for soybean. The identical plot and treatment layout was used in the same area of the same field at both locations for all four years. The daily air temperatures and rainfall were recorded at both locations each year.

Soybean yields were determined at maturity with a plot combine by harvesting the center two rows for the entire plot length from each plot on 29 September 2008, 21 October 2009, 21 September 2010, and 30 September 2011 at Jackson and 2 October 2008, 19 October 2009, 20 September 2010, and 30 September 2011 at Milan. The yields were adjusted to 130 g kg^{-1} moisture content.

2.2. Soil P Testing

A composite soil sample consisting of 10 cores was randomly collected at the 0-15 cm depth from each plot with a 2.5-cm diameter hand soil probe at Jackson and Milan before P treatments were applied on 3 March and 9 April 2008, respectively. After soil samples were air-dried, ground to pass a 2-mm screen, and thoroughly mixed, they were analyzed for basic properties by the University of Tennessee's Soil, Plant, and Pest Center (Nashville, TN). Soil pH was determined in a 1:1 (soil:H_2O) solution (Watson and Brown, 1998). Soil available NH_4^+, NO_3^-, P, K, Ca, and Mg were extracted with the

Mehlich I method [30].

A composite soil sample was also taken from each plot at soybean harvest each year for soil available P which was determined with the same method as stated above. The specific sampling dates were 9 October 2008, 4 November 2009, 1 November 2010, and 1 December 2011 at Jackson and 28 October 2008, 5 November 2009, 5 October 2010, and 6 October 2011 at Milan.

2.3. Analyses of Leaf and Seed P Concentrations

A composite leaf sample composed of 20 most recently fully developed trifoliate leaves with petioles was randomly collected at approximately the V5, R1, and R3 growth stages from each plot at both locations in all four seasons for the determination of total P concentrations. The specific sampling dates were 8 July 2008, 26 June 2009, 14 June 2010, and 21 June 2011 at Jackson and 9 July 2008, 29 June 2009, 17 June 2010, and 13 July 2011 at Milan for V5; 16 July 2008, 6 July 2009, 21 June 2010, and 21 July 2011 at Jackson and 17 July 2008, 7 July 2009, 28 June 2010, and 25 July 2011 at Milan for R1; and 28 August 2008, 27 July 2009, 26 July 2010, and 1 August 2011 at Jackson; 29 August 2008, 23 July 2009, 28 July 2010, and 4 August 2011 at Milan for R3. A composite seed sample was taken at harvest from each plot at both locations during 2008-2010 for analyses of P concentrations. Leaf and seed samples were dried at 65˚C in a forced air oven for at least 3 d and then ground in a Wiley mill (Arthur K. Thomas Co., Philadelphia, PA) to pass a 1-mm screen. Total P in each leaf and seed sample was digested with nitric acid and hydrogen peroxide in a CEM MDS 2100 series microwave (CEM Corporation, Matthews, NC), and the digested solution was analyzed on a Thermo Jarrel Ash 1100 ICP [31].

2.4. Determination of Protein, Oil, and Fatty Acid Concentrations in Seed

Seed samples collected at harvest were also analyzed for protein, oil, and fatty acid concentrations at both locations during 2008-2010. Approximately 25 g of seed from each plot was ground with a Laboratory Mill 3600 (Perten, Springfield, IL) and analyzed with near infrared reflectance [9] [32] using a diode array feed analyzer AD 7200 (Perten, Springfield, IL). Calibrations were initially developed by the University of Minnesota, using Perten's Thermo Galactic Grams PLS IQ software. Protein and oil concentrations were expressed on a dry seed basis [32] [33], while fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids) were expressed on an oil basis. The production of protein or oil was defined as the product of soybean seed yield level and the concentration of protein or oil in the seed. Since the concentrations of fatty acids were expressed on an oil basis, the production of each of these fatty acids was defined as the product of soybean oil production and the concentration of specific fatty acid in the seed oil.

2.5. Statistical Analysis

Analyses of variance were conducted for the concentrations and production of protein, oil, and fatty acids in each individual year at each location with the ANOVA procedure in SAS for Windows V9 (2) (SAS Institute, Cary, NC). A randomized complete block design with four replications was used for all these analyses. Data combined across the three or four years at each location were analyzed under a randomized complete block design with year as a random factor. Treatment or year means were separated with the Fisher's protected LSD test if needed. Probability levels lower than 0.05 were designated as significant.

3. Results and Discussion

Weather conditions were different among the four years at Jackson and Milan (Table 1). It was obvious that 2010 was hotter during May to October, and 2011 was hotter in July, than 2008 and 2009 at both locations. During 2010, both locations received heavy rains totaling 370 mm on 1 and 2 May 2010 but suffered reduced late season moisture. In contrast, the 2008 growing season was generally dry throughout the season at Milan with poor late season moisture at Jackson.

3.1. Soil P Levels

According to the present Tennessee soil-test P interpretations, soil P fertility is categorized as low, medium, high, and very high for soybean when soil-test P concentration is 0 - 9, 10 - 15, 16 - 59, ≥60 mg P kg^{-1} (equivalent to 0 - 18, 19 - 30, 31 - 119, and ≥120 lb P a^{-1}), respectively, with Mehlich I as the extractant [29]. Based on the above criteria, the initial soil P fertility under Mehlich I was high (21.5 mg P kg^{-1}) at Jackson but low (6.9 mg P kg^{-1}) at Milan based on a composite soil sample which was taken from the

Table 1. Monthly average air temperature and rainfall during the growing season at Jackson and Milan, TN from 2008 to 2011.

Location	Month	Temperature				Rainfall			
		2008	2009	2010	2011	2008	2009	2010	2011
		˚C				mm			
Jackson	May	19.9	20.5	22.2	19.9	174.2	191.0	566.4	211.8
	June	25.7	25.7	27.6	26.3	71.4	91.7	163.6	156.2
	July	26.9	24.7	28.0	28.2	159.5	187.5	168.9	95.3
	August	25.6	25.1	28.0	26.7	64.8	76.5	125.0	42.4
	September	22.9	23.1	23.3	20.1	20.1	184.4	28.2	82.8
	October	15.9	14.4	16.4	14.5	80.0	176.3	35.8	28.2
Milan	May	19.2	20.1	21.8	19.7	233.7	229.6	534.7	285.5
	June	25.3	26.0	27.5	26.4	38.6	56.4	82.0	172.7
	July	26.5	24.6	27.6	27.9	79.2	200.9	150.6	36.1
	August	25.1	24.5	27.8	26.4	18.8	56.6	50.0	29.0
	September	22.5	22.4	23.1	21.0	9.9	119.9	9.1	259.3
	October	15.1	13.9	15.9	14.6	65.0	207.8	48.5	27.2

two locations during November to December 2007, prior to the initiation of this study. Soil P concentrations before the treatment imposition in spring 2008 did not differ statistically among the plots assigned to the different P treatments, indicating that the two fields were generally uniform in soil P fertility prior to this study (Table 2).

At Jackson, soil P levels did not differ among the P treatments after soybean harvest in the fall of 2008, 2010, or 2011 (Table 2). However, P effects were significant on post-harvest soil P with application of 40 kg·P·ha^{-1} resulting in the highest soil P level in 2009. At Milan, soil P levels were not different among the P treatments after soybean harvest in the fall of 2008, 2009, or 2010, but differed in the fall of 2011 (Table 2). Soil P concentration was the highest when 40 kg·P·ha^{-1} was applied in 2011.

According to the boundaries of soil-test P with Mehlich I as the extractant for soybean in Tennessee [29], soil P fertility was all in the high category, even under the zero P treatment at both locations in fall 2011 by the end of this study. These ratings were generally identical as those in spring 2008 prior to the treatment imposition of this study. It was unexpected that soil P did not suffer noticeable decrease even under the zero P treatment for continuous four years of soybean production at either location.

Table 2. Phosphorus fertilization effects on soil P concentrations at Jackson and Milan from 2008 to 2011.

Location	Treatment[a]	2008		2009	2010	2011
		Pre-plant	Post-harvest	Post-harvest	Post-harvest	Post-harvest
				mg·kg^{-1}		
Jackson	1	26.9	21.4	17.1b[b]	34.3	25.9
	2	23.0	20.9	20.9b	28.6	26.1
	3	28.6	23.8	18.1b	30.4	20.5
	4	26.5	24.0	17.4b	33.8	33.9
	5	28.8	28.1	29.4a	31.4	30.6
	6	26.0	20.1	18.4b	22.9	26.6
	Sig[c]	ns[d]	ns	*	ns	ns
Milan	1	17.1	24.6	14.5	18.9	16.6d
	2	18.1	25.1	15.8	23.0	21.9bcd
	3	16.5	27.3	15.3	20.4	28.0ab
	4	14.6	28.6	17.6	13.8	26.3bc
	5	20.4	30.0	19.8	18.6	35.1a
	6	16.9	21.4	11.6	18.0	19.1cd
	Sig	ns	ns	ns	ns	**

*Significant at 0.05 probability level; **Significant at 0.01 probability level. [a]Treatments 1, 2, 3, 4, and 5 were the annual P application rates of 0, 10, 20, 30, and 40 kg·P·ha^{-1}, respectively; treatment 6 was the P fertilizer recommended rate based on soil P testing results every year. [b]Means in a column within each location followed by the same letter are not significantly different at P = 0.05 according to the protected LSD. [c]Sig, significance. [d]ns, not significant at 0.05 probability level.

3.2. Leaf P Concentrations

Phosphorus fertilization effects were occasionally significant on soybean leaf P concentrations at the V5, R1, and R3 growth stages in this study (Table 3). At Jackson and Milan, leaf P levels measured at V5 in 2009 and 2011 generally increased with increasing P rate. Overall, P rates seemed to have greater impacts on leaf P at V5 than at R1 and R3 at both locations. As P application rate went up, the increase in leaf P gradually slowed down.

Campbell and Plank (2011) [34] recommended that the range of adequate leaf P concentrations was 3.0 to 6.0 g kg^{-1} at both the early growth and flowering stages for soybean grown in the southern United States. According to these criteria, leaf P concentrations were mostly below the lower limit of the sufficient ranges regardless of growth stage and location in 2008, which was a dry year. In 2009, leaf P concentrations were almost all below the sufficiency ranges at V5 and R1 but above them at R3 irrespective of treatment and location. However, leaf P levels were above the lower limit of the sufficiency ranges at both locations in 2010 and 2011 except R3 at Milan in 2011. Therefore, soybean yield responses to P applications were not expected at any location in any year based on the sufficient leaf P concentrations with the zero P treatment, or no significant improvement in leaf P with P applications.

Table 3. Phosphorus fertilization effects on leaf P concentrations at Jackson and Milan from 2008 to 2011.

Location	Treatment[a]	2008			2009			2010			2011		
		V5[b]	R1	R3	V5	R1	R3	V5	R1	R3	V5	R1	R3
		$g \cdot kg^{-1}$											
Jackson	1	2.60	2.93	2.77	2.35b[c]	2.76	3.21c	3.40	3.57	3.50	3.81bc	3.54b	3.26
	2	2.59	2.88	2.63	2.54b	2.69	3.38abc	3.61	3.66	3.29	3.48c	3.58b	2.97
	3	2.67	3.11	2.71	2.54b	2.81	3.36bc	3.40	3.79	3.54	4.15b	3.63b	3.46
	4	2.52	2.80	2.69	2.53b	2.84	3.43ab	3.83	3.99	3.42	4.13b	4.07a	3.37
	5	2.59	3.21	2.80	3.09a	3.00	3.55a	4.03	4.07	3.64	4.71a	3.98a	3.35
	6	2.59	3.09	2.76	2.72ab	2.89	3.44ab	3.49	3.88	3.51	3.80bc	3.75ab	3.29
	Sig[d]	ns[e]	ns	ns	*	ns	*	ns	ns	ns	**	*	ns
Milan	1	2.26	2.66	2.88	2.48a	2.23	3.50	3.75	3.48	3.39	4.33b	4.18	2.62
	2	2.37	2.72	2.80	2.53a	2.40	3.99	3.87	3.74	3.37	4.30b	4.24	2.87
	3	2.43	2.61	2.69	2.52a	2.35	3.66	3.75	3.46	3.47	4.50ab	4.21	2.76
	4	2.36	2.69	2.97	2.59a	2.26	3.70	3.95	3.46	3.47	4.65a	4.26	2.87
	5	2.43	2.67	2.75	2.64a	2.31	4.13	4.13	3.70	3.35	4.40b	4.20	2.80
	6	2.32	2.50	2.85	2.16b	2.15	3.97	3.84	3.66	3.26	4.47ab	4.35	2.73
	Sig	ns	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns

*Significant at 0.05 probability level; **Significant at 0.01 probability level. [a]Treatments 1, 2, 3, 4, and 5 were the annual P application rates of 0, 10, 20, 30, and 40 $kg \cdot P \cdot ha^{-1}$, respectively; treatment 6 was the P fertilizer recommended rate based on soil P testing results every year. [b]V5, 5-leaf growth stage; R1, beginning flowering stage; R3, beginning pod stage. [c]Means in a column within each location followed by the same letter are not significantly different at P = 0.05 according to the protected LSD. [d]Sig, significance. [e]ns, not significant at 0.05 probability level.

3.3. Seed Yield and P Concentrations

At Jackson, soybean seed yield did not respond to P applications in any year or on the four-year averages (Table 4). However, a trend of increase in seed yield was observed when P application rate went up from 0 to 30 or 40 kg·P·ha^{-1} regardless of year. Seed yields varied among the four growing seasons with higher yields in 2008, 2009, and 2010 than those in 2011.

At Milan, seed yield responded to P application averaged over the four years (Table 4). Applying 10 kg·P·ha^{-1} produced the highest yield out of the six treatments on the four-year averages. Seed yields differed among the four years with higher yields in 2009 and 2010 than those in 2008 and 2011.

Significant responses of seed P concentrations to P applications were observed at both locations in 2010 only (Table 5), which suggests that P treatment effects on seed P level may depend on environmental conditions and is not consistent. There seemed to be a tendency of increase in seed P when P application rate was increased from 0 till 30 or 40 kg·P·ha^{-1} in all three years at Jackson only.

Overall, our results showed that soybean yields did not respond significantly to the P application rates at either location in this study. Soybean has been classified as a poor responder to P fertilization compared with other row crops although responses have been observed in low-testing soils [35]. Large soybean yield responses to P fertilization have been reported [36]-[40], but these responses were frequent observed when soils tested very low or low.

3.4. Protein, Oil, and Fatty Acid Concentrations

Both protein and oil concentrations in seed differed among P application rates at Jackson in 2009 and 2010 and averaged over the three years (Table 5). The fact that significant protein and oil responses to P fertilization in 2009 and 2010 but not in 2008 at Jackson might be attributable to the accumulated treatment effects over the years. At Milan, protein concentrations responded to P rates in 2009 and on the three-year averages; while oil concentrations were not affected by P applications in any year (Table 5). In general, protein concentrations were enhanced with increased P application rates, but oil levels decreased when P rate was increased at both locations. The year effects on protein and oil concentrations were significant at both locations.

It is well known that P deficiency reduces soybean leaf area and the number of leaves, nodes, and branches; P-deficient plants exhibit reduced carbohydrate supply to nodules and decreased nodule weight, number, and functioning, such as reduced nitrogenase activity of the nodule. Therefore, P fertilization increases N fixation when soybean is deficient in P, and thus enhances protein concentration in soybean seed.

Concentrations of the fatty acids in seed oil responded differently to P application rates at both locations (Table 6). At Jackson, the responses of palmitic and oleic acid concentrations to P rates were significant and positive in 2009 and 2010 and on the

Table 4. Phosphorus fertilization effects on seed yield at Jackson and Milan from 2008 to 2011.

Year	Treatment[a]	Jackson	Milan
		$Mg\cdot ha^{-1}$	
2008	1	2.45	2.28
	2	2.55	2.37
	3	2.48	2.22
	4	2.49	2.33
	5	2.61	2.27
	6	2.51	2.29
	Sig[b]	ns[c]	ns
2009	1	2.42	2.92
	2	2.59	2.91
	3	2.50	2.56
	4	2.46	2.90
	5	2.52	2.72
	6	2.61	2.73
	Sig	ns	ns
2010	1	2.74	2.71
	2	2.63	2.99
	3	2.77	2.79
	4	2.83	2.80
	5	2.82	2.69
	6	2.82	2.70
	Sig	ns	ns
2011	1	2.08	2.14
	2	2.19	2.23
	3	2.06	2.18
	4	2.03	2.21
	5	2.29	2.14
	6	2.01	2.22
	Sig	ns	ns
Average	1	2.42	2.51ab[d]
	2	2.49	2.63a
	3	2.45	2.44b
	4	2.45	2.56ab
	5	2.56	2.45b
	6	2.49	2.48b
Year	2008	2.51b	2.29b
	2009	2.52b	2.79a
	2010	2.77a	2.78a
	2011	2.11c	2.19b
Sig	Trt[e]	ns	*
	Year	***	***
	Trt × Year	ns	ns

*Significant at 0.05 probability level; ***Significant at 0.001 probability level. [a]Treatments 1, 2, 3, 4, and 5 were the annual P application rates of 0, 10, 20, 30, and 40 $kg\cdot P\cdot ha^{-1}$, respectively; treatment 6 was the P fertilizer recommended rate based on soil P testing results every year. [b]Sig, significance. [c]ns, not significant at 0.05 probability level. [d]Means in a column within each year, averaged over the four years, or averaged over the six treatments followed by the same letter are not significantly different at P = 0.05 according to the protected LSD. [e]Trt, treatment.

Table 5. Phosphorus fertilization effects on the concentrations of seed P, protein, and oil at Jackson and Milan from 2008 to 2010.

Year	Treatment[a]	Jackson			Milan		
		Seed P	Protein	Oil	Seed P	Protein	Oil
		$g \cdot kg^{-1}$					
2008	1	5.40	437	205	5.50	405	220
	2	5.48	433	205	5.73	403	221
	3	5.55	431	203	5.55	407	220
	4	5.35	430	207	5.45	406	220
	5	5.53	432	205	5.50	404	218
	6	5.58	435	207	5.30	407	219
	Sig[b]	ns[c]	ns	ns	ns	ns	ns
2009	1	5.80	410c[d]	244a	6.04	412b	228
	2	5.80	433b	225c	5.90	441a	225
	3	5.83	432b	232bc	6.05	438a	225
	4	5.95	433b	234b	5.90	433a	226
	5	6.01	441a	233bc	6.03	436a	224
	6	5.90	436ab	238ab	5.84	440a	224
	Sig	ns	***	**	ns	***	ns
2010	1	6.66c	445c	198a	6.22b	420	202
	2	7.09a	448bc	195ab	6.20b	421	203
	3	6.85bc	446c	199a	6.51a	422	201
	4	6.92ab	459a	190c	6.39ab	422	200
	5	6.85bc	454ab	191bc	6.24b	422	204
	6	6.80bc	451bc	199a	6.30b	423	203
	Sig	*	*	**	*	ns	ns
Average	1	5.96	431d	216a	5.92	412b	217
	2	6.12	438bc	208d	5.94	422a	216
	3	6.08	436c	212bc	6.04	422a	216
	4	6.07	441ab	210cd	5.91	420a	215
	5	6.13	442a	210cd	5.92	421a	215
	6	6.12	441ab	214ab	5.81	423a	216
Year	2008	5.48c	433b	205b	5.50c	405c	220b
	2009	5.88b	431b	234a	5.96b	433a	225a
	2010	6.86a	450a	195c	6.31a	421b	202c
Sig	Trt[e]	ns	***	***	ns	***	ns
	Year	***	***	***	***	***	***
	Trt × Year	ns	***	***	ns	***	ns

*Significant at 0.05 probability level; **Significant at 0.01 probability level; ***Significant at 0.001 probability level. [a]Treatments 1, 2, 3, 4, and 5 were the annual P application rates of 0, 10, 20, 30, and 40 $kg \cdot P \cdot ha^{-1}$, respectively; treatment 6 was the P fertilizer recommended rate based on soil P testing results every year. [b]Sig, significance. [c]ns, not significant at 0.05 probability level. [d]Means in a column within each year, averaged over the three years, or averaged over the six treatments followed by the same letter are not significantly different at P = 0.05 according to the protected LSD. [e]Trt, treatment.

Table 6. Phosphorus fertilization effects on the concentrations of fatty acids at Jackson and Milan from 2008 to 2010.

Year	Treatment[a]	Jackson					Milan				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic	Palmitic	Stearic	Oleic	Linoleic	Linolenic
		$g \cdot kg^{-1}$ oil					$g \cdot kg^{-1}$ oil				
2008	1	110	41.1	249	566	68.7	116	43.3	238	556	48.2
	2	110	40.9	249	554	72.4	113	43.2	247	557	51.5
	3	109	40.7	245	564	76.2	116	43.4	242	555	46.9
	4	106	41.1	248	561	71.7	112	43.3	249	554	46.5
	5	110	41.1	248	561	74.7	115	43.4	244	558	43.8
	6	110	40.7	245	566	75.5	115	43.5	245	553	46.3
	Sig[b]	ns[c]	ns	ns	ns	ns	ns	ns	ns	ns	ns
2009	1	112d	40.5	227c[d]	536ab	107a	117b	392	228c	540b	90.6
	2	144ab	41.0	273ab	528c	78.9b	136a	387	276a	552a	109.0
	3	130c	40.6	269b	535abc	80.7b	129ab	407	263ab	540b	93.5
	4	135bc	40.6	282a	531bc	79.1b	141a	391	271ab	539b	104.0
	5	145a	40.8	273ab	541a	79.9b	135a	391	262ab	550a	101.0
	6	144ab	40.5	271ab	538ab	90.4ab	142a	391	256b	546a	98.4
	Sig	***	ns	***	*	*	*	ns	**	*	ns
2010	1	93.4bc	42.5	290bc	539	65.0	99.0	43.5	274	555	67.9d
	2	95.0bc	42.5	283c	551	58.7	97.8	43.1	272	549	96.3a
	3	101a	42.6	278c	540	68.9	101	42.3	265	565	87.3ab
	4	91.2c	42.8	307a	534	62.9	98.9	43.1	278	560	80.7bc
	5	94.1bc	42.9	300ab	533	71.3	98.0	43.2	276	555	76.6bcd
	6	97.0ab	42.4	298ab	543	69.9	99.6	42.6	265	562	74.8cd
	Sig	*	ns	*	ns	ns	ns	ns	ns	ns	**
Average	1	105c	41.4	255d	547	80.4	111	42.0	247d	551	68.9c
	2	115a	41.5	268bc	544	70.0	115	41.7	265ab	553	85.5a
	3	113ab	41.3	264c	546	75.3	115	42.1	257bc	553	76.9b
	4	111b	41.5	279a	542	71.2	117	41.8	266a	551	77.1b
	5	117a	41.6	274ab	545	75.3	116	41.9	261abc	554	73.6bc
	6	117a	41.2	268bc	549	78.6	119	41.7	255cd	554	73.2bc
Year	2008	109b	40.9b	247c	562a	86.1a	115b	43.4a	244c	556a	47.2c
	2009	134a	40.7b	266b	535b	73.2ab	133a	39.3b	259b	545b	99.3a
	2010	95.2c	42.6a	291a	540b	66.1b	98.6c	43.0a	271a	558a	80.6b
Sig	Trt[e]	***	ns	***	ns	ns	ns	ns	***	ns	***
	Year	***	***	***	***	***	***	***	**	*	***
	Trt × Year	***	ns	***	**	*	**	ns	**	*	ns

*Significant at 0.05 probability level; **Significant at 0.01 probability level; ***Significant at 0.001 probability level. [a]Treatments 1, 2, 3, 4, and 5 were the annual P application rates of 0, 10, 20, 30, and 40 $kg \cdot P \cdot ha^{-1}$, respectively; treatment 6 was the P fertilizer recommended rate based on soil P testing results every year. [b]Sig, significance. [c]ns, not significant at 0.05 probability level. [d]Means in a column within each year, averaged over the three years, or averaged over the six treatments followed by the same letter are not significantly different at P = 0.05 according to the protected LSD. [e]Trt, treatment.

three-year averages. Linoleic and linolenic acid levels showed significant responses to P applications in 2009 only. Linolenic acid concentration was reduced with P fertilization in 2009.

At Milan, P fertilization effects on the concentrations of palmitic and linoleic acids were significant and positive in 2009. Oleic acid responded positively to P rates in 2009 and averaged over the three years. Linolenic acid levels showed a significant and positive response to P application up to a certain rate in 2010 and on three-year averages. However, stearic concentration was not influenced by P application regardless of location and year in our study.

The fact that significant responses of several fatty acids to P fertilization in 2009 and 2010 but not in 2008 might be attributable to the accumulated treatment effects over the years at both locations. A larger number of fatty acids in response to P fertilization at both locations in 2009 might be partially resulted from the relatively lower temperatures in July 2009, because lower temperatures reduced soybean P uptake, and thus resulted in greater responses of fatty acids to P fertilization. On the other hand, very low rainfall in September at both locations in 2008 and 2010 might be in part responsible for no fatty acid response in 2008 and fewer significant responses of fatty acids in 2010 to P fertilization.

In general, palmitic and oleic acid concentrations responded to P fertilization positively when the responses were significant in our study. The responses of linolenic acid concentration were positive in 2009 at Jackson and positive in 2010 and on the three-year averages at Milan. There were significant differences in the concentrations of all fatty acids among the three years irrespective of location. It is interesting that protein, oil, and fatty acid concentrations sometimes responded significantly to P applications even when seed yield and/or seed P concentration did not respond to P fertilization.

Abbasi *et al.* (2012) [27] reported that P application increased both protein and oil concentrations in soybean seed. However, Haq and Mallarino (2005) [20] found that P fertilization increased soybean protein and oil concentrations in some trials but decreased protein and oil levels in other trials. Krueger *et al.* (2013) [23] reported that protein or oil concentrations in soybean seed were not affected by P fertilization at most locations. Gaydou and Arrivets (1983) [25], Israel *et al.* (2007) [28], and Krueger *et al.* (2013) [23] found that linoleic acid concentration decreased with P fertilization. Gaydou and Arrivets (1983) [25] and Israel *et al.* (2007) [28] also observed an increase in oleic acid concentration at higher P application rates. Krueger *et al.* (2013) [23] reported that linolenic acid concentration increased with excessively high P fertilization. The different results in seed quality attributes between our study and these prior investigations are possibly related to the differences in soil P fertility levels, cultivar characteristics, and weather conditions among these studies.

Linoleic acid concentration is a key quality attribute in soybean seed for human and animal consumption because it is an important polyunsaturated fatty acid that cannot be synthesized by humans or animals [41]. Therefore, higher levels of linoleic acid in the oil may increase the market value of soybean seed. The results of our study suggest that linoleic acid concentration in soybean seed can sometimes be positively affected by P fertilization.

Soybean breeders have endeavored to create new cultivars with lower linolenic acid concentration in the seed for value added cooking oil with improved health benefits [42] [43]. Our results suggest that indigenous soil P fertility level and P fertilizer application rate should be taken into account in breeding for soybean cultivars with low linolenic acid concentration since they can influence linolenic acid concentration in the seed.

Year exerted significant effects on protein, oil, and fatty acid concentrations at both locations in our study. The fact that the temperatures and rainfall were different among the four years at least partially explained the variability in P fertilization effects on the concentrations of protein, oil, and fatty acids among the three growing seasons.

Our results agree with those of Krueger *et al.* (2013) [23] in that excessive levels of P fertility decrease the quality of some attributes in soybean seed; seed quality attributes varied with treatments, locations, and years, but the variations were generally not consistent. Seed quality attributes are very important traits for soybean producers to manage because many processors place higher prices on soybean seed with desirable quality attributes.

3.5. Production of Protein, Oil, and Fatty Acids

At Jackson, the production of protein, oil, and fatty acids were not affected by P fertilization in any year or on the averages over the three years except linolenic acid in 2009 which was reduced due to P application compared with the zero P control (data not shown). However, the year effects were significant on all the quality attributes except palmitic acid. Protein production was higher in 2010 than those in the other two years; while the production of oil and stearic, oleic, linoleic, and linolenic acids were all higher in 2009 than those in 2008 and 2010 (data not shown).

At Milan, the production of protein, oil, and fatty acids were not influenced by P fertilization in any year except palmitic and oleic acids in 2009 and linolenic acid in 2010 (**Table 7**). In general, P application rate up to a certain level resulted in higher production of palmitic and oleic acids in 2009 and linolenic acid in 2010. Averaged over the three years, there was a significant P effect on all the quality attributes except stearic acid. Obviously, application of 10 $kg \cdot P \cdot ha^{-1}$ resulted in higher production of protein, oil, and palmitic, oleic, linoleic, and linolenic acids than zero P and the higher P application rates as well on the three-year averages. The year effects were consistently significant on all the quality attributes; but the quality attributes responded differently to year. Both protein and stearic acid production were similar for 2009 and 2010, which were higher than those in 2008. The production of oil and palmitic, oleic, linoleic, and linolenic acids were the highest in 2010 but the lowest in 2008.

Basically the production of protein, oil, and fatty acids depend on the seed yield level and the concentrations of these attributes in the seed. Haq and Mallarino (2005) [20]

Table 7. Phosphorus fertilization effects on the production of protein, oil, and fatty acids at Milan from 2008 to 2010.

Year	Treatment[a]	Protein	Oil	Palmitic	Stearic	Oleic	Linoleic	Linolenic
		kg·ha^{-1}						
2008	1	921	501	57.9	21.7	119	279	24.2
	2	955	524	59.2	22.6	129	292	27.2
	3	900	488	56.5	21.2	118	271	22.8
	4	949	514	57.6	22.3	128	285	23.8
	5	917	495	56.9	21.4	121	276	21.8
	6	930	501	57.6	21.8	123	278	23.1
	Sig[b]	ns[c]	ns	ns	ns	ns	ns	ns
2009	1	1205	665	77.8bc[d]	26.1	152c	359	60.1
	2	1282	654	88.6ab	25.3	181a	361	71.0
	3	1123	578	74.0c	23.5	152c	312	54.2
	4	1253	656	92.5a	25.6	178ab	354	68.5
	5	1187	609	82.5abc	23.8	160abc	334	61.2
	6	1201	613	87.0ab	24.0	157bc	335	60.6
	Sig	ns	ns	*	ns	*	ns	ns
2010	1	1137	547	54.1	23.8	150	303	37.1c
	2	1256	606	59.3	26.1	165	333	58.9a
	3	1174	560	56.3	23.7	149	316	48.9b
	4	1180	559	54.1	24.1	155	313	45.1bc
	5	1132	547	53.6	23.6	151	303	41.8bc
	6	1141	549	54.6	23.5	146	308	41.3bc
	Sig	ns	ns	ns	ns	ns	ns	**
Average	1	1088b	571abc	63.2b	23.8	140c	314ab	40.5b
	2	1164a	595a	69.0a	24.7	158a	329a	52.4a
	3	1066b	542c	62.3b	22.8	139c	300b	42.0b
	4	1128ab	576ab	68.1a	24.0	154ab	317ab	45.8b
	5	1079b	550bc	64.3ab	23.0	144bc	305b	41.6b
	6	1091b	555bc	66.4ab	23.1	142c	307b	41.7b
Year	2008	929b	504c	57.6b	21.8b	123c	280c	23.8c
	2009	1209a	629a	83.8a	24.7a	163a	343a	63.0a
	2010	1170a	561b	55.3b	24.1a	153b	313b	45.5b
Sig	Trt[e]	*	*	*	ns	**	*	***
	Year	***	***	***	**	***	**	***
	Trt × Year	ns	ns	ns	ns	ns	ns	ns

*Significant at 0.05 probability level; **Significant at 0.01 probability level; ***Significant at 0.001 probability level. [a]Treatments 1, 2, 3, 4, and 5 were the annual P application rates of 0, 10, 20, 30, and 40 kg·P·ha^{-1}, respectively; treatment 6 was the P fertilizer recommended rate based on soil P testing results every year. Sig, significance. [c]ns, not significant at 0.05 probability level. [d]Means in a column within each year, averaged over the three years, or averaged over the six treatments followed by the same letter are not significantly different at P = 0.05 according to the protected LSD. [e]Trt, treatment.

found that protein and oil production responses to P fertilization tended to follow yield responses; P fertilization that increases soybean yield has infrequent, inconsistent, and small effects on oil and protein concentrations but often increases oil and protein production.

4. Conclusions

Phosphorus application rates had greater impacts on leaf P nutrition at the earlier growth stages. As P application rate went up, the increase in leaf P gradually slowed down. Protein, oil, and fatty acid concentrations in seed responded differently to P fertilization. In general, protein concentrations were enhanced but oil levels decreased with increased P application rate. Palmitic and oleic concentrations responded positively to P application rate up to a certain level. However, the response of linolenic acid concentration was inconsistent (negative or positive). Stearic concentration was not influenced by P fertilization. Application of 10 $kg{\cdot}P{\cdot}ha^{-1}$ resulted in higher production of protein and palmitic, oleic, and linolenic acids than zero P and the higher P application rates as well on the P deficient soil. Excessive P application rates could lower seed yield and the quality of some attributes in seed. Our results suggest that linoleic acid concentration, a key quality attribute in soybean seed for human and animal consumption, can sometimes be enhanced by P fertilization, and thus appropriate P application may increase the market value of soybean production; the indigenous soil P fertility level and P application rate should be taken into account by soybean breeders in breeding for new soybean cultivars with low linolenic acid level since P can affect linolenic acid level in the seed.

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