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Effects of Phosphorus on Seed Oil and Protein Contents and Phosphorus Use Efficiency in Some Soybean Varieties

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ABSTRACT

Phosphorus (P) uptake and utilization by soybean (*Glycine max* (L.) Merrill) is essential for plant growth. Therefore, this experiment was conducted to evaluate the P effects on selected soybean varieties based on the seed oil content (SOC) and the seed protein content (SPC) and to assess the physiological responses associated with changes in shoot P-utilization efficiency (SPUE). The experiment was carried out during 2008 and 2009 with a split-plot design at the Agronomy Department, Kasetsart University, Bangkok, Thailand. The main plots were for tested three P levels in a nutrient solution (0.5, 1.0 and 2.0 m*M* P), with subplots for the three soybean cultivars, CKB1, SJ5 and CM60. The results indicated that at maturity, the P levels of 2.0 m*M* P decreased SPUE by 27% compared to that of 0.5 m*M* P (the control). SOC was not significantly affected by the P level. Relative to the control, the P nutrition levels of 1.0 and 2.0 m*M* P significantly decreased SPC by 4% and 5%, respectively. There were no significant differences in SOC between varieties. The SPC of CKB1 was 8% greater than that of SJ5 but showed no significant difference to that of CM60.

Key words: *Glycine max* (L.) Merrill, phosphorus, seed oil, seed protein content, P use efficiency

INTRODUCTION

Phosphorus is an essential element for growth, development and yield of soybean (*Glycine max* (L.) Merrill). A lack of this element is doubly serious since it may prevent other nutrients from being absorbed by soybean plants (Barkert and Sfredo, 1994). Fertilization with N, P, K and other nutrients can affect yield and many physiological processes that, in turn, can influence grain yield and protein or oil concentration (Kamprath, 1974). Soybean responses to P or K fertilization have been reported in Iowa (Borges and Mallarino, 2000). Supra-optimal N also increased the seed protein content (SPC) of the high protein line variety, but this increase resulted in a decrease in yield (Nakasathien *et al.*, 2000). Phosphorus application through single super phosphate significantly increased the seed yield and oil content (Tanwar and Shaktawat, 2003).

Soybean quality is influenced by nutrient availability and phosphorus has a positive effect upon oil and protein content (Gaydou and Arrivets, 1983). Very high soil phosphate values may depress seed protein and oil contents, or induce zinc and iron deficiencies (Weiss, 1983). Phosphorus application is necessary for high protein and oil yield from soybean seeds (Shah *et al.*, 2001).

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Soybean seed contains approximately 37-41% protein, 18-21% oil, 30-40% carbohydrate and 4-5% ash (Hulse, 1996). Research has shown that genetic success in increasing yield and oil concentration, maintained protein concentration, but high protein cultivars tended to have relatively low oil concentration (Westgate *et al*. 1999; Morrison *et al.,* 2000).

Increases in P- and N-utilization efficiencies were associated with increases in the relative proportions of dry matter, P and N in shoot tissues (Israel and Rufty, Jr. 1988). In evaluating plant biomass production in relation to nutrient supply, the efficiency of utilization of the absorbed nutrient within the plant is considered to be due to the efficiency of nutrient absorption (Siddiqi and Glass, 1981). Varietal differences in response to nutrient levels and plant efficiencies for nutrient uptake and use have been reported for many species. The search for efficient plants in nutrient uptake and use has been stimulated since large genetic variability was reported for these characters within germplasm of several species. Nutrient solution techniques have been used as important tools in short-term experiments to select and identify nutrient-efficient and Al-tolerant plants (Furlani *et al*., 1998).

Responses in oil and protein concentrations of soybean grain to fertilization were infrequent, small, inconsistent and unrelated to more frequent and usually positive grain yield responses (Haq and Mallarino, 2005). Numerous studies investigated fertilization effects on soybean grain yield, but a few focused on oil and protein concentration. The objective of this research was to determine phosphorus fertilization effects on soybean seed oil and protein concentrations and P use efficiency.

MATERIALS AND METHODS

Plant culture

The treatments involved three soybean cultivars, Chakrabhandhu1 (CKB1), SJ5 and Chiangmai 60 (CM60), grown under three rates of P in nutrient solution. The CM60 variety was used as a control to check variation in the experiment. Seeds were pre-germinated on germination paper and saturated with 0.5 m*M* $CaSO_4$ at 28°C and 95% relative humidity for four days before transplanting into recipient lids with coarse perlite. Four-day-old uniform-sized seedlings were transplanted to the (25-L) recipients, according to the experimental design and harvesting-stage. In each recipient, one seedling/hole was suspended in the nutrient solution from lids holding plants of the three cultivars. The experimental design used split plots with three replications. The main plots had different P rates and the sub-plots were based on the soybean cultivars. The P rates consisted of 0.5, 1.0 and 2.0 m*M*. The treatment of 0.5 m*M* P was used as the control P. Fifteen days after transplanting, strings were suspended from the upper part of the greenhouse structure to hold plant shoots and to guide and support them during growth. The following procedures were performed daily or on alternate days: adjustment of nutrient solution levels of the under-table recipients to the 75-L level with deionized water; root separation per cultivar; pH and electrical conductivity monitoring. The 25-L recipients were provided with lids containing nine 2.5 cm-holes to support the plants. The under-table 75-L recipient was provided with a pump to circulate the nutrient solution continuously. Plants were exposed to natural light intensity, temperature, humidity and day-length conditions throughout the entire course of the experiment. The experiment was carried out in a greenhouse of the Agronomy Department, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand from late November 2008 until mid-March 2009.

Nutritional treatments

The base nutrient solution (Hoagland

solution) was made with chemicals and deionized water. The nutrient solution composition maintained during plant growth for macronutrients in mmol L^{-1} was $Ca(NO_3)_2$ = 2.5; KNO_3 = 2.5; and $MgSO_4$ = 1. The nutrient solution composition for micronutrients in μmol L^{-1} was H_3BO_3 = 46.2; $MnCl_2$ = 9.2; $CuSO_4$ = 0.38; $ZnSO_4$ = 2.4; $NaMoO_4$ = 1.2 and for Fe(III)EDTA in mmol L^{-1} was Fe(III)SO_4 = 90; and EDTA = 90. P was added in three doses as KH_2PO_4 (0.5, 1.0 and 2.0 mmol L^{-1}).

The nutrient solution pH was about 5.1 and remained at about 4.8 during plant growth. The electrical conductivity was about 2-4 mS cm^{-1}. The nutrient solution was sampled for chemical analysis at the beginning of the experiment and on four other occasions. The nutrient solution was replenished every 14 days after transplanting using proportional volumes of the balanced stock solutions. Nutrient depletion occurred slowly because of the large volume of solution available to the plants.

Sampling procedures

Three replicates of the control and P-treated plants were harvested at V5, R5 and maturity for biological yield and harvested at maturity for grain yield, oil and protein contents. Plants were separated into leaves, stem, roots, seeds and pod walls and oven-dried at a constant 65°C for 72 h. The dry mass of the plant parts and the P concentration of plant shoots and seeds were measured.

Phosphorus analysis

For determination of total P-concentration, air-dried tissue samples (100 mg) were ashed overnight at 500°C. The ash was dissolved in 3 ml of 4 M HCl and diluted to 25 ml with redistilled water. Appropriate aliquots were analyzed for P by the ammonium-molybdate spectrophotometry method (Israel and Rufty, Jr. 1988; Ryan *et al.*, 2001).

Seed oil and protein content analysis

All samples of seeds were ground to a fineness that provided maximum homogeneity and minimum sampling variation. Oil and protein concentrations were determined in whole grain samples by a near infrared spectroscopy method (Haq and Mallarino, 2005). The calibration process was described by Rippke *et al.* (1996) and was subsequently made into a standard method by the American Association of Cereal Chemistry (1999). Both oil and protein concentrations were adjusted to 130 g kg^{-1} grain moisture concentration.

Statistical analysis

Data were subjected to the analysis of variance (ANOVA) procedure of the Statistical Analysis System and the differences between treatment means were separated by the use of least significant difference (LSD) at the 5% level (IRRISTAT-5.0).

RESULTS

Soybean growth and phosphorus use efficiency

There were no interactions among P levels and soybean varieties. Shoot/root DM at the R5 stage was not significantly different among soybean varieties and P levels. The whole-plant dry weight of CKB1 was 37 % and 27 % greater than that of SJ5 and CM60, respectively (Table 1). The application of P-nutrient concentration rates of 1.0 and 2.0 m*M* P increased the whole-plant dry weight by 48% and 31%, respectively, compared to 0.5 m*M* P (control). The 1.0 m*M* P-nutrient concentration had significantly higher shoot, root and plant dry weight than the others (Table 1).

The plant shoot P uptake of each variety response to P application was approaching the maximum level at 2.0 m*M* external P-concentration (Figure 1). Furthermore, the accumulation of P in the shoots was increased significantly by higher phosphorus levels when

Table 1 Effects of different P levels on dry weight of shoot, root, whole plant and shoot/root ratio of three selected soybean varieties at the R5 stage.

P level/Variety	Shoot	Root	Total DM	Shoot/root DM
	(g/plant)			
P level				
0.5 m*M* P	14.1c	1.35b	15.4c	11.9
1.0 m*M* P	20.5a	2.36a	22.9a	9.0
2.0 m*M* P	18.2b	1.96a	20.2b	9.4
Variety				
CKB1	22.2a	2.54a	24.7a	9.3
SJ5	14.2c	1.42b	15.7c	10.2
CM60	16.4b	1.74b	18.1b	10.29
CV (a) %	12	13	12	16
CV (b) %	8	17	8	18

Note: Within column, means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test (DMRT).

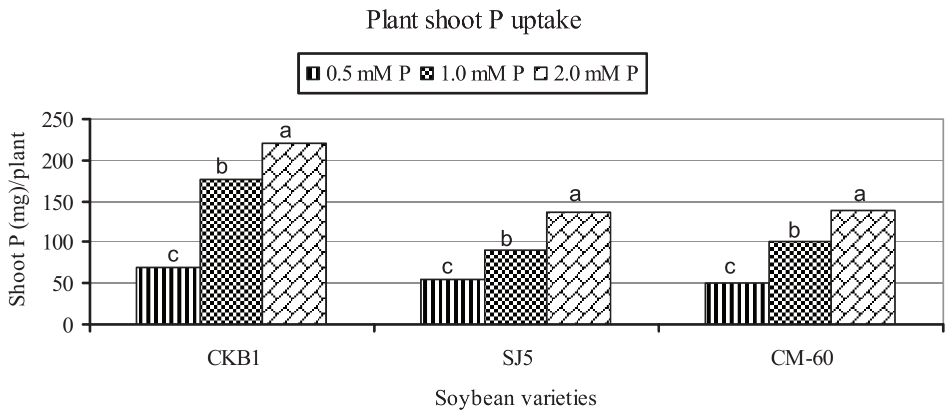

Figure 1 Shoot P uptake of the three soybean varieties grown with different P concentrations at the R5 stage. Note: a, b and c indicate statistically significant difference at the 5% level by DMRT.

compared to that of the control. Maximal P-utilization efficiency (g dry weight^2/mg P), calculated by the procedure of Siddiqi and Glass (1981), occurred in the nutrient P-concentration range of 0.5 to 1.0 m*M* P for plants in an average of soybean varieties and at higher P applications, the shoot P-utilization efficiency decreased significantly (Figure 2A). Phosphorus utilization quotients (g dry weight/mg P) in shoots were also greatest at the lowest P-fertilization level in all soybean varieties at reproductive stage R5 (Figure 2B). A decrease in P-utilization quotients with increasing solution P-concentration was observed in these soybean varieties.

Observation of the effects of different P levels at maturity indicated that there was no significant difference in the dry weight of whole plant, the shoots and in the P utilization quotient among varieties (Table 2). The shoot dry mass of 1.0 and 2.0 m*M* were significantly increased by

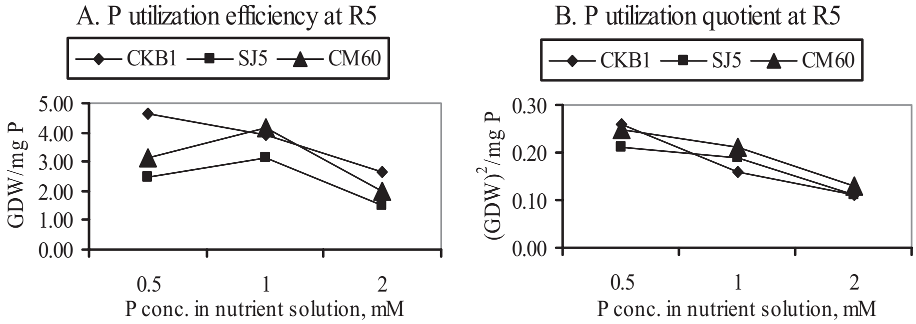

Figure 2 Effect of different P levels on shoot P-utilization efficiencies and P utilization quotient of three soybean varieties at the R5 stage.

Table 2 Effects of different P levels on the shoot P-utilization quotient and P utilization efficiency of three selected soybean varieties at mature stage.

P level/Variety	Total DW (g/plant)	Shoot DW (g/plant)	P-utilization Quotient a)	P-utilization Efficiency b)
P level				
0.5 m*M* P	25.8b	24.6b	0.213a	5.21a
1.0 m*M* P	36.0a	34.4a	0.139b	4.77ab
2.0 m*M* P	33.3a	31.6a	0.117b	3.79b
Variety				
CKB1	34.2	32.5	0.156	4.89a
SJ5	27.1	25.9	0.147	3.72b
CM60	33.8	32.2	0.167	5.17a
CV (a) %	21	21	12	10
CV (b) %	21	22	14	12

a) Shoot P-utilization quotient = plant shoot dry weight/mg P in plant shoot of P
b) Shoot P-utilization efficiency = [(shoot DM)^2/shoot P-content]
Note: Within column means followed by the same letter are not significantly different at the 5% level by DMRT.

40% and 28%, respectively, when compared to that of 0.5 m*M* P (Table 2). Maximal dry matter accumulation was approached in the solution P concentration rate of 1.0 m*M* (Table 2). The SPUE of CM60 and CKB1 were significantly higher than for the SJ5 variety by 39 and 31%, respectively. The P levels of 2.0 m*M* caused a significant decrease in SPUE by 27%, compared to that of 0.5 m*M* P at maturity. The shoot P-utilization quotient (g dry weight/mg P) of 1.0 and 2.0 m*M* were significantly decreased by 35 and 45%, respectively, when compared to that of 0.5 m*M* P (Table 2).

The application of 1.0 and 2.0 m*M* P increased shoot total P by 118 and 146%, respectively, compared to the application of 0.5 m*M* P (Table 3). The plant shoot P and seed P obtained with 1.0 and 2.0 m*M* P were not statistically different. However, the CM60 variety showed significantly higher accumulation of P in seed by 28 and 18% when compared to SJ5 and CKB1 varieties, respectively (Table 3). In addition, there was no significant difference in dry mass between 1.0 and 2.0 m*M* P. Thus, the plant shoot P accumulation response to P application approached the maximum level at 1.0 m*M* P external P-concentration.

Seed oil and protein contents

There were no significant differences in oil content among soybean varieties and P levels (Table 3). SJ5 showed significantly lower protein content than CKB1 and CM60, whereas 0.5 m*M* P produced the highest protein content (Table 3). Furthermore, there was a highly significant difference among the seed protein contents of the soybean varieties. The CKB1 variety was the highest in protein content among the soybean varieties, which was higher than that of SJ5 and CM60 by 8 and 3%, respectively. CKB1 and CM60 had comparable protein contents that were significantly higher than that of SJ5. When averaged across the varieties, the application of 1.0 and 2.0 m*M* P caused significant decreases in the seed protein content by 4 and 5%, respectively, when compared to that of 0.5 m*M* P (Table 3).

DISCUSSION

The results were consistent with previous reports of Israel and Ruffty, Jr. (1988) and Furlani *et al*. (2001). Increases in P utilization efficiencies were associated with increases in the relative proportions of dry matter. In evaluating plant biomass production in relation to nutrient supply, efficiency of utilization of the absorbed nutrient within the plant was considered to be as important as the efficiency of nutrient absorption (Siddiqi and Glass, 1981). The decreasing plant shoot biomass with increasing P fertilization indicated that the plants grown at the lowest P level were the most efficient in using P for production of dry matter. In any experiment with a nutritional variable, plants grown at the lowest nutrient concentrations will inevitably have the highest utilization quotient because of this dilution effect (Jarrell and Beverly, 1981). P concentrations in the dry tissue of shoots and seeds increased with increasing P levels and were inversely proportional to the dry matter yield, which was what was expected due to the dilution effect (Furlani *et al*., 2001). Similar effects for P supply on the utilization efficiency of shoot phosphorus were noted at the R5 and mature stages of soybeans. The decreasing P-utilization quotients with increasing P concentration in the solution indicated that the plants grown at the lowest P levels were the most efficient in using P for the production of dry matter. In contrast, maximal SPUE was attained at a solution P-concentration of 0.5 m*M*, which was similar to the optimum for dry matter production. The decrease in SPUE at 1.0 and 2.0

Table 3 Effect of different P levels on the P content of plant shoot total, seed and seed oil and protein contents of three selected soybean varieties at mature stage.

P level/Variety	Shoot P (mg/plant)	Seed P (mg/plant)	Oil content (%)	Protein content (5)
P level				
0.5 m*M* P	118.6b	47.9b	16.9	40.2a
1.0 m*M* P	258.4a	72.3a	18.1	38.5b
2.0 m*M* P	291.6a	64.4a	17.5	38.3b
Variety				
CKB1	236.1	59.7ab	16.9	40.4a
SJ5	211.8	52.5b	17.3	37.1b
CM60	220.6	72.4a	18.3	39.4a
CV (a) %	13	14	17	2
CV (b) %	16	15	7	2

Note: Within column means followed by the same letter are not significantly different at the 5% level by DMRT.

m*M* P resulted from the accumulation of increasing amounts of P without any additional increase in growth. The P utilization quotient was greatest at the lowest level of P supply and decreased as the P supply was increased.

Observation from this experiment confirmed previous reports of complex environmental effects on soybean oil and protein concentrations (Westagate *et al*., 1999). P fertilization increased seed dry weight and SOC up to the rate of 1.0 m*M* external P-fertilization, but decreased SPC with increased P rates. The soybean quality was influenced by nutrient availability, with P having a positive impact on seed oil and grain yield. Responses by soybean to P or K fertilization have been reported in Iowa (Borges and Mallarino, 2000). Due to the increase in yield and improvement in the nutritive quality of the soybean seed and shoot, P application is necessary to achieve high protein and oil yields from soybean seeds (Shah *et al*., 2001). P has been shown to be an essential element and its application has been shown to be important for growth, development and yield of soybean (Kakar *et al*., 2002). Significant effects on oil and protein content were noted in different levels of P and seed inoculation (Malik *et al*., 2006). P application is necessary for high protein and oil yields from soybean seeds (Shah *et al*., 2001). Excess phosphorus may depress the seed protein and oil content. Very high soil phosphate values may depress the seed protein content and adequate supplies of phosphate are necessary to optimize seed protein content and amino acids in the protein, which is particularly important where soybean is a major protein source (Weiss, 1983). Research has shown that genetic success at increasing both yield and oil concentration has maintained protein concentration, but high protein cultivars tend to have relatively low oil concentration (Morrison *et al.,* 2000). P uptake and utilization by soybean is essential for plant growth and to ensure higher yield and improved quality of the crop.

CONCLUSIONS

Phosphorus utilization efficiencies and dry matter accumulation in the soybean varieties studied increased with increased phosphorus nutrient application up to the rate of 1.0 m*M* P. Increasing the external P concentration to more than 1.0 m*M*, caused a decline in P-utilization efficiencies, as the P concentration in the tissues increased without any additional growth. An increase in P-utilization efficiencies was associated with an increase in the relative proportions of dry matter P in shoot tissues.

Furthermore, the application of P-fertilization rates significantly affected SPC. P fertilization decreased SPC with increasing P rates. Applying external P increased the seed dry weight and SOC up to the rate of 1.0 m*M* P, but decreased SPC with increased P rates was observed. Total oil and protein production responses to fertilization tended to follow grain yield responses. The increase in grain yield and improvement in the nutritive quality of the soybean seed in these experiments indicated that applying P and using selected soybean varieties are necessary for high oil and grain yields from soybean seeds.

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