RUFORUM Working Document Series (ISSN 1607-9345), 2018, No. 17 (1): 637-642.
Available from *http://repository.ruforum.org*

Research Application Summary

Soybean (*Glycine max* (L.) Merr) response to biofix (*Bradyrhizobium japonicum*), trianum (*Trichordema harzianum* t-22) and phosphorus in poorly responsive soils of western Kenya

Chepkoech, C.*[1] & Njoroge, R.

University of Eldoret, Department of Soil Science, P. O. Box 1125-30100, Eldoret, Kenya
***Corresponding author:** chepkoechcaroline93@gmail.com

Abstract

Declining soybean yields in western Kenya due to low soil fertility is a threat to farmers' goals of generating income for improved livelihoods. Though commendable success has been observed after use of both phosphate (P) fertilizers and Biofix (Rhizobium inoculant), there is still a wide gap of attaining the potential yield in poorly responsive soils. This could be an indication that other factors such as soil physiochemical properties might be limiting. A greenhouse experiment was, therefore, conducted in University of Eldoret-Kenya, using two poorly responsive soils from Busia-North and Bungoma-Southwest to assess crop performance when Trianum, a Trichoderma inoculant was added to both Biofox and P fertilizer. While both soils had generally low nutrient status, Bungoma-Southwest soils were moderately acidic (5.4) compared to almost neutral pH in Busia-North (6.2). The experiment layout was a factorial in a completely randomized design, with sixteen treatments each replicated four times. The treatments consisted of (i) absolute control (with no fertilizer application), (ii) sole application of either inoculants or P and (iii) various combination of the three. Results indicated that the use of inoculants together with the P fertlizer improved in soybean yields (biomass) and nodulation in Busia-North soils compared to Bungoma-Southwest soils.

Key words: Inoculants, N-fixation, poorly responsive soils, soil pH, soybean biomass

Résumé

La baisse des rendements du soja dans l'ouest du Kenya en raison de la faible fertilité des sols menace les objectifs des agriculteurs de générer des revenus pour améliorer leurs moyens de subsistance. Bien qu'un succès louable ait été observé après l'utilisation à la fois d'engrais phosphatés (P) et de Biofix (inoculant Rhizobium), il y a encore un large écart à atteindre le rendement potentiel dans des sols peu réactifs. Cela pourrait indiquer que d'autres facteurs tels que les propriétés physico-chimiques du sol pourraient être limitatifs. Une expérience en serre a donc été menée à l'Université d'Eldoret-Kenya, en utilisant deux sols peu réactifs de Busia-North et Bungoma-Southwest pour évaluer la performance des cultures lorsque Trianum, un inoculant Trichoderma a été ajouté à la fois à l'engrais Biofox et P. Bien que les deux sols aient généralement un statut nutritif faible, les sols de Bungoma-Sud-Ouest étaient modérément acides (5,4) par rapport au pH presque neutre de Busia-Nord (6,2). La configuration de l'expérience était factorielle dans une conception complètement randomisée, avec seize traitements chacun répétés quatre fois. Les traitements consistaient en (i) un contrôle absolu (sans application d'engrais), (ii) une application unique soit d'inoculant ou de P et (iii) diverses combinaisons des trois. Les résultats ont indiqué que l'utilisation d'inoculant avec l'engrais P améliorait les rendements de soja (biomasse) et la nodulation dans les sols Busia-Nord par rapport aux sols Bungoma-Sud-Ouest.

Mots clés: Inoculants, fixation de l'azote, sols peu réactifs, pH du sol, biomasse de soja

Introduction

The demand for soybean grain both for food and fodder products has progressively increased over the years in Kenya. The grain is a major source of protein and edible oils. However, the local grain supply seldom meet the demand of 15,000 tonnes and thus prompt annual imports at an average of 5-6 tonnes (FAOSTAT, 2016). In spite of the entire supply gap, soybean production has become a lucrative farming business in western Kenya. Smallholder farmers in the region supply 80% of the 8,000 tonnes that is locally produced (Keino *et al.,* 2015). In fact, this has prompted the setup of a soybean processing plant at Malaikisi in Busia County. Although such an initiative offers a better chance of improved livelihoods in the region, farmers are grabbling with the persistent low yields at an average of 0.5 t ha^{-1} which is six times below a production potential of 3 t ha^{-1} (Mahas*i et al.,* 2011).

Recommendable efforts have been made towards improving soybean production including the application of mineral fertilizers and rhizobia inoculants with remarkable success in responsive soils (Thuita *et al.,* 2012). However, there are widespread cases where some soils do not respond to the former strategy and therefore, grain yields remain considerably low. Such soils are termed as 'poorly responsive soils', and offer no economic benefit to fertilizer use (Njoroge *et al.,* 2017). The same authors indicate 48% occurrence of such soils in western Kenya. For such cases, low yields mainly coincide with poor nodulation (Thuita *et al.,* 2012) Therefore, there is a need to design and evaluate appropriate fertilizer packages for such soils that would not only improve soybean yields but also render fertilizer use profitable.

In this context, we assessed the effect of two inoculants (Rhizobium and Trichoderma), alongside phosphate fertilizer on soybean biomass yield and nodulation. While Rhizobium is commonly known for its effectiveness in biological nitrogen fixation (Zahran, 1999), Trichoderma has an array of benefits; it promotes the production of plant growth hormones such as auxins, gibberellins and cytokinins, induces plant resistance to stress and diseases, solubilizes phosphate ions in the soil and also aids in nitrogen fixation (Zhang *et al.,* 2016).

Materials and Methods

Type of soils used. The soils were sampled from two individual farms in each study area with the consent of the farmers. The two fields had a gentle slope of < 5% and both soils are well drained. Both soils had low nutrient status especially N (<0.1 % total N) and P (<7 mg P ha^{-1}) and were are acidic. Soils from Busia-North are classified as Gleyic Arenosols while those in Bungoma South West are Eutric Cambisols (WRB, 2015).

Experiment design, treatment description, setup and maintenance. The experiment was a 2 3 factorial laid out in a completely randomized design (CRD) resulting in eight treatments per soil type and each replicated four times. The treatments structure consisted of an absolute control, sole application of the inoculants or P fertilizer at 30kg ha^{-1} and combinations of the inoculants with or without P fertilizer. For this study, a pot experiment was set in the greenhouse of Soil Science Department, University of Eldoret greenhouse located at 350 18' E and 000 34' for 10 weeks (27th November 2017 through 20th February 2018). During the experimental set up, 2 kg of dried and sieved soils through 5mm mesh was weighed into plastic. Treatments, where P was not applied, were watered at field capacity; 395ml and 502ml for Busia-North and Bungoma-Southwest soils, respectively. For the remaining pots, P fertilizer was first mixed with soil before watering. The soybean seeds (TGX 1740- 2F) were inoculated with a commercial inoculant (Biofix) which contains Bradyrhizobium japonicum strain before planting two of the inoculated soybean seeds in each pot, at the depth of 2½ cm. Young soybean plants were inoculated with Trianum (*Trichordema harzianum* T-22) 30 days after planting in solution form. Soil moisture was maintained by daily watering at field capacity for two consecutive weeks after which, a day was skipped until the end of the experiment. The pots were kept weed-free by hand weeding.

Data collection. Fresh and dry above ground biomass of soybean plants was recorded at the end of the experiment period (10th week). Numbers of nodules per plant were also recorded and categorized in large (>5mm), medium (2-5mm) and or small (<2mm) sizes. Further, using visual observation, nitrogen fixation was assessed by slicing the nodules into two halves. A nodule showing pink to red pigmentation was declared fixing while those with either white or green pigmentation were regarded as not fixing.

Statistical analysis. The effects of the different treatments on soybean biomass and nodulation were statistically evaluated using a general linear model where soil and fertilizer types were the fixed factors while replicates were considered as random factors. Genstat14th Edition statistical software was used for the analysis. Treatments means and their interactions were compared by using the standard errors of difference (SED) at P= < 0.005.

Results and Dissicusion

Effects of treatments on above ground biomass. There are significance differences in the above ground biomass due to treatments in the two soil types (Figure 1). For soil from Busia-North, sole application of P fertilizer, inoculating the soybean seeds with Biofix and or combining the two significantly increased above ground biomass by at an average of 49% above 9.5g obtained a control. For the same soil, combined use of the inoculants

(*Rhizobium* and *Trichoderma*) and P fertilizer resulted in the largest biomass increase of 6.8 g above the control. While inoculating the plants with Trianum alone has no effect on the soil biomass planted in soil from Busia-North, combining it together with P increased biomass production by 30% above the control. A significant increase of soybean biomass was observed only when P fertilizer was combined with rhizobium inoculation in the Bungoma-Southwest soil. The observed variations in soybean biomass may be attributed to the soil characteristics especially the soil pH. The soil sampled from Bungoma southwest were more acidic (pH of 5.4) compared to that from Busia- North (pH of 6.2). Rhizobia are bacteria species that do not thrive well in acidic conditions (Ferguson *et al.,* 2013). Therefore, their multiplication and survival may have been affected by such soil conditions and hence resulting in low N-fixation that translated into low biomass production for the Bungoma-Southwest soil. In addition, although it is commonly perceived that fungi thrive in acidic conditions, the Trichoderma species for this study requires as pH ranges between 6-6.5. (Benítez *et al.,* 2004) and hence also not being effective in soil from Bungoma- Southwest.

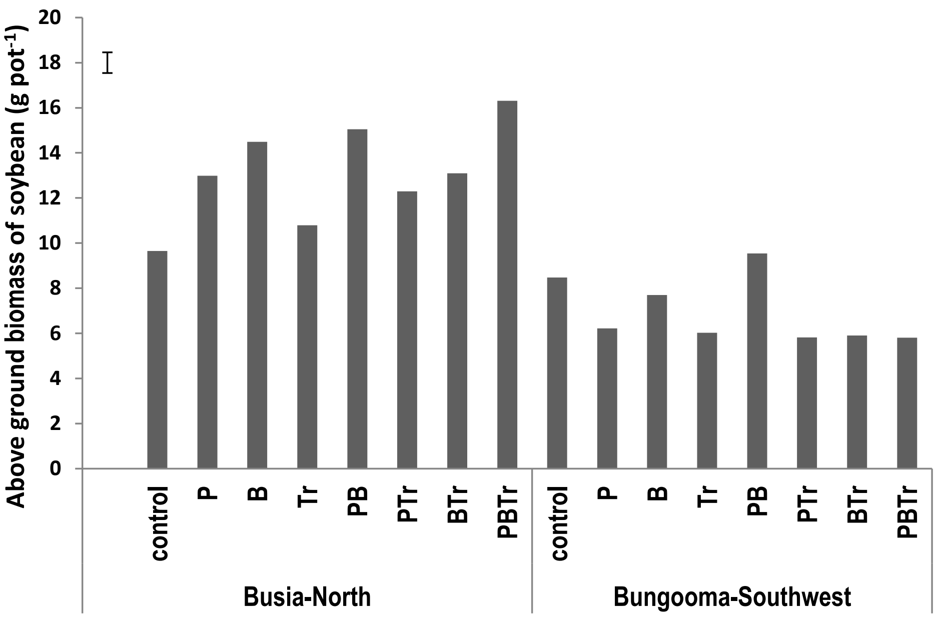

Figure 1. Effect P fertilizer, Biofix and Trianum inoculants on above ground biomass of soybean grown poorly responsive soils. Control= without fertilizer use, P = phosphorus (Triple Super Phosphate fertilizer), B = Biofix inoculant *(Bradyrhizobium japonicum)* and Tr = Trianum inoculant *(Trichoderma harzianum*).

Soybean nodulation. Table 1 shows that majority of the number of nodules were of large and medium sizes in all treatments resulted in except when all the three fertilizers (P, Biofix and Trianum) were used in Busia-North soil. For the same treatment, N-Fixation was 100% throughout the nodule size categories. Use of Trianum or without P application on the same soil had the least frequency of effective N-fixation irrespective of the number and size of the nodules. For the Bungoma-Southwest soil, both nodule numbers and N-fixation were low across the size categories and treatments compared to those observed in the Busia-North soil. For the same soil, combining Biofix and P resulted in the largest number of large and medium sized nodules and that was fixing N. Nodulation was absolutely absent in control and where Trianum was used with or without P for the soil from Bungoma-Southwest. Although sole application of P to the same soil resulted in a few number of nodules across the size categories, all of them were not fixing N. Soybean nodulation and N fixation shows a similar trend as observed with the above ground biomass indicating a synchrony between nutrient use and utilization efficiency.

Table 1. Frequency of N fixation in soybean as observed in three categories of nodule sizes and as effected by use P fertilizer applied together with or without Biofix and or Trianum inoculants

		Nitrogen fixation (%)		
		Large (>5mm)	Medium (2-5mm)	Small (<2mm)
Busia-North	Control	75 (7)	0 (2)	0 (0)
	P	75 (4)	50 (2)	100 (1)
	B	100 (12)	100 (10)	0 (4)
	Tr	100 (2)	100 (1)	0 (0)
	BP	100 (17)	100 (11)	0 (5)
	BTr	100 (8)	0 (3)	100 (1)
	PTr	100 (4)	75 (3)	100 (4)
	PBTr	100 (14)	100 (19)	100 (14)
Bungoma-Southwest	Control	0 (0)	0 (0)	0 (0)
	P	0 (2)	0 (1)	0 (2)
	B	25 (4)	25 (2)	0 (1)
	Tr	0 (0)	0 (0)	0 (0)
	BP	100 (9)	75 (5)	0 (4)
	BTr	50 (4)	100 (3)	10 (1)
	PTr	0 (0)	0 (0)	0 (0)
	PBTr	75 (5)	100 (3)	50 (2)

Numbers indicate percent frequency of N fixation while those in brackets indicate mean of nodules. Control = no fertilizer use, P = phosphorus (Triple Super Phosphate), B = Biofix ((*Bradyrhizobium japonicum*) and Tr = Trianum (*Trichoderma harzianum*).

Conclusion

Combined use of the inoculants and P application improves soybean production in poorly responsive soils of Busia-North soil. For such soils in Bungoma-Southwest soil inoculating seed with Biofix and applying P fertilizer still remain the best viable option. Therefore, use of Trianum in such soils has nod added value. This study indicates that an optimal soil pH is important for biomass production, nodulation and N fixation in soybeans. A field evaluation is recommended to validate the results obtained in this study.

Acknowledgements

Special appreciation goes to the Soil Science Department, University of Eldoret for allowing us to set the experiment using their facilities. We also acknowledge the assistance accorded by the laboratory technicians during data collection. This paper is a contribution to the 2018 Sixth African Higher Education Week and RUFORUM Biennial Conference.

References

Benítez, T., Rincón, A.M., Limón, M.C. and Codon, A.C. 2004. Biocontrol mechanisms of Trichoderma strains. *International Microbiology* 7: 249-260.

FAOSTAT, 2016. soybean production and imports in Kenya (2010-13). http://faostat3.fao.org.

Ferguson, B.J., Lin, M.-H. and Gresshoff, P.M. 2013. Regulation of legume nodulation by acidic growth conditions. *Plant Signaling and Behavior* 8: e23426.

Keino, L., Baijukya, F., Ng'etich, W., Otinga, A.N., Okalebo, J.R., Njoroge, R., Mukalama, J., 2015. Nutrients limiting soybean (*glycine max* l) growth in Acrisols and Ferralsols of Western Kenya. *PloS one* 10: e0145202.

Mahasi, J., Mukalama, J., Mursoy, R., Mbehero, P. and anlauwe, B. 2011. A sustainable approach to increased soybean production in western Kenya. 10th African Crop Science Conference Proceedings, Maputo, Mozambique, 10-13 October 2011. African Crop Science Society.

Njoroge, R., Otinga, A.N., Okalebo, J.R., Pepela, M. and Merckx, R. 2017. Occurrence of poorly responsive soils in western Kenya and associated nutrient imbalances in maize (*Zea mays* L.). *Field Crops Researc*h 210: 162-174.

Thuita, M., Pypers, P., Herrmann, L., Okalebo, R.J., Othieno, C., Muema, E. and Lesueur, D. 2012. Commercial rhizobial inoculants significantly enhance growth and nitrogen fixation of a promiscuous soybean variety in Kenyan soils. *Biology and Fertility of Soils* 48: 87-96.

WRB, I.W.G. 2015. World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps., World soil Resources Reports FAO, Rome.

Zahran, H.H. 1999. Rhizobium-Legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Reviews* 63: 968-989.

Zhang, S., Gan, Y. and Xu, B. 2016. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Frontiers in Plant Science* 7: 1-11.