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Research Application Summary

Soybean (*Glycine max*) and maize (*Zea mays*) production as influenced by rhizobia inoculation and cropping systems in western Kenya

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Abstract

The use of promiscuous varieties and commercial inoculants has been shown to increase soybean production and improve soil fertility. However, most cropping systems are characterized by indigenous bradyrhizobia whose populations are often low or ineffective and can be increased in the presence of the host legume for several seasons. The contribution of a commercial inoculant in soils with low levels of indigenous rhizobia to soybean and maize production in different cropping systems was evaluated in field experiments in three sites in Siaya County in Kenya using a completely randomized block design. Maize and soybean were planted as sole crops or in rotations for four seasons (short rain (SR) 2014, long rain (LR) 2015, SR 2015 and LR 2016). Soybean grain yields increased significantly under rotation when maize and soybean were planted in the LR and SR, respectively. A strong, significant and positive correlation between soybean grain yield and nodule weight was recorded. The inoculated and non-inoculated soybean-maize system recorded a rotational yield gain of 15.7% and 7.3% in LR 2015, 24.9% and 24% in LR 2016, respectively. These findings give a scientific validation of the yield responses and seasonal implications of the choice of crop that the farmer makes in a rotation. Further research on economic profitability and isolation of the native strains and bacterial diversity in the study soils is needed so as to validate the results on nodulation and yields.

Key words: Inoculation, Kenya, native rhizobia, rotational yield gain, Siaya County

Résumé

Il a été démontré que l'utilisation de variétés peu fréquentées et d'inoculants commerciaux augmente la production de soja et améliore la fertilité des sols. Cependant, la plupart des systèmes de culture sont caractérisés par des bradyrhizobies indigènes dont les populations sont souvent faibles ou inefficaces et peuvent être augmentées en présence de la légumineuse hôte pendant plusieurs saisons. La contribution d'un inoculant commercial dans les sols à faibles niveaux de rhizobium indigène à la production de soja et de maïs dans différents systèmes de culture a été évaluée dans des expériences sur le terrain dans trois sites du comté de Siaya au Kenya en utilisant une conception en blocs complètement aléatoire. Le maïs et le soja ont été plantés comme cultures uniques ou en rotation pendant quatre saisons (pluie courte (SR) 2014, pluie longue (LR) 2015, SR 2015 et LR 2016). Les rendements en grains de soja ont augmenté de manière significative pendant la rotation lorsque le maïs et le soja ont été plantés respectivement dans la LR et la SR. Une corrélation forte, significative et positive entre le rendement en grains de soja et le poids des nodules a été enregistrée. Le système soja-maïs inoculé et non inoculé a enregistré un gain de rendement en rotation de 15,7% et 7,3% dans LR 2015, 24,9% et 24% dans LR 2016, respectivement. Ces résultats donnent une validation scientifique des réponses de rendement et des implications saisonnières du choix de la culture que l'agriculteur fait dans une rotation. Des recherches supplémentaires sur la rentabilité économique et l'isolement des souches indigènes et de la diversité bactérienne dans les sols étudiés sont nécessaires afin de valider les résultats sur la nodulation et les rendements.

Mots clés : Inoculation, Kenya, rhizobium indigène, gain de rendement en rotation, comté de Siaya

Introduction

The population of sub-Saharan Africa (SSA) is projected to double in the next 40 years and increases in food production are much needed (FAO, 2017). As the potential to expand agricultural land is limited in many areas with high population densities, sustainable intensification of agricultural production is crucial (Vanlauwe *et al*., 2014). A potential pathway for sustainable intensification is the inclusion of food grain legumes in the cropping systems (Vanlauwe *et al*., 2014). Grain legumes fix atmospheric nitrogen gas (N2) that can contribute to the nitrogen (N) economy of fields, provide other rotational benefits to subsequent crops, produce in situ high quality organic residues with a high N concentration and a low C to N ratio, and thereby contribute to integrated soil fertility management (ISFM) (Ojiem *et al*., 2006). Legumes also have important nutritional value in terms of protein, amino acids and micro-nutrients. Even with these documented benefits of grain legumes, their yields remain disappointingly low in many cropping systems. For instance, Soybean grain yields have been reported to be < 1 Mg ha^{-1} (Mhango *et al*., 2013), compared to other tropical regions which produce >4 Mg ha^{-1}soybean. In western Kenya, for instance, soybean grain yields remains low with average yields of 600 kg ha^{-1} against the potential yield of 3,000 kg ha^{-1}. As an introduced crop, soybean often nodulates poorly in many tropical soils and inoculation with selected commercial *Bradyrhizobium japonicum* strains is frequently necessary for N2 to be fixed.

Owing to challenges associated with inoculum production, handling, and storage, breeding for promiscuity was proposed. However, the use of promiscuous soybean does not yield as expected because considerable evidence indicates that in many locations, indigenous rhizobial populations are present in the soils and they pose a barrier to the benefits of inoculation (Klogo *et al*., 2015). Even though the populations of the indigenous rhizobia have been reported to be low, their availability in sufficient populations in the soils can be increased by including the host legumes into cropping systems for a period of time.

These scenarios present an opportunity to conduct a definitive probe on the competitiveness of a commercial strain in soils with low populations of indigenous rhizobia under different cropping systems using a promiscuous soybean variety. Comparisons between indigenous rhizobia and recommended strains under different cropping systems and seasons are useful in assessing the competition or complementarily of the strains under ideal farmer conditions. Conclusions derived from this study would be used as a platform for advocating for improvements of locally produced inoculants.

Materials and methods

Description of experimental site, layout, design and treatments. On farm experiments were carried out at three sites (Ugunja, Wagai and Ukwala) of Siaya County in Kenya in a randomized complete block design with seven treatments replicated four times per site. The study was carried out in the Short Rains (SR) 2014, LR 2015, SR 2015 and Long Rains (LR) 2016 cropping seasons. The plots measured 4.5 x 5 m (gross plot of 22.5 m^2) with a 1 m inter-plot spacing. The experimental treatments are presented in Table 1.

In plots where the test crop was maize, hybrid variety 306 from Western Seed Company commonly known as IR (Imidazolinone-resistant) maize was used. Two seeds of maize were planted per hill. Two weeks after planting, thinning was done to ensure one plant per hill. The inter row and intra row spacing for maize was 75cm x 25cm, respectively, to give a total of 53 333 plants ha^{-1}. The fertilizer source for continuous mono maize cropping system at planting and top dressing was Mavuno plant and Mavuno top at a rate of 60 kg P ha^{-1} and 60 kg N ha^{-1}, respectively. Top dressing for maize was done at four weeks after planting when the maize had attained the height of about 45 cm. Under the rotational cropping system, maize was planted with Sympal fertilizer (Containing 0:23:15 [N: P_2O_5:K_2O] + 10 CaO + 4 S + 1 MgO + 0.1 Zn) since nitrogen was expected to be supplied to the maize crop from the preceding soybean crop. In plots where the test crop was soybean, a promiscuous soybean variety TGx1740-2F (locally referred to as SB19) with medium maturity rate of 95–100 days was used. Soybean was planted at 50 cm × 5 cm spacing to give a total of 400,000 plants ha^{-1}. Legume fix was the commercial inoculant used in the different cropping systems. It was applied at the rate of 10 g per 1 kg of soybean seed. Legumefix has a peat-based formulation with at least 1×10^9 CFU g^{-1} of *Bradyrhizobium japonicum* strain 532c. Inoculation was done at planting as a seed coating following the instructions given on products by the producing company. Weeding was carried out three times in all the plots in every season.

Table 1. Treatment structure for the different cropping systems, inoculant and fertilizer application in the study sites

Treatment number	Cropping system	Inoculation to soybean	Fertilizer application to maize
1	Continuous soybean	+	-
2	Continuous soybean	-	-
3	Continuous soybean*	+	-
4	Maize-soybean rotation	+	60 kg P
5	Continuous maize	-	60 kg P and 60 kg N
6	Soybean-maize rotation	+	60 kg P
7	Soybean-maize rotation	-	60 kg P

Soil sampling and determination of the physical and chemical properties. Soil samples were taken up to a depth of 20 cm in each replicate for characterization. The soils were submitted for analysis of pH (H_20), Extractable P (mg kg^{-1}), Exchangeable K ($cmol_ckg^1$), Exchangeable Ca ($cmol_ckg^1$), Exchangeable Mg ($cmol_ckg^1$), Mn (mg kg^1), Fe (mg kg^{-1}), Cu (mg kg^{-1}), Zn (mg kg^{-1}), C (%), N (%) and particle size at MEA laboratories limited using methods described by Okalebo *et al*. (2002). A fresh soil sample was used in the estimation of rhizobia in the soils using the most probable number technique.

Assessment of nodulation and above ground biomass accumulation. Soybean samples were collected at mid pod filling stage by destructive sampling from a 0.5 m row long section of the net plot and number of soybean plants within the section were counted and recorded. The fresh weight of the above ground biomass was determined and later dried at 65 °C and the dry weight was recorded.

Harvesting of soybean and maize. At physiological maturity of soybean and maize were harvested within the effective area, omitting the outer lines and the sampled row. Weights were taken at harvest and after oven drying to determine grain yields per plot and extrapolated to hectare basis. The rotational effect was calculated as the yield of maize following a legume (inoculated or non-inoculated) minus the yield following maize.

Statistical analysis. Data from the experiment were entered into an excel spreadsheet, checked for normality of errors and homogeneity of variances prior to the statistical analyses. Data on nodule weight, shoot biomass and grain yields were analyzed per site using two-way analysis of variance (ANOVA) and interaction determined using GENSTAT 14th edition software. When differences among treatments were detected, standard error of means at $P<0.05$ was performed to compare treatment means. Simple linear regression was done for nodule weight and shoot dry weights. The data was then presented in graphs and tables.

Results and discussion

Initial soil characterization at the study sites. The mean soil pH values of the study sites was extremely acidic (pH<4.5) according to grading levels for soil acidity described by Kanyanjua *et al*. (2002). This is a characteristic feature of Ferralsols which impedes the rhizobia from creating the nod factor and form nodules. Under these conditions, important micro nutrients including molybdenum that are cofactors for nitrogen fixation may become unavailable. Soil acidity is associated with Aluminium (Al), Hydrogen (H), Iron (Fe) and Manganese (Mn) toxicities to plant roots in the soil solution and corresponding deficiencies of the available P, Molybdenum (Mo), Ca, Mg and K (Jorge and Arrunda, 1997). These acidic conditions could have resulted in low nodulation by the native soil rhizobia during the SR 2014 season.

The extractable P was 28.7, 23.7 and 26.8 (mg kg^{-1}) for Ugunja, Ukwala and Wagai, respectively. This low P is associated with high levels of P sorption through their reaction with phosphate ions to form insoluble compounds. The infection of a leguminous root by *Bradyrhizobium* and N_2 fixation has high energy requirements. However, there was basal application of P to soybean to counteract these conditions (Tidsale *et al*., 1990). The exchangeable cations were in the ranges of 0.01-0.28, 0.49-1.97 and 0.07-1.65 $cmol_c$ kg^1 for K^+, Mg^{2+} and Ca^{2+}, respectively. These ranges for exchangeable cations in the three study sites could be rated as very low to low according to Okalebo *et al*. (2002). The low levels of exchangeable cations reported in the study sites imply that the soils have very low percent base saturation and high exchangeable acidity on the cation exchange sites.

The total nitrogen (N%) in the study soils ranged from 0.0 8-0.17%.The low total nitrogen reported in the study sites could have resulted to enhanced nodulation and biomass yield by inoculation with Legumefix in the first (SR 2014) season. When the nitrogen levels in the soil are high, nodule formation is negatively affected. These findings are in consistent with Hungria *et al*. (2003) who noted that low level of N in the soil can enhance nodule formation and increase grain yields.

The population estimates of indigenous rhizobia in the soil was 0.364×10^3, 0.328×10^3 and 0.436×10^3 per gram of soil in Ugunja, Ukwala and Wagai, respectively. This study revealed a small amount of the *Bradyrhizobium* in the study soils.

Effects of cropping systems and inoculation on soybean grain yields. During the SR 2014 season, the inoculated treatment performed significantly better than the non-inoculated in all the sites with Wagai site recording significantly higher grain yields in comparison to other sites [1398 kg ha^{-1} in comparison to 988 kg ha^{-1} and 890 kg ha^{-1} for Ugunja and Ukwala, respectively]. The inoculated soybean treatment had the largest mean grain yield of 1198 kg ha^{-1} in comparison to 985 kg ha^{-1} for the non-inoculated soybean treatment (21.6% grain yield increase). This yield increase could be an indication of low populations and ineffective native rhizobia. These findings support those of Thuita *et al*. (2012) who reported higher grain yields and N_2 fixation while working with the commercial inoculants.

On average, the rotational systems performed better than the mono-cropping systems. A possible reason for the better performance of rotational than mono cropping system would be the differences in carbon to nitrogen ratios in the two study crops. Soybean plant has low carbon to nitrogen ratio in comparison to maize. Rotating it with crops of high carbon to nitrogen ratios has been cited in helping a diverse community of soil micro-organisms along with improvement in soil tilth and can lead to more sustainable soil structure and decrease in susceptibility to soil erosion. This is important especially in the tropics where N mineralization occurs too early in the growing season for plants use.

The significant increase in grain yields with the non-inoculated cropping systems performing better than the inoculated system could also be attributed to better adaptation of rhizobia to the local environment. The indigenous rhizobia have been described as being persistent (Fening and Danso, 2002), well adapted to local conditions and therefore can compete successfully at the expense of commercial strains for nodule occupancy and nitrogen fixation. This is especially true when the host legume is included in the cropping system. In our study the host legume was a promiscuous variety and has been shown to nodulate effectively with indigenous rhizobia if their populations are high.

Maize grain yield and rotational benefits under different cropping systems and seasons. Larger maize grain yields were recorded under the rotational system in comparison to continuous mono maize system and could be attributed to other non-N benefits. In our study we did not measure N uptake by maize and the yield increase is likely to be a combination of N and non-N factors (Franke *et al*., 2017). Non-N rotational benefits could have included increased availability of P to maize following legumes, suppression of root nematodes or other benefits (Franke *et al*., 2017).

Crop rotation with legumes improves soil physical, chemical and biological conditions (Giller, 2001). They can improve soil structure, water holding capacity, humus content, and organic carbon content (SOC) of soils (Giller, 2001). Maize performed better during the long rain season and soybean during the short rain season. These findings concur with those of Chianu *et al*. (2011) in western Kenya that farmers consider legumes as the most suitable crop to be grown during the short rain season even as pure stands because maize performs poorly during this cropping season. The findings could further ascertain that growing soybean after maize is a better system than a mono cropping system. The reported findings are in consonance with Zingore (2011) who reported a rotational yield gain of 25% in maize after groundnuts in comparison to continuous maize.

Table 2. Soybean grain (kg ha^{-1}) as affected by cropping systems and inoculation in Siaya County, Kenya

Season	Treatment (Trt)	Ugunja	Ukwala	Wagai	Mean
SR2014	1	1103	938	1554	1198
	2	873	841	1242	985
	Mean	988	890	1398	1090
	SED: Trt =75.1**; Site =91.9***; SiteßTrt =130.0*				
LR2015	1	1003	1602	1874	1493
	2	770	1490	1741	1334
	3	1003	1760	2519	1761
	4	1214	2002	2033	1750
	Mean	998	1714	2042	1585
	SED: Trt = ns; Site = 211.6*; SiteßTrt =ns				
SR2015	1	1653	2358	1569	1860
	2	2365	2219	2974	2586
	3	1523	1665	1597	1479
	6	3067	2142	2866	2692
	7	2508	2458	2977	2648
	Mean	2223	2169	2437	2253
	SED: Trt =241.7***; Site =ns; SiteßTrt =418.7*				
LR2016	1	1357	1510	1592	1486
	2	813	1236	1546	1198
	3	1109	916	1794	1273
	4	1177	1006	1540	1241
	Mean	1114	1167	1618	1300
	SED: Trt =ns; Site =168.7*; SiteßTrt =337.4				

Treatments: 1= Continuous soybean (inoculated); 2 = Continuous soybean (non-inoculated); 3 = Continuous soybean (inoculated only during the LR 2014 season); 4 = Maize-soybean (inoculated); 6 = Soybean-maize (inoculated); 7 = Soybean-maize (non-inoculated); SED = standard error of difference between means of treatments; stars represents significance levels, thus, *,**,***=significant at $p<0.05$, 0.01 and 0.001, respectively; ns=not significant

Conclusions and recommendations

The rotational system resulted in superior and robust increase in grain yields in comparison with the mono cropping system irrespective of whether the inoculant was used or not since the indigenous rhizobia was able to build up. The choice of legume in the rotational system should bear in mind the season since higher rotational benefits are accrued when the legume is included during the SR season. Studies on economic profitability of the different cropping systems and studies to identify the profile and diversity of these native rhizobia that nodulate soybeans in the soils of the study area are recommended.

Table 3. Maize grain yields and the effects of the preceding inoculated or non-inoculated soybean relative to mono cropping system

Season	Treatment (Trt)	Ugunja	Ukwala	Wagai	Mean
SR2014	5	2646	3108	1788	2514
	SED: Site = 259.3**				
LR2015	5	3349	5103	1540	3330
	6	3408	5698	2740	3949
	7	4972	5552	2781	4435
	Mean	3909	5450	2353	3909
	SED: Trt = ns; Site =505.1***; SitexTrt =ns				
	% change	1.7	10.4	43.8	15.7
	% change	32.6	8.1	44.6	24.9
SR2015	4	2207	3413	1171	2263
	5	1792	3086	1220	2032
	Mean	1999	3249	1195	2148
	SED: Trt =ns ; Site=373.0***; SitexTrt =ns				
	% change	18.8	9.6	-4.2	10.2
LR2016	5	2690	4583	925	2732
	6	2732	4123	1991	2948
	7	4214	4561	2005	3593
	Mean	3212	4422	1640	3092
	SED: Trt =ns ; Site =501.9***; Site xTrt =ns				
	% change	1.5	-11.2	53.5	7.3
	% change	36.2	-0.5	53.9	24

Treatment (5 = Continuous mono maize; 6 = Soybean-maize (inoculated); 7 = Soybean-maize (Non-inoculated); Change in grain yield was calculated relative to treatment 5 for the respective season; SED = standard error of difference between means of treatments; stars represents significance levels, thus **, *** = significant at $p<0.01$ and 0.001, respectively; ns=not significant

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