Response of Soybean Seed Protein and Oil Content to Phosphorous Fertilizer and Lime in Western Kenya

Bonface Alkamoi*[1] Lucas Ngode[1] and Julius Ochuodho[2]

School of Agriculture and Biotechnology University of Eldoret,
P.O. Box 1125-30100, Eldoret[1]
* Corresponding author, e-mail: alkamoi@yahoo.com

Department of Seed, Crop and Horticultural Sciences, University of Eldoret
P.O. Box 1125-30100, Eldoret[2]

Abstract

Soybean is an important protein and oil crop that is cultivated because of its uses as human food, animal feed and other industrial uses. The effect of phosphorus on seed protein and oil content had not been well understood. Thus, the objective of this experiment was to study the influence of phosphorus and lime on seed protein and oil content. In this respect a field experiment involving two soybean cultivars (SB 133 and SB 19), three phosphorous fertilizer rates (0, 30, 60 kg P ha^{-1}) and two lime rates (0, 2 tons ha^{-1}) was conducted at the university of Eldoret farm and Busia training centre farm. The treatments were laid out in the field in factorial arrangement in RCBD on 4 m by 3 m experimental plots. Seed samples were obtained from the experimental units and ground to uniform fineness for protein and oil content analysis. The result shows that the effect of phosphorus on seed protein and oil was significant. Seed protein and oil content for both cultivars increased with increase in P fertilizer application to high values at 60 kg P ha^{-1} at Busia and Eldoret. Seed protein was significantly influenced by lime whereas seed oil was not. It was concluded that supplying sufficient amounts of phosphorous fertilizer and lime in acidic soils with low initial P is desirable to improving the soybean seed protein and oil yields.

Keywords: *Glycine max*, phosphorous, lime, seed oil, seed protein.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the very important protein and oil crops in the world. It is used as human food, livestock feed and in several industrial purposes (Myaka *et al*., 2005). It comprises of 18-20 % edible oil, 24-45 % carbohydrates, 38-45 % crude protein and vitamins (Morshed *et al.*, 2008; Wilcox and Shibles, 2001). Its products are high in calcium, phosphorus, fibre and are cholesterol free with low levels of saturated fats (BIDCO, 2005). It is also a multipurpose legume that plays a significant role in improving the soil nutrition through the process of nitrogen fixation. In this regard, it has it fixes between 44 to 103 kg N ha^{-1} annually (Sanginga *et al.*, 2003). This had being attributed to improved yields of intercrop and the following rotated crop (Giller, 2001; Vanlauwe *et al.*, 2006).

In soybean production, provision of essential soil nutrition is crucial for improved crop performance and yield. Phosphorus is an essential element that is involved in several key plant functions that includes, photosynthesis, transformation of sugars and starches, energy transfer, translocation and transfer of genetic characteristics (Silva and Uchida, 2000). It is also involved in improving the process of biological nitrogen fixation by enhancing nodulation *(Kamara et al.,* 2007) and bacterial symbiotic relationship (Gangasuresh *et al.*, 2010).

Soybean has a high demand of phosphorous that is needed for root development, vegetative growth, flower initiation, seed and fruit development (Gangasuresh et al., 2010). It is important for crop maturity, improving yield and quality (Sara *et al.*, 2013) as well as enhancing tolerance to environmental stress (Tsvetkova and Georgiev, 2003). Pod filling and seed development had been identified as the peak P absorption phase estimated at 60 % of total P absorbed in the plant due to high demand for production of protein, seed oils, phospholipids and phytin (Shahid *et al.,* 2009; Silva and Uchida, 2000).

Studies have shown that soybean nutritional composition are positively influenced by both the genetic and environmental factors (Haq and Mallarino, 2005; Krishnan, 2000) which includes the crop nutrition, temperature, photoperiod among other factors during seed development. In other studies, the crop nutrition such as phosphorus, potassium, nitrogen and sulphur have been reported to affect yield, seed protein and oil concentration (Mahmoodi *et al*., 2013; Utsumi *et al.,* 2002). However, other scholars have reported the effect of phosphorous on seed oil and protein as moderate, infrequent and inconsistent (Haq and Mallarino, 2005; Seguin and Zheng, 2006).

However, the supply of phosphorous to plants is limited by soil *p*H levels, such that in acidic soils, it is fixed by highly reactive Aluminium (Al^{+}) and Iron (Fe^{3+}) ions whereas in basic soils, it is precipitated by Calcium (Ca^{2+}) into immobile pools (Gyaneshwar *et al.,* 2002; Hao *et al.*, 2002). Mandal and Khan (1972) observed that fixation of P in the soil complexes as di- and tri-calcium phosphate happens immediately after application rendering about two-thirds of phosphatic fertilizer unavailable. Soil amendment as a measure to release P and improve the soil condition for plant growth in such soils is therefore desirable. In acid soils for instance, lime is applied to neutralize the effect of acidic ions (H^{+}, Fe^{2+}, Al^{3+}, Mn^{4+} and Cu^{2+}) from soil adsorption site and supply significant amounts of Ca^{2+} and Mg^{2+} (Onwonga *et al.,* 2010). It is based on the limited study on the influence of phosphorous on soybean seed oil and protein content when grown in acid soils that this experiment was carried out to provide information on the influence of phosphorous on soybean seed oil and protein.

MATERIALS AND METHODS

The experiment was carried out in Western Kenya at University of Eldoret farm in Uasin Gishu County and in Agricultural Training Centre (ATC) farm in Busia County. Uasin Gishu County lies between longitudes 34°50 and 35°37 East and latitudes 0°30 South and 0°55 North at an elevation of 2120 m above sea level. It falls under the agroecological zone LH 3 (wheat/barley zone).The average rainfall is between 800 - 1000 mm, with an annual mean temperature of about 22 °C (Republic of Kenya, 1997). The soils are classified as Rhodic Ferralsols because it is underlain by murram, well drained, moderately deep, dark red friable clay of petroplinthite (Jaetzold & Schmidt, 1983). Soil analysis showed that it contains 9.11 ppm available phosphorous (Alkamoi, 2017) a value that is lower which is below the critical value of 10 mg P/ kg suggesting P application,(Okalebo *et al.*, 2002) and a *p*H of 4.96. On the other hand, the ATC farm in Busia is located at 0°27'26.91"N and 34° 7'0.94"E, at an elevation of 1130 m above sea level. It receives an annual rainfall range of between 1270 mm to 1790 mm and a mean temperature range of 22° C. The soils are well drained, moderately deep to very deep, reddish brown to yellowish brown, friable clay, over petroplinthite (orthic ferralsols; partly petro-ferric phase; with orthic (acrisols) and about

30% murram (Jaetzold and Schmidt, 1983). The experimental soils were found to be acidic of *p*H 5.41 with 5.00 ppm of phosphorous (Alkamoi, 2017).

Two soybean cultivars were used in the study; SB 133 that contains high oil content of 24.37% and relatively lower protein content of 37.59% and SB 19 which has a high protein content of 42.29% and relatively lower oil content of 19.71%. Phosphorous fertilizer in the form of triple supper phosphate (TSP) fertilizer (0.46.0) was applied at the rate of 0, 30 and 60 P Kg ha^{-1}. Physiolith® lime ($CaCo_3$) was supplied at two levels at 0 and 2 ton ha^{-1} (Okalebo *et al.,* 2002). The experiment was laid out in 2×3×2 factorial arrangement in a randomized complete block design and replicated thrice. The treatment were applied on 4 m by 3 m plots where the soybean cultivars were planted on an inter row spacing of 50 cm and intra row spacing of 5 cm. The field management to ensure the establishment of a competitive crop stand was done until the crop was ready for harvesting.

Seed harvesting

Soybean seeds were harvested when the crop had attained a uniform harvest maturity in a net area of 3 m by 2 m from each treatment plot. The pods were manually separated from the stover, sun-dried, gently hand threshed, cleaned and further dried to a safe storage moisture content of 13 % before processing further for protein and oil analysis.

Determination of seed protein and oil content

The seed samples from each treatment were each ground separately to a fineness that provided maximum floor homogeneity. Nitrogen content in seed was determined by Kjeldahl method where, the nitrogen content in the seed was multiplied by dry matter-based factor of 6.25 to determine total protein content (Williams *et al.*, 1998). The seed oil content was determined by adopting the Soxhlet Ether Extraction procedure, described by AOAC, (2005).

Data analysis

The data on seed protein and oil content was subjected to analysis of variance using R® statistical software version 3.4.3 (Kite-Eating Tree). The statistical significance of the treatments was tested at 5 per cent level of probability using Tukey’s HSD.

RESULTS AND DISCUSSION

The influence of soybean cultivar on seed oil content was significant ($p< 0.001$) when produced in Busia and Eldoret (Figure 1). High seed oil of 20.78 % in Busia and 19.93 % in Eldoret was recorded in SB 133, which differed significantly with the values recorded in SB 19 of 19.31 % in Busia and 18.55 % in Eldoret. The differences might be attributed to be caused by cultivar differences where, SB 133 was categorized as oily and SB 19 as proteinous (TSBF-CIAT, unpublished). The seed composition differences could have been as a result of genotype and environmental interaction thus contributing to the cultivar differences (Haq and Mallarino, 2005).

Seed oil content

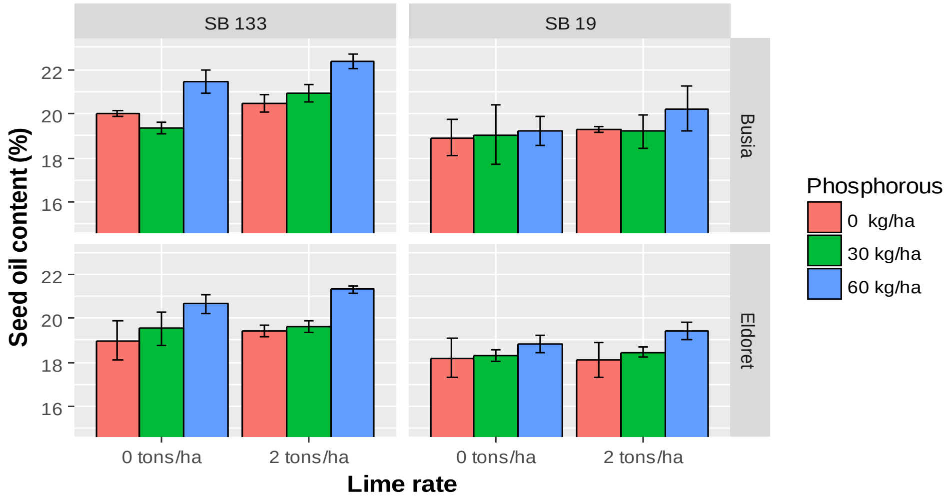

Figure 1: Seed oil content (%) of soybean cultivars SB 19 and SB 133 produced in Busia and Eldoret under different rates of phosphorous fertilizer and lime.

The effect of phosphorous fertilizer on seed oil was significant ($p< 0.05$) at both production sites (Figure 1). Application of phosphorous fertilizer resulted to an increase in seed oil by 0.74 % and 8.70 % in SB 133 when P was applied at 30 kg P ha^{-1} and 60 kg P ha^{-1}. Similarly, there was an increase in SB 19 by 0.67 % and 4.27 % when 30 kg P ha^{-1} and 60 kg P ha^{-1} was applied. The positive response to P application could be accredited to an increase in the availability of P for plant uptake. However, the increase between application at 0 kg P ha^{-1} and 30 kg P ha^{-1} was not significant at both sites, indicated the importance of residual P in the soil. High seed oil content was recorded at 60 kg P ha^{-1} where, SB 133 yielded 21.92 % in Busia and 20.98 % in Eldoret, whereas SB 19 recorded 19.72 % in Busia and 18.13 % in Eldoret. The positive influence of phosphorous fertilizer on seed oil content implies that P fertilization in acidic soils is crucial and effective. Similar results have been reported before by (Devi *et al.*, 2012; Shah *et al.*, 2001; Tanwar and Shaktawat, 2003), where, application of P fertilizer increased seed oil content, thereby suggesting the pertinent role it plays in the synthesis of phospholipids.

However, other scholars such as Shahid et al., (2009) did not report significant influence of P fertilization on seed oil, beside observations indicating an increase of seed oil with P application to a maximum at 100 kg P_2O_5 ha^{-1}. In other studies, Win *et al*., (2010) in Bangkok observed an increase of seed protein with P application to a maximum where further application resulted to a decline. Similarly, Krueger, Susana Goggi, Mallarino, & Mullen, (2013) observed a high seed oil content at 0 kg P ha^{-1} that declined with application of phosphorous fertilizer thus suggesting deleterious effect of P on seed oil when applied at high levels.

Application of lime at both production sites did not produce significant effect on seed oil of the soybean cultivars. However, application of lime resulted to an increase in seed oil for the soybean cultivars at Busia and Eldoret site. The lack of significant response indicated that lime did not dissociate adequately and wanted more time in the soil after application so as to neutralise the acidic cations and release the fixed P for plant uptake. Low soil *p*H allows for a higher saturation of aluminium ions thereby declining the saturation of basic cations such as calcium, magnesium and potassium, thus limiting the availability of essential crop nutrients.

In spite of the study showing a positive trend of increase of seed oil when P fertilizer was applied in acidic orthic ferralsols soils, the contrary scenarios observed from literature shows that further work needs to be done on different soil types and P levels so as to clearly determine the effect of phosphorous on seed oil.

Seed protein content

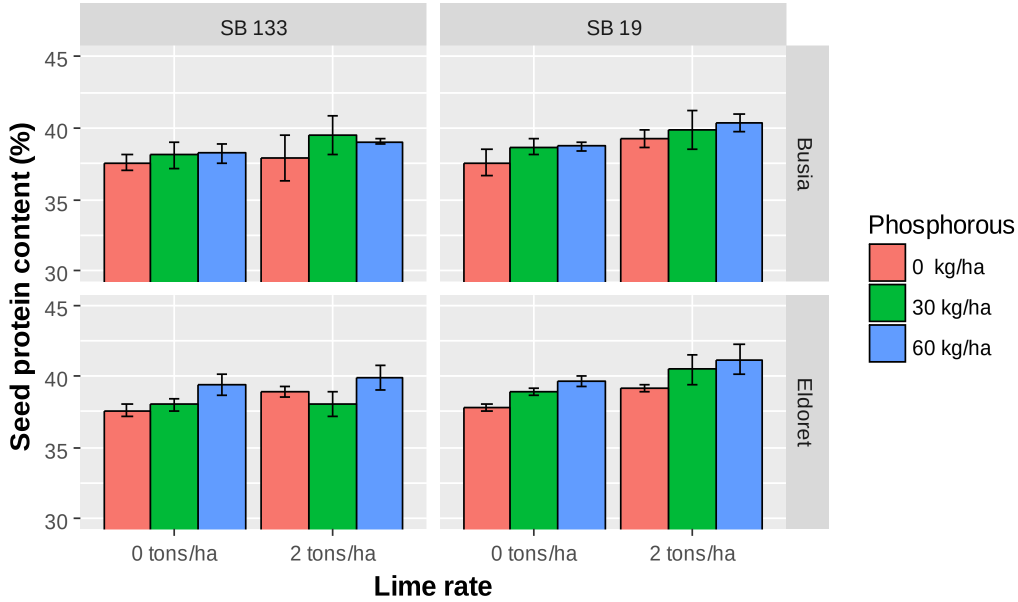

Figure 2: Seed protein content (%) of soybean cultivars SB 19 and SB 133 produced in Busia and Eldoret under different rates phosphorous fertilizer and lime.

Cultivar significantly influenced ($p < 0.05$) seed protein content for the soybean produced in Eldoret, whereas no significant differences were observed for the soybean grown in Busia (Figure 2). High seed protein content of 39.09 % in Busia and 39.52 % in Eldoret were recorded in SB 19 compared to SB 133 that produced 38.40 % in Busia and 38.63 % in Eldoret. These differences could be attributed to cultivar and environmental influences.

The influence of phosphorous fertilizer on seed protein was significant ($p < 0.05$) when the soybean cultivars were produced in Eldoret whereas no significant differences was observed in Busia (Figure 2). Generally, application of P fertilizer was observed to increase seed protein content in SB 19 by 2.71 % and 4.00 % when P was applied at 30 kg ha^{-1} and 60 kg ha^{-1} respectively. Similarly, an increase of seed protein by 1.13 % and 2.99 % was observed in SB 133 when 30 kg ha^{-1} and 60 kg ha^{-1} respectively. The positive influence of P fertilizer in these findings are in agreement with previous work by Devi *et al.*, (2012) in an experiment in India where it was determined that protein content increased with P application up to the highest value (35.20 %) at 80 kg P_2O_5 ha^{-1}. In the same country, (Sara *et al.*, 2013) working on acidic alfisols in North-eastern India observed an increase in protein with P application to maximum of 40.03 % at 90 kg P_2O_5 ha^{-1}. In another study, Shahid *et al.* (2009) reported an increase of seed protein with increased application of phosphorus fertilizer, where maximum of 41.33% seed protein was recorded at 100 kg P_2O_5 ha^{-1}. Analogous results have also been described before by (Deliboran *et al.*, 2011; Kaviani and Kharabian, 2008; Sharma *et al.*, 2007) indicating that application of phosphorous increases the amount of seed protein.

An increase in protein content following P fertilization confirms the pertinent role it plays in seed protein synthesis and crop physiological functions (Silva and Uchida, 2000). Phosphorous had been associated to improving N utilization in plants. It had been reported to improve the activities of *Bradyrhizobium japonicum*, which increases N fixation that is later used by the plants to synthesis proteins (Haru & Ethiopia, 2012; Tsvetkova & Georgiev, 2003). Thus, increasing the amounts of P in the soil improves the relationship between the root bacteria and the leguminous plants which promotes soybean plant's tolerance to environmental stress conditions.

The influence of lime on seed protein was significant (p< 0.05) when the soybean cultivars were grown in Busia and Eldoret (Figure 2). It was observed that liming resulted to an increase in seed protein content in SB 133 by 2.26 % and 1.67 % in Busia and Eldoret respectively. Similarly, the protein content in SB 19 increase with lime application by 3.86 % and 3.84 % when the soybean was produced in Busia and Eldoret respectively. Comparable results had been observed by Tanaka, Mascarenhas, & Miranda, (1991) that reported increased seed protein concentration when lime was incorporated in the study. These results indicate the multiple purposes of liming where it improves the soil condition by reducing soil acidity and enhancing releasing of fixed P. The released P is an essential energy source for nitrogen fixing bacteria and nutrition that supports the plants physiological processes, including seed protein synthesis. However, other studies did not report a significant effect of lime on seed protein when P was applied, such as the work of (Caires *et al.*, 2006) that studied in oxisol (clay-kaolinite-rhodic soils) with a *p*H of 4.6 in Brazil.

CONCLUSION

The results from this study showed that application of phosphorous fertilizer and lime increased the levels of seed oil and protein content of soybean. The application of P fertilizer at 60 kg P ha^{-1} and liming at 2 tons ha^{-1} was observed to produce a higher percentage of seed protein and oil content. It can therefore be deduced and recommended that improving the supply of phosphorous in the soil through application and soil amendment should be done to improve the quality of soybean seed.

ACKNOWLEDGEMENTS

We would like to express gratitude to National Commission for Science, Technology and innovation (NACOSTI) for the research grant, the Chemistry department of the University of Eldoret for their assistance with seed proximate analysis. Special thanks go to the project supervisors, Professor Julius Ochuodho and Dr. Lucas Ngode for their guidance in executing this study and the write up.

REFERENCES

Alkamoi, B., 2017. Influence of phosphorous fertilizer and lime on seed quality of soybean (Glycine max (L.) Merrill) in Western Kenya, (Unpublished Msc. Thesis, University of Eldoret).

AOAC, 2005. Official methods of analysis of association of official analytical chemists, 15th ed, Washington, DC. Arlington, Virginia, USA.
BIDCO, 2005. BIDCO (Business and Industrial Development Cooperation) Oil Refineries LTD. Thika, Kenya.
Caires, E.F., Churka, S., Garbuio, F.J., Ferrari, R.A., Morgano, M.A., 2006. Soybean yield and quality a function oflime and gypsum applications. Sci. Agric. 63, 370–379. https://doi.org/10.1590/S0103-90162006000400008
Deliboran, A., Sakin, E., Aslan, H., Mermut, A., 2011. Effect of water, phosphorous and magenesium doses on the quality and yield factors of soybean (Glycine max L.) in Harran plain conditions. Int. J. Phys. Sci. 6, 1484–1495.
Devi, K.N., Khomba Singh, L.N., Sunanda Devi, T., Nanita Devi, H., Basanta Singh, T., Khamba Singh, K., Singh, W.M., 2012. Response of Soybean [Glycine max (L.) Merrill] to Sources and Levels of Phosphorus. J. Agric. Sci. 4, 44–53. https://doi.org/10.5539/jas.v4n6p44
Gangasuresh, P., Muthulakshmi, E., Muthumari, S., 2010. Synergistic Efficiency of Phosphate solubilizer associated with Nitrogen fixer on the Growth of Soybean (Glycine max). Int. J. Biol. Technol. 1, 124–130.
Giller, K.E., 2001. Nitrogen Fixation in Tropical Cropping Systems: Chapter 1 - Tropical Environments: Climates, Soils, and Cropping Systems. CABI Publ. Wallingford, Oxon, GBR CABI Publ. 2001.
Gyaneshwar, P., Naresh Kumar, G., Parekh, L.J., Poole, P.S., 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245, 83–93. https://doi.org/10.1023/A:1020663916259
Hao, X., Cho, C.M., Racz, G.J., Chang, C., 2002. Chemical retardation of phosphate diffusion in an acid soil as affected by liming. Nutr. Cycl. Agroecosystems 64, 213–224. https://doi.org/10.1023/A:1021470824083
Haq, M.U., Mallarino, A.P., 2005. Response of soybean grain oil and protein concentrations to foliar and soil fertilization. Agron. J. 97, 910–918. https://doi.org/10.2134/agronj2004.0215
Jaetzold, R., Schmidt, H., 1983. Farm management handbook of Kenya, Natural conditions and farm information. Nairobi Kenya.
Jaetzold H., & S., 1983. Natural Conditions and Farm Management Information, in: Farm Management Handbook of Kenya. pp. 37–404.
Kamara, A.Y., Abaidoo, R., Kwari, J., Omoigui, L., 2007. Influence of phosphorus application on growth and yield of soybean genotypes in the tropical savannas of northeast Nigeria. Arch. Agron. Soil Sci. 53, 539–552. https://doi.org/10.1080/03650340701398452
Kaviani, B., Kharabian, A., 2008. Improvement of the nutritional value of soybean [Glycine max (L) Merr.] seed with alteration in protein subunits of glycinin (11S globulin) and β-conglycinin (7S globulin). Turkish J. Biol. 32, 91–97.
Krishnan, H.B., 2000. Biochemistry and Molecular Biology of Soybean Seed Storage Proteins. J. New Seeds 2, 1–25. https://doi.org/10.1300/J153v02n03_01
Krueger, K., Susana Goggi, A., Mallarino, A.P., Mullen, R.E., 2013. Phosphorus and potassium fertilization effects on soybean seed quality and composition. Crop Sci. 53, 602–610. https://doi.org/DOI: 10.2135/cropsci2012.06.0372
Mahmoodi, B., Mosavi, A.A., Daliri, M.S., Namdari, M., 2013. RTICLE The evaluation of different values of phosphorus and sulfur application in yield, yield components and seed quality characteristics of soybean (. Adv. Environ. Biol. 7, 170–176.
Morshed, R.M., Ahman, M.M.R., Ahman, M.A.R., 2008. Effect of Nitrogen on Seed Yield , Protein Content and Nutrient Uptake of Soybean (Glycine max L .). J. Agric. Rural Dev. 6, 13–17.
Myaka, F.A., Kirenga, G., Malema, B., 2005. Proceedings of the First National Soybean Stakeholders Workshop, .
Okalebo, J., Gathua, K., Woomer, P., 2002. Laboratory methods of soil and plant analysis: a working manual, 2nd ed, Tsbf. TSBF-CIAT and Sacred Africa, Nairobi, Kenya.
Onwonga, R.N., Lelei, J.J., Mochoge, B.B., 2010. Mineral Nitrogen and Microbial Biomass Dynamics under Different Acid Soil Management Practices for Maize Production. J. Agric. Sci. 2, 16–30.
Sanginga, N., Dashiell, K.E., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R.J., Tarawali, S., Asafo-Adjei, B., Menkir, A., Schulz, S., Singh, B.B., Chikoye, D., Keatinge, D., Ortiz, R., 2003. Sustainable resource management coupled to resilient germplasm to provide new intensive cereal-grain-legume-livestock systems in the dry savanna. Agric. Ecosyst. Environ. 100, 305–314. https://doi.org/10.1016/S0167-8809(03)00188-9
Sara, S., Morad, M., Reza, C.M., 2013. Effects of seed inoculation by Rhizobium strains on Chlorophyll content and protein percentage in common bean cultivars (Phaseolus vulgaris L.). Int. J. Biosci. 3, 1–8. https://doi.org/10.12692/ijb/3.3.134-141
Seguin, P., Zheng, W., 2006. Potassium, Phosphorus, Sulfur, and Boron Fertilization Effects on Soybean Isoflavone Content and Other Seed Characteristics. J. Plant Nutr. 29, 681–698. https://doi.org/10.1080/01904160600564477
Shah, P., Kakar, K.M., Zada, K., 2001. Phosphorus use-efficiency of soybean as affected by phosphorus, in: Horst, W.J. (Ed.), Plant Nutrition - Food Security and Sustainability of Agro-Ecosystems. Kluwer Academic Publishers, Netherlands, pp. 670–671.
Shahid, M.Q., Saleem, M.F., Khan, H.Z., Anjum, S. a, 2009. Performance Of Soybean (Glycine max L .) Under Different Phosphorus Levels And Inoculation. Pakistan J. Agric. Sci. 46, 237–241.

Sharma, S., Gambhir, S., Munshi, S.K., 2007. Changes in lipid and carbohydrate composition of germinating soybean seeds under different storage conditions. Asian J. Plant Sci. 6, 502–507.
Silva, J., Uchida, R., 2000. Essential Nutrients for Plant Growth :, Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture. College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa.
Tanaka, R.T., Mascarenhas, H.A.A., Miranda, M.A.C., 1991. Effect of liming on soybean protein and oil yield. Better Crop. Int. 7, 9.
Tanwar, S.P.S., Shaktawat, M.S., 2003. Influence of phosphorus sources, levels and solubilizers on yield, quality and nutrient uptake of soybean (Glycine max)-wheat (Triticum aestivum) cropping system in southern Rajasthan. Indian J. Agric. Sci. 73, 3–7.
Tsvetkova, G.E., Georgiev, G.I., 2003. Effect of phosphorus nutrition on the nodulation, nitrogen fixation and nutrient-use efficiency of Bradyrhizobium japonicum – soybean (Glycine max L. Merr.) symbiosis. Bulg. J. Plant Physiol. Special Is, 331–335.
Utsumi, S., Maruyama, N., Satoh, R., 2002. Structure function relation of soybean protein revealed by using recombinant systems. Enzyme Microb. Technol. 30, 284–288.
Vanlauwe, B., Ramisch, J., Sanginga, N., 2006. Integrated Soil Fertility Management in Africa, Principles, Practices and Developmental Process. Tropical Soil Biology and Fertility Institute of the International Centre for Tropical Agriculture, Nairobi. https://doi.org/10.1201/9781420017113.ch18
Wilcox, J., Shibles, R., 2001. Interrelationships among Seed Quality Attributes n Soybean. Crop Sci. 41, 11–14. https://doi.org/10.2135/cropsci2001.41111x
Win, M., Nakasathien, S., Sarobol, E., 2010. Effects of phosphorus on seed Oil and protein contents and phosphorus use efficiency in some soybean varieties. Kasetsart J. - Nat. Sci. 44, 1–9.